Clopidogrel Pharmacokinetics and Pharmacodynamics Vary Widely Despite Exclusion or Control of Polymorphisms (CYP2C19, ABCB1, PON1), Noncompliance, Diet, Smoking, Co-Medications (Including Proton Pump Inhibitors), and Pre-Existing Variability in Platelet Function

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Objectives
This study sought to determine whether known genetic, drug, dietary, compliance, and lifestyle factors affecting clopidogrel absorption and metabolism fully account for the variability in clopidogrel pharmacokinetics and pharmacodynamics.

Background
Platelet inhibition by clopidogrel is highly variable. Patients with reduced inhibition have increased risk for major adverse cardiovascular events. Identification of factors contributing to clopidogrel’s variable response is needed to improve platelet inhibition and reduce risk for cardiovascular events.

Methods
Healthy subjects (n = 160; ages 20 to 53 years; homozygous CYP2C19 extensive metabolizer genotype; no nicotine for 6 weeks, prescription drugs for 4 weeks, over-the-counter drugs for 2 weeks, and no caffeine or alcohol for 72 h; confined; restricted diet) received clopidogrel 75 mg/day for 9 days, at which time clopidogrel pharmacokinetic and pharmacodynamic endpoints were measured.

Results
At steady-state, clopidogrel active metabolite (clopidogrel-AM) pharmacokinetics varied widely between subjects (coefficients of variation [CVs] 33.8% and 40.2% for clopidogrel-AM area under the time-concentration curve and peak plasma concentration, respectively). On-treatment vasodilator stimulated phosphoprotein P2Y12 platelet reactivity index (PRI), maximal platelet aggregation (MPA) to adenosine phosphate, and VerifyNow P2Y12 platelet response units (PRU) also varied widely (CVs 32% to 53%). All identified factors together accounted for only 18% of intersubject variation in pharmacokinetic parameters and 32% to 64% of intersubject variation in PRI, MPA, and PRU. High on-treatment platelet reactivity was present in 45% of subjects.

Conclusions:
Clopidogrel pharmacokinetics and pharmacodynamics vary widely despite rigorous exclusion or control of known disease, polymorphisms (CYP2C19, CYP3A5, ABCB1, PON1), noncompliance, co-medications, diet, smoking, alcohol, demographics, and pre-treatment platelet hyperreactivity. Thus, as yet unidentified factors contribute to high on-treatment platelet reactivity with its known increased risk of major adverse cardiovascular events. (A Study of the Effects of Multiple Doses of Dexiansoprazole, Lansoprazole, Omeprazole or Esomeprazole on the Pharmacokinetics and Pharmacodynamics of Clopidogrel in Healthy Participants: NCT00942175) (J Am Coll Cardiol 2013;61:872–9) © 2013 by the American College of Cardiology Foundation

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Antiplatelet therapy with clopidogrel reduces coronary events in patients with acute coronary syndrome (1). However, platelet inhibition by clopidogrel is highly variable, and patients with reduced platelet inhibition have an increased risk for major adverse cardiovascular events (2). Esterases degrade ~85% of absorbed clopidogrel, leaving only 15% to be converted by the cytochrome P-450 family of enzymes (3,4) and possibly paraoxonase 1 (PON1) (5), to the active metabolite required for inhibition of the platelet adenosine diphosphate (ADP) receptor, P2Y12. Variability in clopidogrel pharmacokinetics and pharmacodynamics has been attributed to absorption (e.g., diet or polymorphisms in the transporter molecule ABCB1 [6–8]), smoking (which alters cytochrome P-450 levels) (9,10), polymorphisms in CYP2C19 (8) and/or PON1 (5), drug–drug interactions (e.g., proton pump inhibitors [PPIs] [11,12] statins), and intrinsic variation in platelet function before exposure to clopidogrel (13–15). However, it remains unclear whether these factors fully explain the variability observed in clopidogrel pharmacokinetics and pharmacodynamics, or if a significant portion of the variability is due to still unknown factors. Here, we report the variability in clopidogrel pharmacokinetics and pharmacodynamics when known factors are rigorously excluded or controlled.

