Pharmacogenomics: application to the management of cardiovascular disease

Julie A. Johnson, Pharm.D.[1], Larisa H. Cavallari, Pharm.D.[2], Amber L. Beitelshees, Pharm.D., MPH[3], Joshua P. Lewis, Ph.D.[3], Alan R Shuldiner, M.D.[3,4], Dan M. Roden, M.D.[5]

[1]Department of Pharmacotherapy and Translational Research and Center for Pharmacogenomics, University of Florida, [2]Department of Pharmacy Practice, University of Illinois at Chicago, [3]Department of Medicine and Program in Personalized and Genomic Medicine, University of Maryland, [4]Geriatric Research and Education Clinical Center, Veterans Administration Medical Center, Baltimore, MD, [5] Departments of Medicine and Pharmacology and Office of Personalized Medicine, Vanderbilt University

Address correspondence to:

Julie A. Johnson, Pharm.D.,
Department of Pharmacotherapy and Translational Research and Center for Pharmacogenomics
University of Florida
Box 100486
Gainesville, Florida, 32610-0486
Email: Johnson@cop.ufl.edu
Phone: 352-273-6007
FAX: 352-273-6121

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Abstract

There have been substantial advances in cardiovascular pharmacogenomics in the past decade. Genetic determinants of response to clopidogrel and warfarin have been defined, resulting in changes to the product label for each drug that suggests use of genetic information to guide therapy. Genetic tests are available, as are guidelines for incorporation of genetic

information into patient care decisions. These guidelines and literature supporting them are reviewed. Significant advances have also been made in the pharmacogenomics of statin-induced myopathy, and response to beta-blockers in heart failure, although the clinical applications of these findings are less clear. Other areas hold promise, including pharmacogenomics of antihypertensive drugs, aspirin, and drug-induced long QT syndrome. The potential value of pharmacogenomics in new drug discovery and drug development is also described. In summary, pharmacogenomics has current applications in management of cardiovascular disease, with clinically-relevant data continuing to mount.

Introduction

Uncovering the causes of inter-patient variability in drug response, and then utilizing that information to the benefit of patients, is at the heart of clinical pharmacology. While the term pharmacogenetics was coined in the 1950s, it is the last decade that has seen an explosion in the research focused on discovering the genetic basis for variable drug efficacy, toxicity and dose requirements. Pharmacogenomics research on cardiovascular drugs has been among the more active areas of investigation within this field. The last decade has seen substantial advances in our understanding of the genetic determinants of response to two commonly used cardiovascular drugs, clopidogrel and warfarin, such that the data on these drugs can now be used in the clinical setting. We highlight the data surrounding these examples, along with other areas of active research in cardiovascular pharmacogenomics that have not yet reached the stage of translation to practice, but hold promise. The data arising from cardiovascular pharmacogenomics research have not only led to potential clinical applications, but have advanced our understanding of the metabolism and or pharmacological mechanisms for a number of drugs. We also highlight the potential for pharmacogenomics research to influence drug discovery and drug development.

Cardiovascular pharmacogenomics and FDA labeling

Despite the growing appreciation of pharmacogenomic markers influencing response to cardiovascular drugs, there are limited examples for which FDA-approved labeling exists. According to the FDA's cataloging of labels, pharmacogenomic biomarkers are included in eight cardiovascular drug or drug combination labels (atorvastatin, carvedilol, clopidogrel, isosorbide/hydralazine, metoprolol, propafenone, propranolol, and warfarin (Table 1). The types of information included in labels are variable in nature and potential value in informing clinical decisions. For example, information ranges from the effect of genetically-influenced metabolism on drug exposure (e.g. hydralazine, carvedilol, metoprolol labels), to information on disease (but

not drug) genetics (e.g. atorvastatin label), to more clinically practical dosing information (e.g. warfarin and clopidogrel labels). Notably, the relative importance of the information seems to be reflected in its label location. For example, the potential for therapeutic failure to clopidogrel in CYP2C19 poor metabolizers is reflected in a boxed warning (among other locations), whereas for other drugs the information is in the clinical pharmacology section.

Among the cardiovascular drugs with pharmacogenomics data included in product labeling, warfarin and clopidogrel contain the strongest labeling. The warfarin label was updated twice (2007 and 2010) to reflect the growing body of knowledge regarding the influence of *CYP2C9* and *VKORC1* gene variations on dose requirements. The first update did not contain actionable information, likely a function of limited data on the clinical utility test information, whereas the second update provided a dosing table with expected dose requirements broken down by *CYP2C9* and *VKORC1* genotype. The clopidogrel label has been updated three times since 2009 to reflect knowledge gained regarding the influence of *CYP2C19* genotype on treatment outcomes. The most recent clopidogrel label update in March 2010 includes a boxed warning specifically advising avoidance of clopidogrel in patients with known genetic polymorphisms of *CYP2C19* and states that physicians should "consider alternative treatment or treatment strategies in patients identified as *CYP2C19* poor metabolizers." The label goes on to state that tests are available to determine a patient's *CYP2C19* genotype and can be used to aid in treatment decisions. It is these two examples for which there are also commercially available genetic tests, guidelines from the Clinical Pharmacogenomics Implementation Consortium (CPIC),(1, 2) and agreement that they are the most actionable among the cardiovascular examples.

Clinical application of pharmacogenomics knowledge: clopidogrel and warfarin

Clopidogrel pharmacogenomics

Clopidogrel inhibits platelet function, thereby preventing recurrent cardiovascular events in patients with acute coronary syndromes (ACS) and/or patients undergoing percutaneous coronary intervention (PCI). Enzymatic modification of clopidogrel, a thienopyridine prodrug, is required in order to produce its bioactive thiol metabolite (SR 26334), which irreversibly binds to the platelet P2Y12 receptor resulting in inhibition of adenosine diphosphate (ADP)-stimulated platelet aggregation for the duration of the platelet's lifespan (~10 days). Wide inter-individual variation of clopidogrel response is well recognized, and recent investigations have shown that this response is highly heritable(3). Single nucleotide polymorphisms (SNPs) in several genes critical for clopidogrel metabolism, transport, and signaling affect pharmacokinetic and pharmacodynamic action have been investigated including several of the cytochrome P450 (CYP) enzymes (e.g. *CYP1A2*, *CYP2C19*, *CYP3A4*, *CYP3A5*), P-glycoprotein (*ABCB1*), paraoxonase 1 (*PON1*), and *P2Y12* (Table 2). While there have been inconsistent findings regarding the effects of polymorphisms in some of these genes on clopidogrel response, there is compelling evidence that variants in *CYP2C19* significantly impact clopidogrel efficacy and recurrent cardiovascular event rates.(4, 5)

CYP2C19 encodes cytochrome P450 2C19, the hepatic enzyme responsible for metabolism of many drugs including clopidogrel, benzodiazepines, and some proton pump inhibitors. Multiple polymorphisms have been identified in *CYP2C19* that result in decreased as well as increased function(6). The most common of these variants include the loss-of-function *CYP2C19*2* variant (c.681G>A; rs4244285), and the gain-of-function *CYP2C19*17* variant (c.-806C>T; rs12248560). In populations of European-, African-, and East Asian-ancestry, the frequency of the loss-of-function *CYP2C19*2* allele is relatively high (15%, 15%, and 29% respectively), as is the gain-of-function *CYP2C19*17* allele (21%, 16%, and 3% respectively). In addition, the frequency of the loss-of-function *CYP2C19*3* variant (c.636G>A; rs4986893) is

considerable in Asian populations, ranging from 2-9%. Other functional variants in *CYP2C19* are rare, generally with frequencies less than 1%.

