The Influence of *CYP2C19* Polymorphisms on the Pharmacokinetics, Pharmacodynamics, and Clinical Effectiveness of $P2Y_{12}$ Inhibitors

Matthew J. Price, MD,[1] Udaya S. Tantry, PhD,[2] Paul A. Gurbel, MD[2]

[1]Scripps Clinic and Scripps Translational Science Institute, La Jolla, CA; [2]Sinai Center for Thrombosis Research, Baltimore, MD

$P2Y_{12}$ antagonists, in combination with aspirin, significantly reduce thrombotic and ischemic events in patients presenting with an acute coronary syndrome and in patients undergoing percutaneous coronary intervention. The thienopyridine clopidogrel is a prodrug that requires bioactivation by the cytochrome P450 (CYP) system in order to exert its antiplatelet effect. Common genetic polymorphisms that reduce the catalytic activity of the CYP2C19 isoenzyme decrease circulating levels of active metabolite, reduce levels of platelet inhibition, and increase the risk of ischemic events in clopidogrel-treated patients. Herein, we review the impact of the CYP2C19 *genotype on the pharmacokinetics and pharmacodynamics of clopidogrel, the association between* CYP2C19 *genotype and clinical outcome, and present the rationale for the implementation of* CYP2C19 *genotyping to individualize antiplatelet therapy in clinical practice.*
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Dual antiplatelet therapy with a combination of aspirin and a $P2Y_{12}$ receptor antagonist is the cornerstone of therapy for patients presenting with acute coronary syndromes (ACS) and for those treated with percutaneous coronary intervention (PCI) and a coronary stent. In particular, clopidogrel (a thienopyridine) has been demonstrated in randomized, placebo-controlled clinical trials to provide significant ischemic benefit in the setting of ACS,[1] PCI for stable or unstable coronary artery disease (CAD),[2,3] ST-elevation myocardial infarction (MI),[4,5] and—according to a post hoc analysis—secondary

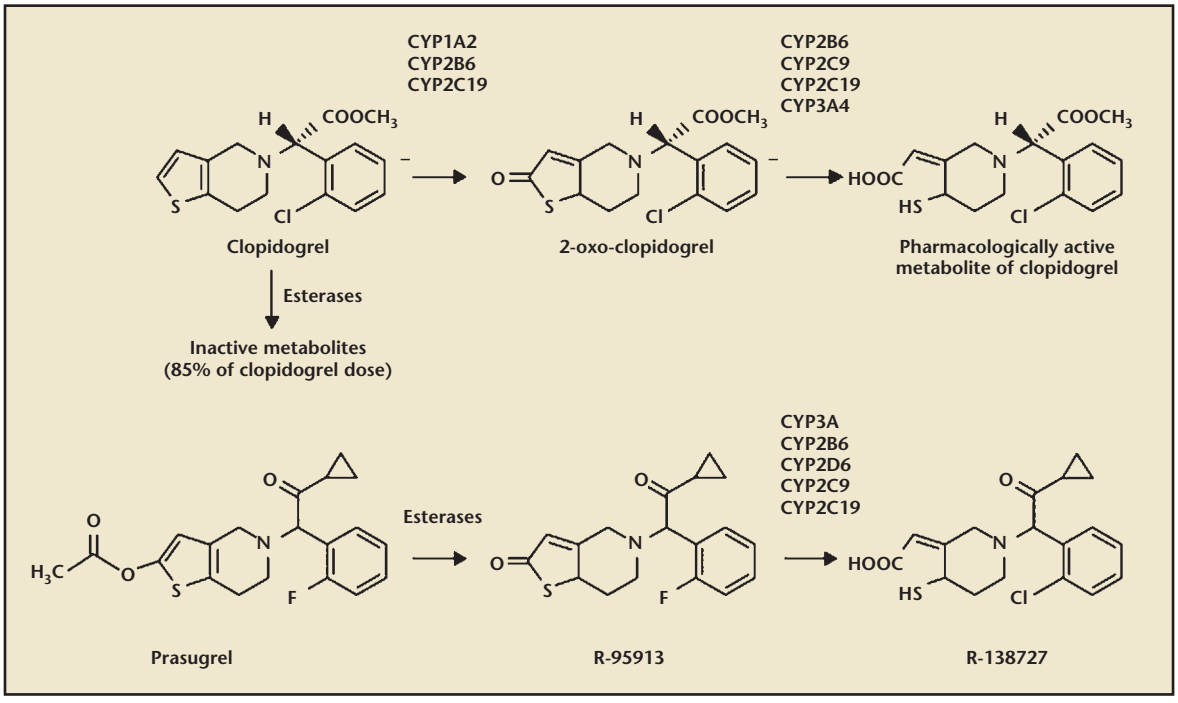
prevention in patients with prior MI, ischemic stroke, or symptomatic peripheral arterial disease.[6] However, it is well established that there is wide interindividual variability in the extent of platelet inhibition provided by clopidogrel.[7-9] Clopidogrel is a prodrug, and genetic variants that influence the catalytic activity of the cytochrome P450 (CYP) isoform CYP2C19 affect the efficiency of active metabolite generation and explain much of this variability. Moreover, these variants have been associated with poor clinical outcomes in ACS and PCI patients treated with clopidogrel. Herein, we review the impact of *CYP2C19* genotype on the pharmacokinetics and pharmacodynamics of clopidogrel, the association between *CYP2C19* genotype and clinical outcome, and the role of novel genotyping technologies in individualizing antiplatelet management in patients undergoing PCI.

