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5-1-2009

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## Recommended Citation

Brazeau, Daniel A. and Brazeau, Gayle A., "Role of the Genomics Revolution in Pharmaceuticals" (2009). *Pharmaceutical Sciences Faculty Publications*. Paper 5.

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## Role of the Genomics Revolution in Pharmaceuticals

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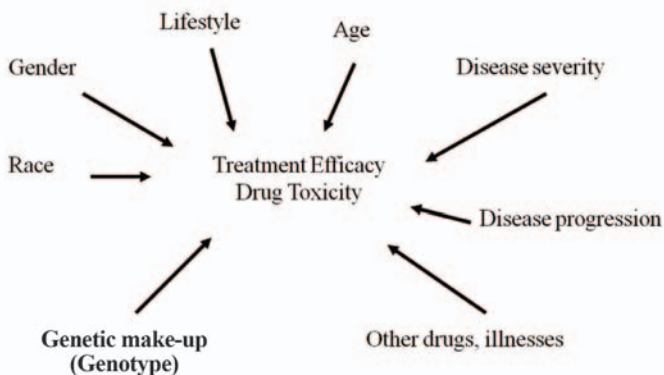
DOI: 10.1309/UDWIGLRLLMUGYN

The genomics revolution, which has seemingly pervaded all of the biological and biomedical sciences, has brought about its most impressive advances in the pharmaceutical sciences. The technological advances in high-throughput DNA sequencing, in assessing gene expression for thousands of transcripts, and in RNA constructs to silence specific genes have not only been the driving force behind genomic studies, but have also contributed to an emerging paradigm shift that is occurring in pharmacology, drug development, and pharmacotherapeutics. The molecular tools now readily available to laboratories have hastened the shift from drug development based largely on chemistry to one based on our growing biological knowledge of the physiological and molecular effects of compounds.<sup>1</sup> It is now becoming possible with these tools to assess at the cellular level the nature of drug action, toxicity, and tolerance. Similarly, our understanding of human disease is being refined, which will lead to more precise therapeutic interventions based on more precise understandings of disease states.

Ironically, the area of genomic-driven advances in pharmacy that enjoys the most public press is also the area where the advances are the least certain. Much has been written about the age of personalized medicine where each patient's genetic makeup will determine an individual-specific course of drug treatment designed to be the most efficacious and safe. Pharmacogenetics, which has been an active area of research for over 50 years,<sup>2</sup> seeks to provide patients efficacious therapeutics with minimal adverse drug reactions based on their genetic makeup (genotype) at one or more genes determining drug metabolism and/or drug transport (drug metabolism or transport pharmacogenetics) or in genes that are the direct targets of drug action (drug target pharmacogenetics). The literature is extensive, including several new journals detailing studies showing gene-drug relationships and the importance of including a patient's genetic makeup in guiding therapeutic decision making.<sup>3-7</sup> However, a significant portion of this body of literature consists of contradictory reports or at the least call into question the utility of genetic testing in guiding therapeutic management. For example, one of the most studied genes in pharmacogenetics, *MDR1* (*ABCB1*), the gene that codes for the ubiquitous drug efflux pump, P-glycoprotein (P-gp), has so far defied any straightforward consensus concerning the importance of genetic polymorphisms in drug disposition and response.<sup>8</sup> Regarding the pharmacogenetics of cytochrome P450 genes (CYP), Nebert and Vesell<sup>9</sup> have cautioned that even recent, much lowered estimates of reductions in adverse drug reactions of 10% to 20% by the extensive genotyping of CYP polymorphisms may be overly optimistic. This constant background of contrary data suggests caution should be used in assessing the importance of pharmacogenetics in managing drug therapies.<sup>10-14</sup>

The many contrary reports, or the authors making them, are not suggesting that there is little to be gained by pharmacogenetics studies and genetic testing. Rather, this data underscores the complexity of the human genome, the extensive genetic diversity among humans, and the nature of gene-gene and gene-environment interactions. This paper summarizes some of these complexities using two of the more successful pharmacogenetic "stories" to highlight the confounding issues involved for all genes that play a role in drug response and efficacy.

