Opinion statement
Pharmacogenomics holds the promise of transforming patient care by allowing providers to tailor therapy to each individual patient based on his or her genetic information. Although no established pharmacogenomic applications in cardiovascular medicine yet exist, there are at least three emerging applications that may ultimately become routine clinical practice; these are related to warfarin, clopidogrel, and statins. Of the three, warfarin pharmacogenomics has been the most rigorously evaluated to date, with several clinical trials either completed or underway. Clopidogrel pharmacogenomics has a growing body of supporting scientific evidence and warrants evaluation in prospective clinical trials. Statin pharmacogenomics remains the least developed application, with controversy surrounding a widely marketed genetic test whose validity has been questioned by recent evidence. Providers are advised to take a “wait and see” approach to pharmacogenomics at the present time, with the expectation that it will be a few years before any cardiovascular pharmacogenomic application is unequivocally proven to be both cost-effective as well as of clinical benefit.

Introduction
Pharmacogenomics, the study of variations of DNA and RNA characteristics as related to drug response, has emerged as a promising area for the clinical application of personal genetic information. Commonly used pharmacologic agents such as lipid-lowering drugs, antiplatelet agents, anticoagulants, and antiarrhythmic drugs have differential effects depending on variation of specific DNA sequences in or near genes. The ultimate objective of pharmacogenomics is the use of “the right dose of the right drug for the right patient” by predicting the therapeutic response as well as any adverse consequences before the drug is administered.

In its present form, pharmacogenomics focuses primarily on the identification of DNA variants that are associated with response to therapy; these DNA variants usually comprise two different versions (known as alleles) at a single location in the genome. In the ideal scenario, patients with different alleles of a given DNA variant (i.e., different genotypes) would
display significantly different responses to a medication. Typically, the DNA variants are located in or near genes that encode enzymes that metabolize the medication in question; one allele may result in increased activity of the enzyme compared to the other allele, resulting in different blood levels of the original medication or of an active metabolite. In some cases, there may be no known biological link between the DNA variant and the medication, only a statistical association between the DNA variant and the patient response to the medication.

Although there are examples of pharmacogenomic applications in routine clinical use for other specialties, in cardiovascular medicine there are not yet any widely accepted uses of pharmacogenomics to guide therapy. In this review, three emerging applications—related to anticoagulants, antiplatelet agents, and lipid-lowering agents—are discussed.

## Treatment

### Pharmacologic treatment

- One practical application for pharmacogenomics is the use of screening tests to identify patients who are more or less likely to respond to medications, either in favorable ways or in adverse ways. A patient with a possible indication for a medication would undergo the screening test, which would identify the genotype of a relevant polymorphism (DNA variant) or set of polymorphisms. This genotype information could then be used to help the provider decide whether the patient's condition is likely to improve from the medication, whether the medication poses a risk and should be avoided altogether, or what specific dose of the medication should be given.

### Warfarin

Warfarin, an anticoagulant widely used for the prevention and treatment of thromboembolic disease, is one of the most challenging drugs in clinical use due to the highly variable responses among patients and even within an individual patient, with a number of factors influencing the responses including age, diet, weight, and use of interacting medications. Patients on warfarin require frequent monitoring of blood clotting activity as measured by the prothrombin time–International Normalized Ratio (INR), particularly in the first few weeks after initiation of the drug when it is unclear what the patient’s ultimate stable therapeutic dosing will be. As such, there is significant risk of either thromboembolism if the warfarin dose is too low or bleeding if the dose is too high.

Polymorphisms in two genes, **CYP2C9** (cytochrome P450 2C9) and **VKORC1** (vitamin K epoxide reductase complex subunit 1), have been demonstrated to account for more than one third of the interindividual variation in stable therapeutic dosing of warfarin [1–5]. Both genes modulate the activity of warfarin. **CYP2C9** encodes the hepatic enzyme responsible for converting warfarin into an inactive form. **VKORC1**—the pharmacologic target of warfarin—controls the function of an enzyme complex that produces the active form of vitamin K. An early trial reported in 2007 evaluated an algorithm that used the **CYP2C9** and **VKORC1** polymorphisms to predict an optimal starting warfarin dose for anticoagulation [6]. When compared to the usual practice (providers
choosing a starting dose using their best clinical judgment), the pharmacogenomic algorithm did not improve the safety of warfarin initiation (as judged by the number of out-of-range INRs during the initiation period, which were unchanged), although it did reduce the numbers and sizes of dosing changes needed to achieve stable therapeutic dosing [6].