Several recent studies have shown that polymorphisms in *CYP2C19* alter clopidogrel active metabolite concentration, residual platelet reactivity, and cardiovascular event rates in patients with ACS and/or patients undergoing PCI. For example, in a genome-wide association study (GWAS) in healthy subjects, *CYP2C19*2* accounted for approximately 12% of the total variation in residual ADP-stimulated platelet aggregation after administration of standard-dose clopidogrel for one week(3). Candidate gene studies have shown that *CYP2C19*2* carriers have lower clopidogrel active metabolite compared to **1/*1* homozygotes(7). Similarly, a growing number of studies have shown that clopidogrel-treated ACS patients carrying the *CYP2C19*2* allele have increased risk of experiencing adverse recurrent cardiovascular outcomes.(4, 5) Mega and colleagues evaluated data from 9,685 patients from 9 independent studies, and reported that patients carrying 1 or 2 copies of the *CYP2C19*2* allele had significantly increased risk of experiencing a composite endpoint consisting of cardiovascular death, myocardial infarction (MI), or stroke (HR = 1.55, 95% CI 1.11-2.17, P = 0.01 and HR = 1.76, 95% CI 1.24-2.50, P = 0.002 for *CYP2C19*2* heterozygotes and homozygotes respectively)(4). Furthermore, in 5,894 patients evaluated for stent thrombosis, carriers of the *CYP2C19*2* reduced-function allele had a significantly increased risk of stent thrombosis (HR = 2.67, 95% CI 1.69-4.22, P < 0.0001 and HR = 3.97, 95% CI 1.75-9.02, P = 0.001 for *CYP2C19*2* heterozygotes and homozygotes, respectively). Taken together, these data convincingly show that *CYP2C19*2* impacts clopidogrel active metabolite concentration, platelet reactivity, and risk for cardiovascular events in a gene-dose dependent manner in ACS and PCI patients treated with clopidogrel. Although fewer studies have investigated the less common loss-of-function *CYP2C19*3* variant, it appears that this variant confers similar increased risk as *CYP2C19*2 (8)*.

Despite the overall consistency of association between *CYP2C19*2* genotype and decreased clopidogrel response, some large well-performed studies failed to show association. These studies included coronary artery disease patients with lower cardiovascular risk or other indications for clopidogrel, e.g., stoke, atrial fibrillation, peripheral vascular disease(9, 10). Thus the clinical utility of *CYP2C19* genotyping may be limited to those coronary artery disease patients at high risk for recurrent events(11).

Similar to *CYP2C19*2*, several studies have evaluated the effect of the gain-of-function *CYP2C19*17* variant on ADP-stimulated platelet inhibition and cardiovascular outcomes in response to clopidogrel therapy, albeit with less consistent results. While some reports indicate that clopidogrel-treated carriers of *CYP2C19*17* have less residual platelet aggregation compared to non-carriers, i.e. greater response(12, 13), others have shown no such effect(14). Similarly, inconsistent results exist regarding the impact of *CYP2C19*17* on recurrent cardiovascular events. Some studies have shown no association between *CYP2C19*17* and stent thrombosis(15) or composite cardiovascular endpoints(3), although the former study did observe that *CYP2C19*17* significantly increased bleeding risk in a gene-dose dependent manner (OR = 1.80, 95% CI 1.03-3.14, for *CYP2C19*17* heterozygotes vs. non-carriers and OR = 3.27, 95% CI 1.33-8.10, for *CYP2C19*17* homozygotes vs. non-carriers). Other studies have observed a significant impact of *CYP2C19*17* genotype on cardiovascular event rates. For example, in 928 high-risk patients with acute MI, *CYP2C19*17* carriers had a 37% reduction in clinically-driven target lesion revascularization and a 22% reduction in major adverse cardiovascular events compared to non-carriers(16). More recently, Paré and colleagues demonstrated in a large study of patients with ACS, that *CYP2C19*17* carriers have significantly reduced risk of experiencing a recurrent cardiovascular event compared to non-carriers (7.7% vs. 10% respectively)(10). Some of the inconsistencies regarding *CYP2C19*17* may be due to

the fact that the *17 variant and the *2 variant are in partial linkage disequilibrium such that individuals carrying the *17 variant are less likely to carry the *2 variant(17).

As a result of these studies and several others, in 2009, the U.S. Food and Drug Administration (FDA) determined that available data have provided compelling evidence that *CYP2C19* genetic variation is a significant predictor of pharmacokinetics, pharmacodynamics, and clinical response. On March 12, 2010, a boxed warning was issued stating that health professionals be aware that some patients may be poor metabolizers of clopidogrel, that genetic tests are available to determine *CYP2C19* status, and that alternative therapy should be considered in these individuals. Despite this warning, the FDA has not mandated *CYP2C19* genetic testing prior to clopidogrel therapy initiation, which has led to confusion among physicians regarding how to clinically implement this information and most effectively treat their patients. Furthermore, recent recommendations by the American College of Cardiology Foundation/American Heart Association state that in the absence of prospective randomized clinical outcomes trials, “the evidence base is insufficient to recommend either routine genetic or platelet function testing at the present time”(18).

With the recent FDA approval of prasugrel (and with ticagrelor available in Europe), neither of which require *CYP2C19* for activation, it may be argued that all patients should be given these agents rather than clopidogrel irrespective of genotype. However, this approach is not cost-effective as clopidogrel is coming off patent in the near future, and also isn’t desirable given the increased risk of fatal and non-fatal bleeding in patients taking prasugrel compared to clopidogrel.

Although prospective randomized trials of genotype-directed anti-platelet therapy are currently being conducted, it will take years before the data become available. In the interim, the Clinical Pharmacogenomics Implementation Consortium (CPIC) of the Pharmacogenomics

Research Network has published guidelines for *CYP2C19* genetic testing and clopidogrel therapy (**Figure 1**).(1) This group is supported by the National Institutes of Health and is not affiliated with any commercial entity. The guidelines provide not only recommendations for use of genetic information to guide clopidogrel therapy, but also provide a comprehensive review of the literature.(1) The CPIC authors note that the most compelling evidence for a relation between *CYP2C19* genotype and clopidogrel response exists in ACS patients who have undergone PCI.

These high-risk patients can be genotyped and categorized as extensive (EM), intermediate (IM), or poor (PM) clopidogrel metabolizers based on **1/*1*, **1/*2*, and **2/*2* genotypes respectively; patients who carry at least 1 copy of the *CYP2C19*17* allele can be categorized as ultrarapid metabolizers (UM's).

The guidelines suggest that patients who are EM's (**1/*1*) or UM's (**1/*17*, **17/*17*) receive the standard dose of clopidogrel, and that patients who are IM's (**1/*2*) or PM's (**2/*2*) be considered for alternative anti-platelet therapy (e.g. prasugrel or ticagrelor) when not contraindicated (**Figure 1**). In addition to *CYP2C19* metabolizer status, physicians should also consider other factors associated with high on-treatment platelet aggregation including age, BMI, diabetes status, and possibly proton pump inhibitor use (most notably omeprazole)(19). At the time of this writing, there are not sufficient data to recommend higher-dose clopidogrel for IM's or PM's, since some small studies show benefit and others do not.

While the guidelines proposed by CPIC regarding genetic variation in *CYP2C19* and anti-platelet therapy cannot possibly encompass the myriad of different clinical situations presented to physicians, they do provide a framework in which to incorporate important and reproducible genetic data into effective individualized anti-platelet therapies. At the time of this writing, prospective randomized clinical trials, comparative effectiveness trials, and studies of

pharmacoeconomics regarding genotype-directed anti-platelet therapies are underway and will likely result in future revisions to the guidelines. In the meantime, however, it seems both prudent and logical to take advantage of all sources of information to most effectively treat patients at high risk for recurrent cardiovascular events.

Warfarin pharmacogenomics

Although in use for nearly 60 years, warfarin remains a difficult drug to manage due to its narrow therapeutic index and the wide inter-patient variability in dose requirements. Warfarin consistently ranks among the leading causes of serious drug-related adverse events, prompting a boxed warning in its labeling regarding bleeding risk. Warfarin interferes with the activation of vitamin K-dependent clotting factors (II, VII, IX, and X) by inhibiting vitamin K epoxide reductase complex 1 (VKORC1). It is usually initiated at a similar dose for all patients, typically 5 mg/day, with dose adjustment according to the international normalized ratio (INR). The problem with this trial-and-error dosing approach is that it often leads to over- or under-anticoagulation during the initial months of therapy when the risk for bleeding is greatest.(20) Warfarin pharmacogenomics aims to enhance our understanding of patient-specific determinants of warfarin response in order to improve dosing accuracy and reduce the risk for adverse sequelae with warfarin therapy.