Prior to considering the complexities of genomes, it is first important to reaffirm the role of environmental factors in individual variation of drug response. There are many environmental factors determining how a given patient will respond to a drug including age, gender, diet, as well other concurrent drug therapies (Figure 1). These nongenetic factors often play a large role in the discrepancies in genotype-phenotype studies.<sup>13</sup> Assessing the proportion of variation in patient response due to environment is absolutely essential before the role of genetics can be considered.



**Figure 1** Many factors, both environmental, patient-specific, and genetic may determine how an individual will respond to a therapeutic agent. Pharmacogenetics seeks to identify that portion of variation in response due to genetic differences among humans.

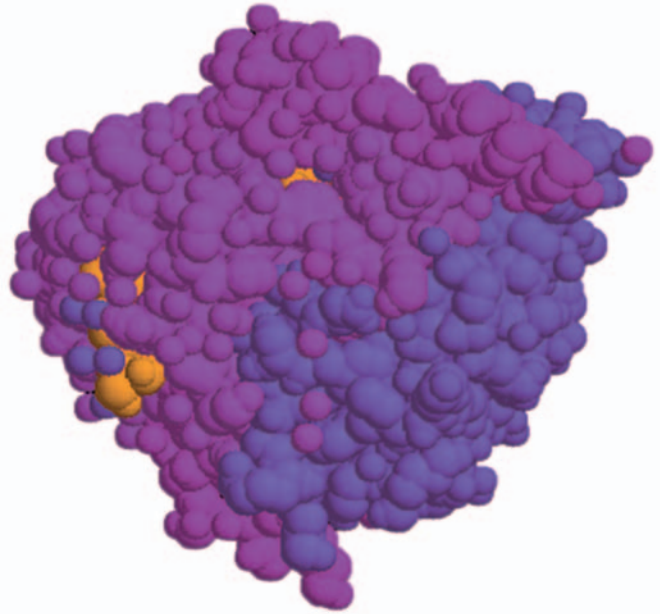
The two pharmacogenetic "success stories" that follow are in part successful because the involved compounds possess two critical properties. These properties are shared by most pharmacogenetics success stories.<sup>9</sup> The first property is one born of the drug itself. Drugs with narrow therapeutic windows are more likely to be pharmacogenetically relevant since even small, genetically-based differences in patient drug metabolism, absorption, distribution, or clearance may result in toxicity. Second, for both groups of compounds there exists at least one critical step in the drug response pathway controlled principally by a single gene—the phenotype is said to be monogenic. Drugs that are metabolized by many genes, or for which there are multiple alternate pathways, are unlikely to elicit varying responses due to polymorphisms in a single gene—the phenotype is said to be polygenic. Similarly, in the same sense that multiple genes lessen the importance of any one gene in patient response, multiple polymorphisms within one gene will tend to lessen the utility of pharmacogenetic studies. The pharmacogenetics of a system can become confusing if the gene responsible for the phenotype has many polymorphisms, all with the similar phenotypic outcomes (referred to as allelic heterogeneity). In such cases, the utility of a single genetic test to guide dosing is small if it is predictive for only a limited subset of patients.

The two examples described here are the pharmacogenetics of thiopurines and warfarin. These compounds both have narrow therapeutic windows and have phenotypes that are determined by one or two genes. They have both been noted repeatedly as classic examples of the important role pharmacogenetics will play in explaining variation in patient response.