Although not an unqualified success, this early study instigated a number of efforts to demonstrate the utility of warfarin pharmacogenomic testing. Several subsequently published small, prospective clinical trials suggested that addition of genetic information could improve the safety and efficacy of warfarin initiation by the numbers (e.g., time to stable therapeutic dosing, amount of time spent in the therapeutic INR range), but none were large enough to be adequately powered to study clinical outcomes [7–9]. The first large warfarin genotyping study, comprising about 4000 individuals, was reported in 2010; the Medco-Mayo Warfarin Effectiveness Study (MM-WES) was designed to test whether the use of genotype information would reduce the incidence of hospitalizations from adverse effects of warfarin, whether bleeding or thromboembolism [10]. A unique feature of this study is that it took place in the “real world,” (i.e., within a community practice setting rather than within the context of a randomized prospective study with strict inclusion and exclusion criteria). About 900 patients who were initiating warfarin therapy submitted DNA samples for determination of CYP2C9 and VKORC1 genotypes. This information was given to their providers (along with a clinical interpretation of the results, but with no subsequent communication with the providers), and the patients were followed for 6 months. Historical controls (from the same community practice setting but who had initiated warfarin therapy the previous year) numbering about 2,700 were used as the primary comparison group.

Remarkably, there was a 31% reduction of hospitalization in the genotyped patients compared to the control patients (P<0.001), with a 28% reduction of hospitalization due to bleeding or thromboembolism [10]. Although there are aspects of MM-WES that can be fairly criticized (e.g., the possibility of selection bias with the lack of randomization and the use of historical rather than contemporaneous controls), the study’s use of patients in the community practice setting and the minimal intervention with respect to both the patients and the providers are clear strengths. Studies of alternative designs are underway, such as the Clarification of Optimal Anticoagulation Through Genetics (COAG) study, a prospective randomized clinical trial comparing a clinical algorithm for determining the dosing for warfarin initiation to a pharmacogenomic algorithm using CYP2C9 and VKORC1 genotypes [11]. This study is planned to include 1200 patients, the largest of its kind to date.

Although additional research studies of various designs such as COAG will be needed to confirm that warfarin genotyping results in clinical benefit in a cost-effective manner, the encouraging early results from MM-WES suggest that anticoagulation will emerge as the first widely adopted pharmacogenomic application in cardiovascular medicine. However, the impending availability of alternative oral anticoagulants, such as dabigatran, that have fixed dosing and do not require INR monitoring could ultimately preempt the use of a pharmacogenomic test for warfarin.
Clopidogrel

Another cardiovascular pharmacogenomic application for the prediction of response to therapy involves the antiplatelet agent clopidogrel, which is now a mainstay of post-acute coronary syndrome (ACS) care, particularly after percutaneous coronary intervention (PCI). Dual antiplatelet therapy with aspirin and clopidogrel carries a class I indication in these patients [12, 13]. It has become clear that patients display variable responses to clopidogrel therapy, linked to the conversion of clopidogrel into its active metabolite by the hepatic cytochrome P-450 2 C19 enzyme; a number of polymorphisms in the CYP2C19 gene encoding this enzyme have been identified, with reduced-function alleles described [14–16].

Three large studies of mostly post-ACS and/or post-PCI patients on clopidogrel therapy—TRITON-TIMI 38 (Trial to assess Improvement in Therapeutic Outcomes by optimizing platelet Inhibition with prasugrel Thrombolysis In Myocardial Infarction 38), FAST-MI (French registry of Acute ST-elevation and non–ST-elevation Myocardial Infarction), and AFIJI (Appraisal of risk Factors in young Ischemic patients Justifying aggressive Intervention)—genotyped the CYP2C19 gene and identified at least one reduced-function allele in about 30% of individuals. In all three studies, carriers of reduced-function alleles experienced significantly higher rates of cardiovascular death, myocardial infarction, and stroke [17–19]. In TRITON-TIMI 38, reduced-function allele carriers also displayed lower plasma levels of the active metabolite of clopidogrel [17], consistent with reduced metabolism of clopidogrel into the metabolite by the cytochrome P-450 2 C19 enzyme. These studies set the stage for a number of additional studies of the effects of polymorphisms in the CYP2C19 gene on cardiovascular outcomes, as well as instigating the release of a “boxed warning” label for clopidogrel by the US Food and Drug and Administration (FDA) indicating that individuals carrying two reduced-function CYP2C19 alleles (termed “poor metabolizers”) experience diminished effectiveness of the drug at standard dosing, and that alternative therapeutic strategies should be considered in these patients.

