USING GENETICS TO DRIVE TREATMENT: A FOCUS ON WARFARIN

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The use of genetics in health care has become a mechanism for identifying risk. In some cases, genetics are diagnostic for particular disease states such as Down syndrome, cystic fibrosis, and familial hypercholesterolemia.[1-3] Patients are commonly asked about family history as a crude way of estimating genetic risk which is used in concert with environmental factors to more fully assess clinical risk. Even with the growing use of imaging and clinical markers that are available to aid in individualizing medical care, patients' responses to drug therapies are variable. The need to predict this variability becomes particularly important for pharmacotherapy that has a narrow therapeutic index or when treating emergent cases where time to a therapeutic endpoint is linked to outcomes.

An emerging area to consider in treatment response are the pharmacogenetics (PGX) of the treatment modality. PGX is the study of the effects that a particular gene has on drug response. Another related term is pharmacogenomics, which evaluates drug response on a larger, genome-wide scale. The concept of PGX dates back over 40 years, and picked up clinical momentum with the completion of the human genome project.[4] The overall idea behind PGX is to maximize drug therapy while reducing side effects.

One drug where PGX has proven beneficial is the anticoagulant, warfarin (Coumadin® or Jantoven™).[5] The evidence supporting the benefits and risks of warfarin has been well documented. The margin of error between the risks and benefits is narrow making warfarin a drug that even with well designed protocols can take weeks to stabilize. This article will review the concepts of PGX, the data surrounding warfarin use, and the key PGX genes which explain the variability in warfarin dosing.

PGX: A Brief Overview

Drug variability driven by PGX is largely due to the pharmacokinetics and the pharmacodynamics of the medication. Genes primarily responsible for drug pharmacokinetics are the metabolizing enzyme genes – namely enzymes of the cytochrome p450 (CYP) enzyme system – and p-glycoprotein which affects the absorption of numerous drugs. Pharmacodynamically, genes that are involved at the drug's site of action are of interest.

More specifically, PGX looks at different genetic polymorphisms, or genetic changes, within a gene and how it may predict drug response. Typically, genetic polymorphisms can include point mutations (a single nucleotide in the sequence of the gene is changed to another nucleotide), insertions (a single nucleotide or more is incorporated into the

INSIDE THIS ISSUE:

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normal gene sequence), and deletions (a single nucleotide or more is removed from the gene sequence). These changes to the genetic code can be neutral in phenotypic effect or they could improve or hamper the function of the coded protein. These genetic changes are often classified as gene*N where N is a particular genetic polymorphism with the designation of 1 given to the functional or normal gene. Examples include CYP3A5*1 and CYP3A5*3, where the *1 represents the normal gene and *3 represents a genetic mutation in intron 3 of the same gene.[6]

Warfarin: A Narrow Therapeutic Index Drug

The use of warfarin is recommended in provoked and idiopathic deep vein thrombosis or pulmonary embolism, after selective major surgeries, heart valve replacement, and prophylactically in atrial fibrillation to help prevent the risk of cardioembolic stroke.[7, 8] Warfarin's efficacy is currently monitored by using the International Normalized Ratio (INR) which is a standardization of the prothrombin time. Warfarin has been linked to beneficial outcomes including reduction in stroke and recurrent venous thromboembolisms (VTEs) with INR's ranging from 2.0-3.5.[8] In addition, warfarin has been linked to an increased rate of serious bleeding with INRs above 4.0.[8, 9] For PGX, the two genes most documented in warfarin dosing are CYP2C9 and a gene impacting warfarin's pharmacodynamic effects, vitamin K epoxide reductase complex subunit 1 (VKORC1).[5, 7]

Warfarin and CYP2C9

Warfarin is extensively metabolized in the liver to inactive metabolites. The metabolism is stereoisomer specific with the 2-5 times more potent

Table 1. Genetic Polymorphisms Associated with Warfarin Dose and Occurrence[12]

Genetic polymorphism	Population	Minor allele frequency
CYP2C9*2	Whites	10-20%
	Blacks	0-2%
	Hispanics	7%
	Asians	0-2%
CYP2C9*3	Whites	6-10%
	Blacks	0-1%
	Hispanics	2%
	Asians	2-4%
VKORC1 1173C>T	Whites	46-48%
	Blacks	13-14%
	Hispanics	Not Reported
	Asians	96%
VKORC1 3730G>A	Whites	33-38%
	Blacks	43-51%
	Hispanics	Not Reported
	Asians	5-10%

