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. 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. 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™). 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
normal gene sequence), and deletions (a single nu-
cleotide or more is removed from the gene se-
quence). 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 ge-
netic changes are often classified as gene*N where N
is a particular genetic polymorphism with the desig-
nation 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 pro-
voked and idiopathic deep vein thrombosis or pulmo-
nary embolism, after selective major surgeries, heart
valve replacement, and prophylactically in atrial fib-
tillation 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 in-
cluding 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 ep-
oxide 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>
<thead>
<tr>
<th>Genetic polymorphism</th>
<th>Population</th>
<th>Minor allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9*2</td>
<td>Whites</td>
<td>10-20%</td>
</tr>
<tr>
<td></td>
<td>Blacks</td>
<td>0-2%</td>
</tr>
<tr>
<td></td>
<td>Hispanics</td>
<td>7%</td>
</tr>
<tr>
<td>CYP2C9*3</td>
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<tr>
<td></td>
<td>Asians</td>
<td>2-4%</td>
</tr>
<tr>
<td>VKORC1 1173C&gt;T</td>
<td>Whites</td>
<td>46-48%</td>
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<td>Asians</td>
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<td></td>
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<td>5-10%</td>
</tr>
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</table>
S-warfarin primarily metabolized by CYP2C9 (the main modulator of \textit{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 \textit{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 \textit{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
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 mercaptopurine and azathioprine and thus the need for lower dosing. Genetic differences in the genes encoding p-glycoprotein (MDR1), the mu opioid 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 potentially 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