Methods

Study design. We performed a randomized, 2-period, crossover design study to assess the effects of PPIs (dexlansoprazole, lansoprazole, esomeprazole, and omeprazole) on steady-state pharmacokinetics and pharmacodynamics of clopidogrel in healthy volunteers, as previously described in detail (12). In the present study, we analyzed steady-state clopidogrel pharmacokinetics and pharmacodynamics in healthy subjects before and after clopidogrel 75 mg/day for 9 days (because repeated daily doses of clopidogrel 75 mg achieve steady-state inhibition of ADP-induced platelet aggregation between days 3 and 7 [16,17]) in the absence of any PPIs. This study was conducted according to the World Medical Association Declaration of Helsinki, the International Conference on Harmonization Harmonized Tripartite Guideline for Good Clinical Practice, and local regulations.

Enrollment criteria. Key enrollment criteria are summarized in Online Table 1. In brief, healthy subjects, homozygous for CYP2C19 extensive metabolizer genotype (i.e., non-carriers of CYP2C19 *2, *3, *4, *5, *6, *7, *8, *9, *10, or *17), aged 18 to 55 years, with a body mass index (BMI) between 18 and 30 kg/m², and free of nicotine for 6 weeks, prescription drugs for 4 weeks, over-the-counter drugs (OTC) for 2 weeks, and caffeine and alcohol for 72 h were enrolled. All subjects found to be CYP2C19 poor, intermediate, or ultra-rapid metabolizer genotypes were deemed screen failures and were excluded.

Treatment period. Enrolled subjects (cohorts of 40 subjects at a time) were confined in a clinical research unit for 10 consecutive days and nights during periods 1 and 2. Subjects fasted ~8 h before clopidogrel dosing. Witnessed dosing of clopidogrel commenced at ~0800 h on days 1 through 9 of both periods.

Diet, fluid, and activity control. During the confinement period, subjects received standardized meals and snacks and refrained from strenuous exercise. All subjects found to be poor, intermediate, or ultra-rapid metabolizers of CYP2C19 were rigorously excluded or controlled.

Abbreviations and Acronyms

ADP = adenosine phosphate
AUCt = area under the time-concentration curve
BMI = body mass index
ClopidogrelAM = clopidogrel active metabolite
Cmax = peak plasma concentration
CVs = coefficients of variation
EM/EM = homozygous
CYP2C19 extensive metabolizer genotype
LTA = light transmission aggregation
MPA = maximal platelet aggregation
OTC drugs = over-the-counter drugs
PON1 = paraoxonase 1
PPI = proton pump inhibitor
Pre-Tx = pre-treatment
PRI = platelet reactivity index
PRP = platelet rich plasma
PRU = P2Y12 reaction units
Rx drugs = prescription drugs
VASP = vasodilator stimulated phosphoprotein