Figure 1. Absolute Differences in Weekly Warfarin Doses From Initial Dose to Final/Stable Dose[5]
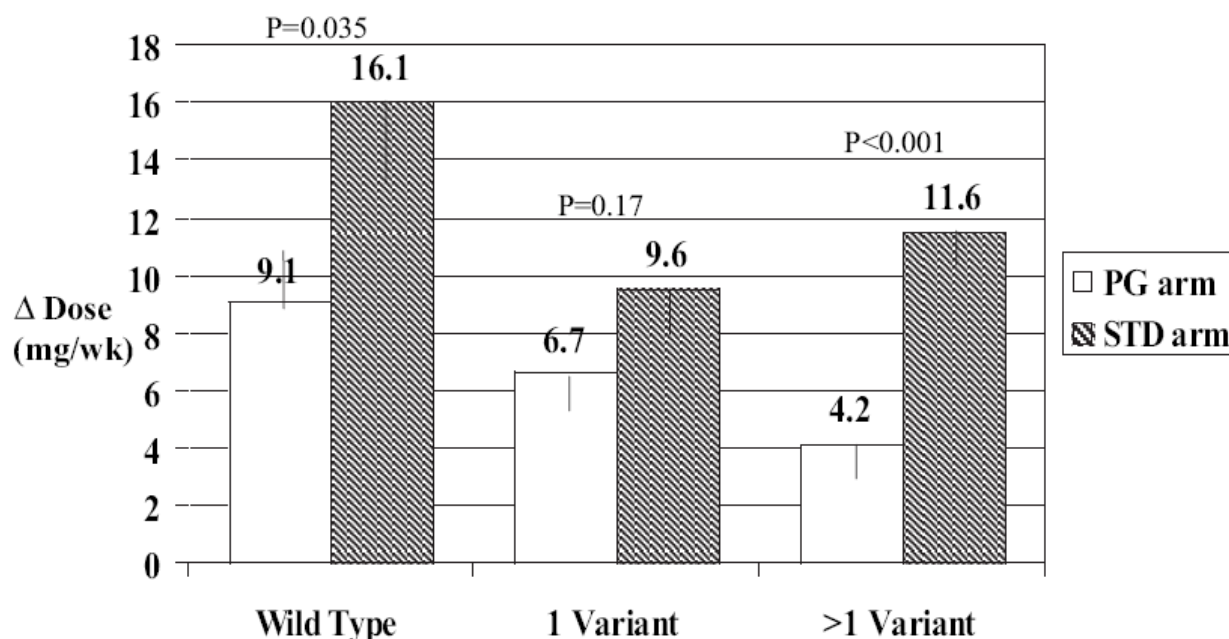
Wild Type refers to the patients that had normal CYP2C9 and VKORC1 genes based on the three main polymorphisms. A variant is the occurrence of mutant or variant allele at any of the three main polymorphisms.

S-warfarin primarily metabolized by CYP2C9 (the main modulator of *in vivo* warfarin activity) and the less potent R-warfarin metabolized by CYP1A2 and CYP3A4.[7] Two common polymorphisms in CYP2C9 are CYP2C9*2, which causes an amino acid change of a cysteine for the normally occurring arginine, and CYP2C9*3, which causes an amino acid change of an isoleucine to a leucine. These two alleles cause a reduction in CYP2C9 activity which would cause less clearance of the more potent S-warfarin. It has been reported that the necessary dose reductions for the *2 and *3 alleles have been as much as 17% and 37% respectively.[10] In a study of 191 patients who were pre-identified with one or both of these CYP2C9 variants, patients had a quicker time to first therapeutic INR and stable anticoagulation of 2.73 days and 18.1 days respectively compared to a standard warfarin initiation algorithm.[11] **Table 1** has the associated allele frequencies for CYP2C9.

Warfarin and VKORC1

Warfarin inhibits the VKOR enzyme on the C1 subunit preventing the regeneration of vitamin K epoxide. This in turn prevents the activation of the coagulant factors II, VII, IX, and X as well as the anticoagulant proteins C and S. There are two main genetic polymorphisms associated with warfarin dosing on the gene encoding the C1 subunit: VKORC1

1173C>T and VKORC1 3730G>A. VKORC1 1173C>T is the main polymorphism supported by the literature and is listed on the current package insert.[7] This particular polymorphism's minor allele is associated with a 44-63% reduction in warfarin dose.[13,14] The minor allele of the VKORC1 3730G>A has been associated with higher maintenance doses of warfarin in some cases by as much as 90%.[13] Allele frequencies for these two polymorphisms are listed in **Table 1**.