Clopidogrel Metabolism

Clopidogrel is a prodrug that requires hepatic conversion into an active metabolite to exert its antiplatelet effect (Figure 1). Approximately 85% of absorbed clopidogrel is hydrolyzed by human carboxylesterase-1 into an inactive carboxylic acid metabolite, and therefore only a fraction of absorbed clopidogrel is available for conversion into the active metabolite by the CYP450 system. Hepatic biotransformation is thought to occur through a two-step oxidative process. In the first step, the thiophene ring of clopidogrel is oxidized to form 2-oxo-clopidogrel. In vitro experiments using enzyme kinetic analysis[10] have demonstrated that this reaction is catalyzed by CYP2C19, CYP1A2, and CYP2B6, with each enzyme contributing approximately 45%, 36%, and 19%, respectively. In the second step, CYP3A4, CYP2B6, CYP2C19, and CYP2C9 catalyze the formation of the active metabolite (*R*-130964), with a relative contribution of 40%, 33%, 21%, and 7%, respectively. The labile active metabolite forms a disulfide bond with the $P2Y_{12}$ receptor as platelets pass through the liver, irreversibly binding and antagonizing the receptor for the platelet's

Clopidogrel is a prodrug that requires hepatic conversion into an active metabolite to exert its antiplatelet effect.

Figure 1. *Comparative metabolism of clopidogrel and prasugrel. Both clopidogrel and prasugrel are prodrugs, requiring biotransformation into their respective active metabolites to exert an antiplatelet effect. Clopidogrel undergoes a two-step process mediated by CYP450 isoenzymes with involvement of CYP2C19 in both steps. A substantial portion of absorbed clopidogrel is shunted into a dead-end pathway by human carboxylesterase-1. Prasugrel undergoes a one-step oxidation after formation of a thiolactone intermediate in the intestine by human carboxylesterase-2. The greater inhibitory effect of prasugrel compared with clopidogrel is believed to be due to differences in the efficiency of the formation of their respective active metabolites. Adapted from The Lancet, Vol. 376, Giusti B, Abbate R, Response to antiplatelet treatment: from genes to outcome, 1278-1281, copyright 2010, with permission from Elsevier.*[48]



lifespan (7-10 days). The sensitivity of active metabolite generation to changes in the catalytic activity of CYP2C19 may be due to the important contribution of this enzyme to both steps of clopidogrel biotransformation. Decreased CYP2C19 function could lead to a bottleneck at the level of hepatic bioactivation, thereby shunting prodrug into the dead-end pathway that leads to the inactive carboxylic acid metabolite.

CYP2C19 Polymorphisms and Predicted Metabolic Phenotype

Several single nucleotide polymorphisms (SNPs) influence the catalytic activity of the CYP2C19 enzyme in a codominant (ie, dose-dependent) manner. These SNPs are described using established common-consensus "star allele" nomenclature. The *CYP2C19*1* allele denotes the lack of known polymorphisms, and therefore is considered to be wild-type (ie, normal function). The *CYP2C19*2* allele results in a splicing defect that produces an abnormal and nonfunctioning protein. It is the most common loss-of-function (LOF) allele, with an allelic frequency of approximately 0.13 in white subjects, 0.18 in black subjects, and 0.30 in Asians. *CYP2C19*3* is the second most common LOF allele, with an allelic frequency of approximately 0.10 in Asians but rarely found in other ethnicities. Less common LOF alleles include *4, *5, *6, *7, and *8, among others. The *17 variant is associated with increased gene transcription and increased catalytic activity of the enzyme. The combination of any two alleles (genotype) can be used to predict the metabolic phenotype of a particular individual: ultra rapid, extensive, intermediate, or poor (Table 1). Genotype frequencies in a population (and in turn, the frequencies of particular phenotypes) can be estimated from the allelic frequency using the principle of Hardy-Weinberg equilibrium: the frequency of homozygotes (2 copies of a particular allele) is the square of the allelic frequency, whereas the frequency of a heterozygote (combination of 2 different alleles) is two times the product of the frequency of the two different alleles. Therefore, approximately 2% of white, 4% of black, and 15% of Asian subjects are CYP2C19-poor metabolizers.

CYP2C19 Polymorphisms and Clopidogrel Pharmacokinetics and Pharmacodynamics

Healthy Subjects

The association between *CYP2C19* genetic variants and the antiplatelet effect of clopidogrel was first described by Hulot and colleagues,[11] who examined the effect of several CYP450 polymorphisms on adenosine diphosphate (ADP)-induced platelet aggregation and vasophosphoprotein (VASP) phosphorylation after a short course of clopidogrel, 75 mg/d, in 28 healthy men volunteers. Eight (28%) of the subjects were heterozygous for the *CYP2C19*2* allele (*1/*2); the mean on-treatment reactivity and the VASP platelet reactivity index were significantly higher in these patients compared with the 20 subjects who were homozygous for *CYP2C19*1* (*1/*1), consistent with a diminished clopidogrel effect in heterozygotes.[11] No subjects in this small cohort possessed 2 copies of the *2 allele. In a slightly larger study of 74 healthy subjects receiving a clopidogrel 300-mg loading dose, *CYP2C19*2* allele carriage (identified in approximately 35% of subjects) was associated with significantly lower exposure to clopidogrel active metabolite (area under the concentration curve [AUC] and maximal plasma concentration [C_{max}]) and less platelet inhibition according to ADP-induced platelet aggregation.[12] In addition, the rate of "poor responders," defined by the investigators as an inhibition of platelet aggregation $<$ 20%, was greater in *CYP2C19*2* carriers. According to a model-based analysis of 162 healthy clopidogrel-treated subjects, carriage of at least one *CYP2C19* LOF allele led to a 32% decrease in the active metabolite AUC and a 9% reduction in the change in maximal ADP-induced platelet aggregation.[13]

Poor metabolizers (*2/*2) are not well represented in these studies due to their low genotypic frequency. Kim and colleagues[14] identified 24 subjects—eight extensive metabolizers (homozygous for *CYP2C19*1*), eight intermediate metabolizers (heterozygous for a *CYP2C19* LOF allele), and eight poor metabolizers

Table 1
Classification of Predicted Metabolic Phenotype According to *CYP2C19* Genotype

CYP2C19 Genotype	Predicted Metabolic Phenotype
*17/*17	Ultrarapid metabolizer
*1/*17	Ultrarapid metabolizer
*1/*1	Extensive metabolizer
*1/*2 -*8	Intermediate metabolizer
*17/*2 -*8	Intermediate metabolizer
*2 -*8/*2 -*8	Poor metabolizer