Trials, CardioSource, Duke Clinical Research Institute (clinical trial steering committees), Slack Publications (Chief Medical Editor, Cardiology Today Intervention), WebMD (CMF steering committees); and is Senior Associate Editor, Journal of Invasive Cardiology. Dr. Bhatt has also received research grants from Amarin, AstreaZeneca, Bristol-Myers Squibb, Eisai, Ethicon, Medtronic, Sanofi Aventis, and The Medicines Company; and he has performed unfunded research with FlowCo, Ptx Pharma, and Takeda. Drs. Lee, Mulford, Wu, and Nadarupati are employees of Takeda Global Research & Development Center, Inc. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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Statistics. Data analyses were performed by A.L. Frelinger III, PhD, Assistant Professor of Pediatrics, Harvard Medical School using GraphPad Prism 5.0 (GraphPad Software, San Diego, California) and SAS 9.2 (SAS Institute, Cary, North Carolina) and independently confirmed by J. Wu, PhD and S. Nudurupati, PhD, Takeda Global Research & Development Center, Inc. using SAS 9.1 (SAS Institute). Descriptive statistics were used to report demographics for all enrolled subjects. Pre- versus post-treatment pharmacodynamic endpoints were compared using paired Student t-test. Partial correlations, $\eta^2$, were calculated to estimate the contribution of demographic, hematologic, and genetic factors to clopidogrel pharmacokinetic and pharmacodynamic endpoints while controlling for study design variables (cohort, period, and treatment group). The effects of selected polymorphisms on clopidogrel pharmacokinetics and pharmacodynamics were evaluated within analysis of variance models with fixed factors for genotype, cohort, period, and treatment group. General linear models with stepwise selection for corrected Akaike’s information criterion and fixed factors for cohort, period, and treatment group were used to select demographic, hematologic, and genetic factors predictive of each of the pharmacokinetic and pharmacodynamic endpoints. Effect size of each factor was estimated using semipartial correlations, $\eta^2$. Log-transformed values of clopidogrel$_{AM}$ AUC$_{t}$, and C$_{max}$ were used for all models.

Results

Subject characteristics and disposition. Five hundred fifty-two subjects were screened and 160 subjects were enrolled, as previously reported (12). All enrolled subjects were genotyped as CYP2C19 *1/*1 extensive metabolizers and had normal hematology, clinical chemistry, and urinalysis results at enrollment. One hundred fifty-six of the 160 enrolled subjects completed the 9 days of treatment with clopidogrel 75 mg and the accompanying pharmacokinetic and pharmacodynamic measurements. Reasons for failure to complete the study were adverse event (n = 2), major protocol violation (n = 1), and pregnancy (n = 1). Subject demographics and baseline characteristics are shown in Table 1.

Variation in clopidogrel pharmacokinetics and pharmacodynamics. Following 9 days of treatment with clopidogrel, interindividual exposure to clopidogrel$_{AM}$ varied widely: AUC$_{t}$ and C$_{max}$ values varied more than threefold from the 10th to the 90th percentile and the coefficients of variation (CV) were large (34% and 40%, respectively) (mean $\pm$ SD: AUC$_{t}$ 41.3 $\pm$ 14.0 ng-hr/ml; C$_{max}$ 39.6 $\pm$ 15.9 ng/ml) (Figs 1A and 1B, Online Table 2). As expected, mean values for clopidogrel pharmacodynamic markers were reduced following 9 days of clopidogrel treatment (Figs. 1C to 1F, Online Table 2). However, as was observed with clopidogrel pharmacokinetics, clopidogrel pharmacodynamics after 9 days of treatment with clopidogrel varied widely (CVs 37%, 53%, 37%, and 32% for VASP platelet reactivity index [PRI], VerifyNow P2Y12 reaction units [PRU], maximal platelet aggregation [MPA] with 5-$\mu$M ADP, and MPA with 20-$\mu$M ADP, respectively (Figs. 1C to 1F, Online Table 2). This variation was not accounted for by day-to-day subject and/or assay variation, which was small as judged by intraclass correlation coefficients of >80% (Online Table 3, Online Fig. 1).

Using the cutpoints suggested by an international consensus group (2), high on-treatment platelet reactivity was present in 62 of 156 (40%) subjects by VASP PRI >50, 15 of 156 (10%) subjects by MPA with 5-$\mu$M ADP, and 8 of 156 (5%) subjects by VerifyNow P2Y12 PRU >235 (Fig. 2). A total of 45% of healthy subjects had high on-treatment platelet reactivity by at least 1 criterion.