Table 3 highlights the genes/SNPs that have been most strongly associated with warfarin dose variability. There are substantial and convincing data from numerous candidate genes studies and genome wide association studies demonstrating that *CYP2C9* and *VKORC1* genotypes impact warfarin dose requirements.(21-24) Specifically, the *CYP2C9**2 (R144C; rs1799853) and *3 (I359L; rs1057910) alleles lead to 40% to 70% reductions in *S*-warfarin clearance and approximately 20% to 40% lower warfarin dose requirements, respectively. These alleles are also associated with increased bleeding risk.(25, 26) The *VKORC1* rs9923231 -1639G>A (or rs9934438 1173C>T) SNP increases sensitivity to warfarin at its target

site, further reducing dose requirements. The -1639G>A variant is in near complete linkage disequilibrium with the 1173C>T variant in all major continental populations,(27) and thus, either may be used to predict warfarin dose requirements. Together with clinical factors (e.g. age, body size, use of amiodarone), the *CYP2C9**2 and *3 alleles and *VKORC1* -1639G>A genotype explain 50% to 60% of the variability in warfarin maintenance dose among Caucasians,(22, 28) but only about 25% among African Americans.(22, 29, 30) Decreased dose prediction in African Americans is secondary to lower frequencies of the *CYP2C9**2, *CYP2C9**3, and *VKORC1* -1639A alleles in this population.(27) However, as shown in **Figure 2**, the racial/ethnic differences in warfarin dose requirements are almost completely explained by differing allele frequencies for *VKORC1* between the major continental populations, such that within a *VKORC1* genotype, doses are similar across population groups.

The *CYP4F2* V433M (rs2108622) variant explains an additional 1% to 2% of the variability in warfarin dose.(31) CYP4F2 metabolizes vitamin K to hydroxyvitamin K, thus limiting the quantity of vitamin K available for clotting factor carboxylation.(32) The 433M variant is common in Caucasians and Asians and associated with lower CYP4F2 activity and higher warfarin dose requirements, and this has now been replicated in several independent studies.(24, 33-35)

Two genome wide association studies demonstrated that the *VKORC1* -1639G>A variant is the major genetic determinant of warfarin maintenance dose in Caucasians, while *CYP2C9**2 and *3 provide lesser contributions to dose.(21, 24) A third genome wide association study in Japanese also showed that *VKORC1* genotype is the strongest predictor of warfarin dose.(34) After controlling for *VKORC1* and *CYP2C9*, the *CYP4F2* V433M genotype emerged as a further predictor of dose requirements in two of these genome-wide studies.(24, 34) Whether the *VKORC1* and *CYP2C9* variants are the primary contributors to warfarin dose requirements in African American populations is unknown. However, genome wide association studies are underway to help address this question.

As noted above, the warfarin labeling was revised in August 2007 to include information on *CYP2C9* and *VKORC1* genotypes as predictors of dose response and recommend dose adjustment in patients with variant genotypes. In early 2010, the labeling was further revised to include specific dosing recommendations according to *CYP2C9* and *VKORC1* genotypes. Several validated dosing algorithms, including that from the International Warfarin Pharmacogenetics Consortium (IWPC) and by Gage and colleagues are also available to assist clinicians with genotype-guided dosing.(22, 28) These algorithms are both available in a user-friendly tool at www.warfarindosing.org. In the Gage algorithm, dose prediction may be refined with the input of previous INR and dose data.(29) **Figure 3** highlights improvement in dose prediction with pharmacogenetics algorithm over the clinical algorithm. The warfarin CPIC guidelines (2) recommend the Gage or IWPC dosing algorithms as the preferred approach for incorporation of genetic information into warfarin dose prediction, based on published data suggesting the superiority of this approach. When electronic access to these algorithms is not available, the dosing table in the warfarin product label is the recommended alternative approach.

Despite the wealth of the data supporting genetic determinants of warfarin dose requirements, recent labeling changes, and the availability of both decision support tools (dosing algorithms) and at least 5 FDA-cleared platforms for warfarin genotyping, genetic testing is not widely embraced in clinical practice. In fact, current consensus guidelines suggest against routine use of genetic data to guide dosing.(20) This is a grade 2C recommendation, indicating that the evidence supporting the suggestion is limited and that patient values and preferences should be taken into account. Similarly, the American College of Medical Genetics does not endorse genetic testing except in cases of unusual warfarin response.(36) As an additional impediment to clinical implementation of genetic testing for warfarin, the Centers for Medicare and Medicaid Services announced in 2009 that coverage for such testing would be denied unless testing is provided in the context of a controlled clinical study.

So why are clinicians and policy makers reluctant to embrace warfarin pharmacogenetics? Barriers to the clinical implementation of genetic testing generally include the need to establish the clinical validity, analytical validity, and clinical utility of testing. In the case of warfarin, the clinical validity is well established, as discussed above. The analytical validity is also well established. However, there are some variants not included on some genotyping platforms that are worth mentioning given their implications for African Americans. The *CYP2C9**5 (D360E), *6 (10601delA), *8 (R150H), and *11 (R335W) alleles occur almost exclusively in African Americans and are associated with reduced metabolism.(37) The *8 allele is as common as other *CYP2C9* alleles combined and correlated with lower warfarin dose requirements in African Americans.(38) Recently, the *CYP2C9* rs7089580, *VKORC1* rs61162043, and calumenin rs339097 gene variants were identified in African Americans and associated with higher maintenance doses in this population.(30, 39) Genome wide association studies in African Americans may reveal other variants with implications for warfarin dosing in this population.

Whether the clinical utility of genetic testing for warfarin has been established is a more debatable question. There is evidence of benefit with genotype-guided dosing from a small clinical trial and a comparative effectiveness study.(40, 41) In the latter, patients were offered free genotyping for the *CYP2C9**2, *CYP2C9**3, and *VKORC1* -1639G>A variants, with results provided to their physician. During the initial 6 months of therapy, those who underwent genetic testing had 30% fewer hospitalizations for any cause and for bleeding or thromboembolism compared to historical controls.(41) These data are tempered by findings from another clinical trial in which patients were randomized to genotype-guided or clinical-based warfarin dosing. Since a large sample size would be required to demonstrate reductions in serious adverse events, investigators focused on INR values outside the therapeutic range as a marker of increased bleeding or thrombotic risk.(42) The percent of out-of-range INRs was similar between dose strategy groups. An exploratory analysis showed a significant benefit with

pharmacogenetic dosing for patients with either multiple variant alleles (who required 3 to 4 mg/day) or the wild-type genotype (who required 6 to 7 mg/day), whereas single variant allele carriers (who required about 5 mg/day) appeared to have no benefit from genotype-guided dosing. This is consistent with findings from the IWPC in which a pharmacogenetic algorithm more accurately predicted low (≤ 3 mg/week) and high (≥ 7 mg/week) warfarin doses than a clinical algorithm, while the two algorithms were similarly predictive of intermediate doses (Figure 3).(22) Based on these data, genotype-guided therapy may not be of benefit to carriers of a single variant allele (approximately 40% of Caucasians), in whom a dose of 5 mg per day (the typical starting dose) would be predicted.

The National Heart, Lung, and Blood Institute-sponsored Clarification of Optimal Anticoagulation through Genetics (COAG) trial is powered to account for the potential lack of benefit with genotype-guided therapy in patients with a single variant. The COAG trial is a prospective, multi-center, randomized, double-blind trial that began in September 2009.(43) It aims to determine whether the percent of time spent within the therapeutic INR range (primary outcome) or the occurrence of an INR >4 or serious event (secondary outcome) during the initial 4 weeks of therapy differs between a pharmacogenetic- and clinical-dosing strategy. The trial is expected to be completed in December 2012. Several other randomized prospective trials addressing the efficacy, safety, and economic implications of warfarin pharmacogenetics are underway in the U.S. and Europe (Clinical and Economic Implications of Genetic Testing for Warfarin Management trial and Genetics Informatics Trial (GIFT) of Warfarin to Prevent Deep Venous Thrombosis, the European Pharmacogenetics of Anticoagulant Therapy trial (EU-PACT) (www.clinicaltrials.gov)).

The need for rapid genotyping is an often cited barrier to implementing warfarin pharmacogenetics. Clinical laboratories often lack the personnel or equipment for rapid genotyping. If samples are sent to an outside facility, results may not be available for several days, at which time INR results are available to guide therapy. Nonetheless, there are data

showing that even with a delay of 4 to 5 days, a pharmacogenetic algorithm incorporating previous INR results and warfarin doses provides more accurate prediction of the warfarin maintenance dose than clinical factors alone.(29) In addition, with continuing technological advances, time to genotyping results will continue to decrease and eventually it is anticipated that the genetic information will be available in the medical record.

In summary, the clinical and analytical validity of warfarin pharmacogenetics is well established, at least for Caucasians. Variants best predicting dose requirements in non-Caucasians are still being investigated. Clinical implementation of genotype-guided therapy is largely hindered by the lack of randomized clinical trial data in a sufficient number of patients to demonstrate beneficial outcomes. However, the data to date provide clear evidence of the ability to better predict the stable warfarin dose requirement with the use of genetic information. Results from on-going clinical trials will help to define the role of warfarin pharmacogenetics in clinical practice. In the meantime, the CPIC guidelines on warfarin provide guidance for incorporation of genetic information for warfarin dose prediction when such information is available.

