Genetic Variants and Antiplatelet Efficacy

Number of Contact Hours – 2.3

Audience: RN/APRN

Pharmacology Hrs: 2.0

Goals and Objectives

Goals

The goal of this article is to focus on potential strategies for personalizing antiplatelet treatment by using pharmacogenomics approach to predict drug response, as well as discussing the plausibility of using it to predict the outcome.

Objectives

Discuss the pharmacogenetics of Aspirin

Discuss the clinical applications of oral anti-coagulants

Identify the four candidate genes of Aspirin

Describe the clinical outcomes of the antiplatelet agents

Describe the process of platelet activation

Discuss the clinical efficacy of Clopidogrel

Introduction

In recent years, substantial effort has been made to better understand the influence of genetic factors on the efficacy and safety of numerous medications. These investigations suggest that the use of pharmacogenetic data to inform physician decision-making has great potential to enhance patient care by reducing on-treatment clinical events, adverse drug reactions, and health care-related costs. In fact, integration of such information into the clinical setting may be particularly applicable for antiplatelet and anticoagulation therapeutics, given the increasing body of evidence implicating genetic variation in variable drug response. While compelling evidence suggests that genetic variants are important determinants of antiplatelet and anticoagulation therapy response, significant barriers to clinical implementation of pharmacogenetic testing exist. Pharmacogenetic testing can provide important information to assist clinicians with prescribing the most personalized and effective antiplatelet and anticoagulation therapy. However, several factors may limit its usefulness and should be considered.

Acute coronary syndromes (ACS) remain life-threatening disorders that are associated with high morbidity and mortality. Dual-antiplatelet therapy with aspirin and clopidogrel has shown to reduce cardiovascular events in patients with ACS. However, there is substantial inter-individual variability in response to clopidogrel treatment in addition to prolonged recovery of platelet reactivity as a result of irreversible binding to P2Y12 receptors. This high inter-individual variability in treatment response has primarily been associated with genetic polymorphisms in the genes encoding for cytochrome (CYP) 2C19 that affect clopidogrel's pharmacokinetics. [1, Rank 5]

Antiplatelet Pharmacogenomics

Dual antiplatelet therapy with aspirin and clopidogrel is currently the standard of care for treating patients with coronary artery disease (CAD) and/or acute coronary syndrome (ACS) undergoing percutaneous coronary intervention (PCI). The next-generation thienopyridine prasugrel, as well as the cyclopentyltriazolopyrimidine ticagrelor, have recently been approved and increasingly serve as alternative antiplatelet agents to clopidogrel. While all of these medications are generally effective, wide interindividual variation in response to these agents, defined by either laboratory response (ie, ex vivo measures of platelet aggregation) or clinical response (cardiovascular endpoints), have been documented. For each of these medications, variable response is, at least in part, heritable. For example, reported heritability estimates suggest that approximately 70% of the variability observed in clopidogrel response, as measured by adenosine diphosphate (ADP)-stimulated platelet aggregation, is attributed to genetic factors. Similarly, it has been shown in both Caucasian and African Americans that heritable factors significantly contribute to on-aspirin platelet responsiveness in molecular pathways directly and indirectly related to cyclooxygenase-1 (COX-1). While no large-scale investigation to date has evaluated the heritability of prasugrel or ticagrelor response, several candidate gene studies have revealed polymorphisms that influence response to these agents, suggesting a nontrivial genetic component. Therefore, identifying the genetic variants that influence response to these medications provides important information regarding pharmacokinetics and pharmacodynamics of these agents and also offers critical insights concerning the potential use of genotype information in prescribing the most effective and individualized antiplatelet therapy. [2, Rank 4]

Platelet Activation

Platelets are activated in response to vascular injury and/or atherosclerotic plaque rupture through a complex network of intra- and intercellular pathways. Activated platelets facilitate cell adhesion, initiate the arachidonic acid (AA) pathway to produce thromboxane A_2 (TXA_2), and excrete adenosine diphosphate (ADP), serotonin and other proteins from their granules, ultimately to form a platelet clot and eventually a thrombus. Given this fundamental role that platelets have in blood loss prevention and vasculature integrity, they

are inherently implicated in cardiovascular diseases such as atherosclerosis, coronary artery disease (CAD) and myocardial infarction (MI), as well as cerebrovascular disease and stroke.

Since platelet activation is influenced, in part, by TXA_2, ADP, serotonin, thrombin, epinephrine and collagen, these biological pathways have been leveraged as potential targets for antiplatelet therapies. The currently approved oral antiplatelet agents include aspirin, clopidogrel, prasugrel and ticagrelor, which are prescribed for prevention of ischemic events among patients with ischemic stroke and symptomatic peripheral artery disease (PAD), and as dual antiplatelet therapy (DAPT) for patients with acute coronary syndromes (ACS). However, variability in patient response to these agents are observed, which can translate to increased risks for adverse cardiovascular events. Potential pharmacogenetic determinants of response variability have been actively studied for all the antiplatelet agents, but none more so than clopidogrel. [3, Rank 3]

The identification of a biologically relevant candidate gene for clopidogrel responsiveness (i.e., cytochrome P450-2C19) and the availability of alternative antiplatelet therapies have provided the opportunity for genotype-directed antiplatelet therapy in selected patient populations. However, despite the enthusiasm for personalized antiplatelet therapy from advocates of this paradigm, the uncertain clinical utility and cost-effectiveness of this approach has made the field of antiplatelet pharmacogenetics highly studied and frequently debated. [4, Rank 4]

Pharmacogenetics of Aspirin

Given its benefit in reducing arterial thrombosis and recurrent cardiovascular events, aspirin (or acetylsalicylic acid) has remained a mainstay in antiplatelet therapy with indications including coronary, cerebral, and peripheral vascular disease. However, despite its general effectiveness in most patients, a considerable number of individuals experience sub-optimal aspirin response, assessed by either laboratory platelet reactivity testing or through its ability to prevent cardiovascular events. This interindividual variability in platelet response to aspirin has been well documented, and patients with high on-treatment platelet reactivity (HTPR) to AA have increased risk of ischemic events. Although estimates of aspirin non-responsiveness are controversial given the lack of standardized definitions, the use of multiple platelet function tests and evaluation of different surrogate endpoints suggest that 5–60% of patients do not adequately respond to aspirin. [5, Rank 4]

Platelet response to aspirin is influenced by several clinical variables (e.g., age, gender, smoking, and non-adherence) and coexisting comorbidities including obesity, diabetes, and hyperlipidemia; however, these factors only explain ~15% of the variability in on-treatment *ex vivo* platelet aggregation. Heritability estimates suggest that 14–39% of the variability in platelet responsiveness to aspirin can be attributed to genetic factors, and potentially through variants that influence both cyclooxygenase-1 (COX1)-dependent and COX1-independent platelet activation pathways.

Aspirin inhibits platelet aggregation primarily by the irreversible acetylation of COX1, which prevents the conversion of AA to TXA_2, a potent platelet agonist. As such, most traditional tests of aspirin response have focused on the COX1 pathway through measurement of AA-stimulated platelet aggregation or circulating thromboxane B_2 levels, the stable inactive metabolite of TXA_2. Using such assays, aspirin leads to near complete inhibition of COX1 in approximately 95% of individuals suggesting that a substantial proportion of the variability in response is mediated by factors outside of the COX1 pathway. While COX1 inhibition is nearly complete, the effect of aspirin on other platelet activation pathways (e.g., collagen, epinephrine, and ADP) is more heterogeneous and may explain, in part, the observed variability in response. Recent studies using collagen-stimulated platelet aggregation have identified novel circulating biomarkers and genetic risk loci associated with response variability. Consequently, while COX1 dependent platelet function assays are the most specific test of aspirin's canonical mechanism of action, recent studies have increasingly used non-COX1-dependent assays to more comprehensively define aspirin response and to identify novel genetic determinants of on-treatment platelet aggregation and cardiovascular outcomes. [6, Rank 3]

Candidate Genes of Aspirin

Most of the initial pharmacogenetic studies of aspirin response variability consisted of relatively underpowered candidate gene studies with different designs, participant selection (i.e., healthy vs. CAD/ACS patients), and primary outcome (i.e., platelet aggregation vs. cardiovascular events). Furthermore, these studies used different aspirin response phenotypes and platelet function tests, which subsequently have been shown to poorly correlate given the lack of standard definitions of aspirin responsiveness and the fact that these assays measure different platelet activation pathways (e.g., AA, epinephrine, and collagen). Although variability in platelet function testing has been previously reviewed, it is important to consider these limitations when assessing the potential roles of the following candidate genes in aspirin response variability. [7, Rank 5]

<u>Cyclooxygenase-1 (COX1)</u>

Given that COX1 is the molecular target of aspirin, multiple studies have evaluated the effect of genetic variants in the *COX1* gene [also known as prostaglandin synthase 1 (*PTGS-1*)] on aspirin response, most commonly involving the linked c.-842A>G (rs10306114) and c.50C>T (rs3842787) variants. Although it was initially reported that healthy individuals with the minor haplotype (c.[-842G;50T]) had better on-treatment inhibition of prostaglandin H_2 and AA-induced platelet aggregation compared to those with the common haplotype (c.[-842A;50C]), subsequent studies on stable CAD patients identified the c.-842G allele to actually be associated with aspirin resistance and non-responsiveness based on AA-induced platelet aggregation and serum TXB_2 levels. Consequently, the available evidence does not support a clinically relevant role for *COX1* variants in aspirin response. [8, Rank 3]