Influence of demographic, hematologic, genetic, and study design factors on clopidogrel pharmacokinetics. Table 2 summarizes the influence of factors related to subject demographics (age, sex, weight), hematology (platelet count, hematocrit), and genetics (polymorphisms of CYP3A5, ABCB1, and PON1), on clopidogrel$_{AM}$ pharmacokinetics (C$_{max}$ and AUC$_{t}$). Only age and baseline weight were significantly associated with clopidogrel$_{AM}$ AUC$_{t}$ and C$_{max}$. However, correlations between clopidogrel$_{AM}$ AUC$_{t}$ and C$_{max}$ and subject weight and age indicated that only 5% to 6% of the variation in clopidogrel$_{AM}$ AUC$_{t}$ and C$_{max}$ was explained by differences in weight and age ($\eta^2$ range: 0.0525 to 0.0639) (Table 2). Neither clopidogrel$_{AM}$ AUC$_{t}$ nor
Cmax were significantly different in men versus women. Polymorphisms in enzymes reported to affect clopidogrel metabolism (5, 6, 8, 18, 19) (CYP3A5*3, ABCB1 C3435T, and PON1 rs662,) were relatively common (Table 1), but were not correlated with clopidogrel AM Cmax or AUCt (Table 2, Online Table 4).

For multivariate analysis, a general linear model with stepwise selection was used to identify demographic, hematologic, and genetic factors that independently predicted clopidogrel pharmacokinetics. The optimal model for predicting clopidogrel AM AUCt accounted for \(110.11\%\) of the variation (adjusted \(r^2\) = 0.1789), and included age (\(\hat{\tau}^2 = 0.0384, p = 0.0082\)) and weight (\(\hat{\tau}^2 = 0.0372, p = 0.0092\)) as factors (Fig. 3, Online Table 5), whereas sex, platelet count, hematocrit, and the indicated polymorphisms in CYP3A5, ABCB1, and PON1 were not significantly associated with clopidogrel AM AUCt. Likewise, the optimal model for predicting clopidogrel AM Cmax accounted for \(~16\%\) of the variation (adjusted \(r^2 = 0.1552\)) and included age (\(\hat{\tau}^2 = 0.0315, p = 0.0179\)) and weight (\(\hat{\tau}^2 = 0.0328, p = 0.0157\)) (Fig. 3, Online Table 5). Thus, for both clopidogrel AM AUCt and Cmax, \(~82\%\) of the variation remained unexplained.

### Influence of demographic, hematologic, and genetic factors on clopidogrel pharmacodynamics

Table 3 summarizes the results of analysis of the influence of clopidogrel AM AUCt and Cmax and factors related to subject demographics, hematology, genetics, and baseline (pre-treatment) VASP PRI on the on-treatment (day 9) VASP PRI. As expected, clopidogrel AM pharmacokinetic endpoints, VASP PRI did not differ significantly when analyzed according to polymorphisms in CYP3A5, ABCB1, and PON1 (Table 3, Online Table 6).

### Table 2 Effects of Demographic, Hematologic, and Genetic Factors on Clopidogrel AM Pharmacokinetics (AUCt and Cmax)

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Clopidogrel AM AUCt</th>
<th>Clopidogrel AM Cmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax</td>
<td>(\hat{\tau}^2)</td>
<td>p Value t</td>
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<tr>
<td>Age (yrs)</td>
<td>0.0639</td>
<td>0.0019</td>
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<td>Weight (kg)</td>
<td>0.0625</td>
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<td>Platelet count</td>
<td>0.0205</td>
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<td>Hematocrit (%)</td>
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<td>0.9050</td>
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<td>Sex (M = 1, F = 2)</td>
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<td>0.9013</td>
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<tr>
<td>CYP3A5*3</td>
<td>0.0249</td>
<td>0.1613</td>
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<tr>
<td>ABCB1 C3435T</td>
<td>0.0109</td>
<td>0.4533</td>
</tr>
<tr>
<td>PON1 rs662</td>
<td>0.0118</td>
<td>0.4217</td>
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*Partial correlation (proportion of variation accounted for by the effect being tested). t p Values are from analysis of variance models using log-transformed values of clopidogrel active metabolite (clopidogrel AM) area under the time-concentration curve (AUCt) and peak plasma concentration (Cmax) with factors for cohort, period, and treatment group.
The optimal multivariable model for predicting VASP PRI (adjusted $r^2 = 0.4755$ for model) (Fig. 3) included clopidogrel$_{AM}$ AUC$_t$ ($\hat{\eta}^2 = 0.3579$, $p < 0.0001$), with very small contributions from platelet count ($\hat{\eta}^2 = 0.0195$, $p = 0.0179$), and pre-treatment VASP PRI ($\hat{\eta}^2 = 0.0187$, $p = 0.0205$).