(homozygous for a *CYP2C19* LOF allele). The authors found a codominant, or gene-dose, effect of *CYP2C19* genotype on clopidogrel pharmacokinetics and pharmacodynamics: the mean peak plasma concentration of clopidogrel prodrug in the poor metabolizers was 1.8- and 4.7-fold higher than that of intermediate and extensive metabolizers, respectively, and poor metabolizers exhibited a significantly lower antiplatelet effect than either the intermediate or extensive metabolizers.[14]

Patients With CAD

The early observations regarding the influence of *CYP2C19* genotype upon the antiplatelet effect of clopidogrel in healthy subjects have been confirmed in larger studies that examined clopidogrel pharmacokinetics and pharmacodynamics in patients with stable and unstable CAD. In 603 patients with non–ST-elevation ACS, *CYP2C19*2* was significantly associated with ADP-induced platelet aggregation, platelet reactivity index according to VASP phosphorylation analysis, and ADP-induced P-selectin expression using both recessive and codominant models.[15] In 707 consecutive patients undergoing elective PCI, patients who carried at least one *CYP2C19*2* allele (31% of the cohort studied) were significantly more likely than wild-types to have high on-treatment reactivity after a clopidogrel 600-mg loading dose.[16] The relative effects of CYP2C19 metabolizer status in clopidogrel- and prasugrel-treated patients were examined in a randomized, double-blind, double-dummy, two-arm parallel-group study in 98 patients with stable CAD.[17] In the clopidogrel-treated patients, the AUC of active metabolite was significantly lower in carriers of a *CYP2C19* LOF allele compared with noncarriers, and carriers exhibited a reduced antiplatelet effect measured by VASP phosphorylation analysis and the VerifyNow® P2Y12 assay (Accumetrics, San Diego, CA).

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CYP2C19*17 *Allele*

The *CYP2C19*17* polymorphism is a promoter variant, causing increased transcription of *CYP2C19* messenger RNA and in turn increased CYP2C19 activity. An allelic frequency of 0.18 in white subjects, 0.18 in black subjects, and 0.04 in Asian subjects has been reported.[18] Therefore, this variant is quite common, because approximately 26% of white subjects are carriers. In patients presenting with non–ST-elevation ACS, *CYP2C19*17* carriage is associated with an increased inhibitory response to clopidogrel.[19] In an observational study of 1524 patients undergoing planned PCI and pretreated with clopidogrel, 600 mg, 25% of patients were identified as *CYP2C19*17* carriers; *CYP2C19*17* genotype had a significant association with platelet aggregation as measured by multiple electrode aggregometry, with carriers of two *17 alleles having the lowest, carriers of one *17 allele having intermediate, and carriers of no *17 alleles displaying the highest levels of on-treatment reactivity.[20] The *2 and *17 alleles appear to have an additive effect on clopidogrel response.[21] However, in a smaller, multicenter, randomized pharmacodynamic study that used several different ex vivo platelet function tests, *CYP2C19*17* allele carriage was not associated with the level of on-clopidogrel platelet reactivity.[22]

In patients presenting with non–ST-elevation ACS, CYP2C19*17 *carriage is associated with an increased inhibitory response to clopidogrel.*

Genome-Wide Association Studies

A basic approach to determine the influence of a genetic variant is to test the association of one or more candidate SNPs with a particular trait or outcome of interest (eg, blood pressure, diabetes mellitus, or platelet reactivity). With this approach, candidate SNPs are selected based upon mechanistic hypotheses. For example, the studies noted above tested the association between SNPs of the CYP450 system and clopidogrel pharmacokinetics and pharmacodynamics. A genome-wide association study is a much more powerful way to identify the genetic determinants of traits, diseases, or outcomes. Genome-wide association studies examine the genetic variation across the entire human genome to identify associations among 500,000 or more SNPs and a particular trait (such as blood pressure, diabetes, or platelet reactivity) in a non–hypothesis-driven fashion, providing substantially more granularity and the ability to identify novel genetic variants that contribute to the particular outcome of interest. The central role of *CYP2C19*2* in the variation in clopidogrel response was confirmed by a genome-wide association study in 429 healthy Amish individuals. In

this homogenous population, a single site in linkage disequilibrium with (ie, corresponding to) *CYP2C19*2* was found to be significantly associated with diminished platelet response ($P = 1.5 \times 10^{-13}$), and accounted for 12% of the variation in platelet aggregation to ADP after clopidogrel treatment. However, clopidogrel response was highly heritable, and therefore it is possible that non-SNPs (eg, copy number variants, insertions/deletions), rare variants, or SNPs not included in the analyses may also contribute to platelet response variability.[23]

CYP2C19 Polymorphisms and Clinical Outcomes

Among the clopidogrel-treated patients with ACS undergoing PCI in the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel–Thrombolysis In Myocardial Infarction (TRITON-TIMI) 38 study, *CYP2C19* LOF allele carriers had a higher rate of recurrent ischemic events compared with noncarriers (hazard ratio [HR] 1.53, 95% confidence interval [CI], 1.07-2.19; $P = .01$), including stent thrombosis (HR, 3.09, 95% CI, 1.19-8.00; $P = .02$).[13] Similarly, Shuldiner and associates[23] demonstrated that carriers of the *CYP2C19*2* allele undergoing PCI had higher cardiovascular (CV) event rates compared with noncarriers (HR 2.42, 95% CI 1.18-4.99; $P = .02$). In a nationwide French registry of 2208 patients with acute MI treated with clopidogrel, patients carrying two LOF alleles had a significantly greater rate of CV events than noncarriers (21.5% vs 13.3%), and among those who underwent PCI, the CV event rate was 3.58 times that of noncarriers.[24] However, in this study, the influence of *CYP2C19* appeared to be restricted to patients with two copies of an LOF allele: carriers of one LOF allele (heterozygotes) had a similar risk of a CV event as patients with no LOF alleles.