<u>Glycoprotein IIIa (GPIIIa)</u>

The glycoprotein IIb/IIIa complex (GPIIb/IIIa) is a critical regulator of thrombosis formation through its ability to bind fibrinogen resulting in platelet-platelet crosslinks. The PIA1/A2 (c.176T>C, p.L59P, rs5918) variant in the *ITGB3* gene that encodes the GPIIIa subunit has been extensively studied as a risk factor for cardiovascular disease and drug response to both aspirin and the GPIIb/IIIa inhibitor abciximab. Collectively, using different platelet function tests and aspirin response definitions, these studies have reported that the PIA2 allele results in increased, decreased, or no change in on-treatment platelet reactivity. [9, Rank 2]

Glycoproteins VI (GPVI), GPIa/IIa, and GPIbα

Given that collagen stimulates platelet aggregation by binding to glycoprotein VI (GPVI) and the glycoprotein Ia/IIa (GPIa/IIa) receptor complex on the platelet surface, these genes have been considered as candidates for aspirin response variability. Pharmacogenetic studies of *GPVI* and aspirin response have led to mixed results. Specifically, the common *GPVI* c.655C>T variant (p.P219S, rs1613662) has been associated with on-treatment platelet function variability in CAD patients. The commonly studied c.759C>T variant of the GPIa gene (*ITGA2*; rs1126643) has been associated with increased risk of stroke, MI, and cardiovascular death; however, studies on platelet aggregation variability after DAPT with aspirin and clopidogrel have also been conflicting and largely not supportive of a clinically meaningful effect on drug response. Given that most of these studies were small and generally underpowered, larger scale replication efforts will be needed to determine the precise roles of these variants on aspirin response. [10, Rank 1]

The *GPIbα* c.-5T>C variant (rs2243093) of the Von Willebrand receptor has also been studied as a candidate for aspirin response variability. Initial studies suggested that this variant altered *GPIbα* mRNA translation and was associated with increased platelet reactivity (as measured by reduced PFA-100 closure time) and increased risk of MI among aspirin-treated CAD patients. In contrast, subsequent studies reported no evidence of association between c.-5T>C and cardiovascular outcomes or aspirin response (including TXB2 levels, collagen-stimulated platelet aggregation and PFA-100 closure time). Taken together, the available evidence does not support a role for the *GPIbα*c.-5T>C variant in aspirin efficacy. [11, Rank 3]

Platelet Endothelial Aggregation Receptor 1 (PEAR1)

The platelet endothelial aggregation receptor 1 (PEAR1) is a type 1 transmembrane receptor that is involved in platelet aggregation through GPIIb/IIIa as well as altered megakaryopoiesis and thrombopoiesis via the PI3K/PTEN pathways. Early genetic studies identified several *PEAR1* variants significantly associated with platelet aggregation in response to multiple agonists before and after aspirin exposure. The most notable *PEAR1*association has been between the intronic rs12041331 variant and *ex vivo* platelet aggregation in response to several platelet agonists (ADP, collagen, epinephrine) as well as pre- and post-antiplatelet therapy treatment (i.e., aspirin and

prasugrel). Furthermore, *PEAR1* rs12041331 significantly reduced 1-year survival in aspirin-treated patients undergoing percutaneous coronary intervention (PCI) and increased rates of MI in an independent cohort of aspirin-treated patients with stable CAD. Paradoxically, the allele that was associated with improved aspirin response, as defined by *ex vivo* platelet aggregometry, was the same allele that resulted in an increased risk of experiencing a thrombotic event. In addition, a recent study did not detect any association between *PEAR1* rs12041331 and clinical outcomes in CAD patients, indicating that additional clinical studies on *PEAR1* and aspirin response are still warranted. [12, Rank 1]

Other Aspirin Candidate Genes

Other commonly investigated aspirin response candidate genes include the TXA2 receptor (TBXA2R), ADP receptors (*P2RY1* and *P2RY12*), coagulation factor XIII (F13A1), and UDP-glucuronosyltransferase 1A6 (UGT1A6); however, their inconsistent results make it difficult to form any firm conclusions.

Aspirin Efficacy

Aspirin is the mainstay antiplatelet agent used for the primary and secondary prevention of MI, stroke, and death. While generally effective, non-responsiveness to aspirin has been well-documented and occurs in approximately 6%–60% of individuals, depending on how it is defined.54 Aspirin irreversibly inhibits prostaglandin G/H synthase 1 (PTGS1, or COX-1) and the conversion of arachidonic acid to thromboxane. Platelet function assays specific for aspirin's effects on platelet COX-1 include serum thromboxane B2 and arachidonic acid-induced platelet aggregation. With adequate dosing and compliance, aspirin is capable of completely inhibiting COX-1 using such assays in >99% of individuals; thus true "aspirin resistance" is rare. However, alternate agonists, such as ADP, collagen, and epinephrine, can produce robust aggregation in the face of complete COX-1 inhibition. These platelet aggregation pathways are sensitive to the effects of aspirin because thromboxane serves in a positive-feedback loop, thus amplifying the downstream signals of these agonists. However, these pathways are not completely dependent on the generation of thromboxane. As a consequence, these "non-COX-1-dependent" platelet function assays demonstrate wide interindividual variability before and after aspirin exposure. Direct measures of platelet COX-1 on aspirin demonstrate little variability and heritability; however, indirect or non-COX-dependent pathways demonstrate significant heritability within families. These findings suggest that there is a significant genomic contribution to the observed variability in platelet aggregation responses to aspirin. Furthermore, the observation that individuals with high levels of residual platelet aggregation on aspirin are also at heightened risk for cardiovascular events suggests that these non-COX-dependent measures of aspirin response may also be clinically significant. [13, Rank 2]

Aspirin is rapidly absorbed after oral administration and has a half-life of 15–20 minutes. With typical daily aspirin dosing (ie, <100 mg/day), there is nearly uniform inhibition of platelet COX-1, suggesting that, for the vast majority of individuals, these dosages are sufficient to inhibit platelet COX-1. However, there are certain populations in which higher aspirin doses may be required. Aspirin undergoes hydrolysis in the plasma as well as in erythrocytes with significant interindividual variability. To identify genetic determinants of plasma hydrolytic activity, a genome-wide association study (GWAS) was performed that identified a genetic variant (rs6445035) in proximity to the butyrylcholinesterase (*BCHE*) gene at genome-wide level of significance, such that each additional copy of the minor allele was associated with a 1.2 nmol/mL/min reduction in aspirin hydrolytic activity but explained 3% of the overall variability in response. Therefore, genetic variation at *BCHE* is unlikely to explain much of the observed variability in aspirin response. [14, Rank 2]

Several observations have been made that suggest that the response to surgical procedures, specifically coronary artery bypass grafting, results in a transient decrease in the in vitro response to aspirin. This transient reduction in the effects of aspirin may be explained by increased transcription of *ABCC4*, which is expressed in platelets and can extrude acetylsalicylate (an organic anion derived from aspirin) out of platelets, thus limiting the amount within platelets that is available to inhibit platelet COX-1. Although genetic variation in *ABCC4* has not been linked to variation in the response to aspirin, expression profiling (ie, RNA or protein levels) may be more suitable biomarkers by which to identify individuals with high levels of this transporter.

In an effort to translate findings related to platelet function measured in the laboratory to clinical outcomes of patients taking aspirin, several groups have attempted to link genetic data with long-term clinical outcomes. In two large-scale investigations, most genetic associations with laboratory outcomes did not translate to differences in risk in clinical outcomes, though these variants were selected prior to recent GWAS findings. In a more contemporary study, the rs12041331 *PEAR1* variant was studied in two independent populations and, whereas carriers of the minor allele had lower levels of platelet function on aspirin, carriers of the minor allele were consistently at higher risk for cardiovascular events. Further, the risk conferred by the rs12041331 *PEAR1* variant seemed to depend on aspirin use, such that the risk in carriers of the minor allele was greatest in those who reported aspirin use. These divergent associations between platelet aggregation results and clinical outcomes with respect to *PEAR1* demonstrate the complexity surrounding use of a laboratory-based assay for studying drug responses and our lack of understanding of the biological processes that occur in vivo. Further, the apparent statistical interaction between aspirin use and *PEAR1* genetic variation demonstrates the complexity of translating genetic findings into clinical outcomes. [15, Rank 3]

A complementary approach to identifying genetic variants that may predict the risk of cardiovascular outcomes in patients treated with aspirin comes from studies

of *LPA*. *LPA* codes for apolipoprotein (a), which forms Lp(a) when linked with low-density lipoprotein particles. Lp(a) is known to be associated with the development of CAD, though it is not known to affect platelet function. A rare variant in *LPA*(rs3798220) was associated with higher concentrations of Lp(a), and carriers had a more than twofold reduction in the risk for cardiovascular disease with aspirin, whereas non-carriers (>95% of Caucasians) had no reduction in a large, placebo-controlled clinical trial. This suggests that the benefits of aspirin in primary prevention may be concentrated in carriers of this rare allele. Thus, this marker could be used to identify individuals who would benefit from low-dose aspirin in the primary prevention of cardiovascular disease.