Evaluation of independent variables that may contribute to clopidogrel's effect on platelet function as measured by VerifyNow P2Y$_{12}$ PRU, VerifyNow percent inhibition, and by MPA with 5- and 20-$\mu$M ADP is shown in Table 3. Clopidogrel$_{AM}$ AUC$_t$, and C$_{max}$, VASP PRI, hematocrit, and sex were each significantly associated with VerifyNow PRU, MPA with 5-$\mu$M ADP, and MPA with 20-$\mu$M ADP (Table 3). Subject age was significantly associated with VerifyNow PRU (Table 3). A modest ($r^2 = 0.2603$), but highly significant ($p < 0.0001$) association, was found between pre-treatment VerifyNow PRU and on-treatment VerifyNow PRU. Pre-treatment MPA to 5-$\mu$M ADP was only weakly associated with on-treatment MPA to 5-$\mu$M ADP (Table 3).

VerifyNow PRU, MPA with 5-$\mu$M ADP, and MPA with 20-$\mu$M ADP were not correlated with polymorphisms in CYP3A5, ABCB1, and PON1 (Table 3, Online Table 7).

The optimal multivariable models for predicting VerifyNow PRU and VerifyNow percent inhibition included factors for clopidogrel$_{AM}$ pharmacokinetics, VASP PRI, hematocrit, and for VerifyNow PRU, pre-treatment VerifyNow PRU. Together these factors explained 65% and 57% of the variation in VerifyNow PRU and VerifyNow percent inhibition, respectively ($r^2 = 0.6500$ and 0.5738, respectively) (Fig. 3, Online Table 8). C$_{max}$, although highly correlated with clopidogrel$_{AM}$ AUC$_t$, was a slightly better predictor of VerifyNow percent inhibition than clopidogrel$_{AM}$ AUC$_t$, and was therefore included in the final model for prediction of VerifyNow percent inhibition. The optimal multivariable model for predicting MPA to 5-$\mu$M ADP included factors for clopidogrel$_{AM}$ AUC$_t$, baseline hematocrit, and pre-treatment MPA with 5-$\mu$M ADP and accounted for 35% of variation in MPA with 5-$\mu$M ADP ($r^2 = 0.3519$) (Fig. 3,
Table 3  
Effects of Individual Demographic, Hematologic, and Genetic Factors on Clopidogrel Pharmacodynamics