Using Warfarin's PGX Data for Dosing

There are several studies combining the CYP2C9*2 and *3 genotypes with the VKORC1 1173C>T in their analyses. The studies have consistently demonstrated that individually these polymorphisms, when combined with age and body weight, add to the predictability in the dosing of warfarin.[15,16]

One recent randomized study evaluated prospective dosing of warfarin based on either a standard dosing algorithm or by PGX guided dosing. PGX guided dosing established a starting dose based on CYP2C9*2, CYP2C9*3, VKORC1 1173C>T, age, weight, and gender.[5] This study evaluated 206 patients whose dose adjustments were done by an unblinded anticoagulation pharmacist. There were no differences in overall out of range INR values between the groups (primary end point); however, in a subset analysis of the combined patients with normal

Figure 2. Average Stable Weekly Maintenance Dose of Warfarin by the Number of Variants[5]
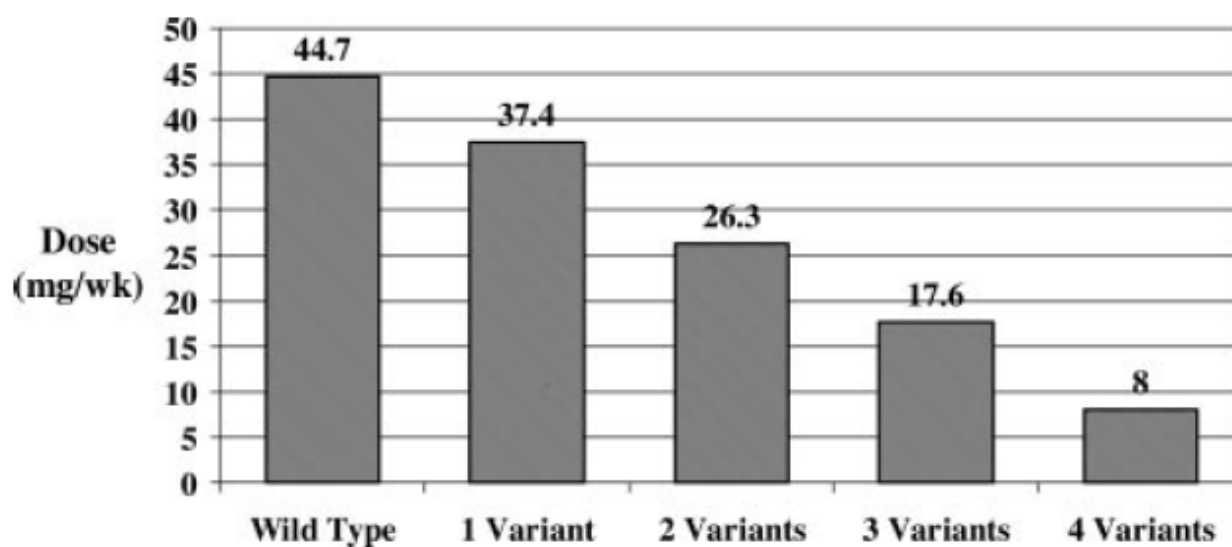
CYP2C9 and VKORC1 and those with multiple variants across those genes, the PGX guided dosing had significantly less out of range INRs. The PGX guided arm, in secondary end point analyses, proved to better predict the stable dose (**Figures 1 and 2**), required less dosing changes, and when doses needed to be adjusted, the PGX arm required less change in the dose.

Other Treatments and PGX

PGX has also proven beneficial in the dosing and treatment choices in several other areas of therapy. In heart failure, β-adrenergic receptor (ADRB) genetics have been associated with survival, cardiac remodeling, and left ventricular ejection fraction with β-blockers.[17] The ability of hydrochlorothiazide to lower blood pressure and reduce hard outcomes such as myocardial infarction and stroke have been linked to genetic differences in the adducin 1 (ADD1) gene.[18] In oncology therapeutics, thiopurine S-methyltransferase (TPMT) genetic mutations have been associated with increased toxicity to agents such as 6-mercaptopurine and azathioprine and thus the need for lower dosing. Genetic differences in the genes encoding p-glycoprotein (MDR1), the mu opiod receptor (OPRM1), and a CYP metabolizing enzyme (CYP2D6) are linked to opiate efficacy and side effects in pain management.[19, 20] In Alzheimer's, apolipoprotein E (APOE) genetics of drugs such as tacrine, donepezil, and rivastigmine have been linked to clinical response.[21] These examples merely touch the surface of the studied PGX interactions and begin to lay the groundwork for a poten-tially new way to dose medications.

Summary

Variability in patient response to therapy makes it difficult to correctly treat patients and know what therapy and dose is most appropriate to initiate. PGX is an emerging area of study that can aid in increasing treatment effectiveness over a shorter amount of time. Warfarin and the genes encoding its metabolizing enzyme, CYP2C9, and its site of action, VKORC1, is an exciting application of PGX research. CYP2C9*2 and *3 polymorphisms have been associated with reduced enzyme activity, and *in vivo,* a reduced warfarin maintenance dose. Likewise, VKORC1 1173C>T has been associated with less warfarin to achieve adequate anticoagulation. The use of PGX information is gaining clinical recognition as the FDA has approved the use of PGX data on warfarin's package insert with a subsequent approval of a lab test (Verigene® Warfarin Metabolism Nucleic Acid Test) to assess these genetic polymorphisms.[22] The role of PGX in clinical therapy will continue to grow beyond its current applications in heart failure, hypertension, oncology, pain management, and dementia.