Pharmacogenomic clinical implementation: programs and approaches

One approach to use genotype data in clinical practice is to order the genetic test as other laboratory/diagnostic tests are ordered. However, as discussed above, this presents certain barriers, including the clinician needing to remember to order the test, turn-around time, genetic test costs, and clinician uncertainty about what to do with the genetic information, among others. As genotyping and sequencing technologies advance, it is expected that whole genome sequencing will eventually replace single genetic tests. The cost for whole genome sequencing is expected to fall below the $1000 mark in the next few years. Thus the possibility of completion of whole genome sequencing at a single point in a person's life, with the data

available for use thereafter, now appears feasible. An example of such an approach was recently published.(44) Thus, an alternate vision is to embed genotypic information in an electronic medical record, to be accessed as needed, with point-of-care decision support provided when a prescription is written for a drug with responses known to be modulated by genetic variants. Clopidogrel and warfarin are examples where As a first step to enabling this long-term vision for genomic medicine, the PREDICT (Pharmacogenomic Resource for Enhanced Decisions in Care and Therapy) project at Vanderbilt University Medical Center is implementing preemptive genotyping for patients likely to receive drugs with responses known to be modulated by pharmacogenomic variants. The initial target population is patients scheduled for cardiac catheterization, 40% of whom ultimately receive clopidogrel. The program was launched in Sept. 2010, and as of April 28, 2011, 1769 patients had been genotyped, 42 with the CYP2C19*2/*2 genotype and 342 with the CYP2C19*1/*2 genotype. Alerts are then generated to the clinician ordering clopidogrel to provide guidance relative to the *CYP2C19* genotype. Genotyping is performed in a CLIA-approved environment using the Illumina VeraCode platform (184 variants thought to be relevant to drug responses), allowing extension of the data to other drug-gene pairs, for use as the clinical need arises.

A similar implementation program is also currently under-way as a network-wide project within the NIH-funded Pharmacogenomics Research Network (PGRN). The Translational Pharmacogenomics Project involves six institutions who are implementing programs similar to the PREDICT project at Vanderbilt, and among other things, this implementation project will define the challenges and successes associated with a pharmacogenomics clinical implementation program. Large-scale implementation of pharmacogenomics will also require the engagement of regulatory agencies, policy makers, insurers, and other stakeholders

Compelling, but not clinically actionable examples in cardiovascular pharmacogenomics

Heart failure pharmacogenomics – opportunities in drug development?

There is limited literature on the pharmacogenomics of the various treatment modalities for heart failure, with the exception of beta-blockers, for which there are numerous studies.(45) As noted in Table 1, the two most commonly used beta-blockers in heart failure, metoprolol and carvedilol, have FDA labeling around polymorphisms in *CYP2D6.* The CYP2D6 enzyme is a major metabolic pathway for both of these drugs (more so with metoprolol than carvedilol) and there are clear data for the differences in plasma drug concentration by *CYP2D6* genotype. However, data are limited suggesting the different drug concentrations have clinical relevance. For example a study of metoprolol succinate during the titration period in heart failure patients showed the expected differences in plasma metoprolol concentration by *CYP2D6* genotype, but there were no differences by *CYP2D6* genotype in the patients with symptomatic worsening or decompensation during the titration phase.(46) Thus, while the evidence is clear for a pharmacokinetic effect on these drugs based on CYP2D6 genotype, this does not appear to translate into important differences in response or adverse effects.

The most compelling beta-blocker pharmacogenomics data in heart failure fall neatly into the pathway candidate gene paradigm, and the relevant pathway is highlighted in **Figure 4**. Significant genetic associations in this pathway arise from the beta1- and beta2-adrenergic receptor genes (*ADRB1 and ADRB2*), the alpha 2C-adrenergic receptor gene (*ADRA2C)* and the G-protein couple receptor kinase 5 gene (*GRK5*).

Best studied is *ADRB1,* which has two common, nonsynonymous polymorphisms (Ser49Gly and Arg389Gly), which have been documented in numerous in vitro mutagenesis, ex vivo, and human studies to be functional.(47) The strongest literature is with Arg389Gly, where the Arg form of the receptor couples more efficiently to G protein, leading to greater down-stream signaling. Some, but not all studies in heart failure suggest Arg389Arg patients have greater improvement in left ventricular ejection fraction with beta-blocker treatment.(45, 47) Studies of the association of this polymorphism with clinical outcomes also suggest an association. The

BEST trial, which compared bucindolol to placebo, found that Arg389Arg patients had significant benefits from bucindolol (reduced mortality and hospitalizations) while in Gly389 carriers no significant benefit over placebo was observed.(48) A population cohort study found significant better outcomes in Arg389Arg patients treated with high dose, versus low dose or no beta-blocker. Other cohort studies however have not observed significant differences in outcomes by genotype.(47) An important difference in the studies with positive associations versus absence of association is the analysis approach. Those with no observed association included cohorts where all or nearly all patients were treated with a beta-blocker, and comparisons across *ADRB1* genotypes showed no differences. However, studies comparing treated versus untreated patients, within genotype have more consistently shown differences in outcomes.(47) This suggests the Arg389Arg genotype may have negatively influence outcomes, with the negative effect of this genotype offset by the beta-blocker.

A four amino acid insertion-deletion polymorphism in *ADRA2C*, which influences norepinephrine release through negative feedback mechanisms,(47) has also been associated with beta-blocker efficacy. In BEST, *ADRA2C* deletion carriers did not obtain benefit from bucindolol that was observed in insertion homozygotes.(49) It is postulated that the negative effect of the deletion allele on bucindolol efficacy is related to greater sympatholysis in deletion carriers; where sympatholytic effects are unique to bucindolol among the beta-blockers tested in heart failure.(49) In contrast, a study of metoprolol, which is a beta1-selective blocker, found that patients who were *ADRB1* Arg389Arg and *ADRA2C* Del carriers had the greatest improvement in ejection fraction, a surrogate for improved outcomes.(50) These seemingly disparate findings in fact align well when one considers the functional mechanism of the polymorphism and the differences in pharmacological properties between metoprolol and bucindolol.

Finally. several studies have documented a pharmacogenomic effect with the Leu41Gln variant of *GRK5.* A series of elegant in vitro and animal studies suggest the Leu41 allele blunts the effects of catecholamines, and has been called an endogenous beta-blocker.(51) The first

study of this SNP in humans showed that Leu41 carriers not treated with beta-blockers had outcomes that were significantly better than those of Gln41Gln beta-blocker untreated patients, and similar to Gln41Gln beta-blocker treated patients.(51) Studies of two other populations suggested beta-blocked benefit was confined to Gln41Gln individuals.(51, 52)

Collectively, these studies suggest genetic variability in the adrenergic signaling pathway have important influence on the benefits of beta-blockers in heart failure. They suggest there are certain genotype groups deriving minimal benefit from beta-blocker therapy. However, the consensus-guideline-driven use of beta-blockers in all patients with systolic heart failure makes clinical application of this information difficult, since it would mean withholding beta-blocker treatment, and, unlike clopidogrel, there are not alternatives.

However, these data may prove beneficial in other ways in heart failure – specifically in drug development. A number of promising drug classes have failed to document efficacy in heart failure in the last 10-15 years in late Phase 3 clinical trials.(45) This suggests the need to target drug development in heart failure to those most likely to benefit from therapy that is in addition to the standard ACE inhibitor, beta-blocker, diuretic, digoxin regimen. Pharmacogenomic data may be one way to target therapy in heart failure drug development. One example might be a study that enrolls only patients who are *ADRB1* Gly carriers, or *GKR5* Leu41 carriers, then tests the novel therapy against placebo in the background of the standard therapies. Since the data suggest these genotype groups might derive minimal benefit from beta-blockers, then it should be easier to document benefit from the novel therapy. By contrast those with genotypes responsive to beta-blockers might already be obtaining the maximal benefit possible from pharmacological therapies, and so their inclusion in trials impairs the ability to document efficacy. This approach is not one that has been employed to date, but is discussed.