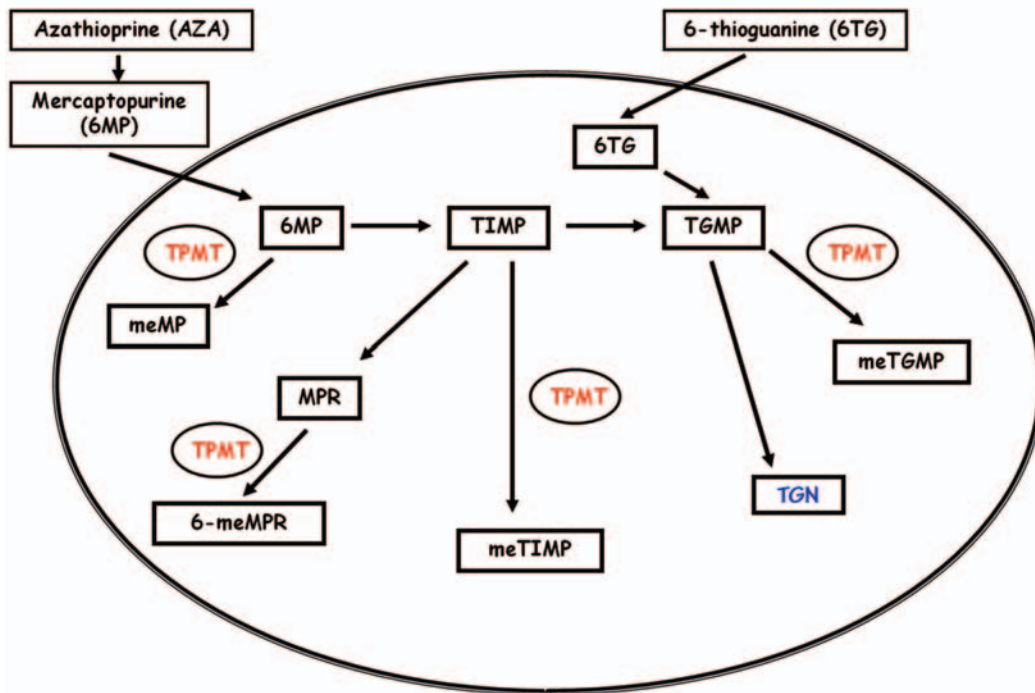
We will use them here to elucidate other features of both drugs and genetics that are important in understanding the limitations and complexities of pharmacogenetics.

**Pharmacogenetics of Thiopurine S-methyltransferase (TPMT)**

Thiopurines are among the first line treatments for childhood acute lymphoblastic leukemia (ALL), organ transplant recipients, inflammatory bowel disease, and autoimmune diseases.<sup>14</sup> Thiopurine S-methyltransferase catalyzes the S-methylation of a number of chemotherapeutic prodrugs such as 6-mercaptopurine (6-MP), 6-thioguanine (6-TG), and azathioprine (AZA). In the cell, 6-MP and AZA are converted into thioinosine monophosphate (TIMP) and ultimately into thioguanosine monophosphate (TGMP). TGMP is ultimately converted into cytotoxic nucleotide analogs (TGN), which inhibits DNA and RNA synthesis. The S-methylation of 6-MP to methylmercaptopurine (meMP) is an inactivation pathway leading to the ultimate clearance of 6-MP and 6-TG (Figure 2). High TGN accumulation has been linked to hematopoietic toxicity and results in low patient tolerance to thiopurine therapy. The genetic role of polymorphisms in TPMT and resulting enzyme activity was first noted in red blood cells of healthy volunteers.<sup>16</sup> Three groups were indentified with high, intermediate, and low enzyme activities. These three groups



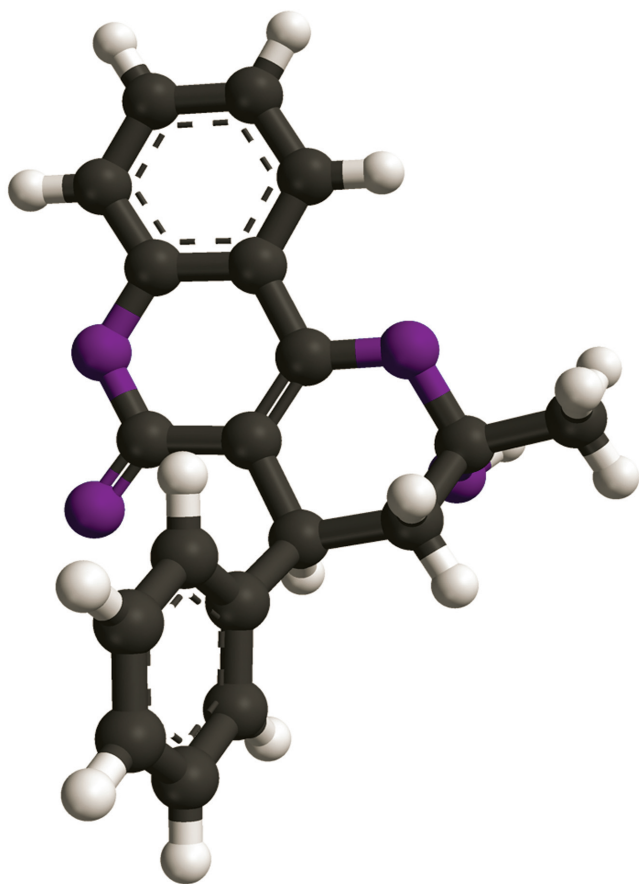
have now been shown to represent individuals carrying either zero, one, or two variant alleles for TPMT. Thus, hematopoietic toxicity, the phenotype of interest, is determined in part by a single gene (monogenic). Interestingly, another enzyme,