References

1. Antonarakis SE, Lyle R, Dermitzakis ET, Reymond A, Deutsch S. Chromosome 21 and down syndrome: from genomics to pathophysiology. Nat Rev Genet 2004;5:725-38.
2. Castellani C, Cuppens H, Macek M, Jr., et al. Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. J

Cyst Fibros 2008;7:179-96.

3. Nicholls DP, Cather M, Byrne C, Graham CA, Young IS. Diagnosis of heterozygous familial hypercholesterolaemia in children. Int J Clin Pract 2008.

4. Evans DA, Clarke CA. Pharmacogenetics. Br Med Bull 1961;17:234-40.

5. Anderson JL, Horne BD, Stevens SM, et al. Randomized trial of genotype-guided versus standard warfarin dosing in patients initiating oral anticoagulation. Circulation 2007;116:2563-70.

6. Langaee TY, Gong Y, Yarandi HN, et al. Association of CYP3A5 polymorphisms with hypertension and antihypertensive response to verapamil. Clin Pharmacol Ther 2007;81:386-91.

7. Bristol-Myers Squibb. Coumadin (warfarin sodium) package insert. Princeton, NJ:2007 Nov.

8. The Seventh ACCP Consensus Conference on Antithrombotic Therapy. The Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy: Evidence-Based Guidelines. CHEST 2004;126:163S-696S.

9. Hylek EM, Go AS, Chang Y, et al. Effect of intensity of oral anticoagulation on stroke severity and mortality in atrial fibrillation. N Engl J Med 2003;349:1019-26.

10. Sanderson S, Emery J, Higgins J. CYP2C9 gene variants, drug dose, and bleeding risk in warfarin-treated patients: a HuGEnet systematic review and meta-analysis. Genet Med 2005;7:97-104.

11. Caraco Y, Blotnick S, Muszkat M. CYP2C9 genotype-guided warfarin prescribing enhances the efficacy and safety of anticoagulation: a prospective randomized controlled study. Clin Pharmacol Ther 2008;83:460-70.

12. Entrez SNP: dpSNP Build 129. In.

13. Herman D, Peternel P, Stegnar M, Breskvar K, Dolzan V. The influence of sequence variations in factor VII, gamma-glutamyl carboxylase and vitamin K epoxide reductase complex genes on warfarin dose requirement. Thromb Haemost 2006;95:782-7.

14. D'Andrea G, D'Ambrosio RL, Di Perna P, et al. A polymorphism in the VKORC1 gene is associated with an interindividual variability in the dose-anticoagulant effect of warfarin. Blood 2005;105:645-9.

15. Miao L, Yang J, Huang C, Shen Z. Contribution of age, body weight, and CYP2C9 and VKORC1 genotype to the anticoagulant response to warfarin: proposal for a new dosing regimen in Chinese patients. Eur J Clin Pharmacol 2007;63:1135-41.

16. Borgiani P, Ciccacci C, Forte V, Romano S, Federici G, Novelli G. Allelic variants in the CYP2C9 and VKORC1 loci and interindividual variability in the anticoagulant dose effect of warfarin in Italians. Pharmacogenomics 2007;8:1545-50.

17. Muthumala A, Drenos F, Elliott PM, Humphries SE. Role of beta adrenergic receptor polymorphisms in heart failure: systematic review and meta-analysis. Eur J Heart Fail 2008;10:3-13.

18. Arnett DK, Claas SA, Glasser SP. Pharmacogenetics of antihypertensive treatment. Vascul Pharmacol 2006;44:107-18.

19. Campa D, Gioia A, Tomei A, Poli P, Barale R. Association of ABCB1/MDR1 and OPRM1 gene polymorphisms with morphine pain relief. Clin Pharmacol Ther 2008;83:559-66.

20. Lotsch J, Skarke C, Liefhold J, Geisslinger G. Genetic predictors of the clinical response to opioid analgesics: clinical utility and future perspectives. Clin Pharmacokinet 2004;43:983-1013.

21. Cacabelos R. Pharmacogenomics and therapeutic prospects in dementia. Eur Arch Psychiatry Clin Neurosci 2008;258 Suppl 1:28-47.

22. FDA. FDA Clears Genetic Lab Test for Warfarin Sensitivity. September 17, 2007.