WARFARIN PHARMACOGENOMICS IN A HISPANIC POPULATION: A CANDIDATE SNP STUDY

By

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As members of the Master's Committee, we certify that we have read the thesis prepared by ***Justin B. Kaye***, titled ***Warfarin Pharmacogenomics in a Hispanic Population: A candidate SNP study*** and recommend that it be accepted as fulfilling the thesis requirement for the Master's Degree.

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Abstract

Background: Warfarin remains one of the most widely prescribed anticoagulants but is also a leading cause of adverse drug reactions. Genotype-guided warfarin dosing algorithms enable accurate dose estimation, potentially leading to improved safety and efficacy. However, genotype-guided dosing algorithms were developed primarily in populations of European descent and limited data are available regarding single nucleotide polymorphisms (SNPs) that significantly influence warfarin dose in Hispanic populations.

Research Aim: The objective of this study was to determine whether clinical factors and SNPs previously associated with stable warfarin dose variability in populations of European and Hispanic descent accurately predicted warfarin stable dose in a Hispanic population.

Study Design: Self-reported Hispanic and Latino patients on stable warfarin dose (defined as the same dose for at least two clinic visits separated by at least two weeks) were recruited.

Methods: Candidate SNPs, including *CYP2C9**2/*3, *VKORC1*-1639G>A, *CYP4F2**3, and *NQO1**2, were genotyped and clinical data were collected using a survey and the electronic medical record. Stepwise linear regression was performed to determine variables that significantly predicted square root of weekly warfarin dose.

Results: A total of 76 patients of primarily Mexican American ancestry participated. All SNPs were within Hardy-Weinberg Equilibrium. The final stepwise regression model incorporated six variables, which explained 71% of the variability in warfarin weekly dose requirements. Significant predictors included weight (R^2=0.287, p<0.0001), age (R^2=0.143, p<0.0001), amiodarone use (R^2=0.067, p=0.0005), and prior stroke (R^2=0.025, p=0.02). Significant SNPs included *VKORC1*-1639A (R^2=0.152, p<0.0001), and *CYP2C9**2/*3 (R^2=0.032, p=0.02). *CYP4F2*3* and *NQO1**2 did not significantly impact warfarin dose requirements despite previously published associations in Hispanic populations.

Conclusion: These findings suggest that clinical and genetic predictors of warfarin weekly dose requirements are similar among populations of European descent and Hispanic populations with Mexican American ancestry. These results require replication and validation in independent cohorts with similar ethnicity, but advance our understanding of influences on warfarin dose variability among different race/ethnic groups.

Background

Warfarin, also known as Coumadin, is the most prescribed oral anticoagulant in the United States (US) at 34 million prescriptions per year.[1] Warfarin is primarily prescribed to prevent thrombosis in patients with atrial fibrillation, heart valve replacement, or risk factors for thromboembolic disease.[2]

The functional discovery of warfarin has an interesting background. The Wisconsin Alumni Research Foundation (WARF) were tasked with establishing why local dairy cows were suddenly dying from the common dehorning procedure used for safety reasons. Following further investigation, the research team determined the cattle were eating spoiled sweet clover hay which contained a compound known as Dicoumarol.[3] The blood-thinning compound was further derived into a more potent form called 'warfarin' and marketed as an effective rodenticide. Eventually, the blood thinning effects of warfarin were found to be reversed with vitamin K and its transition to clinical care took place in 1954.[3] By 1978, warfarin inhibition of vitamin K epoxide reductase enzyme (VKORC1) was found to be the drug's mechanism of action that caused thinning of the blood.[4] Initial strategies to dose warfarin were largely based on clinical observation where population average doses to achieve stable warfarin therapy were approximately 5 mg/day.[5]

As of today, warfarin remains a mainstay in anticoagulation therapy. Despite its effectiveness in reducing the risk of clotting, warfarin also causes severe bleeding and has been listed in the top 10 of the FDA's Adverse Event Reporting System for the past several decades.[6] Warfarin's propensity to cause bleeding is partly due to the high inter-individual variability in dose response. Clinical observations of warfarin therapy have

noted patients may require more than 10 fold differences in dose to achieve the same therapeutic anticoagulation.[5]

Patients who begin warfarin therapy require close monitoring of their blood clotting level, which is measured by the International Normalized Ratio (INR).[7] For most patients on long-term therapy, the INR goal is to reach a stable therapeutic blood clotting level between the levels 2 and 3, with healthy individuals having an INR of approximately 1. Patients with INR levels above 3 have increased risks of major hemorrhage while patients with INR levels below 2 experience increased risk of thromboembolism.

Many factors are known to influence warfarin dose response and thereby interfere with the stability of therapeutic INR levels. Recent studies have found clinical and genetic factors that contribute to the variation in warfarin response between patients.[8] Clinical factors known to affect warfarin dose response include age, gender, diet (i.e. leafy greens with high Vitamin K), interacting drugs, and body mass index. Genetic factors associated with warfarin dose variability include variants in genes that encode for proteins involved in warfarin metabolism and the vitamin K cycle. Instead of prescribing all patients a fixed starting dose of 5mg/day, which can lead to adverse events in some patients, clinical and genetic information known to affect warfarin dose may be harnessed to more accurately predict the right dose.

The first half of this manuscript will detail the molecular aspects of vitamin K contribution to clotting, the enzymes that influence warfarin pharmacology, variants in genes that effect warfarin dose, and the considerations regarding race/ethnicity in genotype-guided warfarin dosing algorithms. The second half will describe the methods

and findings of my thesis project regarding the influence of warfarin dose requirements of 5 candidate single nucleotide polymorphisms (SNPs) in a clinical study of Hispanic patients on stable warfarin doses. Lastly, future directions and conclusions will be offered to explain the real-world impact the study will bring toward improving warfarin management to patients of Hispanic descent.

Vitamin K Cycle and contribution to clotting

Vitamin K is a necessary cofactor in converting upstream clotting factors to their active form. These vitamin K-dependent clotting proteins include factors II (prothrombin), VII, IX, and X.[9] Once activated, the clotting factors contribute to both the intrinsic and extrinsic clotting cascades that will result in the formation of fibrin, the end product of the coagulation cascade. Fibrin contributes to stopping excessive bleeding that takes place in vascular endothelial cells by forming a stable mesh network around platelets. Individuals acquire the fat soluble vitamin K compound in two forms.[9] Vitamin K1, also known as Phylloquinone is a natural molecule that is synthesized in green leafy plants such as cabbage, cauliflower, and spinach. Vitamin K2, or Menaquinone, is a form that is made by commensal gut flora and serves as an added vitamin K supplement to the dietary intake from green leafy vegetables.