Despite the recent advances in the genetics underlying the response to aspirin, there are many hurdles to overcome before these can be implemented in clinical practice. For example, although the associations with *PEAR1* and platelet function are sound, the divergent results with clinical outcomes demands further explanation. Gene expression profiling of peripheral blood RNA is now a clinically available diagnostic test, thus facilitating measurement of genes represented by the ARS; however, it is not yet clear how treatment would be modified on the basis of these data to improve clinical outcomes. Direct platelet function testing using point-of-care devices is available, and although these testing platforms can detect inadequate levels of platelet inhibition on aspirin, no study to date has consistently shown an association of test results with cardiovascular events on aspirin. Therefore, although aspirin has been a mainstay of treatment for patients at risk for cardiovascular disease, there will continue to be uncertainty regarding the tailoring of aspirin therapy in the clinical setting. [16, Rank 4]

Clopidogrel Genetic Variants

Clopidogrel is a second generation thienopyridine that undergoes hepatic biotransformation to an active metabolite, which binds irreversibly to the $P2Y_{12}$ receptor and inhibits ADP-mediated platelet activation and aggregation. The majority of clopidogrel (~85%) is hydrolyzed to inactive metabolites by esterases, including carboxylesterase 1 (CES1), leaving only ~15% available for transformation to the active metabolite. Two sequential oxidative reactions by the cytochrome P450 (CYP450) system form the active metabolite: the first involving CYP1A2, CYP2B6 and CYP2C19, and the second involving CYP2B6, CYP2C9, CYP2C19, CYP3A4 and CYP3A5. Clopidogrel and aspirin administered as DAPT reduces cardiovascular death and ischemic events in ACS patients and those undergoing PCI. However, wide interindividual variability in ex vivo platelet aggregation is common among DAPT-treated patients, and some still experience thrombotic events. Importantly, patients with persistent HTPR to ADP are at increased risk for adverse cardiovascular events. Other clinical factors implicated in clopidogrel response variability include age, co-medications, diabetes, disease activity, renal failure, and cardiac failure. [17, Rank 3]

In order to identify variants that influence clopidogrel response variability, a number of candidate genes in the clopidogrel pharmacokinetic and pharmacodynamic pathways have

been studied. Among them, the most robust association has been with the common *CYP2C19*2* loss-of-function allele (c.681G>A; rs4244285), which was initially reported in 2006 to be significantly associated with HTPR in healthy subjects. [18, Rank 3]

ABCB1

The *ABCB1* gene encodes the well-described multidrug resistance protein 1 (MDR1), an ATP-dependent efflux transporter important in the bioavailability of multiple endogenous and xenobiotic compounds including clopidogrel. As clopidogrel is absorbed from the intestinal lumen via duodenal enterocytes, MDR1 immediately transports a portion of the drug back into the lumen, resulting in decreased clopidogrel bio-availability. While *ABCB1* is highly polymorphic, significant attention has focused on the effect of a three-SNP haplotype, tagged by the C3435T SNP (rs1045642), on clopidogrel metabolite level, platelet reactivity, and cardiovascular events. Prior investigations have shown that the T-allele of the C3435T variant is relatively common (allele frequency ranges from 10%–60% depending on race/ethnicity) and results in increased MDR1 expression, thereby potentially leading to increased clopidogrel extrusion

Early investigations of the *ABCB1* C3435T variant revealed that PCI patients who were homozygous for the T-allele had significantly less clopidogrel prodrug and active metabolite levels compared to C-allele carriers when given either a 300 or 600 mg loading dose.In clopidogrel-treated ACS PCI patients of the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel-Thrombolysis in Myocardial Infarction (TRITON-TIMI) trial, *ABCB1* T-allele homozygotes had a 72% increased risk of a composite endpoint consisting of cardiovascular death, MI, or stroke. However, a recent meta-analysis consisting of over 10,000 clopidogrel-treated patients, primarily with ACS (89%) and/or undergoing PCI (74%), was conducted in order to evaluate the effect of the C3435T variant on several cardiovascular outcomes. When comparing 3435 T-allele homozygotes to C-allele carriers, while there was moderate evidence of a relationship between this variant and short-term (<30 days) recurrent ischemic events (*P*=0.02, odds ratio [OR] =1.41, 95% CI: 1.06–1.87), no evidence of association was observed between the C3435T variant and overall recurrent events (*P*=0.07, OR =1.15, 95% CI: 0.99–1.33), stent thrombosis (*P*=0.37, OR =0.79, 95% CI: 0.47–1.32), or bleeding (*P*=0.82, OR =0.98, 95% CI: 0.79–1.21). While there is a growing evidence base supporting a potential role for the *ABCB1* C3435T variant in clopidogrel efficacy, the inconsistencies of these findings make the use of this variant in genotype-directed therapy or other clinical applications, at this time, premature. [19, Rank 2]

CYP2C19

After its absorption, several enzymes contribute to hepatic metabolism of clopidogrel, resulting in both biologically active and inactive derivatives. Previous investigations have shown that CYP2C19 is the major contributor regarding generation of the bioactive metabolite. Consistent with this observation, both loss-of-function (LOF) and gain-of-

function (GOF) genetic variants in *CYP2C19* have been most consistently associated with clopidogrel efficacy. There are several LOF variants in *CYP2C19* that contribute to altered clopidogrel response. CYP2C19*2 (rs4244285), which results in a cryptic splice site in exon leading to a premature stop codon, is the most common of these variants, with approximately 20%–30% of Caucasians and Africans and 60% of Asians carrying at least one copy of this allele. Other LOF variants (ie, *CYP2C19*3–*8*) are generally rare in most populations, thus limiting their potential clinical utility, with perhaps the exception of *CYP2C19*3* (rs4986893), which is substantially more common in Asian populations, with an allele frequency ranging from 5%–9%. The most comprehensively evaluated GOF variant is *CYP2C19*17*(rs12248560). *CYP2C19*17* resides in the promoter region of this gene and has been implicated in altered clopidogrel response and increased bleeding risk (see the last two paragraphs of the current section) through its ability to increase transcription of *CYP2C19*. [20, Rank 3]

There is now a convincingly large body of evidence to suggest that the *CYP2C19*2* variant significantly impacts clopidogrel pharmacokinetics and pharmacodynamics. The *CYP2C19*2*-specific higher platelet aggregation post-clopidogrel exposure was due to altered clopidogrel metabolism resulting in a reduction of circulating clopidogrel active metabolite. These findings have been subsequently replicated in multiple investigations of patients with cardiovascular disease, leaving little doubt regarding the relationship between *CYP2C19*2* and clopidogrel pharmacokinetics as well as pharmacodynamics.

Some of the earliest of these investigations reported that clopidogrel-treated patients who carried at least one copy of the *CYP2C19*2* allele were significantly more likely to experience a major adverse cardiovascular event (MACE) compared to individuals who were homozygous for the *CYP2C19*1*allele. In these studies, the authors also observed an approximately two- to threefold increase in the incidence of stent thrombosis in *CYP2C19*2* allele carriers compared to noncarriers, an observation that has subsequently been replicated by other groups. Given the potential clinical utility of these findings, multiple investigations were subsequently performed and revealed a generally consistent relationship between *CYP2C19*2* genotype and on-clopidogrel MACE. [21, Rank 4]

In the last few years, several meta-analyses have revealed important insights regarding the effect of the *CYP2C19*2* variant in clopidogrel-treated patients. Furthermore, these studies may explain, at least in part, the inconsistencies observed in the investigations described in the previous paragraph. Together, these results suggest that the effect of the *CYP2C19*2* variant on clinical outcomes may be indication-specific. It is well established that high-risk patients who undergo PCI derive the most benefit from clopidogrel therapy compared to other indications. Therefore, it is reasonable to speculate that the impact of the *CYP2C19*2* variant may be more pronounced in PCI patients, particularly in those who may experience stent thrombosis. Indeed, as reviewed in depth previously, nearly all meta-analyses conducted to date have shown a strong association between *CYP2C19*2* genotype and risk of stent thrombosis. On the other hand, large-scale evaluations of lower-risk and/or

non-PCI patients, such as the populations used in the CURE and ACTIVE-A trials, have not reproducibly shown an effect of *CYP2C19*2* on cardiovascular outcomes. The results of these meta-analyses have 1) provided robust evidence of a relationship between *CYP2C19*2* genotype and risk of stent thrombosis, and 2) highlighted the importance of clinical indication in studies of *CYP2C19* genetic variability and clopidogrel efficacy. [22, Rank 3]