Following these seminal observations, numerous subsequent studies and two meta-analyses have demonstrated the significant association between *CYP2C19* polymorphisms and poor clinical outcomes in patients treated with dual antiplatelet therapy.[25] In a meta-analysis involving 11,959 patients enrolled in 10 studies (4 of which reported stent thrombosis),[26] *CYP2C19*2* carriers displayed a 30% increase in the risk of major CV adverse clinical events compared with noncarriers (odds ratio [OR] 1.29, 95% CI, 1.12-1.49; $P < .001$). In addition, carriage of at least one *CYP2C19*2* allele was associated with increased mortality (OR 1.79, 95% CI, 1.10-2.91; $P = .019$) and increased stent thrombosis (OR 3.45, 95% CI, 2.14-5.57; $P < .001$). A gene-dose effect was observed, with carriers of two LOF alleles having increased ischemic risk compared with carriers of one LOF allele; this effect was more pronounced with regard to stent thrombosis. However, this observation is limited by the low frequency of LOF homozygotes (< 3% of all patients studied). A collaborative meta-analysis of nine clinical studies involving 9684 patients (91% of whom underwent PCI and 55% of whom had ACS)[27] demonstrated a significantly increased risk of the composite endpoint of CV death, MI, or stroke among carriers of one *CYP2C19* LOF allele (HR 1.55, 95% CI, 1.11-2.17; $P = .01$) and 2 *CYP2C19* LOF alleles (HR, 1.76; 95% CI, 1.24-2.50; $P = .002$) as compared with noncarriers. A significantly increased risk of stent thrombosis in carriers of one and two *CYP2C19* LOF alleles was also observed (HR 2.67, 95% CI, 1.69-4.22, $P = .0001$; and HR 3.97, 95% CI, 1.75-9.02; $P = .001$, respectively). Thus, the results of these meta-analyses indicate an apparent gene-dose effect of *CYP2C19* LOF allele carriage on CV events that is more pronounced among patients undergoing PCI and particularly evident with respect to the occurrence of stent thrombosis.

The influence of *CYP2C19* genotype on outcomes is less apparent in populations treated with clopidogrel for indications other than PCI. In the genetic substudy of the Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events (ACTIVE)-A, which was a randomized comparison of aspirin and clopidogrel compared with aspirin alone for the prevention of thromboembolic events in atrial fibrillation, the primary outcome was similar in carriers and noncarriers of *CYP2C19*2*.[28] Similarly, in the Clopidogrel in Unstable Angina to Prevent Recurrent Ischemic Events (CURE) trial, in which only 14% of patients presenting with ACS underwent PCI, there was no difference in ischemic outcomes according to *CYP2C19* genotype.[28] A genetic analysis of Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance (CHARISMA) trial showed no apparent risk in clopidogrel-treated patients who were carriers of *CYP2C19* LOF alleles[29]; however, because clopidogrel had no effect on ischemic outcomes in the overall study population, no pharmacogenetic interaction would be expected.[27]

The influence of CYP2C19 *genotype on outcomes is less apparent in populations treated with clopidogrel for indications other than PCI.*

CYP2C19*17 *Allele*

Unlike the *CYP2C19* LOF alleles, the influence of the gain-of-function *CYP2C19*17* allele on clinical outcome in patients treated with clopidogrel is less studied. Sibbing and coworkers[20] studied the impact of *CYP2C19*17* on bleeding in 1524 patients undergoing PCI after pretreatment with clopidogrel, 600 mg. In a multiple logistic regression model, a gene-dose effect of *CYP2C19*17* on 30-day TIMI bleeding was observed (compared with no *17 carriage, OR for one *17 allele 1.85, 95% CI, 1.19-2.86; OR for 2 *17 alleles 3.41, 95% CI, 1.42-8.17; $P = .006$).[20] An effect of *17 carriage on ischemic events was not observed. In contradistinction, in the genetic substudy of CURE, clopidogrel had a significantly more pronounced effect on ischemic events compared with placebo in carriers of at least one *CYP2C19*17* allele compared with noncarriers (HR with clopidogrel among carriers 0.55, 95% CI, 0.42-0.73; HR among noncarriers 0.85, 95% CI, 0.68-1.05; $P = .02$ for the interaction). A potential effect of *CYP2C19*17* carriage on bleeding events was detected in the Platelet Inhibition and Patient Outcomes (PLATO) genetic substudy. The rate of major bleeding was significantly greater in clopidogrel-treated patients with a *17 allele compared with patients without any *17 or LOF alleles (11.9% vs 9.5%; $P = .02$). However, there were no significant interactions of bleeding rates with any *CYP2C19 *17* or LOF alleles within or between the treatment groups; that is, noncoronary artery bypass graft bleeding consistently increased with ticagrelor compared with clopidogrel, irrespective of *CYP2C19* polymorphisms.[30]

Other Genetic Variants

ABCB1

P-glycoprotein is an ATP-dependent efflux pump encoded by the *ABCB1* gene (Figure 2). It is expressed in intestinal epithelial cells and increased expression or function can influence the bioavailability of drugs that are its substrate. Three categories of gene expression can be predicted based upon allele carriage of the *ABCB1* 3435C→T SNP: high (C/C), intermediate (C/T), and low (T/T). Inhibition of *p*-glycoprotein reduces the bioavailability of clopidogrel, and healthy subjects with low expression (T/T) have decreased pharmacodynamic effect.[31] However, the influence of *ABCB1* polymorphisms on clopidogrel pharmacodynamics has been inconsistent across studies, with several studies finding no relationship between *ABCB1* variants and the antiplatelet effect of clopidogrel.[22,32,33]