These pharmacogenomic data are however currently being used in another way in the development of bucindolol, which does not have FDA approval. Specifically, ARCA Biopharma (Broomfield, CO) has made a new drug application (NDA) to the FDA, to seek approval for use

of bucindolol in *ADRB1* Arg389Arg patients. The FDA denied the initial NDA, and requested a randomized controlled trial. The company has plans to launch in late 2011 a superiority trial in 3,200 *ADRB1* Arg389Arg patients who will be randomized to metoprolol CR/XL or bucindolol.(53) To our knowledge, this represents the first example of pharmacogenomically-guided drug development in cardiovascular disease.

Serious adverse drug effects and pharmacogenomics

The idea that risk for an unusual, rare, serious adverse drug effect could include a genetic component goes back to hemolytic anemia during treatment with anti-malarials in World War II. In this case, the risk for this unexpected drug effect was much higher among African American subjects and is associated with glucose-6-phosphate dehydrogenase deficiency. Other adverse effects are "expected" in that they represent sensitivity to a drug's known pharmacologic effects: such as serious bleeding during anticoagulant therapy. Notably, even these adverse effects may include a genomic component. In recent years, there are a number of examples where serious adverse drug events that have been described as idiosyncratic now have a clear genetic component. These include the hypersensitivity reaction to abacavir, severe skin reactions to carbamazepine, and drug-induced liver injury from lumiracoxib – all of which link to markers in HLA.(54)

Serious adverse drug events associated with cardiovascular drugs, or affecting the cardiovascular system (e.g. statin-induced myopathy and rhabdomyolysis; drug-induced long QT and Torsades de Pointes) however do not appear to link to HLA.

Muscle toxicity during treatment with HMG-CoA reductase inhibitors (statins) Rhabdomyolysis is very rare with statins, while varying degrees of muscle aches and creatine kinase (CK) elevations are commoner. All are known to be more common with higher (vs lower) statin doses, and thus presumably represented drug-concentration-related adverse effects. As such,

candidate gene studies examined the role of drug metabolism and transport pathways and suggested that variants in CYP3A5(55) and the uptake transporter OATP1B1 (encoded by *SLCO1B1*)(56) modulate risk. The Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH) consortium examined CK values in over 12,000 subjects assigned to low- or high-dose simvastatin (20 and 80 mg/day). There were 8 possible cases of muscle toxicity in 6033 patients treated with the low dose, but 98 possible cases among the 6031 assigned the high dose. A genome-wide association study comparing 85 patients with muscle toxicity in the high dose arm to 90 controls in the high dose arm not developing toxicity identified a single SNP (rs4363657) at genome-wide significance in *SLCO1B1*; this SNP tags a known non-synonymous variant (Val174Ala)(57). Among patients homozygous for the CC risk allele (2.1% of the study population), 18.6% developed muscle toxicity over 5 years, compared to 0.63% in the low-risk (TT) group, that comprised 73% of the population; the odds ratio was 16.9, and risk in the heterozygotes was intermediate. This result was replicated in a separate cohort in the original publication, and has been replicated in two other studies,(58, 59) including one that used simply staying on statins as an endpoint. In the latter study, Val174Ala also predicted stopping atorvastatin but not pravastatin. These findings emphasize how environmental factors (notably drug dose and duration of therapy) and genetics interact to produce a drug response phenotype.

This represents an example of a pharmacogenomic marker that appears to modulate risk and yet its predictive value, and thus clinical utility, remains to be defined.

Drug-induced long QT syndrome

The congenital long QT syndrome (LQTS) is a rare genetic disease recognized first in the 1950s and 1960s. Affected individuals displayed marked QT interval prolongation, recurrent syncope, and risk for sudden death due to a morphologically distinctive polymorphic ventricular

tachycardia termed “torsades de pointes”. Exposure to certain drugs can also produce similar ECG findings, and this adverse drug effect has therefore been termed drug-induced LQTS (diLQTS). QT-prolonging antiarrhythmics are by far the most commonly implicated drugs and 1-5% of patients exposed to sotalol, dofetilide, or quinidine develop drug-induced LQTS.(60) The adverse effect is seen but much less frequently with drugs used for non-cardiovascular indications, and these include antihistamines, antibiotics, antipsychotics, and methadone. A common feature in the diLQTS is that risk is increased with higher doses or plasma concentrations of most culprit drugs. In some cases, genetically-determined variable drug metabolism has been implicated; risk may be higher among CYP2D6 poor metabolizers treated with thioridazine, a CYP2D6 substrate. The thioridazine product label describes this risk. Similarly, CYP-based drug interactions can increase risk; a very prominent example was diLQTS during treatment with the antihistamine terfenadine. Terfenadine prolongs QT interval but ordinarily undergoes extensive presystemic CYP3A4-mediated clearance to its antihistamine metabolite fexofenadine, which does not prolong QT. When CYP3A4 is inhibited (e.g. by ketoconazole or similar strong inhibitors) or in patients taking terfenadine overdose, the parent drug appears in plasma, and prolongs QT and can cause torsades de pointes. Because of this adverse effect, and because of the development of fexofenadine as a non-QT-prolonging antihistamine, terfenadine was withdrawn from the US market in 1997. The diLQTS can occur at low doses and concentrations in susceptible individuals; in addition, diLQTS is commoner at low rather than high doses of quinidine. The explanation is thought to be that at low doses the drug produces arrhythmogenic effects on cardiac repolarization while at higher ones, it produces electrophysiologic effects that inhibit repolarization-related arrhythmias.

Mutations in 13 different genes, encoding ion channels or proteins modulating ion channel function, have been identified in families with the congenital LQTS. While initial studies of the congenital syndrome focused on patients with obvious QT prolongation and recurrent episodes

of syncope, subsequent studies in families have identified incomplete penetrance of the clinical phenotype. Thus, one hypothesis that is being explored is that patients with diLQTS represent subclinical cases of the congenital syndrome, in which drug exposure exposes the full blown clinical phenotype.

A number of small studies (up to approximately 100 patients) have examined the frequency of subclinical congenital long QT syndrome gene mutations in patients with diTdP.(61, 62) Small studies have identified mutations in the 5 major congenital long QT syndrome disease genes in 10-20% of subjects with diLQTS. More recently, one small study in Japan suggested a higher incidence, approximately 40%,(63) and a study using targeted next-generation sequencing to screen all 13 congenital long QT syndrome disease genes and other arrhythmia susceptibility genes in 31 patients identified rare variants predicted to be deleterious to protein function in 20 (64.5%).(64) Thus, there seems little doubt that congenital LQTS mutations contribute to risk for diLQTS, but the extent to which they explain the risk is uncertain. Arrhythmias in the congenital syndrome are often adrenergically-triggered, but polymorphisms in the $beta_1$- and $beta_2$-adrenergic receptor genes were not associated with diLQTS.(64)

Preliminary data have been reported using intensive candidate gene and GWA approaches for analysis of risk for diTdP. The cases were accrued at multiple US and European sites using common definitions, and controls were either large healthy populations or patients starting QT prolonging antiarrhythmics for clinical indications, and not developing long QT intervals. The candidate gene study examined ~1500 single nucleotide polymorphisms (SNPs) in 18 candidate genes, and identified variants in I_{Ks} channel genes as risk variants. The GWA identified multiple associated genomic regions, none of which included obvious candidate genes.(64)

Thus, there has been substantial effort to identify the genes placing patients as risk for drug-induced long QT syndrome and beyond genes associated with congenital long QT there are few

striking findings, and at this point no genomic markers are sufficiently robust for prediction of risk of development of drug-induced long QT syndrome.

Promising areas in cardiovascular pharmacogenomics research

Pharmacogenomics of antihypertensive drugs

There are several areas of research in cardiovascular pharmacogenomics that hold promise, but do not yet have the level of replicated data of the examples above. The pharmacogenomics of antihypertensive drugs is one of these, and studies range from testing genetic associations with blood pressure (BP) lowering, adverse metabolic effects, and long-term sequelae of hypertension, like death, stroke and myocardial infarction.

Among the first line antihypertensive drug classes, those with the greatest body of literature are the beta-blockers and thiazide diuretics. Similar to the data on beta-blockers in heart failure, there are interesting beta-blocker pharmacogenomics data in hypertension, particularly with the *ADRB1* gene. Consistent with the data in heart failure, the Arg389Gly and Ser49Gly polymorphisms have been associated with differential BP lowering in a number of studies, with Ar389Arg genotype or the Ser49/Arg389 haplotype associated with the best antihypertensive response.(47, 65) The Ser49Arg389 haplotype has also been associated with improved outcomes (particularly lower death rate) in atenolol treated hypertensives (compared to those treated with verapamil).(65) This finding is also consistent with the heart failure literature, where the genotype/haplotype appears to be associated with risk and beta-blockers offset that risk.