**Figure 2** The inactive prodrugs, 6-mercaptopurine (6-MP), 6-thioguanine (6-TG), and azathioprine (AZA), are activated by multiple enzymes. After uptake, 6-MP and AZA are converted into thioinosine monophosphate (TIMP). 6-TG is converted into thioguanosine monophosphate (TGMP). Thiopurine methyltransferase (TPMT) is the major enzyme involved in the inactivation pathway for all three thiopurines to methylmercaptopurine (meMP), 6-methylmercaptopurine (6-meMP), methylthioguanosine monophosphate (meTGMP), and methyl-thioinosine monophosphate (meTIMP). Cytotoxic effects of thiopurine drugs occur when cytotoxic nucleotide analogs (TGN) are incorporated into DNA or RNA stopping synthesis. Adapted from [www.pharmgkb.org/index.jsp](http://www.pharmgkb.org/index.jsp).<sup>15</sup>

xanthine oxidase (XHD), also plays a role in inactivation of thiopurines. However, in hematopoietic tissue, XHD is not expressed<sup>17</sup> so the polymorphisms that must certainly exist in XDH do not confound the phenotypic response of interest; namely, hematopoietic toxicity.

Polymorphisms in TPMT have been shown to play a role in explaining individual variation in response to thiopurine drug therapy. Low TPMT activity is associated with hematopoietic toxicity in patients treated with standard doses of 6-MP, 6-TG, or AZA.<sup>18</sup> Twenty-nine variant alleles have been identified in TPMT as of 2008. Of these variant alleles, four have been most studied (for review see Wang and Weinshilboum<sup>19</sup>) with regard to their effects on decreased TPMT activity—*TPMT*\*2, \*3A (most common in Caucasians), \*3B, and \*3C (most common variant in African Americans). The most common variant allele, *TPMT*\*3A, is associated with low TPMT activity though no differences