Notably, none of these three initial studies were performed in placebo-controlled clinical trials, so it was unclear whether the presence of reduced-function CYP2C19 alleles affects the relative reduction of cardiovascular risk by clopidogrel compared to placebo. This question was explored using data from participants in the CURE (Clopidogrel in Unstable Angina to Prevent Recurrent Events) and ACTIVE A (Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events A) trials, whose primary designs were to compare the effects of clopidogrel versus placebo on cardiovascular outcomes [20••]. Using genotype data from the participants, the investigators found that the relative risk reduction seen with clopidogrel treatment was similar for carriers of reduced-function CYP2C19 alleles and non-carriers; it was also noteworthy that within the clopidogrel arm, carriers had no significant difference in risk from non-carriers. These findings undermine the notion that CYP2C19 genotype testing could be useful for identifying which patients would receive increased or
decreased benefit from the standard dosing of clopidogrel therapy. However, very few of the patients in either trial underwent percutaneous coronary intervention (PCI) with stent placement (14.5% in CURE), highlighting that clopidogrel was largely being used as chronic therapy in lower-risk individuals [20].

A different pharmacogenetic study of participants in the PLATO (PLATelet inhibition and patient Outcomes) trial, in which 64% underwent PCI with stent placement, compared the effects of ticagrelor versus clopidogrel in carriers of reduced-function CYP2C19 alleles and non-carriers [21]. Irrespective of genotype, ticagrelor resulted in superior outcomes compared to clopidogrel, with similar relative risk reductions seen in the two groups. No difference was seen in outcomes on ticagrelor between carriers and non-carriers; although carriers experienced a higher event rate on clopidogrel than non-carriers within 30 days of initiation of therapy, in the long term there was no difference in the event rate on clopidogrel. This study suggested that 1) the effect of reduced-function CYP2C19 alleles is more relevant in the acute setting rather than the long term, and 2) as suggested by CURE and ACTIVE A, CYP2C19 genotype testing may not be useful for deciding which therapy to administer to which patients, at least for lower-risk patients.

To assess whether reduced-function CYP2C19 alleles may be more relevant in higher-risk patients (i.e., those who have undergone PCI with stent placement), a meta-analysis of nine clopidogrel pharmacogenomics studies comprising mostly PCI patients was performed [22]. This large study of almost 10,000 participants taking clopidogrel found that carriers of reduced-function CYP2C19 alleles experienced a 57% increase in risk of cardiovascular death, myocardial infarction, or ischemic stroke compared to non-carriers. The increased risk was seen in both carriers of two reduced-function CYP2C19 alleles (76% increase) as well as in carriers of just one reduced-function CYP2C19 allele (55% increase), with evidence of a dose-dependent effect. The difference was particularly notable with respect to stent thrombosis, with an almost tripling of risk of this outcome in reduced-function CYP2C19 allele carriers. Although no non-clopidogrel individuals were included in this meta-analysis, the large difference in risk of stent thrombosis argues strongly for the reduced efficacy of clopidogrel in carriers when used in post-PCI patients and lends justification for the FDA “boxed warning” label.

Unlike with warfarin, there have not yet been any clinical trials assessing whether the use of CYP2C19 genotypes improves clinical outcomes. Such studies will be needed to determine if routine post-ACS genotyping of CYP2C19 will be cost-effective and benefit patients. Therapeutic strategies that could be tested include 1) giving carriers of two reduced-function CYP2C19 alleles or, perhaps, one reduced-function allele a higher dose of clopidogrel than non-carriers, or 2) giving carriers other (more expensive) thienopyridines such as prasugrel and ticagrelor.