<table>
<thead>
<tr>
<th>Predictors</th>
<th>VASP PRI</th>
<th>VerifyNow PRU</th>
<th>VerifyNow % Inh</th>
<th>MPA 5-µM ADP</th>
<th>MPA 20-µM ADP</th>
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<tr>
<td></td>
<td>(\eta^2)</td>
<td>p Value (\dagger)</td>
<td>(\eta^2)</td>
<td>p Value</td>
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<td>ClopidogrelAM AUC(\text{\textsubscript{T}})</td>
<td>0.3820</td>
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<td>ClopidogrelAM C(\text{\textsubscript{max}})</td>
<td>0.2329</td>
<td>&lt;0.0001</td>
<td>0.2374</td>
<td>&lt;0.0001</td>
<td>0.3320</td>
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<td>Pre-treatment VASP PRI</td>
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<td>Pre-treatment VerifyNow PRU</td>
<td></td>
<td>0.2603</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment VerifyNow % Inh.</td>
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<td></td>
<td>0.0250</td>
<td>0.0540</td>
<td></td>
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<td>Pre-treatment MPA 5-µM ADP</td>
<td></td>
<td></td>
<td>0.0269</td>
<td>0.0458</td>
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<td>Pre-treatment MPA 20-µM ADP</td>
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<td></td>
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<tr>
<td>VASP PRI</td>
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<td>0.3771</td>
<td>&lt;0.0001</td>
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<td>Hematocrit (%)</td>
<td>0.0010</td>
<td>0.7201</td>
<td>0.2699</td>
<td>&lt;0.0001</td>
<td>0.0881</td>
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<td>Sex (M = 1, F = 2)</td>
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<td>0.6581</td>
<td>0.2241</td>
<td>&lt;0.0001</td>
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<td>Age (yrs)</td>
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<td>0.2300</td>
<td>0.0450</td>
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<td>Weight (kg)</td>
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<td>0.1114</td>
<td>0.0131</td>
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<td>Platelet count</td>
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<td>0.6309</td>
<td>0.0021</td>
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<td>CYP3A5 × 3</td>
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<td>0.0075</td>
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<td>ABCB1 C343ST</td>
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<td>0.0021</td>
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<td>PON1 rs662</td>
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<td>0.0024</td>
<td>0.8417</td>
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</table>

\(\dagger\)Partial correlation (proportion of variation accounted for by the effect being tested).  \(\dagger\) Values are from analysis of variance models using log transformed values of clopidogrelAM AUC\(\text{\textsubscript{T}}\) and C\(\text{\textsubscript{max}}\) with factors for cohort, period, and treatment group.

Online Table 8). Likewise, the optimal model to predict MPA with 20-µM ADP included factors for clopidogrelAM AUC\(\text{\textsubscript{T}}\), VASP PRI, sex, and pre-treatment MPA with 20-µM ADP and accounted for 44% of variation in MPA with 20-µM ADP (\(r^2 = 0.4357\)) (Fig. 3, Online Table 8).

**Discussion**

The main findings of this study are the following. 1) Despite the rigorous elimination of variation in CYP2C19 polymorphisms, compliance, diet, nicotine, and prescription and nonprescription medications (including PPIs and statins), clopidogrel pharmacokinetics and pharmacodynamics still varied widely. 2) Differences within the study population with respect to age, weight, sex, platelet count, hematocrit, and polymorphisms in CYP3A5, ABCB1, or PON1 accounted for only ~18% of the variability in clopidogrelAM AUC\(\text{\textsubscript{T}}\), and C\(\text{\textsubscript{max}}\), leaving ~82% of the variability unexplained. 3) These factors, together with each subject’s clopidogrelAM AUC\(\text{\textsubscript{T}}\) and C\(\text{\textsubscript{max}}\) accounted for only ~48% of the variability in ADP-induced signaling through P2Y\(\text{\textsubscript{12}}\) as measured by VASP phosphorylation, leaving ~52% of the variability unexplained. 4) Differences in the preceding factors, clopidogrelAM AUC\(\text{\textsubscript{T}}\), and C\(\text{\textsubscript{max}}\) and P2Y\(\text{\textsubscript{12}}\) signaling together accounted for 35% to 65% of the variability in platelet aggregation in platelet-rich plasma or whole blood, leaving 35% to 65% of the variability unexplained. 5) Pre-treatment variability in platelet reactivity was a significant, albeit minor, contributor to on-treatment platelet reactivity. 6) Variation in clopidogrel pharmacokinetics and pharmacodynamic parameters were not associated with polymorphisms in CYP3A5, ABCB1, or PON1. Because patients with reduced platelet inhibition during clopidogrel treatment have an increased risk for major adverse cardiovascular events (2), the present findings, summarized diagrammatically in Figure 3, are important. For example, the presently described wide variability in clopidogrel pharmacokinetics and pharmacodynamics independent of known factors (including single nucleotide polymorphisms, noncompliance, diet, smoking, and co-medications) provides a pharmacological explanation for the relative lack of effect of clopidogrel in nonsmokers (10) and strongly suggests that therapeutic strategies based on the CYP2C19\(^*2\) polymorphism (20) will not eliminate the increased risk for major adverse cardiovascular events in patients with high on-treatment platelet reactivity.

