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Commentary

Genotyping

One Piece of the Puzzle to Personalize Antiplatelet Therapy

Paul A. Gurbel, MD,* Udaya S. Tantry, PhD,* Alan R. Shuldiner, MD,† Dean J. Kereiakes, MD‡

Baltimore, Maryland; and Cincinnati, Ohio

The loss-of-function hepatic cytochrome P450 (CYP) 2C19*2 allele has been associated with reduced clopidogrel active metabolite generation and higher ex vivo platelet reactivity to adenosine diphosphate. Independently, in post hoc analyses, CYP2C19*2 has been associated with worse clinical outcomes during clopidogrel therapy. The controversy surrounding the diminished effectiveness of clopidogrel in poor metabolizers, those having 2 loss-of-function alleles, has been recently highlighted in the "boxed warning" issued by the U.S. Food and Drug Administration. However, much of the variation in clopidogrel response is not explained by the CYP2C19*2 allele (the most frequent loss-of-function allele), and other factors, both genetic and nongenetic, are likely to be important contributors. High on-treatment platelet reactivity to adenosine diphosphate during clopidogrel therapy is a well-documented predictor of recurrent ischemic events in the percutaneous coronary intervention population. While platelet function is dynamic in individual patients because of the influence of variable external factors, the influence of the CYP2C19*2 allele is intrinsically constant. Thus, it may be reasonable to consider both genotyping and platelet function measurement to assess ischemic risk and to guide antiplatelet therapy. Prospective clinical trials to test new algorithms for optimal personalized antiplatelet therapy are needed to provide the evidence base required for the routine adoption of genotyping into clinical practice. (J Am Coll Cardiol 2010;56: 112–6) © 2010 by the American College of Cardiology Foundation

In this issue of the *Journal*, Damani and Topol (1) propose routine genotyping alone to personalize dual-antiplatelet therapy. Here we summarize what we know and what we should know before using routine genotyping alone for personalized antiplatelet therapy.

What We Know

The current "one size fits all" antiplatelet regimens recommended by the American Heart Association, American College of Cardiology, and European Society of Cardiology guidelines are associated with about 10% recurrent ischemic event rates (2). Multiple studies have clearly demonstrated that platelets play a major role in the genesis of both periprocedural and long-term atherothrombotic events, including myocardial infarction and stent thrombosis (2). Adenosine diphosphate (ADP) is an important secondary agonist released in response to other agonists (thromboxane A_2, collagen, thrombin, and shear) that amplifies platelet activation and aggregation. Persistent activation of the glycoprotein IIb/IIIa receptor and subsequent stable thrombus generation at the site of vessel wall injury is highly dependent on continuous ADP-mediated $P2Y_{12}$ receptor signaling. Therefore, the addition of the $P2Y_{12}$ receptor blocker clopidogrel to aspirin has been associated with a significant reduction in major cardiovascular events in high-risk patients. However, nonresponsiveness and high on-treatment platelet reactivity (HPR) measured by ex vivo assays of platelet function have been overwhelmingly associated with increased ischemic event occurrence in clopidogrel-treated patients (2).

Pharmacokinetic studies indicate that clopidogrel is converted into its active metabolite by hepatic cytochrome P450 (CYP) isoenzymes in a 2-step oxidation process involving primarily CYP2C19, CYP1A2, and CYP2B6 isoenzymes in the first step and CYP2C19, CYP2C9, CYP2B6, and CYP3A4 isoenzymes in the second step. The active metabolite (R130964) covalently binds to the platelet $P2Y_{12}$ receptors to irreversibly inhibit ADP-stimulated platelet aggregation. Both CYP2C19 and CYP3A4 have been suggested as major isoenzymes involved in the metabolic activation of clopidogrel (3).

From the *Sinai Center for Thrombosis Research, Sinai Hospital of Baltimore, Baltimore, Maryland; †Division of Endocrinology, Diabetes and Nutrition, Department of Medicine, University of Maryland School of Medicine, Baltimore, Maryland; and ‡The Christ Hospital Heart and Vascular Center/The Lindner Research Center at The Christ Hospital, Cincinnati, Ohio. Dr. Gurbel has received research grants from AstraZeneca, Portola Pharmaceuticals, Pozen Inc., and Sanofi-Aventis, and is a consultant for and has received honoraria from AstraZeneca, Portola Pharmaceuticals, Pozen Inc., Sanofi-Aventis, Bayer AG, Eli Lilly and Company, Daiichi-Sankyo, and Schering-Plough. Dr. Kereiakes has received research grants from Daiichi-Sankyo, Abbott Vascular, Amylin Pharmaceuticals, and Boston Scientific; has received consulting fees from Eli Lilly and Company, Devax, Boston Scientific, Abbott Vascular, Medpace, and REVA Medical Inc.; and is on the Speakers Bureau of Eli Lilly and Company.