A final issue is whether it would be more useful to determine the CYP2C19 genotype or to empirically administer the standard dose of clopidogrel to a patient in the acute setting, followed by measurement of platelet function [23]. In principle, the degree of platelet function is more
directly relevant to cardiovascular endpoints such as stent thrombosis and so should be more predictive of the endpoints than genotype. Assuming wide availability and similar expense, platelet function testing would be preferable to genotype testing, especially because it could be used to monitor in real time the effects of increasing the dose of clopidogrel or using an alternative thienopyridine. One counter-argument is that as genomic medicine becomes commonplace, each patient’s genetic information will become part of his or her medical record, and the \textit{CYP2C19} genotype will already be known when the patient presents with acute coronary syndrome; if the patient carries reduced-function alleles, the providers would be alerted to avoid using the standard dosing of clopidogrel and to proceed directly to an alternative.

Another area of active investigation concerns potential interactions between thienopyridines and proton pump inhibitors (PPIs), particularly the potential for the latter to inhibit the effectiveness of the former. It is plausible that polymorphisms will be found to modify the interactions between the two classes of medications and, if such polymorphisms are characterized, that pharmacogenomic testing may have a future role in selecting particular drugs from the classes and doses of the drugs so as to mitigate undesirable interactions in individual patients [24].

\textit{Statins}

Statins and other lipid-modifying medications are some of the most widely prescribed drugs in the world, used for the primary and secondary prevention of cardiovascular disease. As such, there is enormous interest in identifying DNA variants that might help predict both the efficacy as well as adverse effects from statins in individual patients.

A genome-wide association study published in 2008 showed that individuals carrying two reduced-function alleles of a polymorphism in the \textit{SLCO1B1} gene, which encodes an organic anion transporter that regulates the hepatic uptake of statin drugs, have 17 times the risk of statin-induced myopathy than non-carriers [25]. The large difference in relative risk (though not absolute risk, given the rarity of statin-induced myopathy) conferred by the variant alleles suggests that a screening test could be helpful in predicting which patients are at risk of getting myopathy or other statin-induced adverse effects before they are started on statins [26]. Clinical trials to assess this potential pharmacogenomic application are just now getting underway.

The Trp719Arg (W719R) variant of the \textit{KIF6} (kinesin-like family 6) gene was identified in an analysis of 116 polymorphisms as potentially being associated with incident coronary heart disease in both European Americans and African Americans in the Atherosclerosis Risk in Communities (ARIC) study [27]. Spurred by the possibility that the \textit{KIF6} variant might signify a novel molecular pathway contributing to the pathogenesis of coronary disease, investigators subsequently performed post hoc analyses in longitudinal cohort studies as well as the placebo arms of several randomized clinical trials. Of note, in each individual study the association between the \textit{KIF6} variant and the primary cardiovascular endpoint was found to be either non-significant or of borderline
statistical significance after adjustment for multiple testing (i.e., using a more stringent \( p \) value threshold than \(<0.05\) when multiple polymorphisms or multiple associations were being tested, as was the case in most of these studies) [27–32]. Only upon pooling of data from multiple studies, best exemplified by a meta-analysis of seven studies suggesting a roughly 20% increase in risk for \( KIF6 \) variant carriers, was a solidly significant \( p \) value achieved [33•].

Contradicting these pooled analyses, a recent large case–control study with 17,000 cases with coronary disease and almost 40,000 controls attempted to replicate the \( KIF6 \) W719R variant association with disease [34••]. This study found minimal evidence for an association, placing an upper limit of about 2% on the increase in risk for \( KIF6 \) variant carriers. Of note, the case–control design used for this study was susceptible to biases that potentially weakened its power to detect any effect of the \( KIF6 \) variant on disease, such as inclusion of non-fatal disease cases but not fatal disease cases (the latter of which are included in cohort studies and randomized clinical trials), or the inclusion of more patients taking statins (who would be protected against disease) in the control group than in the case group. Nonetheless, it is telling that similar case–control studies on coronary disease were able to discern strong associations for well-established lipid genes that are unequivocally linked to disease [35, 36], such as \( LDLR, PCSK9 \), and \( LPA \)—suggesting that \( KIF6 \), if truly a causal gene for coronary disease, is of less importance to the disease process.