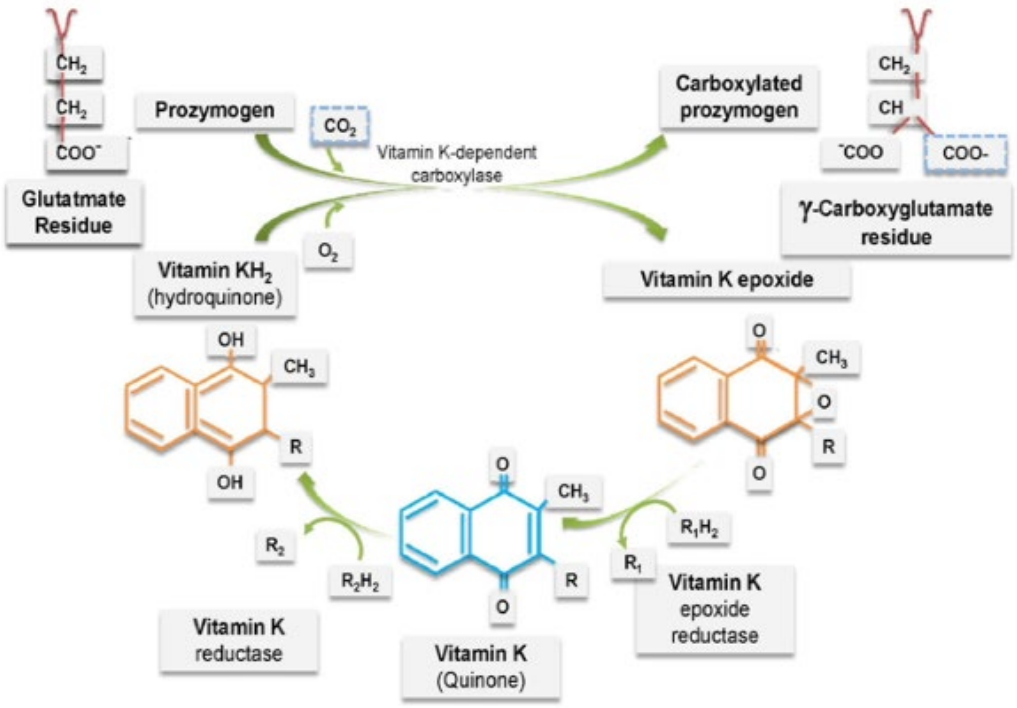
Following its intake, vitamin K will be absorbed along with fat chylomicrons into the bloodstream. From the bloodstream, vitamin K is transported into the liver along with low-density lipoprotein, where it can serve as a cofactor in the carboxylation and activation of the previously mentioned clotting factors. The clotting factors responsible for activating the downstream coagulation cascade are synthesized in the liver in the form of inactive precursor proteins known as prozymogens. To become active, these

clotting factors must be post-translationally modified through the carboxylation of glutamic acid residues. This reaction involves the conversion of glutamate (Glu) to carboxyglutamate (Gla) and is dependent on the enzyme gamma-glutamyl carboxylase (GGCX).[9]

The carboxylation reaction mechanism takes place in the endoplasmic reticulum membrane space and involves a propeptide recognition site (amino acid 495-513) within the GGCX enzyme binding to a conserved 18 amino acid region within the clotting factor proteins.[10] These conserved regions are located upstream of domain regions that contain the target glutamate (Glu) residues. Upon binding of the GGCX propeptide, a conformational change occurs which leads to increased affinity and binding of GGCX to vitamin K hydroquinone cofactor. The vitamin K hydroquinone cofactor is necessary in that it serves as the electron donor molecule. Following electron donation, vitamin K hydroquinone is oxidized to vitamin K2,3-expoxide. The electron donor transfer allows GGCX enzyme to catalyze the exchange of hydrogen on the glutamate residue for a carboxyl group, resulting in carboxyglutamate (Gla). This process will continue where the next unmodified glutamate (Glu) residue will take position in the GGCX enzyme active site and with the help of vitamin K hydroquinone cofactor, additional carboxylation of Glu residues will take place. When all of the Glu residues have been converted to Gla residues, the propeptide sequence of GGCX will decrease in affinity and the release of the active site from the clotting factors will occur. The activated clotting factors will then be transported to the plasma membrane of the hepatocyte through the Golgi transport system where they are exocytosed into the vascular system.[10]

Following the carboxylation of glutamic acid residues present in the peptide chains, the clotting factors will essentially contain two negative carboxyl groups on the peptide side-chain. The addition of the negative charge, provided by Gla, allows the clotting factor proteins to become active and induces their ability to bind calcium in the blood.[10] The addition of calcium to the peptide chains, allows the clotting factor proteins to bind endothelial cell membrane components such as phospholipids. The binding of endothelial cells enables the clotting factors to localize to an area in the vascular system where damage to cell membranes and hemorrhaging is present. As a result, fibrin can be formed and the production of stable clots to stop bleeding will occur. A schematic of the vitamin K cycle is shown in Figure 1.

Figure 1. The vitamin K cycle



Furie B. N Engl J Med. 2013; 369(24):2345-6

Molecular mechanisms of important enzymes involved in vitamin K activation cycle

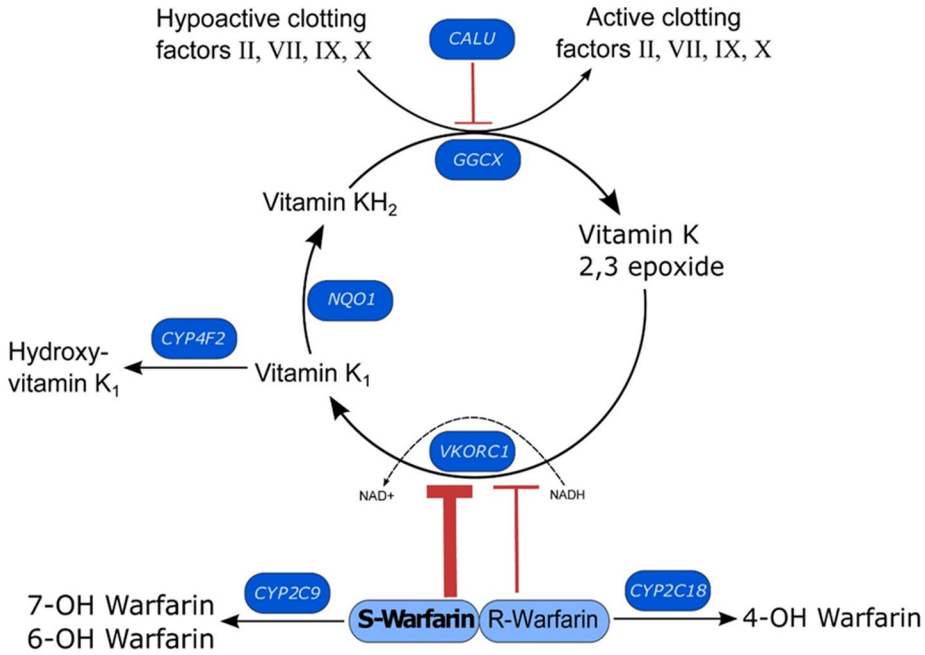
In order for the continued activation of clotting factors to respond to future bleeding events, oxidized vitamin K2,3-epoxide must be recycled back to its reduced hydroquinone form through the vitamin K cycle.[9] It is well known in the event of diminished vitamin K presence or activity, either through hereditary or environmental conditions like malabsorption, carboxylation of glutamate residues on clotting factors will not take place. Due to the inactivation of clotting factors, severe bleeding events can take place and may be reversed with high doses of vitamin K. However, in normal processing of the vitamin K cycle, the oxidized form, vitamin K2,3-expoxide, will be reduced to vitamin K1 (quinone form), which is catalyzed by vitamin K epoxide reductase (VKORC1) enzyme.

Upon uptake into hepatocytes or from reduction by VKORC1, vitamin K1 quinone, which represents the natural form in blood such as Phylloquinone or Menaquinone, will be further reduced to vitamin KH_2, a reduced quinone form known as hydroquinone. In this reaction, two hydrogens are added to the carbonyl oxygens located on the quinone group. The reduction and transfer of the hydrogen atoms that initiates the change from K1 to KH_2 can be catalyzed by either VKORC1 (in this case vitamin K reductase) or NAD(P)H quinone dehydrogenase 1 (NQ01) and can be additionally facilitated by the donation of hydrogens in dithiol groups such as glutathione. Through this feedback-loop cycle, oxidized vitamin K can be recycled back to the active hydroquinone form KH_2 where it can participate as a necessary cofactor in the activation of additional clotting factors through carboxylation of glutamate peptide

residues by gamma-glutamyl carboxylase (also called vitamin K-dependent carboxylase).[9] The integrated vitamin K cycle process is shown above in Figure 1.