While the *CYP2C19*2* variant has been the most extensively studied polymorphism with regard to clopidogrel response variability, substantial effort has been made to understand the impact of the relatively common *CYP2C19*17* GOF allele. In a retrospective study consisting of 598 non-ST elevation clopidogrel-treated ACS patients, researchers found that individuals who carried the CYP2C19*17 variant had significantly better clopidogrel response as assessed by vasodilator stimulated-phosphoprotein (VASP) platelet reactivity index. However, subsequent follow-up investigations regarding the impact of this variant on the formation of clopidogrel active metabolite, platelet aggregation, and cardiovascular outcomes have had mixed results. Similarly, currently available meta-analysis data are also inconsistent. For example, two meta-analyses performed in 2012 provided evidence that the *CYP2C19**17 was significantly associated with decreased rates of adverse clinical outcomes but increased rates of adverse bleeding. In contrast, an independent systematic review and meta-analysis revealed that carriers of the *CYP2C19*17* variant did not significantly influence risk of experiencing a composite cardiovascular endpoint or stent thrombosis. [23, Rank 4]

PON1

A genetic variant in paraoxonase 1 (*PON1*), the common Q192R missense variant (rs662), is a major determinant of clopidogrel efficacy. In fact, it was observed that PON1, a well-described hepatic esterase, was critical in converting 2-oxo-clopidogrel into the bioactive thiol metabolite and that the 192Q allele was significantly associated with reduced clopidogrel pharmacokinetics and increased occurrence of stent thrombosis in CAD PCI patients (OR =3.6, 95% CI: 1.6–7.9, *P*=0.003). They extended these findings in an independent prospective cohort consisting of 1,982 ACS patients and found that *PON1* 192Q-allele homozygotes were significantly more likely to experience fatal or nonfatal definite stent thrombosis (HR =10.2, 95% CI: 4.3–71.4, *P*<0.001) as well as a composite cardiovascular endpoint consisting of vascular death, nonfatal MI, and nonfatal stroke (OR =3.9, 95% CI: 2.1–7.2, *P*<0.001). Consistent with decreased clopidogrel response, it was also observed that *PON1* 192Q-allele homozygotes had lower risk of major bleeding (HR =0.4, 95% CI: 0.2–0.8, *P*=0.006). [24, Rank 2]

As a result of this investigation, several groups evaluated the role of the *PON1*Q192R variant on clopidogrel efficacy. Interestingly, however, nearly all replication efforts failed to observe an association between the Q192R variant and clopidogrel response, as assessed by either platelet reactivity or occurrence of on-treatment cardiovascular events. Given that several

factors, including statistical power may explain, at least in part, the discrepant results observed between the investigation and subsequent replication studies, members of the PON1 Study Group recently conducted a systematic review and meta-analysis of summarized data in order to evaluate the impact of *PON1* Q192R on platelet reactivity and recurrent ischemic events. Consistent with the findings of the individual replication efforts, no evidence of association was observed between *PON1* Q192R and platelet reactivity, regardless of the laboratory method used (global mean standardized difference =0.10, 95% CI: −0.06 to 0.25, *P*=0.22). Moreover, analysis of eleven independent investigations that evaluated the impact of this polymorphism on MACE risk revealed no difference in event rate by Q912R genotype (OR =1.28, 95% CI: 0.97–1.68, *P*=0.08).43 While more recent investigations suggest that *PON1* Q192R influences relative platelet inhibition instead of on-clopidogrel platelet reactivity and that genetic variability in this gene is associated with clinical outcomes in PCI patients through mechanisms independent of clopidogrel treatment, currently available information do not convincingly support a role of *PON1* in clopidogrel pharmacogenetics. [25, Rank 1]

CES1

CES1 is the primary enzyme responsible for converting clopidogrel, 2-oxo-clopidogrel, and the bioactive thiol metabolite into biologically inactive carboxylic acid derivatives. In fact, up to 85% of therapeutically administered clopidogrel may be degraded by CES1 activity in the liver. Given the important role of this enzyme in clopidogrel metabolism, it is not difficult to speculate that variability in CES1 function and/or expression may have important clinical implications. To date, however, few studies have evaluated the effect of genetic variants in *CES1* on clopidogrel response. In 566 participants of the Pharmacogenomics of Anti-Platelet Intervention (PAPI) Study, it was observed that an LOF missense polymorphism resulting in glycine-to-glutamic acid substitution at position 143 (G143E, rs71647871) significantly impacts clopidogrel pharmacokinetics and pharmacodynamics. In fact, compared to CES1*143G* allele homozygotes, individuals who carried the 143E allele had significantly higher circulating levels of clopidogrel active metabolite (19.0 versus 30.3 ng/mL, respectively, *P*=0.001) as well as greater inhibition of ADP-stimulated platelet aggregation (43% versus 29% of baseline, respectively, *P*=0.003). Researchers extended these findings in 350 CAD patients and observed that *CES1* 143E allele carriers had significantly better clopidogrel response as assessed by ADP-stimulated platelet aggregation compared to 143G homozygotes (on-clopidogrel maximal platelet aggregation =25% and 45%, respectively, *P*=0.03). Furthermore, 0% of *CES1* 143E allele carriers experienced a cardiovascular event at 1 year compared to 13.7% in patients who carried two copies of the 143G allele; however, this comparison was not statistically significantly (*P*=0.44), possibly due to the relatively low power of the analysis. The G143E variant completely inhibited the hydrolysis of both clopidogrel and 2-oxo-clopidogrel. In addition, the latter investigation also evaluated the role of other genetic variants on CES1 enzymatic function and it was observed that the D260fs mutation significantly impacted CES1 activity, while the G18V, S82L, and

A269S variants did not. It is important to note that, while these initial investigations evaluating the role of genetic variation in *CES1* on clopidogrel response have yielded some interesting results, *CES1* is a highly polymorphic gene. Given its critical role in clopidogrel metabolism, further studies that more comprehensively evaluate the effect of genetic variation in this gene on clopidogrel response seem warranted. [26, Rank 1]

Other Variants

While CYP2C19 is the most important enzyme in the bioactivation of clopidogrel, several other CYP enzymes contribute to its metabolism, including CYP2B6, CYP2C9, CYP3A4/5, and CYP1A2, albeit to a lesser extent. In some early studies of clopidogrel pharmacogenetics, nominal evidence of association was observed between polymorphisms in these genes (eg, *CYP1A2*1F* and *CYP2C9*2/3*) and clopidogrel response. However, subsequent replication efforts have had mixed results. Given the lesser role of genetic variation in these enzymes in clopidogrel activation, it has been speculated that redundant mechanisms of metabolism make the overall effect of these variants relatively small. These gene variants have been reviewed in detail previously. Taken together, while genetic variation in these genes may contribute to variable clopidogrel response, the current evidence is not strong enough to support the use of genotype information in other CYP genes for the purpose of tailoring antiplatelet therapy. [27, Rank 2]

Genetic variants in genes responsible for clopidogrel pharmacodynamics have also been implicated in altered clopidogrel response. Previous investigations suggest that a two-SNP haplotype in the *P2RY12* gene consisting of the G52T (rs2046934) and T744C (rs2046934) leads to higher P2Y12 receptor expression and higher platelet reactivity in clopidogrel-treated subjects. However, inconsistent findings in replication efforts have called the validity of this association into question. Similarly, polymorphisms in the *ITGB3* gene, which encodes the beta subunit of the well-described glycoprotein IIb/IIIa receptor, have been associated with clopidogrel response and cardiovascular events including stent thrombosis in some investigations but not others. Taken together, these investigations suggest that, if variants in *P2RY12* or *ITGB3* are truly associated with clopidogrel response, their effects are small and not likely to be of clinical utility. [28, Rank 1]

Clopidogrel Clinical Efficacy

Given its effectiveness and relatively low cost compared to its alternatives, clopidogrel remains one of the most widely prescribed antiplatelet medications to prevent recurrent ischemic events in patients with ACS, myocardial infarction, and/or who are undergoing PCI. Despite its wide use, however, it has been consistently shown that approximately 4%–30% of patients do not respond adequately to this drug, resulting in high on-treatment platelet reactivity (HTPR) and increased rates of cardiovascular events. In fact, pharmacodynamic investigations have shown that laboratory measures of ex vivo ADP-stimulated platelet reactivity, a widely used surrogate marker of clopidogrel response, vary substantially among clopidogrel-treated patients. Moreover, PCI patients on clopidogrel who exhibit HTPR are

more likely to experience a recurrent adverse clinical event, seemingly regardless of clinical presentation. While several clinical and demographic factors that influence clopidogrel efficacy have been determined (eg, age, body mass index, diabetes, diet, smoking, drug–drug interactions [eg, proton pump inhibitors], etc), the proportion of variation in drug response they collectively account for is relatively modest. In order to better understand variability in clopidogrel response, genetic evaluation of multiple candidate genes has been conducted, revealing several single nucleotide polymorphisms (SNPs) that may significantly influence clopidogrel response. [29, Rank 4]