There are also interesting data with thiazide diuretics. One of the polymorphisms most consistently associated with thiazide response is the *ADD1* Gly460Trp polymorphism, a documented functional polymorphism.(64, 66, 67) While not all studies have documented an *ADD1* association with thiazide response, work in this area led to the development of a novel antihypertensive drug class, which targets alpha adducin (the protein encoded by *ADD1*) and

ouabain, and the Phase 2 study data on the BP lowering efficacy of the drug are quite impressive.(68) This highlights not only the possibility of defining genetic determinants of response through pharmacogenomics research, but also identification of novel drug targets.

Another candidate gene of interest with thiazides is *NEDD4L*, which contains a documented functional SNP, plays a role in sodium reabsorption, and has been associated BP response and clinical outcomes with thiazide treatment.(66, 69) Finally, the only published genome-wide association study for antihypertensive response discovered a SNP on chromosome 12 associated with thiazide response in African Americans,(70) which was recently replicated in an independent cohort. There are no known genes in the region involved in thiazide response, but an interesting candidate is *FRS2*, whose encoded protein is involved in fibroblast growth factor signaling, which plays a role in vascular smooth muscle cell regulation. Since the vascular mechanisms for BP lowering with thiazides are not well-defined, this highlights the potential for pharmacogenomics research to identify novel mechanisms of action. It may also represent a novel drug target.

There is a limited, but interesting body of data on the calcium channel blockers (CCB). The majority of studies center on outcomes associated with CCB therapy, relative to calcium signaling genes, including the protein target's gene, *CACNA1C,* along with *CACNB2*, and *KCNMB1*.(71-73) While these findings require replication, they involve strong biological candidates and *CACNB2* has been documented from GWAS to be a hypertension gene. Of interest is that despite a relatively large number of studies with ACE inhibitors (or angiotensin receptor blockers), there are limited examples of convincing genetic associations with response to these drugs.

Aspirin Pharmacogenomics

Dual antiplatelet therapy with aspirin and clopidogrel is the most commonly used antiplatelet regiment for patients with acute coronary syndromes. While considerable advances in clopidogrel pharmacogenomics have resulted in potential clinical applications (see above), the pharmacogenomics of aspirin response remains a promising yet poorly understood area of investigation. Variability of aspirin response is well-documented, and heritability estimates suggest that genetic factors contribute moderately to residual platelet reactivity post-aspirin treatment(74). Despite these data, and a large number of candidate gene studies, genetic variation in relatively few genes have reproducibly been associated with aspirin response. Some of the more intensively studied candidate genes include cyclooxygenase 1, purinergic receptors P2Y1 and P2Y12, and several platelet glycoproteins (e.g. GPIIb-IIIa, GPVI, GPIa, and GPIb). Variants in few if any of these genes have reproducibly been shown to be associated with aspirin response. A recent systematic review by Goodman and colleagues(75) of 31 studies evaluating 11 genes revealed that the GPIIIa PIA1/A2 polymorphism was associated with aspirin resistance in healthy subjects (P = 0.009; OR = 2.36, 95% CI 1.24 – 4.48) but not in the combined group of both healthy subjects and patients with cardiovascular disease (P = 0.40; OR = 1.14, 95% CI 0.84 – 1.54). Polymorphisms in cyclooxygenase 1, P2Y1, P2Y12, and GP1a were not associated with aspirin resistance(75).

Several factors contribute to inconsistencies among studies. Assessment of aspirin resistance has been difficult not only because of varying methodologies used in measuring platelet function but also differences in the definition of aspirin resistance itself. The problem of phenotype assessment heterogeneity is compounded by small sample sizes and incomplete coverage of variation in the candidate genes studied. Future studies must focus on more comprehensive genome-wide approaches in large numbers of well-phenotyped individuals.

Statin pharmacogenomics.

There has been extensive work on the genetic predictors of LDL cholesterol lowering but limited numbers of genes have emerged, and they generally explain relatively small percentages of the LDL response. The genes with the strongest data include that which encodes the protein target, *HMGCR* and the LDL receptor gene (*LDLR)*, which are both very strong biological candidates.(64) A nonsynonymous SNP in *KIF6* has been suggested as a predictor of outcomes with statin therapy, and a commercially available test is available. However, unlike most of the examples raised in this review, the potential role of *KIF6* in coronary disease or statin response is unknown, and recent data have called this association into question. Overall, with the exception of the predictors of statin muscle toxicity, it appears that identification of SNPs that are sufficiently predictive of statin response to have clinical utility will remain a challenge.

Conclusions

Pharmacogenomics research of cardiovascular drugs has led to examples with clinically-actionable findings (warfarin and clopidogrel) that not only have the potential to improve management of patients prescribed these drugs, but has advanced our understanding of their pharmacokinetics and pharmacodynamics. Guidelines for the use of genetic information to guide warfarin and clopidogrel therapy have been published, and in the future, genetic information may be available within the medical record. This will obviate the need to order specific genetic tests, and likely enhance the pace of translation to practice in cardiovascular pharmacogenomics. A drug transporter polymorphism has been strongly implicated in statin-induced myopathy, and while this may not be predictive enough to use clinically, it has enhanced our understanding of this potentially serious adverse drug event. Beta-blocker pharmacogenomics research in heart failure has clearly implicated various components of the adrenergic signaling pathway, and these data have the potential to influence future drug development in heart failure. Areas of active investigation that hold promise include

antihypertensive and aspirin pharmacogenomics. The clinical utilization of pharmacogenomics in cardiovascular disease holds promise and clinical implementation has begun in certain centers. The potential implementation approaches are described here, and certain challenges are discussed. The findings from the vanguard centers that are leading clinical implementation will provide important insight into the challenges and future directions for pharmacogenomics.

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Figure Legends

Figure 1. Clinical Pharmacogenetics Implementation Consortium guidelines for initiating antiplatelet therapy in coronary patients based on cytochrome P450 2C19 genotype. ACS: acute coronary syndrome; PCI: percutaneous coronary intervention; CYP2C19: cytochrome P450 2C19. Adapted from Scott, et al.(1)

Figure 2. Median warfarin dose requirements in Asians, Caucasians, and African Americans, overall and by VKORC1 genotype; based on data from the International Warfarin Pharmacogenetics Consortium (IWPC).(29) The cyan bars indicate the median warfarin dose in each race group overall. The yellow, blue, and pink bars show the dose within each race group by the VKORC1 -1639 AA, AG, and GG genotypes, respectively. As shown, overall warfarin dose requirements are lower in Asians and higher in African Americans compared to Caucasians. This racial difference in dose is largely explained by a higher frequency of the VKORC1 -1639 AA (low dose) genotype in Asians, AG (intermediate dose) genotype in Caucasians, and GG (high dose) genotype in African Americans, resulting in similar doses by race within genotype. Prepared from data in Limdi, et al.(27)

Figure 3. Percent of patients whose actual warfarin dose fell within 20% of the predicted dose according to either the clinical or pharmacogenetic dosing algorithm derived by the International Warfarin Pharmacogenetics Consortium (IWPC).(22) The pharmacogenetic algorithm was more predictive of actual dose requirements for those requiring ≤21 mg/week or ≥49 mg/week of warfarin. For individuals requiring intermediate doses, the clinical and pharmacogenetic algorithms were similarly predictive of dose requirements. The percent of patients in each dose group are shown at the bottom. Adapted from Klein, et al. (22)

Figure 4. Schematic of adrenergic receptor signaling in the heart. From Johnson and Liggett.(47) Abbreviations: α1ARs – alpha1-adrenergic receptors; α2ARs – alpha2-adrenergic

receptors; AC – adenylyl cyclase; β1AR – beta1-adrenergic receptors; β2AR – beta2-adrenergic receptors; cAMP – cyclic adenosyl monophosphate; EPI – epinephrine; Gαs – G protein alpha subunit, stimulatory; Gαi - G protein alpha subunit, inhibitory; G protein alpha subunit q, GRK – G protein receptor kinase; IP3 – inositol triphosphate; DAG – diacylglycerol; NE – norepinephrine; PLC – phospholipase C

Table 1. Cardiovascular drugs with pharmacogenomic labeling (as of May 2011)