Additional enzymes that also play a role in the regulation of the vitamin K cycle include Cytochrome P450 family 4 subfamily F member 2 (CYP4F2) and calumenin (CALU). CYP4F2 contributes as a counter regulator to VKORC1 by oxidizing excess vitamin K1 into a hydroxy-vitamin K derivative.[11] The proposed function of CYP4F2 is to serve as a way to limit the excess accumulation of vitamin K1, possibly resulting from diets high in vitamin K. Calumenin protein is found in the endoplasmic reticulum where GGCX and VKORC1 also localize. CALU may play a role in regulating the activity of GGCX through the inhibition of both GGCX and VKORC1.[12] The contribution of these essential enzymes to the vitamin K cycle is shown in Figure 2.

Figure 2. Genes coding for important enzymes involved in the Vitamin K cycle and warfarin metabolism



Kaye et al. Pharmacotherapy 2017; 37: 1150–63

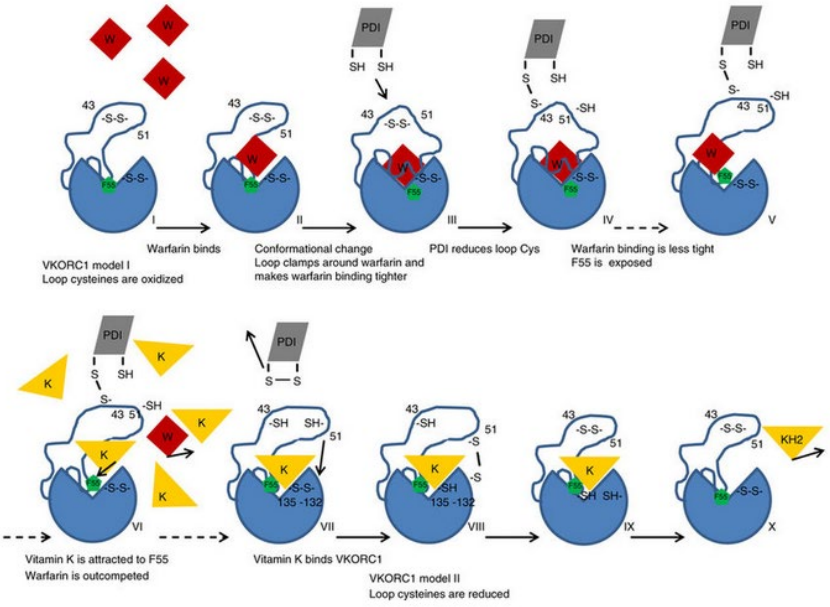
Warfarin pharmacological effects on VKORC1 and warfarin metabolism

Warfarin elicits is anticoagulant effect by preventing the reduction of the oxidized vitamin K2,3-epoxide form to the reduced vitamin K1 form through inhibition of vitamin K epoxide reductase (VKORC1). This prevents the formation of vitamin KH_2 cofactor and thus its participation with gamma-glutamyl carboxylase (GGCX) in activating clotting factors. A recent study by Czogalla et al. presented a mechanism for VKORC1 inhibition where warfarin competes with vitamin K2,3-epoxide for the same Phe55 phenyl side chain located in a hydrophobic pocket active site within VKORC1.[13] When VKORC1 is in an oxidized conformation, warfarin has greater affinity to bind, which induces a further conformational change in which Phe55 will localize deeper in the hydrophobic pocket, making it less likely for vitamin K2,3-epoxide to bind. In this conformational state, warfarin is tightly bound to VKORC1 and supported by cysteine-loop clamps that hold warfarin in the active site.

The presence of protein disulfide isomerase located in the endoplasmic reticulum will reduce the cysteine loops and cause VKORC1 to shift to a reduced conformation where the loop clamps on warfarin will be loosened and allow the release of warfarin and the exposure of Phe55. The reduced form of VKORC1 and exposed active site of VKORC1 will attract the binding of vitamin K2,3-epoxide. Next, the reduced cysteine loops will transfer electrons to the active site where vitamin K2,3-epoxide will be reduced to vitamin K1 quinone. vitamin K1 quinone can also bind to VKORC1 in the same manner and will be reduced to vitamin KH_2. The proposed molecular pathway of VKORC1 inhibition followed by competitive binding by vitamin K2,3-epoxide is shown in Figure 3.[13] This recent proposed mechanism of VKORC1 binding by competitive

inhibition parallels clinical observation where high doses of vitamin K can effectively reverse the effects of over-anti coagulation by warfarin.

Figure 3. Warfarin and vitamin K compete for binding to Phe55 in VKORC1



Czogalla et al. Nat Struct Mol Biol 2017; 24: 77–85

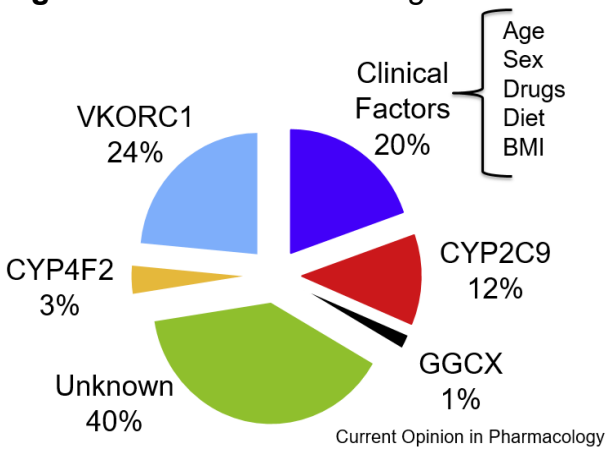
The metabolism of the more potent S-enantiomer of warfarin to an inactive hydroxy metabolite is carried out by cytochrome P450 family 2 subfamily C member 9 (CYP2C9).[14] Upon oral intake, warfarin is hydrolyzed to its R and S enantiomer forms in the gut and absorption along with fat chylomicrons into the systemic circulation occurs at the level of the small intestine. Warfarin enters the liver during first past metabolism and exerts its anticoagulant effect on VKORC1 and in addition metabolizes to an inactive state by CYP2C9 enzymes, which are primarily located in the liver.

<u>Variants in vitamin K cycle genes that affect warfarin dose response</u>

Both enzymes, VKORC1 and CYP2C9, directly interact with warfarin and thus are the main contributors that can influence warfarin dose and warfarin anticoagulation response in patients receiving warfarin therapy.[15] Inherited single nucleotide

polymorphisms (SNPs) in the genes that encode VKORC1, CYP2C9 and variants in additional genes involved in the vitamin K cycle such as *NQ01*, CYP4F2, *GGCX*, and *CALU* are known to be one of the primary causes for the wide variability in interpatient warfarin dose requirements.[16] It is estimated that clinical factors account for ~20 percent and genetic factors account for ~40 percent of the variation in warfarin dose response.[17] The remaining 40 percent is still unknown and may include the contribution of factors such as epigenetics, insertions, deletions, duplications, proteomic, and metabolomic interactions. The estimated percent contributions of these factors to warfarin dose response is shown in Figure 4.