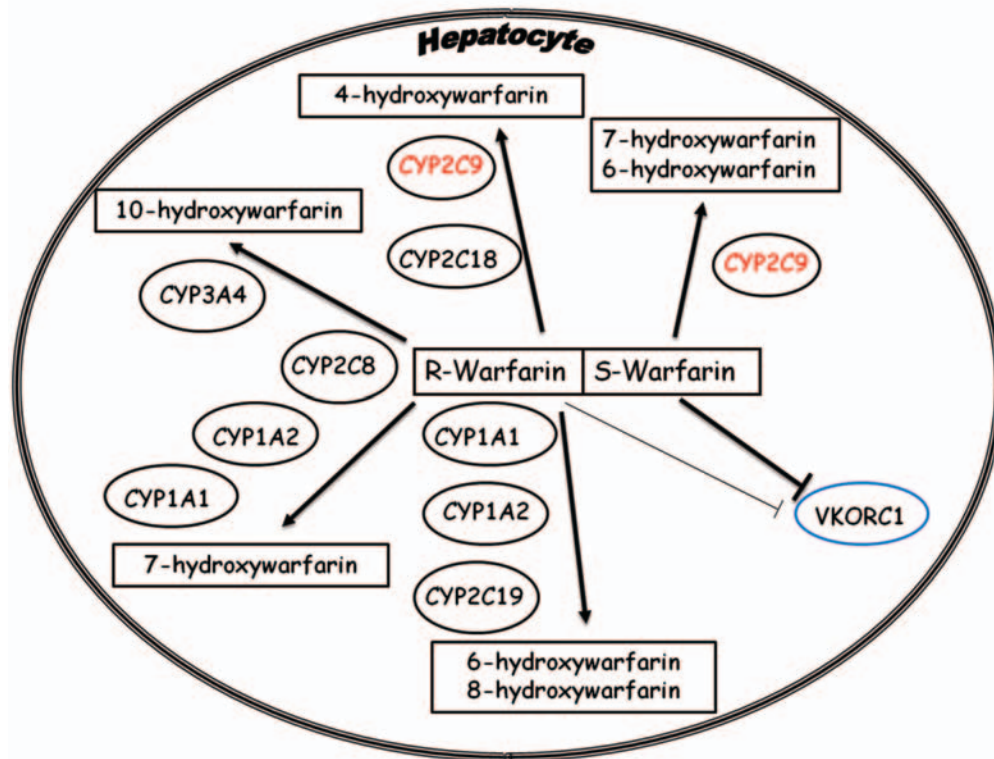
are noted in gene expression compared with the wildtype allele.<sup>20</sup> *TPMT*\*2 shows loss of catalytic activity though no difference in mRNA concentrations.<sup>21</sup> The low enzyme activity observed for both variants is likely due to greater rate of degradation.<sup>20,22</sup> Most importantly, while other variants have been identified (*TPMT*\*4, \*5, \*6, and \*7), they appear to be rare and therefore unlikely to confound the relationship between phenotype and genotype, at least among the populations studied to date. Note, however, that the four common variant alleles do not explain all the side effects associated with thiopurine therapy.<sup>23</sup> As is often the problem for many other pharmacogenetic cases, there are other variant alleles

for TPMT whose frequencies and, therefore, importance in other populations is yet unknown. In a review examining the role of genetic variation in TPMT mediated adverse drug reactions, van Aken and colleagues found that 78% of the adverse drug reactions could not be accounted for by a limited number of polymorphisms generally examined in TPMT.<sup>23</sup> These authors point out the need for further studies identifying additional variant alleles in other ethnic groups and for the need for continued careful clinical monitoring of adverse drug reactions.

## Pharmacogenetics of Warfarin

Warfarin is a commonly prescribed oral anticoagulant for the prevention and treatment of myocardial infarction, ischemic stroke, venous thrombosis, and atrial fibrillation. Warfarin has a narrow therapeutic window with large interpatient variation. Insufficient dosing may prevent thromboembolism while over dosing may cause risk of bleeding events. Warfarin is a very effective antagonist of the vitamin K epoxide reductase complex (VKORC1), a critical enzyme in the vitamin K-dependent clotting pathway. Warfarin is delivered as a racemic mixture of the R and S stereoisomers. The stereoisomers are metabolized by different members of the cytochrome P450 phase 1 enzymes. S-warfarin is the more potent inhibitor of VKORC1 by 3- to 5-fold and accounts for 60% to 70% of the anticoagulation response (for review see Yin and Miyata<sup>24</sup>). This is critical in terms of warfarin's pharmacogenetics since S-warfarin is largely metabolized by a single enzyme (CYP2C9) and thus behaves as a monogenic trait. In contrast, R-warfarin is metabolized by a number of CYP enzymes, mainly CYP3A4, and to a lesser degree CYP 1A1, 1A2, 2C8, 2C9, 2C18, and 2C19. If R-warfarin were the most active agent, it is unlikely this drug would be important pharmacogenetically since with so many different genes involved in its metabolism, no single set of polymorphisms would be useful predictors of therapeutic outcome.