The genes responsible for clopidogrel transport, metabolism, and action were obvious choices for pharmacogenetic candidate gene studies. Clopidogrel is an oral, second-generation thienopyridine prodrug that, following rapid absorption by the duodenum, is metabolized through a two-step conversion by hepatic cytochrome P450 enzymes, primarily CYP2C19, resulting in a biologically active thiol metabolite. Approximately 15% of clopidogrel prodrug is converted into the active metabolite, while ~85% is degraded into inactive carboxylic acid derivatives by hepatic esterases, most notably carboxylesterase 1 (CES1). In circulation, the active thiol metabolite irreversibly binds to and inactivates the P2Y12 receptor on the surface of platelets, leading to inhibition of ADP-induced platelet activation and aggregation. [30, Rank 3]

Next-generation P2Y12 inhibitors (prasugrel and ticagrelor)

The known variability in the response to clopidogrel spurred the development of novel P2Y12 inhibitors to overcome this limitation of clopidogrel. Prasugrel, which, like clopidogrel, is administered as an inactive prodrug that must be converted to an active metabolite (R-138727), also irreversibly inhibits the platelet P2Y12 receptor. However, unlike clopidogrel, due to its chemical structure, the vast majority of the parent compound is converted into an active metabolite, thus achieving higher concentrations of active metabolite compared to clopidogrel and greater platelet P2Y12 receptor inhibition. Ticagrelor is a novel, non-thienopyridine, P2Y12 receptor antagonist that is administered as an orally active drug, thereby overcoming the bioactivation process. In addition, ticagrelor, a cyclopentyltriazolopyrimidine, is the first in its class of non-thienopyridines that reversibly inhibits the platelet P2Y12 receptor. Ticagrelor can also be metabolized into an active metabolite (AR-C124910XX) and has concentrations that are roughly one-third that of the parent compound. [30, Rank 5]

Though both prasugrel and ticagrelor achieve consistently high levels of inhibition of the platelet P2Y12 receptor, there remains significant variability when examining agonists other than ADP, such as collagen. Furthermore, in patients treated with prasugrel, variability in ex vivo platelet function is associated with the risk of cardiovascular events. This suggests that, as with clopidogrel, ex vivo platelet function testing may be a useful biomarker for identifying those who may (or may not) receive the full benefits of these novel agents. Although there have not been genome-wide approaches applied to the response to

prasugrel, several candidate gene approaches have been used. Because prasugrel is a prodrug that is dependent on hepatic CYPs for bioactivation, several genetic association studies involving *CYP2C19*, *CYP2C9*, *CYP2B6*, and *CYP1A2* have been performed. While most suggest that genetic variation at these loci are not important for prasugrel response, another study has shown that CYP2C19**2* and CYP2C19**17* significantly impact platelet reactivity index VASP in prasugrel-treated patients undergoing coronary stenting. Two regions of the *PEAR1* locus appear to be associated with the extent of platelet inhibition after prasugrel exposure. These findings have not yet been replicated or extended to clinical outcomes in patients treated with prasugrel. Because ticagrelor is administered as an orally active drug, we should not expect genetic variation in *CYP2C19* to influence the response to ticagrelor. To confirm this, investigators have shown that *CYP2C19*2* does not influence platelet aggregation or clinical outcomes in patients treated with ticagrelor, unlike patients treated with clopidogrel. In an effort to identify genetic variants beyond *CYP2C19* that are associated with the response to ticagrelor, a recent GWAS was conducted on levels of ticagrelor and its active metabolite from the PLATO clinical trial. Genetic variants in linkage disequilibrium with the *SLC01B1*5* loss of function variant were associated with higher ticagrelor and active metabolite levels. Further, a genetic variant (rs61361928) in *UGT2B7* was associated with ticagrelor active metabolite levels. None of the variants in *SLCO1B1* nor *UGT2B7*, however, were associated with bleeding or ischemic events in the ticagrelor-treated arm. [31, Rank 4]

Prasugrel Genetic Variants

Prasugrel is a third generation thienopyridine administered with aspirin as DAPT for the management of ACS patients undergoing PCI. Prasugrel is hydrolyzed by carboxylesterases to yield thiolactone (R-95913), which undergoes hepatic bioactivation by CYP3A4, CYP2B6, CYP2C9, CYP2C19 and CYP2D6, to generate its active metabolite (R-138727). Like clopidogrel, the prasugrel active metabolite antagonizes the $P2Y_{12}$ receptor and impairs ADP-mediated activation. Prasugrel is rapid-acting and generates a higher level of active metabolite compared to clopidogrel, resulting in a more potent and effective platelet inhibition; however, the increased efficacy is counterbalanced by an increased risk for major bleeding. Despite the advantages of prasugrel over clopidogrel, HTPR has also been reported among prasugrel-treated PCI patients, which was associated with higher rates of thrombotic events.

Given that prasugrel undergoes CYP450-mediated hepatic bioactivation, initial pharmacogenetic studies on prasugrel response focused on *CYP450* variant alleles. Prasugrel pharmacokinetics and pharmacodynamics were initially tested for association with *CYP450* variants among healthy subjects; however, no significant relationship was detected for either active metabolite exposure or pharmacodynamic response. A small study of CAD patients also failed to detect a significant difference in prasugrel active metabolite exposure or pharmacodynamic responses based on *CYP2C19*genotype status. Notably, the large TRITON-TIMI 38 trial included a pharmacogenetic substudy of prasugrel-

treated ACS patients with planned PCI and genotyped 54 alleles in six *CYP450* genes. No significant effects on prasugrel pharmacokinetics or pharmacodynamics were identified, nor were any *CYP450* variants associated with clinical outcomes. Considering most studies use ADP-induced platelet aggregation as a measure of platelet function, a subsequent clinical study was performed to assess the influence of *CYP2C19* alleles on prasugrel response as determined by platelet reactivity index (PRI) from vasodilator-stimulated phosphoprotein (VASP) analysis. Interestingly, similar to clopidogrel, *CYP2C19*2* carriers had a significantly higher PRI and risk of HTPR than noncarriers, which remained consistent with a subsequent study on prasugrel maintenance therapy response by PRI VASP. [32, Rank 1]

In addition to the *CYP450* genes, a few other candidate genes have been interrogated for association with prasugrel response. For example, the *ABCB1* gene was also genotyped in the TRITON-TIMI 38 pharmacogenetic substudy; however, it was not significantly associated with any outcomes in prasugrel-treated ACS/PCI patients. Interestingly, the *PEAR1* gene was genotyped in a small study of healthy Han Chinese subjects, which reported a significant association between selected *PEAR1* variants and ADP-induced platelet aggregation; however, the extremely small sample size of the study (n=36) indicates that these findings are preliminary. [33, Rank 3]

Ticagrelor Genetic Variants

Ticagrelor is a cyclopentyl-triazolo-pyrimidine agent that is an allosteric ADP antagonist that does not require hepatic bioactivation to generate an active metabolite; however, after oral administration and absorption, it is degraded to its primary active (ARC124910XX) and inactive (AR-C133913XX) metabolites through CYP3A4/5-mediated metabolism. Consequently, the ticagrelor label recommends avoiding coadministration with strong CYP3A inhibitors and inducers among patients with ACS. Although this also suggests that *CYP3A4* and/or *CYP3A5* variant alleles may potentially influence ticagrelor efficacy, pharmacogenetic studies have yet to prove this hypothesis. Additionally, the role of CYP3A4 in generating the active ticagrelor metabolite may also be responsible for the reported drug interaction between ticagrelor and statins. Since ticagrelor is both a CYP3A4 substrate and inhibitor, its use results in higher serum concentrations of simvastatin and lovastatin when coadministered, as these drugs are also metabolized by CYP3A4. This interaction may be responsible, in part, for the mortality benefit observed with ticagrelor compared to clopidogrel in the PLATO trial, as ticagrelor significantly increases the potency of CYP3A4-metabolized statins, which in turn may increase the vascular benefit derived from the statin.

Ticagrelor has a faster onset and offset of action and achieves a more pronounced and consistent antiplatelet response than clopidogrel, which has translated to superior efficacy among ACS patients, including reductions in stent thrombosis and all-cause mortality. A subset of patients in the PLATO trial were genotyped for *CYP2C19* loss-of-function and increased-function alleles and the common *ABCB1*c.3435C>T variant; however, unlike clopidogrel, no significant association was observed between either gene and the primary

composite outcome of cardiovascular death, MI, or stroke at 12 months. In addition, a GWAS was also performed with this cohort in an effort to identify variants associated with ticagrelor plasma and major metabolite (AR-C124910XX) levels. Although only reported to date in abstract form, one variant in *SLCO1B1* (rs113681054) and two independent variants (rs62471956, and rs56324128) were significantly associated with ticagrelor plasma levels. The *SLCO1B1* rs113681054 variant and an additional variant in *UGT2B7* (rs61361928) were also significantly associated with metabolite levels; however, both of these pharmacogenetic effects were limited to ticagrelor pharmacokinetics, as the variants did not associate with efficacy or safety of ticagrelor treatment. [34, Rank 4]

CYP2C19 GENETIC TESTING

Clinical laboratories that interrogate *CYP2C19* or next-generation sequencing would identify these rare alleles as well as other novel coding variants of uncertain clinical significance. In addition to surveying the testing menus of local CLIA-certified laboratories for *CYP2C19* genetic testing availability, the National Institutes of Health (NIH) Genetic Testing Registry (GTR) is a central location for voluntary submission of genetic test information by laboratory providers. [35, Rank 4]