Figure 4. Factors contributing to variation in warfarin response



Baker et al. Curr Opin Pharmacol 2016; 27: 38–42

In terms of *CYP2C9* SNPs that affect warfarin dose, variants *CYP2C9**2 and *CYP2C9*3* were reported to decrease CYP2C9 activity in comparison to the wild-type (normal) allele *CYP2C9**1.[18] The reduced activity of CYP2C9 in carriers of these variants were confirmed, in that homozygous patients with genotypes (*2/*3 or *3/*3) required up to a 28 percent reduction in mean weekly dose compared to wild-type patients (*1/*1).[19] These variants reduce the ability of CYP2C9 to metabolize warfarin and the patients who were carriers of these variant alleles became more warfarin

sensitive and required lower doses of warfarin to achieve stable anticoagulation. Regarding *CYP2C9**2, a DNA nucleotide base pair change from cytosine to thymine at position 430 in the exon 3 coding region resulted in an amino acid substitution of arginine for cysteine. In *CYP2C9**3, a nucleotide substitution of adenine for cytosine at position 1075 in the exon 7 coding region resulted in an amino acid substitution of isoleucine for leucine. These non-synonymous missense mutations were not located directly within the active site of CYP2C9, however, recent proteomic modeling studies have shown the amino acid substitutions cause a decreased binding affinity of warfarin toward CYP2C9.[19]

The presence of *CYP2C9**2 and *3 variants induce a conformational change where hydrogen bonding interactions between CYP2C9 and warfarin are disrupted in a proportional manner to the number of variants. For example, as shown in Figure 5, a total of either 3, 1, or no hydrogen bonds will be present in patients who have a wild-type (*1/*1), heterozygous (*1/*2 or *1/*3) or homozygous (*2/*3) *CYP2C9* genotype, respectively. These findings provide further molecular-based evidence to suggest the variants cause a reduction in metabolism of warfarin due to the decreased affinity binding to warfarin. Additional *CYP2C9* variants such as *CYP2C9**5,*6,*8, and, *11 alleles have also been identified that cause reduced activity and increased sensitivity to warfarin.[20]

Figure 5. Protein-ligand interactions with reference to C*YP2C9* variants

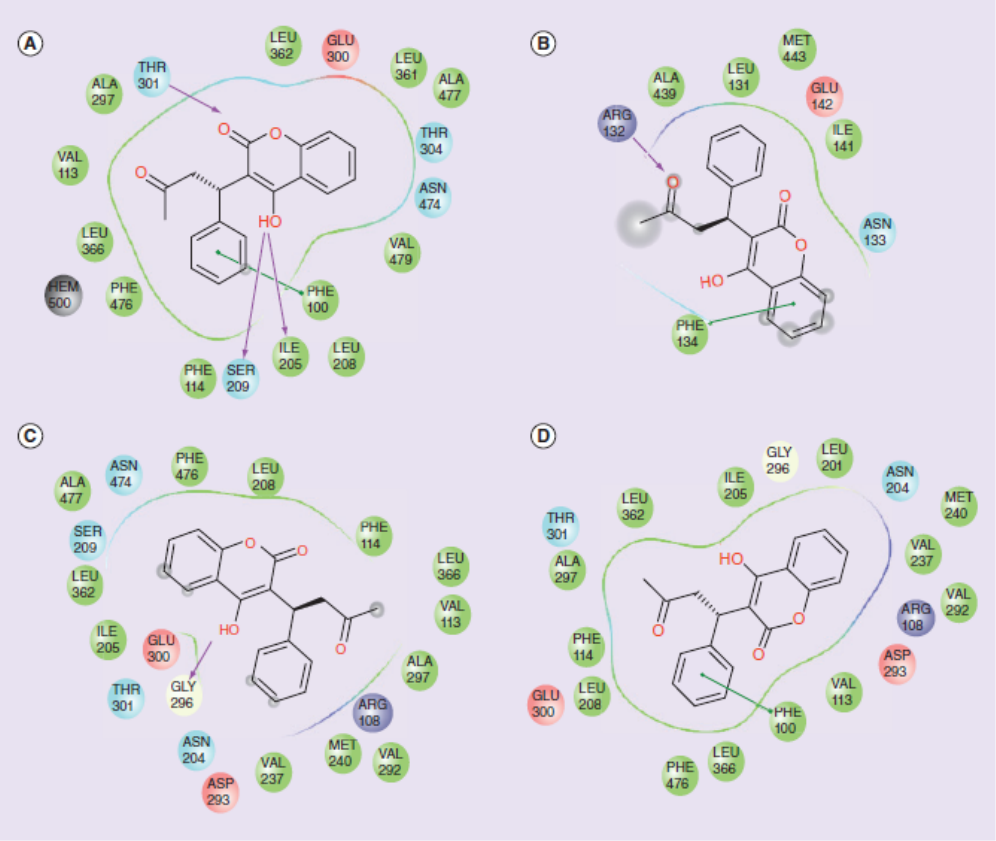


Figure 3. Protein–ligand interactions with reference to *CYP2C9* variants. **(A)** Warfarin showing two hydrogen bonds with protein backbone, one hydrogen bond with the protein side chain and one π–π interaction with F100 of the wild-type of protein (*CYP2C9*1*). **(B)** Warfarin exhibits only one hydrogen bond with the protein side chain and one π–π interaction in *CYP2C9*2*. **(C)** Warfarin exhibits only one hydrogen bond with the protein side chain and one π–π interaction in *CYP2C9*3*. **(D)** Warfarin exhibits no hydrogen bonding with the protein and π–π interaction is retained in *CYP2C9*2/*3*.

Pavani et al. Pharmacogenomics 2015; 16: 393–400

In terms of *VKORC1* polymorphisms, *VKORC1-1639G>A* has been identified as a primary variant that contributes to warfarin dose response. In contrast to the *CYP2C9* variants, which confer a reduction in activity, the variant in *VKORC1* is located in an upstream promoter region of the gene and instead affects the expression and the abundance of VKORC1 protein. Individuals who are carriers of the -1639A allele have decreased VKORC1 protein and thus reduced maintenance warfarin dose requirements compared to the wild-type -1639G allele. The clinical impact is that individuals heterozygous for the A allele required 13 mg less warfarin per week, while homozygous individuals required 23 mg less warfarin per week than those with the GG genotype.[21]

These findings were substantiated in a second study that resulted in a 2-fold lower expression of *VKORC1* mRNA in carriers of the -1639A allele.[21] The exact molecular mechanism showing how a guanine to adenine substitution may reduce expression has yet to be illustrated. However, this substitution creates an E-box binding site in the promoter region of *VKORC1* where E-box binding proteins can bind and suppress transcription, and thus abundance of the protein.[21]

Additional SNPs found to affect warfarin dose, although in a less substantial manner to VKORC1 and CYP2C9, include polymorphisms in *CYP4F2*, *NQO1*, *GGCX*, and *CALU*. The *CYP4F2*3* variant leads to a V433M amino acid change and reduced enzyme activity.[11] The loss of activity confers the limited ability to oxidize vitamin K1 and remove excess vitamin K from the vitamin K cycle, which results in more vitamin K able to become activated and initiate clotting. This is confirmed by the increased resistance to warfarin therapy and higher doses of warfarin needed to achieve stable anticoagulation in patients with the *CYP4F2*3* allele. The finding of no measured differences in mRNA expression levels in human hepatocytes between wild-type and variant allele carriers further suggests the V433M amino acid substitution does not affect transcription but rather enzyme activity.[22]

A cytosine to thymine substitution in the exon region encoding NQO1 causes a proline to serine substitution in the amino acid position 187, which is denoted as *NQO1*2* allele.[23] The amino acid change has been shown to destabilize and reduce the function of the allele and has been confirmed in homozygous carriers who require reduced warfarin maintenance dose.[24] A loss of function variant has also been reported in the gene *CALU*, which corresponds to a 11% to 15% higher warfarin dose

requirement.[25] The presence of the *GGCX* (CAA) 16/17 repeat variant resulted in a gain of function and an increase in warfarin dose requirements compared to the wild-type form.[26]

Warfarin Pharmacogenomics and consideration of race/ethnicity

Based on the mounting evidence that genetic variation influences warfarin dose response, interest in using genetic tests to personalize warfarin dosing has increased greatly. Due to the wide inter-individual variation in dose response to reach therapeutic INR levels, shifting from a 'one-size fits all' dosing strategy to a dosing strategy that incorporates personal genetic and clinical data makes clinical sense. The development of genotype-guided warfarin dosing algorithms may allow improved warfarin dose prediction and increase the safety and efficacy during the early course of warfarin therapy. Researchers focused on warfarin pharmacogenomic implementation have developed several common algorithms. Algorithms developed by the International Warfarin Pharmacogenomics Consortium (IWPC) and Gage et al. primarily serve as the recommended warfarin dosing guidelines.[15,27] However, these algorithms were developed in populations of primarily European ancestry and at the time of development, only included the genetic variants *CYP2C9*2/*3* and V*KORC1-1639A* along with clinical data.