To date, more than 50 variants in CYP2C9 have been described in human populations; at least 24 are nonsynonymous substitutions resulting in proteins with altered amino acids sequences. Two variants, CYP2C9\*2 and \*3, are the most common and most extensively studied. Patients with CYP2C9\*2 and/or CYP2C9\*3 variants metabolize warfarin more slowly; thus, traditional dosing regimens may lead to bleeding events or longer times to achieve stable drug concentrations versus dose during which bleeding events may occur. Other polymorphisms that occur at much lower frequencies have not been evaluated. Importantly, frequencies of CYP2C9\*2 and \*3 vary considerably among ethnic populations. Among Caucasians, \*2 and \*3 frequencies vary from 8% to 20% and 6% to 10%, respectively. Unfortunately, in respect to their utility as general predictors of patient response to warfarin therapy, CYP2C9\*2 and \*3 are largely absent in Asian populations and are rare in African-American populations with frequencies ranging from 1% to 4%. Once again, the distribution of clinically important alleles among human populations is important and limits the universal application of data gathered from one ethnic group. This problem cannot be overstated. The advancements to be derived from pharmacogenetics are dependent upon the cataloging of all relevant variants in human populations and the development of large-scale genetic screening technologies to identify these alleles.



**Figure 3** Warfarin is a natural product and is given as a racemic mixture of the R and S stereoisomers of the drug. The stereoisomers are metabolized by a number of phase 1 enzymes, though significantly the S isomer is metabolized by a single enzyme, CYP2C9. In contrast, the less active R-warfarin is metabolized mainly by CYP3A4, but also CYP1A1, CYP1A2, CYP2C8, CYP2C9, CYP2C18, and CYP2C19. Warfarin, particularly the S isomer, is a potent inhibitor of vitamin K epoxide reductase complex (VKORC1), a critical enzyme in the vitamin K-dependent clotting pathway. Adapted from [www.pharmgkb.org/index.jsp](http://www.pharmgkb.org/index.jsp).<sup>15</sup>

There is yet another player in the warfarin pharmacogenetics story. The target of warfarin (VKORC1) also has variant alleles that affect how patients respond to therapy. This is an example of drug target pharmacogenetics, which, unlike pharmacogenetics of genes involved in drug metabolism or drug transport, often results in differences in the pharmacodynamics of response. Mutations in VKORC1 have been identified in vitamin K-deficiency disorders and warfarin resistance. There are at least five important variants for VKORC1 as well as numerous, less-common variants. Fortunately, these variants can be grouped into four haplotypes—chromosomal segments within which the DNA sequence is invariant or constant among most human populations. These four haplotypes (VKORC1\*1, \*2, \*3, and \*4) include most of the common SNPs that contribute to interpatient variation in warfarin dosing in Caucasians. VKORC1\*1 is considered the reference sequence and is the likely ancestral haplotype. Individuals with the VKORC1\*2 haplotype (also confusingly referred to as haplotype group A) require lower warfarin doses. This haplotype is common in Asians and Caucasians and rare in African populations.<sup>25</sup> VKORC1\*3 and VKORC1\*4 (referred to as haplotype group B) require a higher warfarin dose. VKORC1\*3 is the most common haplotype in African populations and is also common in Caucasians.

Recently, another CYP gene has been identified that has a clinically important impact on the ability of patients to reach stable warfarin dosing. An allele in CYP4F2 that is at moderate frequency in Asians and Caucasians (~30%) though

low in African-Americans (7%) results in higher warfarin doses to stable dosing. Thus, the warfarin story becomes less perfect. The metabolic role of CYP4F2 is, as yet, unknown.

## Summary

Neither of the two examples presented here is the perfect pharmacogenetics story, though they may be the best examples to date. Our goal is to highlight these two examples as a means of describing some of the problems that are common to many pharmacogenetic cases. The pharmacogenetic literature contains many examples of confusing, or even contradictory, studies that arise due to unknown environmental factors that result in poor outcomes; drugs whose metabolism/transport are affected by multiple genes in multiple pathways; and clinically important genes that have many rare allelic variants with similar phenotypic variation in the frequencies of allelic variants among ethnic groups that mask the role of any one variant.

These issues are common to most gene/drug dynamics and do not preclude the importance of pharmacogenetic studies. They do call for more realistic assessments of the role of genetic testing for the practicing clinician as this field develops. LM

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