Drug	Gene/biomarker	Label sections
Atorvastatin	LDLR	Warnings and precautions; clinical pharmacology; Clinical studies
Carvedilol	CYP2D6	Drug interactions; clinical pharmacology
Clopidogrel	CYP2C19	Boxed warning; Dosage and administration; Warnings and precautions; Drug interactions; Clinical pharmacology
Isosorbide dinitrate/hydralazine	NAT1; NAT2	Clinical pharmacology
Metoprolol	CYP2D6	Precautions; Clinical pharmacology
Propafenone	CYP2D6	Clinical pharmacology
Propranolol	CYP2D6	Precautions; Drug interactions; Clinical pharmacology
Warfarin	CYP2C9; VKORC1	Dosage and administration; Precautions; Clinical pharmacology

http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm

Table 2. Level of Evidence Linking Candidate Gene Genotype[1] to Clopidogrel Response

Gene	Chromosome	Genetic Variant	Effect on Protein Structure	Effect on Protein Function or Expression	Level of Evidence[2]
CYP2C19	10	rs4244285 (*2)	Splicing Defect	Decreased	High
CYP2C19	10	rs4986893 (*3)	W212X	Decreased	Moderate
CYP2C19	10	rs12248560 (*17)	None	Increased	Moderate
CYP1A2	15	rs762551 (*1F)	None	Increased	Weak
CYP2C9	10	rs1057910 (*3)	I359L	Decreased	Weak
CYP3A4	7	rs2242480 (*1G)	None	Increased	Weak
CYP3A5	7	rs776746 (*3)	Splicing Defect	Decreased	Weak
ABCB1	7	rs1045642 (C3435T)	I1145I	Decreased	Moderate
P2Y12	3	H2 Haplotype	None	Gain-of-Function	Weak
PON1	7	rs662	Q192R	Increased	Weak

[1]Additional polymorphisms exist in each candidate gene. Selected genes were investigated in at least 3 independent publications.

[2]Level of evidence is based on previously published criteria. Definitions: **High**, Consistent evidence from several well-designed, well-conducted studies; **Moderate**, Evidence is sufficient to determine effects, but the strength of evidence is limited to by the number, quality, or consistency of the individual studies; generalizability; or indirect nature of the evidence; **Weak**, Evidence is inconsistent

and/or insufficient to assess the effects on health outcomes because of limited number or power of studies, important flaws in their design or conduct, gaps in the chain of evidence, or lack of information. From Scott, et al.(1)

Table 3. Minor allele frequencies for important variants in warfarin pharmacogenetics(26, 30, 37, 38)

Allele	Caucasians	Asians	African American
*CYP2C9**2 rs1799853	0.12	0	0.02
*CYP2C9**3 rs1057910	0.06	0.03-0.04	0.01
*CYP2C9**5 rs28371686	0	0	0.01
*CYP2C9**6 rs9332131	0	0	0.01
CYP2C9 *8 rs7900194	0	0	0.06
*CYP2C9**11 rs28371685	0	0.01	0.02
CYP2C9 rs7089580	ND	ND	0.23
VKORC1 rs9923231 (-1639G>A)	0.40	0.90-0.94	0.10
VKORC1 rs61162043	ND	ND	0.47
CYP4F2 rs2108622 (V433M)	0.23	0.24-0.25	0.09
CALU rs339097	0	0.01-0.02	0.16

ND, not determined

Figure 1.

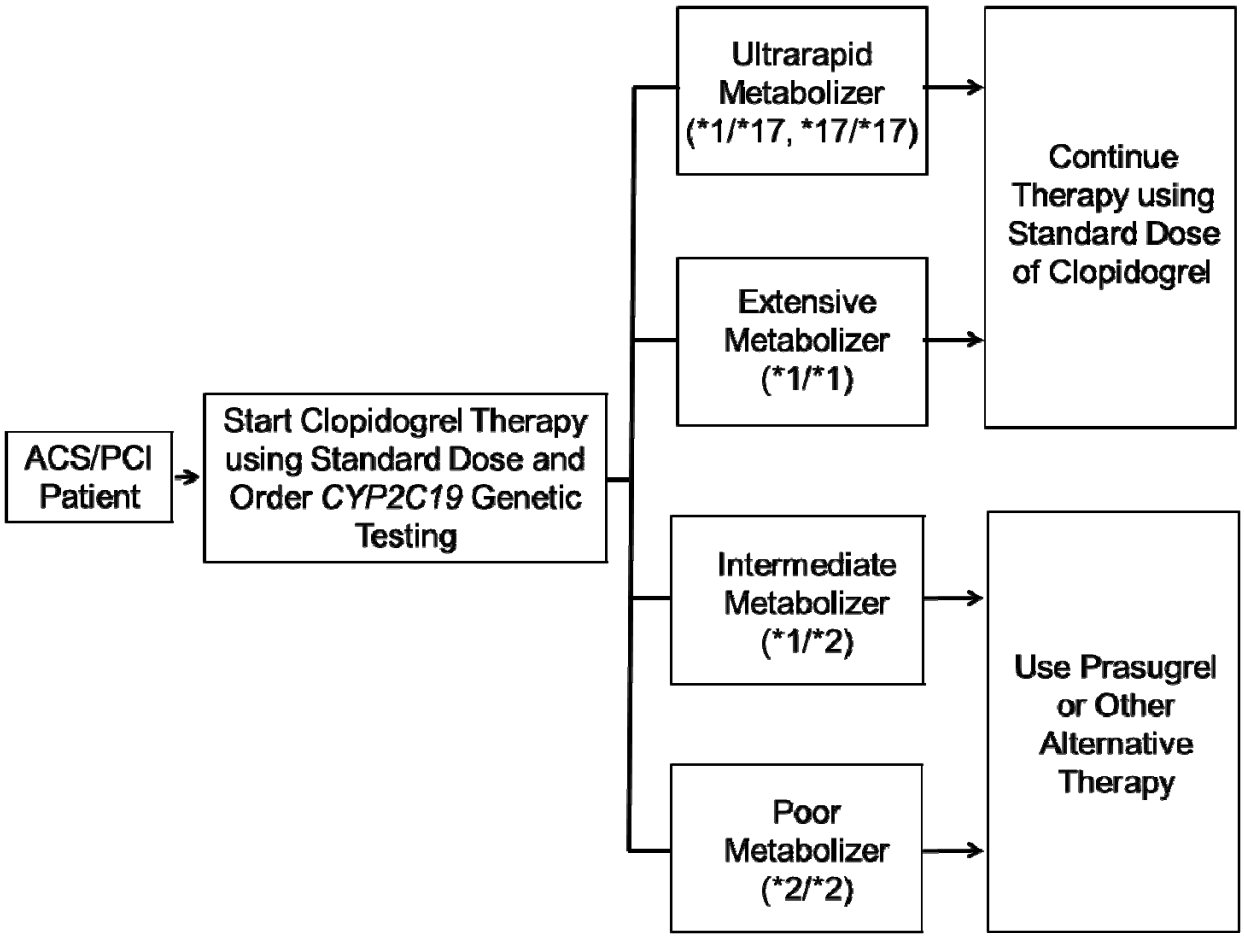

ACS/PCI Patient
Start Clopidogrel Therapy using Standard Dose and Order *CYP2C19* Genetic Testing
Ultrarapid Metabolizer (*1/*17, *17/*17)
Extensive Metabolizer (*1/*1)
Intermediate Metabolizer (*1/*2)
Poor Metabolizer (*2/*2)
Continue Therapy using Standard Dose of Clopidogrel
Use Prasugrel or Other Alternative Therapy

Figure 2.

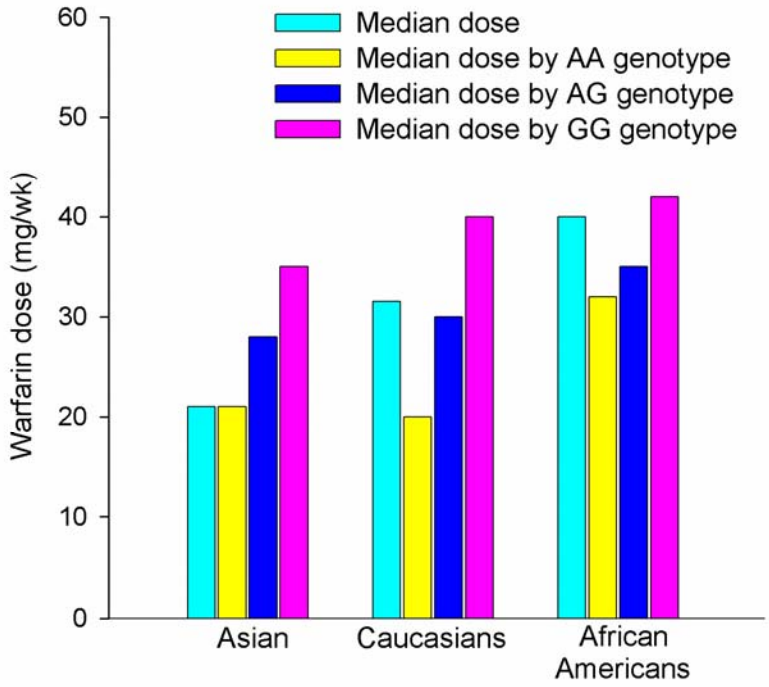

Median dose
Median dose by AA genotype
Median dose by AG genotype
Median dose by GG genotype
Warfarin dose (mg/wk)
60
50
40
30
20
10
0
Asian
Caucasians
African Americans

Figure 3.