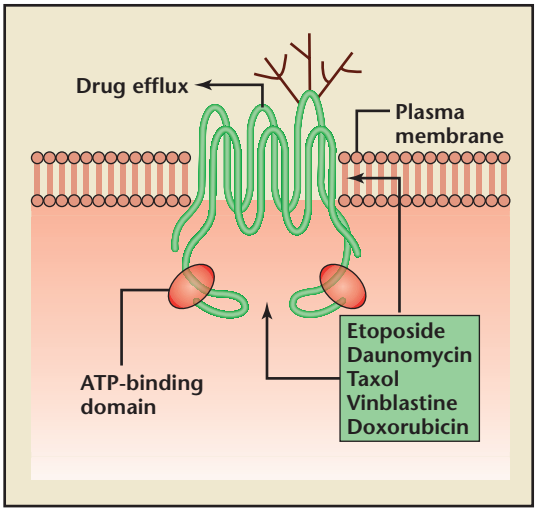

Figure 2. *P-glycoprotein as a transmembrane drug efflux pump. The multidrug resistance gene* MDR1*, which encodes the cell-surface molecule P-glycoprotein (PGP), can confer resistance to a wide variety of drugs. PGP transports drugs out of the cell, which is a process that requires the presence of two ATP-binding domains. These domains are a defining characteristic of this family of ATP-binding cassette (ABC) transporters. The exact mechanism of drug efflux is not well understood, but might involve either direct transport out of the cytoplasm or redistribution of the drug as it transverses the plasma membrane. Some cytotoxic drugs that are known substrates for PGP include etoposide, daunomycin, taxol, vinblastine, and doxorubicin. PGP is modified by sugar moieties (black) on the external surface of the protein. Reprinted by permission from Macmillan Publishers Ltd: Nat Rev Cancer,[48] copyright 2002.*

. . . the influence of ABCB1 *polymorphisms on clopidogrel pharmacodynamics has been inconsistent across studies, with several studies finding no relationship between* ABCB1 *variants and the antiplatelet effect of clopidogrel.*

ABCB1 *Genotype and Clinical Outcomes*

In the French Registry of Acute ST-Elevation and Non-ST-Elevation Myocardial Infarction (FAST-MI) study,[24] clopidogrel-treated patients with low *ABCB1* expression (T/T) had a significantly higher rate of death, MI, or stroke than wild-type patients (C/C) (15.5% vs 10.7%, HR 1.72, 95% CI, 1.2-2.47; $P = .007$). Patients with 2 *CYP2C19* LOF alleles and at least one *ABCB1* 3435C→T allele had the highest risk of CV events (HR 5.31, 95% CI, 2.13-13.20; $P = .009$). Similarly, in the TRITON-TIMI 38 genetic substudy, clopidogrel-treated patients with low *ABCB1* expression (T/T) had a 72% increased risk of CV death, MI, or stroke compared with patients with intermediate (C/T) and high (T/T) expression (12.9% vs 7.8%, HR 1.72, 95% CI, 1.22-2.44; $P = .002$). *ABCB1* genotype had no effect on CV events in prasugrel-treated patients.[31] Unlike that observed in the FAST-MI and TRITON-TIMI 38 studies, in the large genetic substudy of PLATO, CV event rates in clopidogrel-treated patients were highest in patients with the high *ABCB1* expression (C/C).[30] Given these conflicting findings, further investigation is required to better

understand the impact of *ABCB1* polymorphisms on clinical outcomes in clopidogrel-treated patients.

Effects of Alternative Antiplatelet Treatment Strategies in Carriers of *CYP2C19* LOF Alleles

A recent boxed warning for clopidogrel mandated by the US Food and Drug Administration (FDA) advises physicians to consider alternative treatment or treatment strategies in patients identified as CYP2C19 poor metabolizers (Table 2). Several studies have examined the pharmacokinetic and pharmacodynamic effects and associated outcomes of such alternative approaches.

Pharmacokinetic and Pharmacodynamic Effects

Prasugrel

Prasugrel is a thienopyridine like clopidogrel, but its biotransformation into the active metabolite differs substantially (Figure 3). Hydrolysis by intestinal human carboxylesterase-2 (and to a lesser extent, human carboxylesterase-1) rapidly forms a thiolactone precursor (*R*-95913) that is then oxidized in a single CYP-dependent step to the active metabolite (*R*-138727).[34] CYP3A4/5 and CYP2B6 are major contributors to this reaction, whereas CYP2C19 and CYP2C9 play a minor role; oxidation by intestinal CYP3A also occurs. The bioactivation of prasugrel is more efficient than that of clopidogrel because there is no competing metabolic pathway to an inactive metabolite. Moreover, the different CYP isoforms can compensate for one another.[17] The more rapid and intense inhibition provided by prasugrel is primarily the result of this more efficient process, as the inhibitory potency of the prasugrel and clopidogrel active metabolites are similar.[35]

Model-based analyses of the influence of CYP2C19 in 238 healthy subjects found no significant associations between the pharmacokinetic and pharmacodynamic responses to prasugrel and either carriage of a *CYP2C19* LOF allele or CYP2C19 metabolic phenotype.[36] In a randomized study of patients with stable CAD, *CYP2C19* genotype did not affect prasugrel active metabolite formation or the magnitude of platelet inhibition during either the loading or maintenance phase after prasugrel, 60 mg, followed by 10 mg daily. Moreover, the AUC of prasugrel active metabolite was greater than that of clopidogrel irrespective of *CYP2C19* genotype.[17] In addition, polymorphisms of the other isoforms of the CYP450 system appear to have no influence on prasugrel pharmacokinetics or pharmacodynamics.[12,17,36]

Ticagrelor

Ticagrelor, a cyclopentyltriazolopyrimidine, is a reversibly binding oral $P2Y_{12}$ receptor antagonist that interacts with the $P2Y_{12}$ receptor at a ligand-binding site separate from that for ADP or the thienopyridines, and therefore antagonizes ADP-mediated $P2Y_{12}$ receptor activation noncompetitively.[37] Unlike the thienopyridines, ticagrelor is not a prodrug and does not require bioactivation to exert its antiplatelet effect. The parent compound is metabolized primarily by CYP3A isoenzymes into a metabolite that has a similar potency in inhibiting the $P2Y_{12}$ receptor. A randomized study of 174 aspirin-treated patients with stable CAD compared the pharmacodynamic effect of clopidogrel (600-mg loading dose followed by 75 mg/d) with ticagrelor (180-mg loading dose followed by 90 mg/d). *CYP2C19* genotype significantly influenced the antiplatelet effect of clopidogrel, but not ticagrelor.[22] Similar to prasugrel, platelet reactivity in ticagrelor-treated patients was consistently lower than clopidogrel-treated patients irrespective of *CYP2C19* genotype.