The clinical utility of the IWPC and Gage algorithm were examined and both showed improved accuracy in predicting stable warfarin dose compared to fixed-dosing and clinical dosing methods. However, as mentioned, these clinical studies consisted of a patient population that was predominately European. Moreover, in a landmark study published in the New England Journal of Medicine including a more diverse patient

population, the performance of genotype-guided dosing in predicting stable warfarin dose was significantly lower than clinically-guided dosing in patients who self-identified as African American (time in therapeutic range was 35.2% vs 43.5%, respectively p=0.0003).[28] The findings from this clinical study suggested using European genetic data to guide dosing may potentially harm patients with African ancestry and these genotype-guided European based algorithms may not be effective in predicting warfarin dose for African-American patients. The inaccuracy of these algorithms are likely to extend to other diverse populations.[29]

Moving forward, the consideration of race/ethnicity in the implementation of genetic-guided dosing will be critical. Recent studies included a severe under-representation of non-European groups including Hispanic, American Indian/Alaska Native, and to a lesser extent, African Americans.[29] Furthermore, non-white populations, in particular African Americans and Hispanics, experience greater sub-optimal warfarin management and greater warfarin-related adverse events compared to European patients.[30,31] These findings suggest diverse populations potentially have the most to gain from genetic studies that are focused on discovering novel variants that affect warfarin dose requirements in other race/ethnic groups.

Race/ethnicity-based genetic diversity contributing to warfarin dose variability

In addition to the wide inter-patient variability in warfarin dose needed to achieve stable anticoagulation, the average warfarin dose required also differs by race/ethnicity. The daily doses needed to achieve stable INR levels is estimated at 5.1 mg in European patients, 5.7 mg in patients with African ancestry, 4.4 mg in Hispanic patients, 3.4 mg in Asian patients, and 4.5 mg in American Indian/Alaska Natives (AI/ANs).[32] Because

variants will have biologically similar effects on warfarin dose across populations, one of the primary drivers responsible for the differences in warfarin dose requirement between race/ethnic groups may be attributed to how common warfarin variants are found within different populations. For example, the warfarin sensitive allele V*KORC1-1639A* is found at a frequency of 88% in Asian populations compared to a frequency of 5% in populations with African ancestry (Figure 6). These differences may partially contribute to the lower average daily warfarin dose requirements observed in Asian populations and higher average daily warfarin dose requirements observed in African American populations.

The presence of race-specific variants may also contribute to differences in warfarin dose requirements between race/ethnic groups. For example, *CYP2C9**2 and *3 warfarin sensitive variants are the most common in Europeans while warfarin sensitive variants *CYP2C9**5,*6,*8, and *11 exist in individuals with African descent but are essentially non-existent in the other populations as shown in Figure 6.[33]

Figure 6. Genetic variants that contribute to warfarin dose and their frequencies by race

Allele	rs Number	Warfarin Dose	Minor Allele Frequency (%) [a, b]				
			European	Latino	African	Asian	AI/AN [c]
*CYP2C9*2*	rs1799853	↓	10	7	2	<1	5
*CYP2C9*3*	rs1057910	↓	7	4	<1	3	3
*CYP2C9*5*	rs28371686	↓	<1	<1	2	<1	–
*CYP2C9*6*	rs9332131	↓	<1	<1	1	<1	–
*CYP2C9*8*	rs7900194	↓	<1	<1	5	<1	<1
*CYP2C9*11*	rs28371685	↓	<1	<1	2	<1	<1
CYP2C9 1878GT	rs7089580	↑	22	12	21	1	–
CYP2C G>A	rs12777823	↓	15	11	25	31	–
VKORC1-1639G>A [d]	rs9923231	↓	39	41	5	88	60
VKORC1-8191A>G	rs61162043	↑	61	57	46	12	–
*CYP4F2*3* (V433M)	rs2108622	↑	29	24	8	21	32
GGCX (CAA) 16/17	rs10654848	↑	<1	–	3	–	–
*NQO1*2*	rs1800566	↑	21	33	18	42	–
CALU T>C	rs339097	↑	<1	1	14	1	–

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Hispanic Specific variants that affect warfarin dose response

Hispanic individuals represent one of the most diverse race/ethnic groups. This genetic diversity is illustrated through the wide variation in individual admixture proportions of European, Native American, and African ancestry, which is largely tied to geographical location.[34] In the limited warfarin genetic studies aimed at evaluating genetic contributions to warfarin dose in Hispanics, the addition of the more prevalent *CYP4F2*3* and *NQO1*2* variants improved the explanation of warfarin dose variability by 10 percent.[35] In addition, the performance of algorithms developed in populations of Hispanic ancestry explained warfarin dose variability more accurately than the European-based IWPC or Gage algorithm when more common Hispanic variants such as *CYP4F2*3* were incorporated.[36]

Interestingly, a study of Hispanic Mexican Americans found *NQO1*2* allele carriers had an increased warfarin dose requirement, which was in contrast with earlier findings that showed the variant caused a decreased warfarin dose requirement in European and Asian patients.[35] Because the biological explanation agrees on how *NQO1*2* variants result in lower warfarin dose requirements, as described previously, it is suggested that *NQO1*2* may be inherited with an unknown causative variant that is not present in other race/ethnic groups. Given the rich diversity in differences in Hispanic ancestry, genetic studies limited to a majority population may fail to capture variants that predict warfarin dose for this population. For example, genotype-guided warfarin algorithms in Hispanic patients with greater percentages of Native American ancestry may be less effective due to the limited genetic knowledge and the possibility of missing important variants that affect warfarin dose in Native American populations. In addition, the majority of Hispanic-specific studies that are developing algorithms primarily focus on replicating associations of known variants rather than focusing on identifying novel variants that may improve prediction of warfarin dose.

Thesis Hypothesis

The vast majority (> 80%), of warfarin genotype-guided studies have been performed in populations of European ancestry.[29] The implementation of genotype-guided algorithms to improve the prediction of optimal warfarin dosing for patients is encouraging, however, the current genetic factors primarily encompass traditional variants (SNPs) found predominately in European populations. This critical underrepresentation of diverse populations in warfarin studies may contribute to a lack

of identification of important variants specific to race/ethnic groups that may be non-existent in European populations.