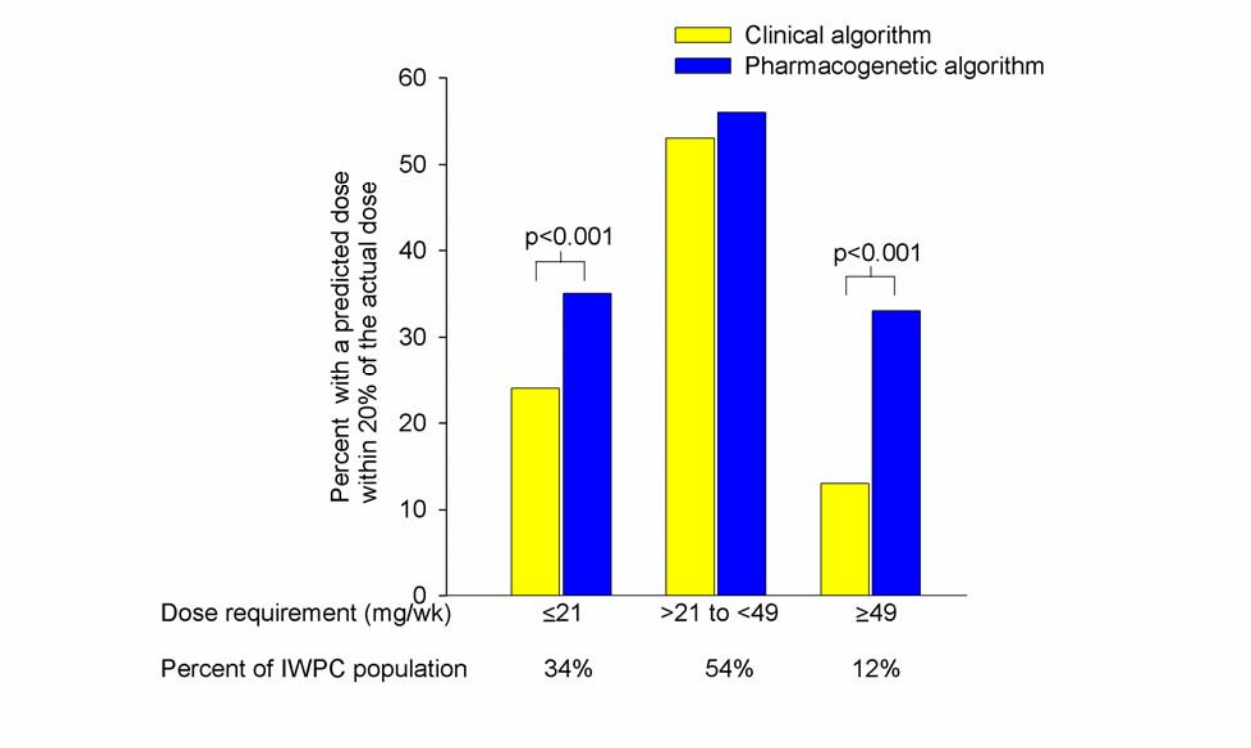
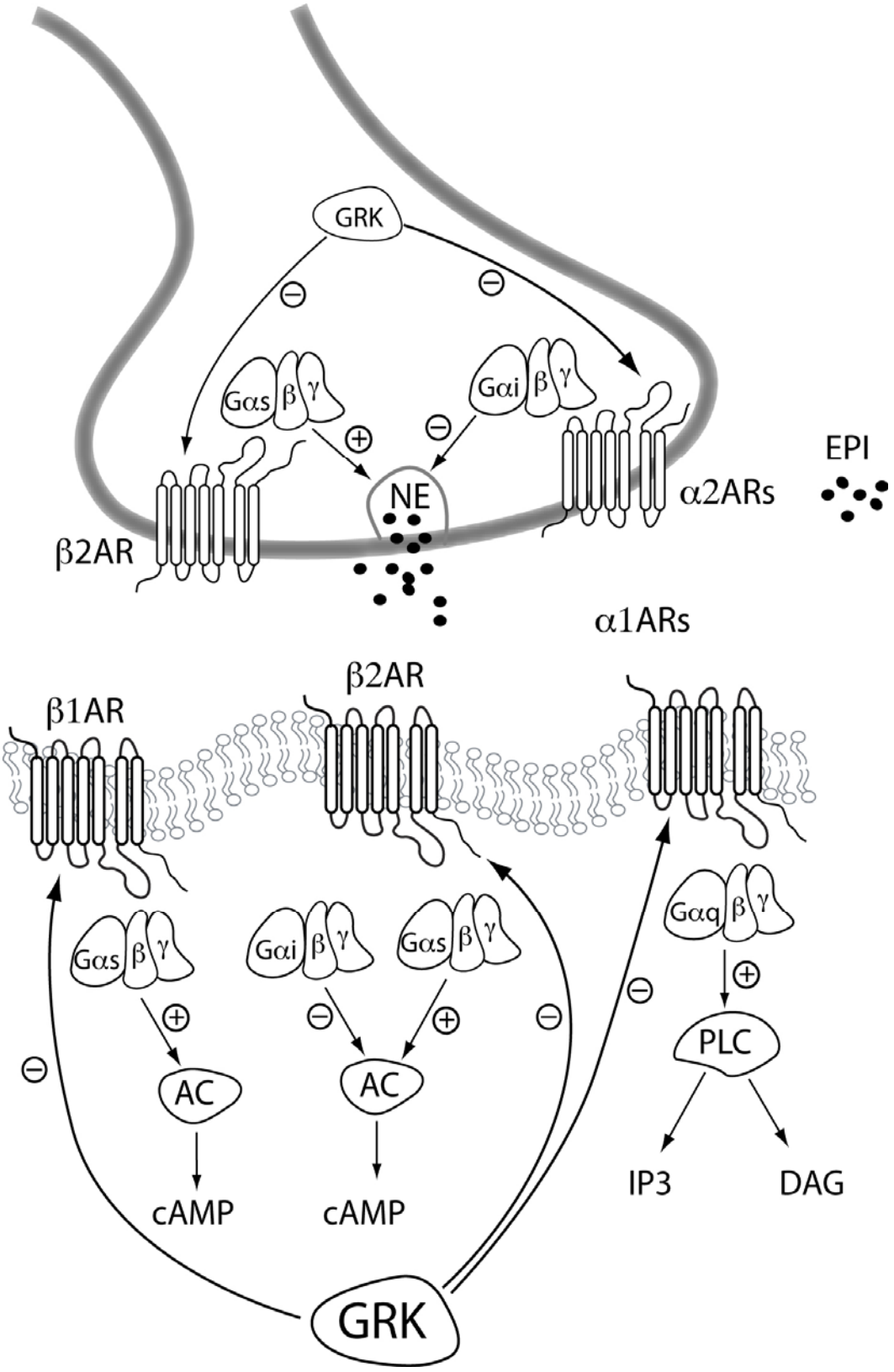

Clinical algorithm
Pharmacogenetic algorithm
Percent with a predicted dose within 20% of the actual dose
60
50
40
30
20
10
0
p<0.001
p<0.001
Dose requirement (mg/wk)
≤21
>21 to <49
≥49
Percent of IWPC population
34%
54%
12%

Figure 4.


GRK
Gαs β γ
Gαi β γ
NE
EPI
α2ARs
β2AR
α1ARs
β2AR
β1AR
Gαs β γ
Gαi β γ
Gαs β γ
Gαq β γ
AC
AC
PLC
cAMP
cAMP
IP3
DAG
GRK