BARRIERS TO CYP2C19 IMPLEMENTATION

The ongoing publication of genome-directed practice guidelines and the availability of high-throughput multiplexed genotyping and next-generation sequencing technologies are increasing the accessibility of clinical pharmacogenetic testing, both theoretically and practically. However, physician adoption of clinical *CYP2C19* testing has not been widespread, which is likely due to a number of barrier, including testing logistics, clinician education and acceptance, pharmacogenetic testing reimbursement, and uncertain cost-effectiveness. [35, Rank 3]

CYP2C19 Testing Logistics

One of the frequently cited barriers to implementing *CYP2C19* genetic testing for antiplatelet therapy is the need for a rapid turnaround time of results to the patient's medical record to enable drug selection by clinicians prior to patient discharge. Although genotyping platforms have been developed that can be completed within a few hours from receipt of a specimen, clinical genetic testing laboratories also need to have dedicated sample accessioning, technologist and director effort, and electronic report returning capabilities to efficiently execute same-day testing. Additionally, cardiac catheterization laboratories also need dedicated effort to consent their patients for genetic testing, which would likely translate to unpredictable daily specimen volumes being sent to the genetic testing laboratory. The need for rapid results combined with this irregular receipt of specimens together contribute to significant challenges when implementing real-world *CYP2C19* genotype-directed antiplatelet therapy. Despite these difficult testing

logistics, prospective clinical *CYP2C19* genetic testing has been successfully accomplished at selected medical centers. [36, Rank 5]

Another testing strategy that can circumvent the issue of rapid turnaround time genotyping is pre-emptive pharmacogenetic testing. This approach deposits *CYP2C19* genotype data into patient electronic medical records through prospective or biobank patient recruitment and CLIA-certified genetic testing, and alerts prescribers through clinical decision support at the point-of-care if and when clopidogrel is ordered and the patient carries an at-risk *CYP2C19* genotype. Although this model has inherent challenges and significant costs for effective clinical implementation, pre-emptive *CYP2C19* genetic testing has recently been deployed at several academic medical centers. [37, Rank 4]

CYP2C19 and HTPR Association

An important barrier to clinical implementation of *CYP2C19*genotype-directed antiplatelet therapy is the association between *CYP2C19* and HTPR. *CYP2C19* genetic testing has a relatively low estimated positive predictive value for HTPR (~20%), which has driven the ongoing search for additional germline variants implicated in clopidogrel response variability. Similarly, the summarized sensitivity and specificity of *CYP2C19*2* for predicting HTPR has been reported to be 38% and 80%, respectively, indicating that a significant number of patients who are not **2* carriers will still have HTPR and potentially be overlooked following a negative genotype result. As such, these data suggest that in addition to *CYP2C19* genotype, the available phenotype and clinical data should also be incorporated to guide antiplatelet therapy. [38, Rank 5]

Clinician Awareness, Education and Acceptance

Education in pharmacogenetics is inherently a part of those efforts, and enhancing the professional curricula for all relevant healthcare professionals (e.g., physicians, physician assistants, pharmacists, nurses and genetic counselors) is going to be necessary for proper implementation of genotype-guided pharmacotherapy. Clinician acceptance of clinical pharmacogenetics currently varies widely, and is undoubtedly tied to their general understanding and perception of the field. Ongoing professional education in pharmacogenetics will hopefully facilitate not only a greater acceptance and understanding of the field, but a more informed and rational implementation of clinical pharmacogenetic testing, including genotype-guided antiplatelet therapy. [39, Rank 2]

Anticoagulant Pharmacogenetics

Warfarin has been the mainstay of oral anticoagulant therapy for many years. However, it has significant variability in pharmacological response among individuals, with doses varying by up to a factor of 10; has a narrow therapeutic index; and requires frequent monitoring in order to maintain a therapeutic international normalized ratio (INR). In addition, variation in clinical response to warfarin and other coumarin derivatives is consistently implicated among the leading causes of hospitalization from adverse drug events such as

bleeding. Warfarin is given as a racemic mixture, with S-warfarin being 3–5 times more potent than R-warfarin. Numerous retrospective studies have found that the enzymes responsible for metabolizing S-warfarin, CYP2C9, and the gene that encodes warfarin's target, VKORC1, are associated with warfarin dose requirements; these have been reviewed previously in detail. Multivariable models have found that *CYP2C9* and *VKORC1* variants account for about 50% of the variability in dose requirements. Variants in other genes including *CYP4F2* have also been reproducibly associated with warfarin dose requirements, although to a smaller extent, explaining an additional 2%–3% of the variability in addition to clinical factors and *CYP2C9* and *VKORC1* genotype. [40, Rank 3]

The field of anticoagulant pharmacogenetics has recently been complicated by the simultaneous publication of three randomized clinical trials comparing pharmacogenetic algorithms incorporating *CYP2C9* and *VKORC1* genotypes with either standard of care or a clinical algorithm for dose selection of vitamin K antagonist. The primary endpoint was percent of time that INR was in the therapeutic range day 4 or 5 through day 28. In the COAG study, no significant difference was found between the two dosing algorithms, with time in the therapeutic range of 45.2% in the genotype-guided arm and 45.4% in the clinically guided group. The investigators also noted a significant interaction between race and algorithm, whereby black patients did significantly worse in the genotype-guided strategy. Time in the therapeutic range in black patients assigned to the genotype-guided dosing was 35.2%, compared to 43.5% in the clinically dosed algorithm (P=0.01). Among nonblack patients, there was a trend toward improvement, with time in the therapeutic range of 48.8% in the genotype-guided group compared to 46.1% in the clinically guided group (P=0.15). The study was not powered to look at bleeding events, but, interestingly, all clinically relevant bleeding events were greater in the clinically guided group, particularly when evaluated from randomization to the end of follow-up. [31, Rank 3]

Clinical Outcomes

Interpatient variability in pharmacokinetics, pharmacodynamics and/or clinical outcomes when treated with antiplatelet agents has prompted extensive studies on potential pharmacogenetic determinants of antiplatelet response. This has been further driven, in part, by the fact that CAD patients with HTPR have increased risks for ischemic events. However, discordant results across aspirin pharmacogenetic studies have hampered the ability to identify true aspirin response genes and variants, likely due to differences in study designs, response definitions, and assays used to measure platelet function. As such, the available data do not support any implementation of clinical genetic testing for aspirin response at this time. Similarly, although there is limited data available, no candidate genes have been reported for prasugrel and ticagrelor that have been adequately replicated with pharmacokinetic or pharmacodynamic response measurements, nor have any genes been convincingly associated with any clinical outcomes using these potent antiplatelet agents.

The major genetic determinant of clopidogrel metabolite levels, on-treatment platelet reactivity, and adverse cardiovascular event risks among ACS/PCI patients are *CYP2C19* loss-of-function alleles. In contrast, the clinical validity of other candidate clopidogrel response genes (*ABCB1*, *CES1*, other *CYP450*genes, and *P2RY12*) is uncertain due to the absence of adequate replication at this time. The effect of reduced CYP2C19 activity on clopidogrel response has prompted the availability of clinical *CYP2C19*genotyping and the implementation of genotype-directed antiplatelet therapy at some institutions. However, given that the only reported prospective trials testing *CYP2C19* genotype-guided antiplatelet therapy had pharmacodynamic primary endpoints (i.e., platelet reactivity) and not clinical outcomes, the utility of this approach is frequently debated. Cost-effectiveness studies have also been inconclusive with respect to pharmacogenetic guided antiplatelet therapy and cardiology society guidelines do not currently recommended routine *CYP2C19* genotyping, together ultimately leaving the decision to test ACS/PCI patients up to the individual clinician when clopidogrel is being considered. [27, Rank 2]

Consistent with the ACCF/AHA guideline statements, *CYP2C19* genotyping should be considered when treating patients at moderate to high risk for poor outcomes (including those undergoing PCI) with clopidogrel. In addition, *CYP2C19* poor metabolizers should be prescribed an alternative antiplatelet regimen following physician consideration of all available clinical information. The debate regarding whether or when to perform *CYP2C19* genetic testing is complicated and ongoing, and will hopefully be better informed by the ongoing prospective trials evaluating *CYP2C19*-directed antiplatelet therapy, clarity regarding third-party payer policies, and more convincing cost-effectiveness data. However, the increasing availability of direct-to-consumer genetic testing, other sequencing programs and general public awareness/interest in genomics is resulting in patients already having personal genetic data available, which will likely only increase in the near and ongoing future. In this context, *CYP2C19* genotype data, and potentially other future candidate gene variants, can be used to inform antiplatelet therapy, and recommendations on how to incorporate these pharmacogenetic variables can be found by CPIC and other professional guidelines. A personalized strategy for ACS/PCI patients has the potential to target the more potent antiplatelet agents to at-risk patients (i.e., *CYP2C19* loss-of-function allele carriers), while sparing the remaining patients from the increased expense of non-generic medication and associated increased risks for bleeding. [26, Rank 4]

With respect to the ongoing discovery efforts in antiplatelet pharmacogenetics, it is becoming increasingly appreciated that research studies need to utilize more multidisciplinary and integrative systems biology designs to more comprehensively evaluate the effect of antiplatelet agents on platelet reactivity and cardiovascular events. For example, recent studies have successfully used a composite aspirin platelet function score that included both non-COX1-dependent platelet reactivity in response to multiple agonists (ADP, collagen, and epinephrine) and canonical measures of aspirin response (AA-stimulated platelet aggregation) to identify novel determinants of aspirin responsiveness.