In a prospective clinical study, we examined candidate gene SNPs in *CYP2C9, VKORC1, CYP4F2,* and *NQO1* in 76 self-identified Hispanic patients to determine the influence of these variants on warfarin stable dose requirements. The variants selected included the primary European-based warfarin predictors *VKORC1-1639A* and *CYP2C9* *2/*3 alleles.[15,37] In addition, SNPs *CYP4F2**3 and *NQO1*2* shown previously to influence dose in a Hispanic population, were examined along with non-genetic variables known to predict warfarin dose.[36,38] We hypothesize incorporation of SNPs *VKORC1-1639A* and *CYP2C9* *2/*3 in a Hispanic population will have similar contributions to the European-based Gage model in explaining variability in warfarin dose requirements. Furthermore, the addition of SNPs *CYP4F2**3 and *NQO1*2* will improve warfarin dose prediction above and beyond the Gage algorithm, which only considered the common *VKORC1-1639A* and *CYP2C9* *2/*3 variants.

Methods

A total of 76 self-reported Hispanic and Latino patients on stable doses of warfarin were recruited between October 2016 and March 2018. Recruitment took place at the Banner University Medical Center-Tucson Sarver Heart Center Coumadin Clinic and El Rio Community Health Center in Tucson, Arizona. Subjects were provided written informed consent approved by the University of Arizona Internal Review Board. Inclusion Criteria were 1) at least 18 years of age; 2) ability to give informed consent; 3) therapeutic INR for at least 2 consecutive clinic visits (separated by at least two weeks);

and 4) self-identifies as Hispanic or Latino. Exclusion criteria were 1) less than 18 years of age; 2) unable to give informed consent and 3) severe hepatic impairment.

Demographic data was obtained in an interview with the participant as well as retrospectively from the electronic health record and included age, gender, height, weight, race/ethnicity, national origin, smoking status, alcohol status, INR measurements, target INR range, concurrent medications, and indication for warfarin therapy as shown in Table 1.

Table 1. Study Population Characteristics in Hispanic Patients treated at Banner Medical Center/El Rio Community Health Center in Tucson, Arizona

Study Population Characteristics	**Total (n=74)[a]**
Stable warfarin dose (mg/week)	
Mean	33.8 (14.8)
Range	9.5-70
Age (years)	
Mean	65.8 (16.9)
Range	26-91
Weight (lbs.)	
Mean	200.0 (64.4)
Range	98-477
Height (inches)	
Mean	65.4 (3.64)
Range	55-74
BMI	
Mean	32.7 (9.58)
Range	19-77
Gender	
Male	41 (55.4)
Race/Ethnicity	
Hispanic/Latino	74 (100)
White	18 (24.3)
American Indian/ Alaska Native	4 (5.41)
Indication(s)	
Tobacco status	9 (12.2)
Alcohol status	10 (13.5)
Deep vein thrombosis	23 (31.1)
Pulmonary embolism	13 (17.6)
Stroke	7 (9.46)
Atrial fibrillation	38 (51.4)

Prosthetic Valve	16 (21.6)
Concomitant medications	
Statin	41 (55.4)
Amiodarone	4 (5.41)
Sulfamethoxazole	1 (1.35)

BMI= body mass index.
[a] Continuous data presented as mean (±SD), dichotomous data presented as n (%).

A 30-milliliter mouthwash sample was obtained from each patient during a routine clinic visit. Genomic DNA was extracted and purified from the mouthwash sample using Puregene© Blood Kit from QIAGEN following the manufacturer's protocol. The DNA was quantified by a NanoDrop spectrophotometer and fluorescent staining of dsDNA using PicoGreen® dsDNA Quantification Kit. Following DNA purification, genotyping of *VKORC1*, *CYP2C9*, *CYP4F2* and *NQO1* at 5 variable regions (2 SNPs in *CYP2C9*) were performed using TaqMan analysis at the University of Arizona Genetics Core.

In order to determine the most significant predictors of warfarin dose requirements, a linear-regression analysis was performed using Statistical Analysis System (SAS) software version 9.4 (SAS® Institute Inc., Cary, NC, USA). The outcome variable, average weekly warfarin dose in milligrams was transformed by taking the square root of the average weekly warfarin dose. The transformation was incorporated to help stabilize the variance in warfarin dose and subscribe to the assumptions of normality and linear-regression. The square root normalization of warfarin dose was also performed to reflect the accepted approach used by previous generated genotype-guided warfarin dosing models.[37] To further ensure the outcome variable followed normal distribution, one patient was excluded due to having an outlier weekly warfarin dose requirement of over 100 milligrams. Following square root transformation and

exclusion of the dose outlier, three goodness of fit normality tests were performed including Kolmogorov-Smirnov (p>0.15), Cramer-von Mises (p=0.217) and Anderson-Darling (p=0.22). All three normality distribution tests were greater than p-value of 0.05 which provided confidence that the outcome variable data (square root of weekly warfarin dose) was normally distributed.

To observe how individual variables affected the square root of stable weekly warfarin dose, a simple linear-regression analysis was initially performed. Non-genetic (i.e. age, gender, height, weight, smoking status, alcohol status, concurrent medications, and indications for warfarin therapy) and genetic (i.e. *VKORC1-1639A, CYP2C9* *2/*3, *CYP4F2**3, and *NQO1*2)* variables were each assessed one by one (n=23 variables). Linear-regression outputs determined each variable's association (p-value), effect size (regression coefficient), and percentage of variation in warfarin dose explained by the predictor variable (R-Square).

Following simple linear-regression analysis, a multiple stepwise regression analysis was performed. The stepwise model automatically controlled for co-variance and selected the most significant variables (0.1 p-value cutoff) from the total pool of non-genetic and genetic variables available to give a final linear-regression pharmacogenetic model. This ensured a potential in clinical bias was avoided and the selection of predictor variables were solely based on the strength of significance. Stepwise modeling began with the intercept and added individual variables that were most significant until the predetermined p-value threshold of 0.1 was reached. During each additive step, variables could also be deleted from the model if they rose above

the 0.1 p-value. The *CYP2C9* variable was defined as either being a *1 or *3 carrier, which resulted in a total of 21 variables analyzed.

Using the pharmacogenetic model developed by the stepwise selection model, we compared the warfarin dose predictive performance relative to two other warfarin pharmacogenetic studies specific to Mexican Americans.[38,39] Additionally, we compared the performance of these Hispanic specific algorithms relative to the European-derived Gage model.[15] The performance of the algorithms was measured by R-square, indicating the variation in warfarin stable dose requirements explained by the clinical and genetic variables.

Results

To assess the accuracy of SNP genotyping, 32 of 76 samples were genotyped as duplicates across 5 SNPs, which resulted in 100 percent concordance. Next, a Hardy-Weinberg equilibrium analysis was performed to determine the expected distribution of SNPs within the patient population. The Chi- Square (X^2) test for all 5 SNPs showed no significant difference between the expected and observed genotype frequencies, which further increased the confidence that the genotyped data was correct. The allele and genotype frequency distributions with Chi-Square results are shown in Table 2.