Alternative Clopidogrel Dosing Regimens

Increasing the clopidogrel loading and maintenance dosages could potentially increase active metabolite generation in patients with decreased CYP2C19 catalytic activity by providing more substrate for clopidogrel bioactivation. Pharmacokinetic and pharmacodynamic studies that did not assess genotype have in general found that larger loading doses provide higher circulating

Table 2
Boxed Warning for Clopidogrel

1. The effectiveness of clopidogrel depends on activation to an active metabolite by the cytochrome P450 (CYP) system, principally CYP2C19.
2. Poor metabolizers treated with clopidogrel at recommended doses exhibit higher cardiovascular event rates following acute coronary syndrome or percutaneous coronary intervention than patients with normal CYP2C19 function.
3. Tests are available to identify a patient's *CYP2C19* genotype and can be used as an aid in determining therapeutic strategy.
4. Consider alternative treatment or treatment strategies in patients identified as CYP2C19 poor metabolizers.

Adapted from Plavix® [package insert].[42]
Plavix is manufactured by a Bristol-Myers Squibb/Sanofi Pharmaceuticals partnership.

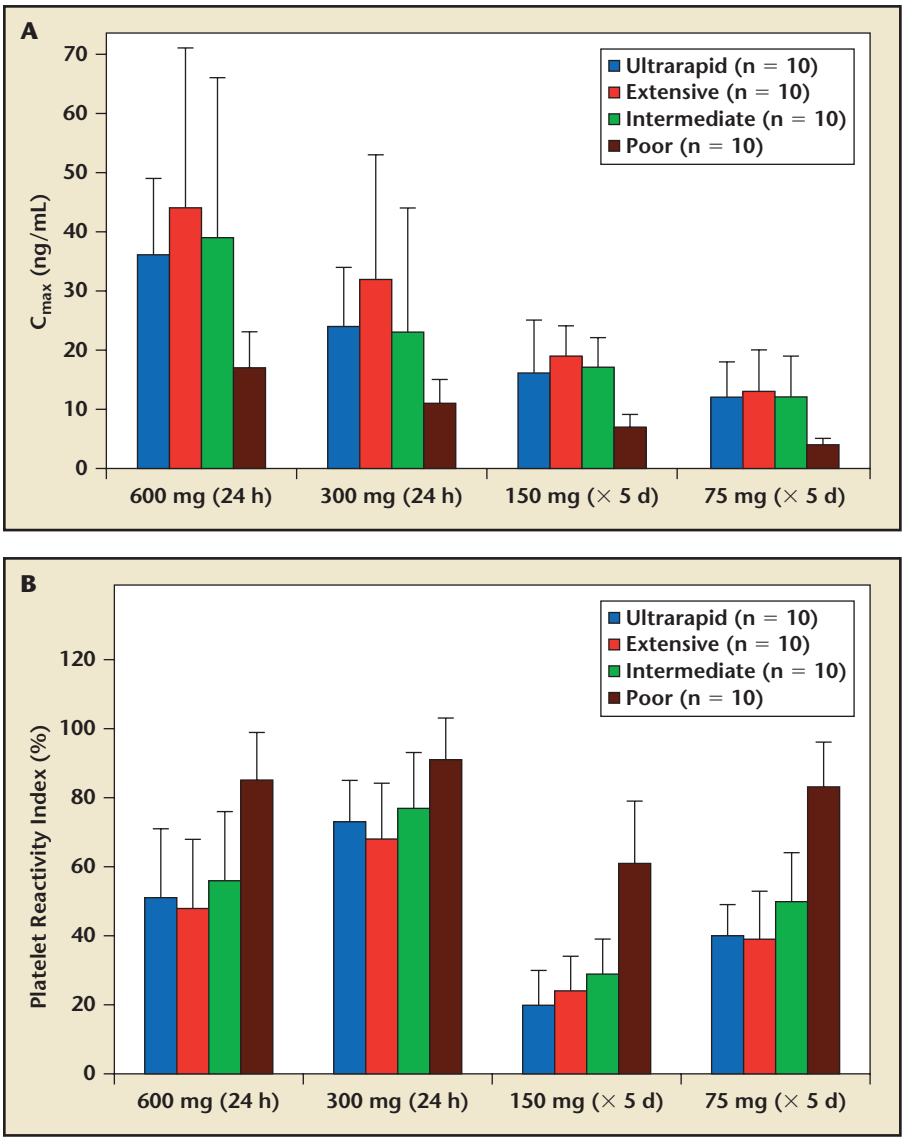

Figure 3. *Pharmacokinetics and pharmacodynamics of standard- and high-dose clopidogrel in healthy subjects according to CYP2C19 metabolic phenotype.* **(A)** *Maximal plasma concentration of clopidogrel active metabolite after a 600-mg or 300-mg loading dose and 75 mg/d or 150 mg/d for 5 days, stratified by CYP2C19 metabolic phenotype.* **(B)** *Antiplatelet effect of the different clopidogrel regimens according to vasophosphoprotein phosphorylation (VASP) analysis. VASP analysis indirectly measures the activity of the $P2Y_{12}$ receptor, the target of clopidogrel. The smaller the platelet reactivity index, the greater the $P2Y_{12}$ inhibition. Adapted from Plavix® [package insert].*[42]

levels of active metabolite and more rapid and intense platelet inhibition.[38,39] Furthermore, a maintenance dose regimen of 150 mg/d is associated with greater platelet inhibition than a dose of 75 mg/d.[40,41] The antiplatelet effect of a higher dose specifically in *CYP2C19* LOF allele carriers has been examined in a few small, observational studies. A genetic substudy of a single-center randomized trial of 60 patients undergoing PCI examined the association of *CYP2C19* genotype and platelet inhibition after 1) either a single clopidogrel 600-mg or a double 600-mg loading dose, and 2) maintenance clopidogrel, 75 mg/d or 150 mg/d. Double loading and clopidogrel, 150 mg/d, provided greater levels of platelet inhibition compared with 600 mg/d and 75 mg/d, respectively, among the 19 patients who were carriers of a *CYP2C19* LOF allele.[33] However, this increased dosing strategy may not "normalize" the antiplatelet effect of clopidogrel: carriers of *CYP2C19*2* or **3* treated with clopidogrel, 150 mg/d, were significantly more likely to have high on-treatment (platelet) reactivity at 30-days compared with noncarriers in an observational study of 126 patients undergoing PCI.[32]