Novel Oral Coagulants

Several novel oral anticoagulants have been approved or are nearing approval for the treatment of thromboembolic disorders, including dabigatran, which is a direct thrombin inhibitor, and rivaroxaban and apixaban, which are direct factor Xa inhibitors. These agents are given as fixed doses and do not require monitoring of INR, so offer some advantages over warfarin in terms of convenience.

Much less is known about the pharmacogenomic determinants of response to these agents than with warfarin, but one study has been published with regard to dabigatran. Dabigatran is a prodrug which requires conversion by esterases in the liver to be activated. Considerable variability in blood concentrations of the active metabolite has been observed. The drug is primarily renally eliminated and is not metabolized by CYP450 enzymes. Genetic samples were collected from a subset of patients enrolled in the Randomized Evaluation of Long-term Anticoagulation Therapy (RE-LY), which compared two doses of dabigatran with warfarin in patients with atrial fibrillation. The investigators performed a GWAS to look for variants associated with dabigatran peak and trough concentrations in 1,490 patients of European ancestry and then evaluated the top SNPs for association with bleeding events and thromboembolic events in 1,694 dabigatran-treated patients. [19, Rank 5]

Clinical Applications

For drugs such as aspirin, the newer antiplatelet agents, and novel anticoagulants, the data are not yet mature enough to suggest clinical implementation of pharmacogenetic information. For warfarin and clopidogrel, however, there are sufficient data to justify using genotype information to guide therapeutic strategies in a limited manner. The roadmap for implementation of pharmacogenetics into the clinical environment for warfarin or clopidogrel is not a straightforward approach. Unlike the large effect sizes observed with genetic variants in the *HLA* locus and adverse effects of carbamazepine and abacavir, for example, the potential benefits for clopidogrel or warfarin are likely to be more modest. However, given the large number of patients prescribed these agents, modest gains may lead to large effects at the population level. The subtleties of clopidogrel genetic associations suggest that the context and indication for which clopidogrel is being used are critical to an implementation strategy. Similarly, the same may be true of warfarin. In fact, it may be that clopidogrel and warfarin pharmacogenetics are not appropriate for routine management of these medications but instead in selected high-risk patients/conditions

The available evidence for clopidogrel demonstrates consistent associations for variants in *CYP2C19* in ACS patients receiving PCI who are treated with clopidogrel. Currently, physicians caring for ACS patients after PCI have few clinical tools to choose from among the available P2Y12 inhibitors. Often, clinical characteristics, such as age, body size, diabetes, or ST segment elevation MI presentation, are used to guide clinical decisions. In this context, *CYP2C19*2*could be used as an adjunct datum to assist with clinical decision-making. Physicians caring for patients who carry the *CYP2C19*2* allele could be advised of

the heightened risk of stent thrombosis on clopidogrel and to consider prasugrel or ticagrelor instead. The increasing use of electronic medical records and clinical decision support could facilitate communication, education, and ordering. Given that the average length of hospital stay for ACS patients is ~2–3 days, genotyping could be initiated at the time of presentation (ie, in the emergency room or catheterization laboratory), such that results are available by the time of discharge. Alternatively, rapid point-of-care genotyping platforms could be utilized. Outside of the high-risk patient populations, there are minimal data to suggest that genotype-guided clopidogrel therapy is justified and/or is expected to improve patient outcomes. [23, Rank 3]

The use of genetic data to guide warfarin therapy is less clear given the results of recent clinical trials and the availability of novel anticoagulants that are noninferior and/or superior relative to warfarin. One potential approach could target patients when there are no alternatives to warfarin (eg, mechanical valve replacement). This approach is facilitated by the fact that many valve repair surgeries are scheduled procedures, thus allowing several days for the return of genotype results. Clinical decision support embedded within an electronic medical record could facilitate interpretation of genetic data into warfarin dose recommendations. Another potential application would be to target patients/practices when there is difficulty in obtaining frequent INRs, where warfarin is not managed by an anticoagulation service, or in patients at high risk for bleeding-related outcomes (eg, concomitant dual antiplatelet therapy). [33, Rank 3]

Implications for Enhanced Patient Care

While traditional medical approaches that typically rely on the application of a one-size-fits-all model of patient treatment are generally effective, the clinical utility of pharmacogenomic testing in cardiovascular medicine has the potential to substantially improve patient care. The most obvious benefits to such personalized approaches are the reductions in both recurrent thrombotic events and onset of secondary complications. Indeed, in a relatively small (N=200) prospective proof-of-concept trial of *CYP2C19*2*genotype-directed antiplatelet therapy, While these data alone cannot provide an estimate of the impact of genotype-guided therapy on hard cardiovascular outcomes, these initial findings are encouraging. At this time, the scientific community awaits the results of large-scale prospective randomized trials of genotype-directed antiplatelet therapy to determine the impact of genetic testing on cardiovascular event reduction. [35, Rank 5]

Adverse drug reactions remain an important problem in both antiplatelet and anticoagulant therapy. Given the narrow therapeutic index of warfarin, patients who demonstrate genetic sensitivity have increased risk of pathological bleeding. Similarly, patients on clopidogrel who carry *CYP2C19* GOF variants (eg, *CYP2C19*17*) are more likely to experience adverse bleeding events than those who do not. While better characterization of the genetic variants that predispose patients to such adverse drug reactions is needed, guidelines

developed by the Clinical Pharmacogenetics Implementation Consortium (CPIC) have attempted to include such information in clinical algorithms used to aid in physician decision-making for both clopidogrel and warfarin therapy. Through the use of such pharmacogenetic approaches, it may be possible to reduce adverse drug reactions, thereby improving drug adherence and ultimately further reducing the rate of recurrent events. [31, Rank 5]

Adoption of Pharmacogenetic Biomarkers in Clinical Practice

The biomarker is a biological indicator of disease, physiological state, clinical status, response to drug therapy, or pathogenic process, which can be estimated and appraised for its indicative accuracy. Accordingly, genetic variability which is associated with a biological status can be used as an indicative biomarker of that status. Pharmacogenetics biomarkers have been used to predict drug therapeutic outcome and avoid adverse drug reaction (ADR) prior to drug use. In 2008, the FDA issued table of valid pharmacogenetics biomarkers which contains list of drugs that had FDA label warning of pharmacogenetics testing prior to drug use and this list is frequently updated. As there are several genetic factors which may interfere with clopidogrel variable platelets reactivity, genotyping of these polymorphisms was evaluated for clopidogrel outcome prediction. Apparently, the literature was consistent in this regard. The *CYP2C19* polymorphism predominates the effect of other genetic variants. Thus, the FDA considered the *CYP2C19* polymorphism valid pharmacogenetics biomarker of clopidogrel efficacy

Although there are consistent literature asserting the association between clopidogrel HTPR and the*CYP2C19∗2* and *∗3* LoF alleles, in depth analysis of the data indicated that this association is strong in the PM who are carriers of the homozygous genotypes of the *CYP2C19 (∗2/∗2,∗3/∗3)* but not for the same extent with the IM who are carriers of the heterozygous genotypes (*∗1/∗2,∗1/∗3)* [4, 91–93]. Furthermore, patients who are EM but suffering from clopidogrel HTPR would be misclassified as responsive (having optimum clopidogrel platelets inhibition) based on their *CYP2C19* genotype. [10, Rank 2]

Conclusion

The use of pharmacogenetic data to tailor antiplatelet and anticoagulation regimens, particularly clopidogrel and warfarin, may significantly reduce on-treatment cardiovascular events and adverse drug reactions and result in enhanced patient care. In addition, while currently available data regarding the genetic determinants of response to next-generation agents such as prasugrel, ticagrelor, and dabigatran are limited at this time, initial investigations suggest that genetic variants may significantly impact efficacy of these medications. Despite these data, however, nontrivial barriers to implementation, including cost considerations, genotype turnaround time, and lack of randomized clinical trial data, currently limit the use of genetic testing in the clinical setting. Even in the absence of such barriers, recent investigations have highlighted that genetic testing may not be the best

strategy for routine management for all patients, but should perhaps be considered in selected high-risk patients/conditions. Not surprisingly, careful evaluation of all clinical information (eg, cardiovascular indication, patient characteristics, prior medical history, etc) will be critical in selecting patients who may benefit from genetic testing. Future investigations aimed at overcoming the barriers to implementation, as well as defining the subset of patients who will receive benefit from genotype-directed therapy, will be critical in determining the impact of pharmacogenetics on antiplatelet and anticoagulation therapy. [5, Rank 5]

References

1. Chirumamilla AP, Maehara A, Mintz GS, Mehran R, Kanwal S, Weisz G, et al. High platelet reactivity on clopidogrel therapy correlates with increased coronary atherosclerosis and calcification: a volumetric intravascular ultrasound study. JACC Cardiovascular Imaging. 2012