Table 2. Genotype and Allele frequency distributions in Hispanic Patients treated at Banner Medical Center/El Rio Community Health Center in Tucson, Arizona

Genotype/allele	**n (%)**	**Chi-Square X^2**	**X^2 p-value**
***VKORC1* -1639G>A**		1.42	0.23
Genotypes			
GG	18 (24.3)		
GA	42 (56.8)		
AA	14 (18.9)		
Allele Frequency			
G	78 (52.7)		

A	70 (47.3)		
CYP2C9*2		0.48	0.49
Genotypes			
*1/*1	63 (85.1)		
*1/*2	11 (14.9)		
Allele Frequency			
*1	137 (92.6)		
*2	11 (7.4)		
CYP2C9*3		0.06	0.81
Genotypes			
*1/*1	70 (94.6)		
*1/*3	4 (5.4)		
Allele Frequency			
*1	144 (97.3)		
*3	4 (2.7)		
CYP4F2		0.007	0.93
Genotypes			
*1/*1	40 (54.0)		
*1/*3	29 (39.2)		
*3/*3	5 (6.8)		
Allele Frequency			
*1	109 (73.6)		
*3	39 (26.4)		
NQO1		2.01	0.16
Genotypes			
*1/*1	37 (50.0)		
*1/*2	34 (46.0)		
*2/*2	3 (4.0)		
Allele Frequency			
*1	108 (73.0)		
*2	40 (27.0)		

Chi-Square values (≥3.841) or Chi-Square p-values (<0.05) are not consistent with Hardy-Weinberg equilibrium expectations

The weekly therapeutic warfarin dose ranged from 9.5 mg/week to 70 mg/week. The mean weekly warfarin dose was 33.8 mg/week (Table 1) and the derived mean daily warfarin dose of 4.8mg/day was similar to previous daily dose averages in Hispanic populations at 4.4mg/day.[32] The mean age was 65.8 years (range 26-91) and 55 percent of the patients were male (n=41). The most common indications for warfarin therapy included atrial fibrillation (38%, n=38), and deep vein thrombosis (23%, n=24)

and the most common concomitant medication was statin use (55%, n=41). In addition to self-identifying as Hispanic or Latino, four patients self-identified as having American Indian/Alaska Native ancestry.

In terms of genetic variants, 47 percent of the participants (n=47.3) were carriers of the warfarin sensitive *VKORC1*A allele. The frequencies of warfarin sensitive *CYP2C9* SNP alleles were 7.4% (n=11) for *2 and 2.7% (n=4) for *3 carriers. The warfarin resistant *CYP4F2*3* and *NQO1**2 SNP alleles had frequency distributions of 26.4% (n=39) and 27% (n=40), respectively. These minor allele frequencies had similar distributions compared to previous Hispanic populations.[29] In addition, the VKORC1-*1639A, CYP2C9* *2/*3, and *CYP4F2**3 alleles were similar in distribution to previous European populations.[29] As described previously, all genetic variants were in Hardy-Weinberg equilibrium.

The simple linear-regression analysis depicted in Table 3 resulted in a total of 9 variables that were significant predictors of warfarin dose (p-value <0.05) which included weight, BMI, height, age, statin use, amiodarone use, atrial fibrillation indication, *VKORC1*-1639G>A, and *CYP2C9* *3/*2 alleles. The clinical effect on warfarin dose requirement, measured by the partial regression coefficient, from each variable aligned with the expected direction shown in previous studies.[37,40] The largest individual clinical contributors to warfarin dose variability were weight (R-Square= 28.67%), age (R-Square=28.23%), and statin use (R-Square=23.21%). The variability in warfarin dose response explained by genetic variables were *VKORC1 -1639G>A* (R-Square=15.1%), and *CYP2C9*2/*3* (R-Square=5.98%). The individual clinical contributions to explaining warfarin dose variability were relatively higher than previous studies, while the genetic

variables followed the proportional contributions in that *VKORC1 -1639G>A* had a greater impact on warfarin dose variability.

Table 3. Simple Linear Regression analysis of square root of weekly warfarin dose (mg/week) in Hispanic Patients treated at Banner Medical Center/El Rio Community Health Center in Tucson, Arizona

Variable	P-Value	Partial regression coefficient	R-Square
Weight (lbs.)	<0.0001	0.0106	0.2867
BMI	<0.0001	0.0611	0.2076
Age	<0.0001	-0.0403	0.2823
Statin use	<0.0001	-1.2275	0.2321
Atrial Fibrillation	0.0002	-1.0657	0.1765
VKORC1 -1639G>A	0.0006	-0.7543	0.1510
Amiodarone use	0.0021	-1.9773	0.1227
Height (inches)	0.0076	0.1085	0.0950
CYP2C9 variant **2* or **3* carrier	0.0358	-0.7737	0.0598

n= 74; *VKORC1* was included as an additive predictor with three levels (coded as 0 for GG, 1 for AG, and 2 for AA); *CYP2C9* *2 or *3 carrier status was combined into one variable (coded as 0 for *1/*1 and 1 for *1/*2 or *3).

To account for co-variance, a multiple stepwise regression analysis was performed. The variables to enter the stepwise model in order of greatest significance included weight, *VKORC1-1639G>A*, age, amiodarone status, *CYP2C9 *3/*2*, and stroke indication. During the analysis no variables were excluded from the stepwise model, therefore the final model contained 6 significant predictor variables of warfarin dose. A summary of the stepwise selection algorithm model is shown in Table 4 with the individual partial R-square (R^2) contributions that explain warfarin dose variability, adjusted R^2 value, partial regression coefficient that explain the individual clinical effect on warfarin dose, and the significance of association to warfarin dose (p-value). The highest partial R^2 contributions included weight which explained 28.7%, *VKORC1-1639G>A* which explained 15.2%, and age which explained 14.3% of variability in warfarin dose in the study population. Overall, the 6 variables in the pharmacogenetic

model were responsible for explaining a total R^2 of 70.6% of the variability in warfarin dose ($p<0.0001$; Table 4). The linear regression model equation for predicting the square root of weekly warfarin dose is shown below:

$$\sqrt{warfarin\ dose}\left(\frac{mg}{week}\right) = 7.627 + (0.00598 * Weight) - (0.03236 * Age) - (0.63506 * CYP2C9) - (0.8686 * VKORC1A) - (1.30079 * amiodarone) - (0.69797 * stroke\ indication)$$

Table 4. Multiple Stepwise regression model of square root of weekly warfarin dose (mg/week) in Hispanic Patients treated at Banner Medical Center/El Rio Community Health Center in Tucson, Arizona

Step	Variables	Partial R^2	Adjusted R^2 after entry	Partial regression coefficient	p-value
1	Weight (lbs.)	0.287	0.2867	0.00598	<0.0001
2	*VKORC1 -1639G>A*	0.1524	0.4391	-0.86860	<0.0001
3	Age (years)	0.1426	0.5817	-0.03236	<0.0001
4	Amiodarone status	0.0674	0.6491	-1.30079	0.0005
5	*CYP2C9 variant *2 or *3 carrier*	0.0315	0.6805	-0.63506	0.0118
6	Stroke Indication	0.0252	0.7057	-0.69797	0.0193

Final model R^2: 0.7057; n= 74; *VKORC1* was included as an additive predictor with three levels (coded as 0 for GG, 1 for AG, and 2 for AA); *CYP2C9* *2 or *3 carrier status was combined into one variable (coded as 0 for *1/*1 and 1 for *1/*2 or *3).

Discussion

To date, only two studies have evaluated the impact of pharmacogenetics on warfarin dose in Mexican Americans.[38,39] A comparison of the clinical and genetic contributions to warfarin dose variability, in terms of R^2, in our cohort vs. the Hispanic American specific Cavallari et al. and Bress et al. cohort, as well as the traditional European-derived Gage cohort is shown in Table 5.