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Multiple lines of evidence show that clopidogrel response variability is due largely to variability in active metabolite generation (4). There are at least 25 single-nucleotide polymorphisms (SNPs) of the gene encoding the CYP2C19 isoenzyme (5). The most widely analyzed and most frequent SNPs are CYP2C19*2, a G→A mutation in exon 5 producing an aberrant splice site leading to the complete absence of CYP2C19 activity, and *17 (-806C>T), a regulatory region variant that has been associated with increased expression and enzymatic activity. The *2 loss-of-function and *17 gain-of-function alleles are in linkage disequilibrium ($D' = 1$, $r^2 = 0.04$), resulting in 3 observed haplotypes and 6 possible diplotypes, which may be grouped into 3 enzymatic activity phenotypes (6,7) (Fig. 1).

Recently, variation in ADP-stimulated platelet aggregation in response to clopidogrel was evaluated in a genome-wide association study in healthy subjects (6). Remarkably, a cluster of 13 SNPs within and flanking the CYP2C18-2C19-2C9-2C8 cluster on chromosome 10q24 (out of about 400,000 SNPs analyzed genome-wide) was strongly associated with clopidogrel response ($p = 10^{-12}$ to 10^{-7}). Further mapping identified the CYP2C19*2 variant, which accounted for most or all of the 10q24 association signal. In a replication study involving patients undergoing percutaneous coronary intervention, carriers of the CYP2C19*2 allele had higher cardiovascular event rates compared with noncarriers (hazard ratio [HR]: 2.42; $p = 0.02$) (6). Candidate gene studies also support an important role of CYP2C19 reduced-function alleles in clopidogrel nonresponsiveness and adverse clinical outcomes. In healthy volunteers, a 32.4% relative reduction ($p < 0.001$) in plasma exposure to the active clopidogrel metabolite and a relative reduction of approximately 25% in mean platelet aggregation ($p < 0.001$) was observed in carriers of at least 1 CYP2C19 reduced-function allele compared with noncarriers (8). Among patients with acute coronary syndromes undergoing stenting and treated with clopidogrel in the TRITON–TIMI 38 (Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel–Thrombolysis In Myocardial Infarction 38) study, CYP2C19 reduced-function allele carriers had a higher rate of recurrent ischemic events (HR: 1.53; $p = 0.01$), including stent thrombosis (HR: 3.09; $p = 0.02$), compared with noncarriers (8). Similarly, Sibbing et al. (9) demonstrated that CYP2C19*2 carriers had a significantly higher cumulative 30-day incidence of stent thrombosis compared with CYP2C19 wild-type homozygotes (HR: 3.81; $p < 0.007$). In a collaborative meta-analysis of various clinical trials involving 9,684 patients, Mega et al. (10) recently demonstrated that CYP2C19*2 allele carriers had a higher risk of major adverse clinical event occurrence compared with noncarriers (HR: 1.61; $p < 0.001$). Similarly, risk was greater in heterozygotes compared with wild type (HR: 1.50; $p = 0.016$) and in homozygotes compared with wild type (HR: 1.81; $p = 0.004$) (10).

CYP2C19 genotyping is currently available through a number of commercial laboratories. However, the turnaround time is often on the order of several days. Because a large number of events happen within the first several hours after percutaneous coronary intervention, for personalized antiplatelet therapy to be optimally applied, rapid and accurate point-of-care CYP2C19 genotyping will be neces-

Abbreviations and Acronyms

ADP = adenosine diphosphate
CYP = hepatic cytochrome P450
HPR = high on-treatment platelet reactivity
HR = hazard ratio
SNP = single-nucleotide polymorphism

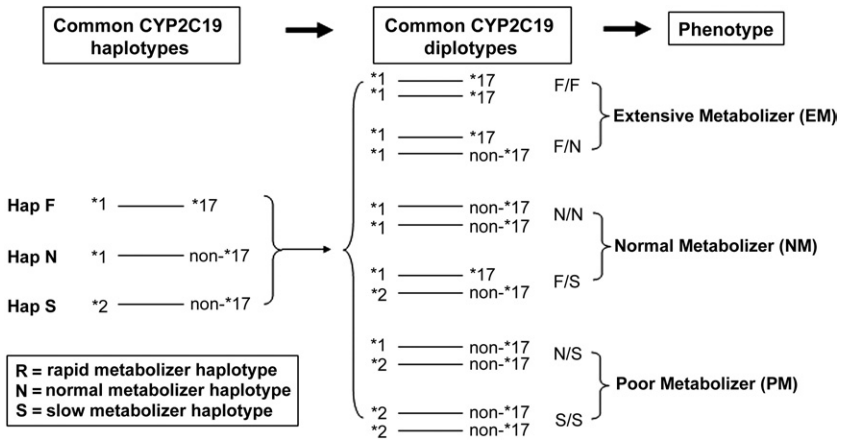

Figure 1 Linkage Disequilibrium and CYP2C19 Haplotypes

Because of linkage disequilibrium between the hepatic cytochrome P450 (CYP) 2C19*2 loss-of-function (slow metabolizer) and *17 gain-of-function (fast metabolizer) variants, only 3 (of 4 possible) haplotypes exist. These 3 haplotypes result in 6 diplotypes, which define 3 main phenotypes: extensive metabolizers, normal metabolizers, and poor metabolizers.