The Food and Drug Administration has issued a “black box warning” for clopidogrel (17,21) that alternative treatment should be considered in patients identified as CYP2C19 poor metabolizers based on the CYP2C19 polymorphism. However, our study demonstrates that even in healthy homozygous CYP2C19 extensive metabolizer subjects free of nicotine, alcohol, prescription, and OTC medications, and with witnessed compliance to study medication, there is a high frequency of poor response to clopidogrel (45% had high on-treatment platelet reactivity by at least 1 criterion). In the present study, rigorous measures were taken to eliminate or control potential contributors to clopidogrel variability, thereby allowing us to determine if the observed variation might be explained by measurable demographic factors. However, clopidogrelAM pharmacokinetics varied widely despite these controls and adjustments for demographics (Fig. 1, Online Table 2), leaving the source of ~82% of the variability unknown. Likely contributors to this variation in clopidogrel pharma-
cokinetics include genetic factors (other than CYP2C19 polymorphisms) and nongenetic sources of variation in clopidogrel absorption and metabolism. Multiple studies confirmed a role for CYP2C19*2 in both the laboratory and clinical response to clopidogrel (2). A genome-wide association study found that variation in platelet function following clopidogrel administration was highly heritable (22), but the CYP2C19*2 polymorphism accounted for (only) ~12% of the variation in platelet aggregation (22). The high heritability estimate suggests that additional genetic variants may contribute to clopidogrel response variability. Bouman et al. (5) proposed that PON1 plays a major role in clopidogrel metabolism and that a common polymorphism in PON1, rs662, affects the rate of clopidogrelAM formation. However, other investigators found no significant effect of the PON1 rs662 polymorphism on clopidogrel pharmacokinetics or pharmacodynamics (23–26). In the present study, the PON1 rs662 polymorphism was not significantly associated with clopidogrel pharmacokinetic or pharmacodynamic endpoints (Tables 2 and 3, Online Tables 4, 6, and 7). This finding adds to and complements previous findings (23–26), because in the present study, possible confounding factors (subject health, co-medications, smoking, diet, and CYP2C19 genotype) were eliminated or controlled by study design. Likewise, and in contrast to some reports (6,8,18,19), in the present, rigorously controlled study, no associations were observed between polymorphisms in ABCB1 (C3435T) or CYP3A5 (*3) and clopidogrel pharmacokinetic or pharmacodynamic endpoints (Tables 2 and 3, Online Tables 4, 6, and 7).

Considering the small number of biochemical steps between ADP binding to P2Y12 and changes in VASP phosphorylation (27), a surprising result of the present study is that the measured clopidogrel pharmacokinetics explain only ~48% of the variability in clopidogrel pharmacodynamics as measured by the VASP assay. Potential explanations for this phenomenon include quantitative or qualitative differences in signaling molecules between P2Y12 and VASP, such as Gs, adenylyl cyclase, or protein kinase A.