Table 5. Performance of Hispanic Mexican American and Gage European-derived Warfarin Pharmacogenetic Dosing Algorithms

Study algorithm	Race/Ethnicity	n	Genetic Variables	Clinical Variables	Genetic & Clinical Algorithm (R^2)	Clinical Variables (R^2)
Banner/El-Rio Cohort	Mexican American-Tucson	74	*VKORC1-1639A CYP2C9 *2, *3*	Age, weight, amio, stroke	71%	53%
Cavallari et al. 2011[39]	Mexican American-Chicago	55	*VKORC1-1639A CYP2C9 *2, *3*	Age, BSA, VTE	55%	17%
Bress et al. 2012[38]	Mexican American-Chicago	50	*VKORC1-1639A CYP2C9 *2, *3*	Age, BSA, Afib	58%[a]	36%
Gage et al. 2008[15]	White (83%) AA (15%) Mixed (2%)	1015	*VKORC1-1639A CYP2C9 *2, *3*	Age, BSA, target INR, amio, race, smoking, VTE	53%	17%

BSA= body surface area; amio= amiodarone; VTE= venous thromboembolism; Afib= atrial fibrillation; INR= international normalized ratio; *VKORC1*= vitamin K epoxide reductase complex subunit 1; *CYP*= cytochrome P450.
[a] Addition of *CYP4F2*3* and *NQO1*2* in the Bress et al. algorithm improved total model R^2 to 68%.

Incorporation of the primary warfarin predictors *VKORC1-1639A* and *CYP2C9* *2/*3 improved the R^2 warfarin dose prediction over prediction based solely on clinical factors in our cohort as well as the Cavallari and Bress Hispanic American cohorts. These improvements in prediction were similar to the well-replicated European-based Gage cohort, which suggests using this algorithm may be appropriate in Mexican American populations. The study performed by Cavallari et al., found the common European *VKORC1* and *CYP2C9* genotypes were sufficient in predicting warfarin dose requirements. When the genotypes were combined with clinical variables, the factors explained 55% of the variability in warfarin dose which corresponds with data in

Europeans, where these variables explain an estimated 50-60% of the dose variability (Gage is 53% shown in Table 5).[15,28] However, the study did not look into identifying ethno-specific variants that may further contribute to warfarin dose response. As shown in the study by Bress et al., the addition of *CYP4F2*3* and *NQO1*2* genotypes improved the overall explanation in dose variability in Hispanic Americans from 58% to 68% (Table 5).[38]

Herein, we selected the candidate gene variants in *VKORC1*, *CYP2C9*, *CYP4F2*, *NQO1,* and additional clinical factors known to effect warfarin dose in Hispanic populations. The final stepwise regression model in our cohort was able to explain 71% of the variability in warfarin dose response (Table 4 and 5). Although our overall R^2 prediction value has the highest performance in explaining variability in warfarin dose, the genetic contribution at 18% was relatively low in comparison and may suggest the need to identify additional genetic contributors associated with warfarin dose. The only significant genotypes to make it into the final model were *VKORC1* -1639G>A and *CYP2C9**2/*3 which is in line with the findings of Cavallari et al. and the larger European-based IWPC and Gage algorithm studies.[15,37,39] However, this statement is made with the caveat that in addition to our cohort only the Bress et al. study considered the additional *NQO1**2 and *CYP4F2**3 allele variants.[38]

In contrast to Bress et al., the *NQO1* and *CYP4F2* genotypes in our cohort were non-significant and were not included in the stepwise model despite the similar prevalence of these variants (minor allele frequencies (MAF), *NQO1*2* MAF is 27% in both cohorts; *CYP4F2*3* MAF is 26% in our cohort vs 23% in Bress cohort). Although our cohort did not find significant warfarin dose associations with these two SNPs, most

of the highly associated variables that made it in the final stepwise model reflect the expected variables that explain warfarin dose as validated in previous studies.[15,41]

The clinical effect on increasing or decreasing dose requirements for each variable (Partial regression coefficient in Table 4) also aligns with the expected direction seen in the previous warfarin pharmacogenetic literature.[37,40] Most notably, although the *NQO1*2* allele did not make it into to the final model, there was an increased effect on warfarin dose requirements as similarly observed in the Bress et al. study.[38] This may indicate the differences in statistical significance could be due to a false positive association in the Bress et al., or a false-negative in our findings since our cohort did not replicate the association. Nevertheless, the increase in effect of *NQO1*2* on warfarin dose requirements in both cohorts is in contrast to non-Hispanic populations, which may suggest alternate causative variants have yet to be determined in Hispanic populations that properly predict dose requirements.

In terms of limitations, our study cohort was relatively small with a sample size of 74 patients. The final prediction variables and their effect on warfarin dose generated from the final stepwise model have also not been replicated and validated in an independent population. Patients self-reported their race/ethnicity and we did not conduct a genotype-based estimation of ancestry. In addition, a direct comparison between our Hispanic population and previously published literature is difficult considering the highly diverse nature of this ethnic group. Lastly, the stepwise model characterized the indication of stroke as a significant clinical predictor which has not been found as a significant factor in explaining dose in previous studies. This variable may be characterizing the fact that our cohort has a higher mean age at 66 years as

stroke is more common in elderly patients. The association could reflect a lower dose requirement which is positively correlated with age.

Future Directions

The potential to improve patient outcomes by more accurately predicting the correct warfarin dose requirement remains high. It is a reasonable rationale that accurate predictions during warfarin initiation should reduce the time it takes to arrive at a therapeutic INR level and thus reduce the risks of warfarin-related adverse events such as major bleeding or thrombosis. A recent large multi-site clinical trial of 1650 patients published in JAMA (GIFT trial), found this to be the case, in which genotype-guided dosing more accurately predicted warfarin dose compared to clinically-guided dosing. This led to a reduction in adverse events of primary outcomes in major bleeding and venous thromboembolism.[40]

Because of the high susceptibility Hispanic patients encounter in terms of warfarin related adverse events[30,31], the potential to better understand how genetic variation contributes to warfarin variability in Hispanic populations is critical. To meet these goals, our next step in the clinical study will involve a similar candidate SNP study as part of a broader Hispanic Warfarin Pharmacogenetic Consortium. The replication studies will be conducted at clinical academic institutions in Florida, Illinois, Puerto Rico, and Brazil, which will provide further evidence as to whether a non-Hispanic pharmacogenetic dosing strategy or a more Hispanic-specific algorithm is appropriate to guide warfarin initiation.

In addition, due to the unique admixture within Hispanic and Latino populations, we plan to properly define the genetic structure of our cohort by genotyping ancestry

informative markers to estimate Native American, European, and African ancestry proportions. Ancestry may be included as a variable in a final revised dosing model along with clinical and genetic factors to further improve dose prediction. Because our study only evaluated clinical and genetic factors derived from one Hispanic American specific study (Bress et al.) and a study with the majority population of European ancestry (GAGE model), we will evaluate the potential of additional or undefined variants that may contribute to explaining warfarin dose.[15,38] A genome-wide association study will be conducted to identify novel associations with warfarin stable dose requirements across a broad range of genomic variation.

Conclusions

In this present study we aimed to identify whether clinical factors and SNPs previously associated with stable warfarin dose variability in populations of European and Hispanic descent, accurately predicted dose in a Hispanic population. Findings from our linear regression model indicate genetic and clinical predictors of stable warfarin dose variability may be similar among populations of European and Hispanic ancestry. The recent updated Clinical Pharmacogenomics Implementation Consortium (CPIC) guidelines for genotype-guided warfarin dosing reflect our findings in terms of dosing recommendations based on race/ethnicity.[41] These recommendations for pharmacogenetic dosing of warfarin recommend we treat patients with African ancestry differently based on their SNP data and all other race/ethnic groups the same. Our results suggest that this is a valid approach in that Hispanics and European patients may potentially be treated similarly using classic SNP data from the common IWPC and Gage algorithms. These findings from our cohort study further add to the understanding

of how genetic variation influences warfarin dose in Hispanic American populations. We hope this clinical study will serve to help guide pharmacogenetic warfarin dosing strategies that focus on improving warfarin therapy and patient outcomes. Further studies will be needed to replicate and validate these findings in other Hispanic populations.

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