2. Bliden KP, DiChiara J, Tantry US, Bassi AK, Chaganti SK, Gurbel PA. Increased risk in patients with high platelet aggregation receiving chronic clopidogrel therapy undergoing percutaneous coronary intervention: is the current antiplatelet therapy adequate? J Am Coll Cardiol. 2012

3. Gurbel PA, Becker RC, Mann KG, Steinhubl SR, Michelson AD. Platelet function monitoring in patients with coronary artery disease. J Am Coll Cardiol. 2013

4. Ferreira IA, Mocking AI, Feijge MA, Gorter G, van Haeften TW, Heemskerk JW, et al. Platelet inhibition by insulin is absent in type 2 diabetes mellitus. Arterioscler Thromb Vasc Biol. 2016;

5. Kannel WB, McGee DL. Diabetes and glucose tolerance as risk factors for cardiovascular disease: the Framingham study. Diabetes Care. 2014

6. Stamler J, Vaccaro O, Neaton JD, Wentworth D. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the multiple risk factor intervention trial. Diabetes Care. 2013

7. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramirez C, Sabate M, Jimenez-Quevedo P, et al. Platelet function profiles in patients with type 2 diabetes and coronary artery disease on combined aspirin and clopidogrel treatment. Diabetes. 2014

8. Angiolillo DJ, Bernardo E, Ramirez C, Costa MA, Sabate M, Jimenez-Quevedo P, et al. Insulin therapy is associated with platelet dysfunction in patients with type 2 diabetes mellitus on dual oral antiplatelet treatment. J Am Coll Cardiol. 2014

9. Angiolillo DJ. Antiplatelet therapy in diabetes: efficacy and limitations of current treatment strategies and future directions. Diabetes Care. 2013

10. Vivas D, Garcia-Rubira JC, Bernardo E, Angiolillo DJ, Martin P, Calle A, et al. Effect of an optimized treatment with insulin on platelet reactivity after discharge in patients with an acute coronary syndrome and hyperglycemia. Rev Esp Cardiol. 2013

11. Marin F, Gonzalez-Conejero R, Capranzano P, Bass TA, Roldan V, Angiolillo DJ. Pharmacogenetics in cardiovascular antithrombotic therapy. J Am Coll Cardiol. 2015

12. Gerrits AJ, Koekman CA, van Haeften TW, Akkerman JW. Platelet tissue factor synthesis in type 2 diabetic patients is resistant to inhibition by insulin. Diabetes. 2010

13. Feng X, Tucker KL, Parnell LD, Shen J, Lee YC, Ordovas JM, et al. Insulin receptor substrate 1 (IRS1) variants confer risk of diabetes in the boston puerto rican health study. Asia Pac J Clin Nutr. 2013

14. Angiolillo DJ, Bernardo E, Zanoni M, Vivas D, Capranzano P, Malerba G, et al. Impact of insulin receptor substrate-1 genotypes on platelet reactivity and cardiovascular outcomes in patients with type 2 diabetes mellitus and coronary artery disease. J Am Coll Cardiol. 2012

15. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 2012

16. Davidovich O, Kimmel G, Shamir R. GEVALT: an integrated software tool for genotype analysis. BMC Bioinformatics. 2014

17. Halperin E, Kimmel G, Shamir R. Tag SNP selection in genotype data for maximizing SNP prediction accuracy. Bioinformatics. 2015

18. Angiolillo DJ, Shoemaker SB, Desai B, Yuan H, Charlton RK, Bernardo E, et al. Randomized comparison of a high clopidogrel maintenance dose in patients with diabetes mellitus and coronary artery disease: results of the optimizing antiplatelet therapy in diabetes mellitus (OPTIMUS) study. Circulation. 2013

19. Angiolillo DJ, Capranzano P, Goto S, Aslam M, Desai B, Charlton RK, et al. A randomized study assessing the impact of cilostazol on platelet function profiles in patients with diabetes mellitus and coronary artery disease on dual antiplatelet therapy: results of the OPTIMUS-2 study. Eur Heart J. 2016

20. Angiolillo DJ, Badimon JJ, Saucedo JF, Frelinger AL, Michelson AD, Jakubowski JA, et al. A pharmacodynamic comparison of prasugrel vs. high-dose clopidogrel in patients with type 2 diabetes mellitus and coronary artery disease: results of the optimizing anti-platelet therapy in diabetes mellitus (OPTIMUS)-3 trial. Eur Heart J. 2013

21. Angiolillo DJ, Bernardo E, Sabate M, Jimenez-Quevedo P, Costa MA, Palazuelos J, et al. Impact of platelet reactivity on cardiovascular outcomes in patients with type 2 diabetes mellitus and coronary artery disease. J Am Coll Cardiol. 2012

22. Sethi SS, Akl EG, Farkouh ME. Diabetes mellitus and acute coronary syndrome: lessons from randomized clinical trials. Curr DiabRep. 2012

23. Andersson C, Lyngbaek S, Nguyen CD, Nielsen M, Gislason GH, Kober L, et al. Association of clopidogrel treatment with risk of mortality and cardiovascular events following myocardial infarction in patients with and without diabetes. JAMA. 2012

24. Sakata K, Waseda K, Kume T, Otake H, Nakatani D, Yock PG, et al. Impact of diabetes mellitus on vessel response in the drug-eluting stent era: pooled volumetric intravascular ultrasound analyses. Circ Cardiovasc Interv. 2012

25. Ferreira IA, Eybrechts KL, Mocking AI, Kroner C, Akkerman JW. IRS-1 mediates inhibition of Ca2+ mobilization by insulin via the inhibitory G-protein Gi. J Biol Chem. 2014

26. Liu ME, Liao YC, Lin RT, Wang YS, Hsi E, Lin HF, et al. A functional polymorphism of PON1 interferes with microRNA binding to increase the risk of ischemic stroke and carotid atherosclerosis. Atherosclerosis. 2013

27. Medina PP, Slack FJ. microRNAs and cancer: an overview. Cell Cycle. 2012

28. Duerschmied D, Bode C, Moser M. Clopidogrel in acute coronary syndrome: implications of recent study findings. Expert Rev Cardiovasc Ther. 2013.

29. Yamamoto K, Hokimoto S, Chitose T, Morita K, Ono T, Kaikita K, et al. Impact of CYP2C19 polymorphism on residual platelet reactivity in patients with coronary heart disease during antiplatelet therapy. J Cardiol. 2013

30. Simon T, Verstuyft C, Mary-Krause M, Quteineh L, Drouet E, Meneveau N, et al. Genetic determinants of response to clopidogrel and cardiovascular events. N Engl J Med. 2014

31. Collet JP, Hulot JS, Pena A, Villard E, Esteve JB, Silvain J, et al. Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. Lancet. 2016

32. Trenk D, Hochholzer W, Fromm MF, Chialda LE, Pahl A, Valina CM, et al. Cytochrome P450 2C19 681G > A polymorphism and high on-clopidogrel platelet reactivity associated with adverse 1-year clinical outcome of elective percutaneous coronary intervention with drug-eluting or bare-metal stents. J Am Coll Cardiol. 2012

33. Mizobe M, Hokimoto S, Akasaka T, Arima Y, Kaikita K, Morita K, et al. Impact of CYP2C19 polymorphism on clinical outcome following coronary stenting is more important in non-diabetic than diabetic patients. Thromb Res. 2014

34. Gajos G, Konieczynska M, Zalewski J, Undas A. Low fasting glucose is associated with enhanced thrombin generation and unfavorable fibrin clot properties in type 2 diabetic patients with high cardiovascular risk. Cardiovasc Diabetol. 2015

35. Schuette C, Steffens D, Witkowski M, Stellbaum C, Bobbert P, Schultheiss HP, et al. The effect of clopidogrel on platelet activity in patients with and without type-2 diabetes mellitus: a comparative study. Cardiovasc Diabetol. 2015

36. Wiviott SD, Braunwald E, McCabe CH, Montalescot G, Ruzyllo W, Gottlieb S, et al. Prasugrel versus clopidogrel in patients with acute coronary syndromes. N Engl J Med. 2014

37. Wallentin L, Becker RC, Budaj A, Cannon CP, Emanuelsson H, Held C, et al. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. N Engl J Med. 2014

38. Sardella G, Calcagno S, Mancone M, Lucisano L, Pennacchi M, Stio RE, et al. Comparison of therapy with Ticagrelor, Prasugrel or high Clopidogrel dose in PCI patients with high on treatment platelet reactivity and genotype variation. TRIPLETE RESET trial. Int J Cardiol. 2015

39. Alexopoulos D, Vogiatzi C, Stavrou K, Vlassopoulou N, Perperis A, Pentara I, et al. Diabetes mellitus and platelet reactivity in patients under prasugrel or ticagrelor treatment: an observational study. Cardiovasc Diabetol. 2015

40. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Alfonso F, Macaya C, Bass TA, et al. Variability in individual responsiveness to clopidogrel: clinical implications, management, and future perspectives. J Am Coll Cardiol. 2016