Irrespective of whether the \( KIF6 \) W719R variant is associated with coronary disease, early evidence suggested that the variant might be useful for a pharmacogenomic application—predicting a patient’s response to statin therapy. In a subset of the WOSCOPS (West of Scotland Coronary Prevention Study) trial, carriers of the \( KIF6 \) variant experienced greater protection against coronary heart disease with statin therapy compared to non-carriers of the \( KIF6 \) variant, although the difference was of borderline statistical significance after adjustment for multiple testing, and this particular study employed a case–control design [29]. In contrast, in the CARE (Cholesterol and Recurrent Events) trial, there was a smaller (and non-significant) difference in response to statin therapy between \( KIF6 \) variant carriers and non-carriers, although the trend still favored the carriers [29]. A similar non-significant difference in response to statin therapy was observed in the PROSPER (Prospective Study of Pravastatin in the Elderly at Risk) trial [37]. In a non–placebo-controlled trial, the PROVE IT-TIMI 22 (Pravastatin or Atorvastatin Evaluation and Infection Therapy: Thrombolysis in Myocardial Infarction 22) study, \( KIF6 \) variant carriers obtained significantly greater benefit from intensive statin therapy (atorvastatin, 80 mg daily) compared to moderate statin therapy (pravastatin, 40 mg daily) than did non-carriers [31], consistent with the findings from WOSCOPS.

On the basis of this data, a commercial test (\( KIF6 \)-StatinCheck from Berkeley HeartLab, Inc., San Francisco, CA) has been developed and widely marketed to health care providers as a tool to help decide whether patients should be started on statin therapy, with the premise being that non-carriers of the \( KIF6 \) W719R variant are less likely to benefit from a
statin drug. Of note, this test had neither been reviewed nor approved by the FDA at the time of this writing, nor had the FDA released a “boxed warning” label for statin therapy (as it had done for clopidogrel) to highlight the possibility of non-responders. Nonetheless, the company has reported performing nearly 200,000 KIF6-StatinCheck tests since it became available, with an estimated cost of about $100 for the test.

This is despite the possibility that the findings with the KIF6 W719R variant, both with respect to its association with coronary disease as well as its utility as a predictor of response to statin therapy, are false positives. As stated above, the largest study to date (albeit of a case-control design) failed to replicate the association of the variant with disease. Furthermore, a recent analysis of the prospective, randomized HPS (Heart Protection Study) clinical trial (simvastatin, 40 mg daily vs placebo), numbering more than 18,000 individuals (making it far larger than the pharmacogenomic studies in WOSCOPS, CARE, PROSPER, and PROVE IT-TIMI 22 described above) failed to see a difference between KIF6 variant carriers and non-carriers in their response to therapy, with both groups receiving significant benefit from statin therapy [38••].

Thus, the largest available datasets of two different study designs have failed to reproduce the original positive findings with KIF6, which has led to considerable skepticism about the utility of the KIF6-StatinCheck test [39].

For many observers, also problematic is the lack of biological plausibility for the KIF6 gene being a contributor to coronary disease and response to statin therapy. Unlike CYP2C9 and VKORCI, which have been firmly established to be involved in warfarin activity, and CYP2C19, with its involvement in clopidogrel metabolism, there is little biological information about the function of KIF6 besides being a member of the kinesin superfamily of proteins that are involved in intracellular transport, much less a credible hypothesis as to how it might affect statin activity.

Thus, KIF6 appears to be an example of a pharmacogenomic application where commercial interests and hype outpaced the careful scientific evaluation of the application. The field is eagerly awaiting further analyses of other large randomized clinical trials that should provide the definitive answer on whether the KIF6-StatinCheck test could be clinically useful, or whether the test has been inappropriately performed on tens of thousands of patients, many of whom may have been incorrectly told by their physicians that they would not benefit from statin therapy—a cautionary tale of the danger of premature adoption of a pharmacogenomic application in the face of insufficient evidence.

Disclosure

The author reports no potential conflict of interest relevant to this article.
References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance


This community-based study demonstrated that genotyping of patients started on warfarin reduced the risk of hospitalization over the next 60 days.


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The analysis of genetic data from more than 18,000 participants in the Heart Protection Study found no significant difference in the benefit conferred by statin use in carriers of the KIF6 W719R variant compared to non-carriers.