The pharmacokinetic and pharmacodynamic effects of increased-dose clopidogrel were also examined in a crossover study of 40 healthy subjects, 10 from each of the different CYP2C19 metabolizer groups (ultrarapid, extensive, intermediate, and poor metabolizers).[42] Subjects were administered a clopidogrel 300-mg load followed by 75 mg/d for 5 days and a 600-mg load followed by 150 mg/d for 5 days. The antiplatelet effect of clopidogrel was measured with light transmittance aggregometry and VASP phosphorylation analysis. Compared with the other groups, poor metabolizers had the lowest peak plasma concentrations of clopidogrel active metabolite and the least platelet inhibition irrespective of dose. Among the poor metabolizers, active metabolite concentration and platelet inhibition were greater with the higher- compared with the lower-dose regimen. According to VASP phosphorylation analysis, clopidogrel, 150 mg/d, in poor metabolizers did not appear to achieve the level of inhibition provided by clopidogrel, 75 mg/d, in intermediate or extensive metabolizers (61 ± 18%, 50 ± 16%, and 39 ± 14%, respectively; a smaller value indicates greater platelet inhibition).

Several observational studies have reported an association between high on-clopidogrel platelet reactivity and thrombotic events after PCI.[8,43,44] The influence of *CYP2C19* genotype upon the incremental antiplatelet effect of clopidogrel, 150 mg/d, in patients with high on-treatment reactivity to standard dosing was examined in a pilot observational study of 41 patients with CAD. The decrease in platelet reactivity was numerically smaller in carriers of a *CYP2C19* LOF allele compared with noncarriers, but this did not reach statistical significance; the antiplatelet effect of clopidogrel, 150 mg, was negligible in the small number of poor metabolizers. However, in an observational study of 73 patients with high on-clopidogrel reactivity, *CYP2C19*2* carriage did not influence the ability of repeated daily clopidogrel, 600 mg, loading to "normalize" platelet reactivity. The influence of *CYP2C19* genotype on the antiplatelet effect of clopidogrel, 150 mg/d, in patients with high on-treatment reactivity to standard dosing will be further explored in the Genotype Information and Functional Testing (GIFT) study (www.clinicaltrials.org identifier NCT00992420).

Clinical Outcomes

As noted above, *CYP2C19* polymorphisms do not affect the pharmacokinetics or pharmacodynamics of prasugrel or ticagrelor. The influence of *CYP2C19* genotype on the clinical effectiveness of prasugrel and ticagrelor were examined in genetic substudies of the TRITON-TIMI 38[36] and PLATO[30] trials, respectively. Among genotyped patients treated with prasugrel in the TRITON-TIMI 38 trial, carriers and noncarriers of *CYP2C19* LOF alleles had similar rates of CV death, MI, or stroke (HR 0.89, 95% CI, 0.60-1.13; $P = .27$) and definite/probable stent thrombosis (HR 0.58, 95% CI, 0.13-2.69; $P = .48$).[36] In the genetic substudy of the PLATO trial, the rate of CV death, MI, or stroke at 30 days among the patients treated with clopidogrel was 37% higher in carriers of at least one CYP2C19 LOF allele compared with noncarriers (5.7% vs 3.8%; $P = .028$). Ticagrelor was associated with a reduced occurrence of CV events compared with clopidogrel among *CYP2C19* LOF carriers (8.6% vs 11.2%, HR 0.77, 95% CI, 0.60-0.99; $P = .038$) and noncarriers (8.8% vs 10.0%, HR 0.86, 95% CI, 0.74-1.01; $P = .06$).[30]

Rationale for *CYP2C19* Genotyping and Individualized Antiplatelet Therapy

The goal of antiplatelet therapy after PCI is to maximize ischemic benefit while minimizing the risk of bleeding. Intensive antiplatelet therapy provides the greatest net clinical benefit in patients with the highest ischemic risk, although it provides little ischemic benefit and potential net clinical harm in patients at low ischemic risk. *CYP2C19* genotype has emerged as a strong risk factor for ischemic and thrombotic events after PCI, and may serve as the foundation of an individualized approach to antiplatelet therapy to maximize net clinical benefit. This type of strategy has been validated by the FDA. The FDA warning regarding clopidogrel summarizes the link between CYP2C19 poor metabolizer genotype, lower active metabolite levels, decreased antiplatelet effects, and worse clinical outcomes in patients with PCI and ACS treated with clopidogrel (Table 1). It further presents the option of alternative approaches using different dosing strategies or agents to provide the intended antiplatelet effect in these patients. Although poor metabolizers (carriers of 2 *CYP2C19* LOF alleles) are the focus of the FDA warning, intermediate metabolizers (carriers of 1 *CYP2C19* LOF allele), which represent approximately 25% of the white population, also are a high-risk group, displaying lower active metabolite levels, decreased antiplatelet effects, and > 2.7-fold increased risk of stent thrombosis after PCI.[27] An American College of Cardiology Foundation (ACCF)/American Heart Association (AHA) Clinical Alert[45] reinforces this concept, stating that the FDA warning serves "to emphasize that clinicians should use this knowledge to make decisions about how to treat individual patients." This expert consensus further states, "genetic testing . . . may be considered before starting clopidogrel therapy in patients believed to be at moderate or high risk for poor outcomes. This might include, among others, patients undergoing elective high-risk PCI procedures (eg, treatment of extensive and/or very complex disease)." Therefore, it appears reasonable to integrate *CYP2C19* genotype into clinical decision making, taking into account other ischemic risk factors as well as the risk of bleeding with more intensive antiplatelet therapy. The availability of rapid, point-of-care genotyping technology such as the Verigene® System (Nanosphere, Northbrook, IL) will markedly enhance the practicality

CYP2C19 genotype has emerged as a strong risk factor for ischemic and thrombotic events after PCI, and may serve as the foundation of an individualized approach to antiplatelet therapy to maximize net clinical benefit.

of adopting such a genotype-driven approach in the acute setting. As the genetic basis of on-clopidogrel platelet reactivity variability beyond *CYP2C19* becomes clearer, future testing strategies may entail the identification of multiple polymorphisms.