ClopidogrelAM AUCt and Cmax values also had an unexpectedly small influence on variation in platelet aggregation measured by LTA or VerifyNow, accounting for <40% of the variation in platelet aggregation assays (Table 3). This may be explained in part by the fact that platelet aggregation is well downstream from the platelet ADP receptor, P2Y12 (the molecular target for clopidogrel’s active metabolite) and is dependent on glycoprotein IIb-IIIa (integrin αIIbβ3) receptor density, fibrinogen, platelet concentration, and cell–cell contact. VASP phosphorylation is distal to signaling through P2Y12, but upstream from glycoprotein IIb-IIIa activation and platelet aggregation (Fig. 3). Because signaling through P2Y12, as measured by VASP phosphorylation, varied from that predicted on the basis of clopidogrelAM pharmacokinetics, we evaluated the ability of VASP PRI to independently predict platelet aggregation measured by 5- and 20-μM ADP-induced LTA and the VerifyNow P2Y12 assay. VASP PRI accounted for ~4% and ~14% of the variation in VerifyNow PRU and VerifyNow percent inhibition, respectively, and ~2% of the variation in MPA to 20-μM ADP (Fig. 3, Online Table 8) independent of pharmacokinetics and other predictors. Thus, variation in signaling between P2Y12 and VASP phosphorylation accounts for a small portion of the variation in these platelet aggregation endpoints; a portion of the remaining variation may be due to variation in signaling distal to VASP.

Approximately 35%, 44%, and 65% of the variation in 5- and 20-μM ADP-induced LTA, and the VerifyNow P2Y12 assay, respectively, could be explained by identified factors in the present study (Fig. 3). In an analysis of the POPular (Do Platelet Function Assays Predict Clinical Outcomes in Clopidogrel-Pretreated Patients Undergoing Elective PCI) study, Bouman et al. (28) demonstrated that, in addition to the CYP2C19*2 genotype, high on-treatment platelet reactivity could be independently predicted by clinical factors (demographics, disease, drug use, etc.). When these clinical factors were combined with CYP2C19*2 polymorphism as predictors, 13.0%, 15.2%, and 20.6% of the variability in 5- and 20-μM ADP-induced LTA, and the VerifyNow P2Y12 assay, respectively, could be explained (28). However, the level of clopidogrelAM, arguably the most critical determinant of the response to clopidogrel, was not measured in POPular. Thus, in POPular (unlike the present study), it was not possible to estimate the total proportion of clopidogrel response variation that could be explained by all known factors.

We, and others, have previously reported that pre-treatment platelet reactivity is a predictor of platelet reactivity during clopidogrel treatment (13–15). Here, we demonstrate that when known contributors to variation in clopidogrel’s pharmacodynamic response are removed, intrinsic pre-treatment platelet reactivity remains a significant, albeit modest (Table 3), predictor of on-treatment variation, accounting for ~2% of variation in platelet reactivity as measured by 3 independent assays. Finally, the proportion of variance due to error in these measures may be nontrivial, although the very high intraclass correlations (>0.8) (Online Table 3) suggests intra-individual variability (which includes assay error) is low.

**Study limitations.** First, this study was conducted in confined healthy volunteers, not patients, but this enabled us to rigorously control for co-medications, diet, smoking, clopidogrel compliance, etc., and to exclude known disease as a contributor to variability. Furthermore, the presently described high degree of variability in clopidogrel response in healthy volunteers would likely only be higher in patients with activated platelets due to acute coronary syndromes or additional co-morbidities such as diabetes mellitus. Second, for uniformity, this study included only homozygous CYP2C19 extensive metabolizers; consequently, our conclusions are limited to this population.
Conclusions

Steady-state clopidogrel pharmacokinetics and pharmacodynamics vary widely despite exclusion or control of known factors: polymorphisms (CYP2C19, CYP3A5, ABCB1, PON1), noncompliance, diet, smoking, co-medications (including PPIs and statins), alcohol, demographic factors, and pre-treatment platelet hyperreactivity. Identifiable factors account for only 18% of the variation in clopidogrel AM pharmacokinetics and only 35% to 65% of the variation in on-treatment platelet reactivity measured by VASP, LTA, and VerifyNow. Sources of the remaining variations are unclear, but they may be future therapeutic targets, given that patients with high on-treatment platelet reactivity are at increased risk of major adverse cardiovascular events.

References

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For an expanded Methods section and the supplemental Figure and Tables, please see the online version of this article.