Laboratory Assays Available to Identify *CYP2C19* Variants in Clinical Practice

Currently available assays that are available to provide *CYP2C19* and *ABCB1* genotype include the TaqMan® assay (Applied Biosystems, Carlsbad, CA), the AmpliChip® CYP450 (Roche Diagnostics, Indianapolis, IN), and the INFINITI™ Analyzer assay (AutoGenomics, Carlsbad, CA) among others.[13,46] Limitations of many genotyping approaches include the need for expensive laboratory infrastructure, batch processing, and a separate DNA isolation step. This often results in the requirement for genotyping to be performed by a specialty outside laboratory as well as substantial turnaround times for results reporting. Because many events occur in the hours to days after presentation with ACS and PCI, antiplatelet therapy decisions are optimally made acutely at the time of hospitalization, and therefore many of these genotyping platforms are not practical for rigorous clinical use. More rapid and less infrastructure-intensive technology may be clinically advantageous. The Verigene System is a point-of-care assay that is based on gold nanoparticle probe technology. It is a cartridge-based assay that requires 1 mL of whole blood and allows for rapid random access testing in the individual patient without the need for a separate DNA isolation step. This enables rapid, multitarget detection of SNPs, including those of *CYP2C19*, within 2 to 3 hours. The feasibility of the Verigene System has been confirmed in a study of patients undergoing PCI that found that the results were concordant with those obtained from the traditional TaqMan assay (P.A. Gurbel, MD, unpublished data, 2011). The clinical utility of rapid genotyping using the Verigene System in thienopyridine-naïve patients undergoing PCI is being examined in the Thrombocyte Activity Reassessment and Genotyping for PCI (TARGET-PCI) trial (www.clinicaltrials.org identifier NCT01177592).

The Verigene System is a point-of-care assay that is based on gold nanoparticle probe technology. It is a cartridge-based assay that requires 1 mL of whole blood and allows for rapid random access testing in the individual patient without the need for a separate DNA isolation step.

Conclusions

Genetic variation in *CYP2C19* contributes substantially to the pharmacokinetic and pharmacodynamic variability of clopidogrel, most likely due to the critical role of CYP2C19 in the biotransformation of clopidogrel into its active metabolite. Several single nucleotide polymorphisms, the most common being *CYP2C19*2*, result in an LOF of this enzyme. There appears to be a gene-dose effect, in which carriers of one and two *CYP2C19* LOF alleles (intermediate and poor metabolizers, respectively)

Main Points

- Clopidogrel is a prodrug that requires biotransformation into an active metabolite to exert its antiplatelet effect; the cytochrome P450 (CYP) isoenzyme, CYP2C19, plays a central role in this process.
- Variants (polymorphisms) of the *CYP2C19* gene (loss-of-function alleles) result in reduced CYP2C19 activity, lower active metabolite levels, and decreased platelet inhibition in clopidogrel-treated patients.
- Clopidogrel-treated patients who carry one or two *CYP2C19* loss-of-function alleles are at substantially higher risk of cardiovascular events, including stent thrombosis, than patients who are not carriers.
- Alternative antiplatelet agents, such as prasugrel, cilostazol, and ticagrelor, and alternative clopidogrel dosing strategies can improve platelet inhibition in patients who are *CYP2C19* loss-of-function allele carriers.
- According to the American College of Cardiology Foundation/American Heart Association Clinical Expert Consensus, *CYP2C19* genotyping "may be considered before starting clopidogrel therapy in patients believed to be at moderate or high risk for poor outcomes. This might include, among others, patients undergoing elective high-risk PCI procedures."
- A genotype-guided approach to antiplatelet therapy in the acute setting will be facilitated by the upcoming introduction of point-of-care, single-sample rapid genotyping platforms that can be integrated into routine clinical practice.

form incrementally lower levels of active metabolite and in turn display higher levels of on-clopidogrel platelet reactivity. *CYP2C19* variants do not affect active metabolite levels or the antiplatelet effect of prasugrel or ticagrelor; small studies suggest that higher doses of clopidogrel may increase platelet inhibition in *CYP2C19* LOF allele carriers. The effect of *CYP2C19* LOF alleles on clinical outcomes in clopidogrel-treated patients is most apparent in the PCI population, and it is in this setting that genotyping provides the most useful risk stratification. Consistent with their influence on clopidogrel pharmacodynamics and pharmacokinetics, *CYP2C19* LOF alleles also have a gene-dose effect on cardiovascular events, where patients who have two copies of CYP2C19 LOF alleles (poor metabolizers) have the worst clinical outcomes. This risk is highlighted in the boxed warning included in the clopidogrel label. An ACCF/AHA Clinical Alert recommends that "genetic testing . . . may be considered before starting clopidogrel therapy in patients believed to be at moderate or high risk for poor outcomes. This might include, among others, patients undergoing elective high-risk PCI procedures."[45] Rapid genotyping technology—providing a result locally within hours rather than centrally within days—will make a genotyping more clinically practical in the acute setting. The TARGET-PCI trial will examine the clinical efficacy of an individualized antiplatelet strategy in elective PCI patients that incorporates routine rapid genotyping in thienopyridine-naïve patients. The advent of generic clopidogrel may make individualized antiplatelet strategies economically attractive in the setting of PCI for ACS given the cost of newer $P2Y_{12}$ antagonists. Therefore, the PCI population represents an ideal setting for the potential adoption of pharmacogenetics to guide clinical decisions in daily practice. ■

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