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First Analysis of the Relation Between *CYP2C19* Genotype and Pharmacodynamics in Patients Treated With Ticagrelor Versus Clopidogrel

The ONSET/OFFSET and RESPOND Genotype Studies

Udaya S. Tantry, PhD; Kevin P. Bliden, MBA; Cheryl Wei, PhD; Robert F. Storey, MD; Martin Armstrong, PhD; Kathleen Butler, MD; Paul A. Gurbel, MD

Background—The influence of cytochrome P450 (CYP) *2C19* genotype on platelet function in patients treated with ticagrelor versus clopidogrel is unknown.

Methods and Results—*CYP2C19* (*1, *2, *3, *4, *5, *6, *7, *8, *17) genotyping was performed in patients with coronary artery disease treated with ticagrelor (180-mg load, 90 mg BID) (n=92) or clopidogrel (600-mg load, 75 mg/d) (n=82). All patients received 75 to 100 mg/d aspirin. Platelet function was measured by aggregometry, VerifyNow P2Y₁₂ assay, and vasodilator-stimulated phosphoprotein-phosphorylation assay at predose, 8 hours postloading, and maintenance. In each treatment group, patients were categorized according to *2C19* genotype carrier status (loss-of-function, gain-of-function) and metabolizer status. Kruskal-Wallis test was used to compare platelet function among these categories for each treatment, and Wilcoxon rank sum test was used to compare platelet function between the clopidogrel and ticagrelor groups for each category. There was no statistically significant influence of genotype on platelet function during aspirin therapy alone. Ticagrelor exhibited lower platelet reactivity than clopidogrel by all assays irrespective of *2C19* genotype or metabolizer status ($P<0.01$). Loss-of-function carriers had greater platelet reactivity during clopidogrel therapy. The influence of genotype on platelet reactivity was greatest during clopidogrel maintenance and best demonstrated by the VerifyNow P2Y₁₂ assay.

Conclusions—This report is the first to demonstrate the superior pharmacodynamic effect of ticagrelor compared with clopidogrel irrespective of *CYP2C19* genotype. Whereas *CYP2C19* genotype influenced the antiplatelet effect of clopidogrel, there was no effect of *CYP2C19* genotype during ticagrelor therapy.

Clinical Trial Registration—URL: <http://www.clinicaltrials.gov>. Unique identifiers: NCT00642811 and NCT00528411. (*Circ Cardiovasc Genet.* 2010;3:00-00.)

Key Words: platelet function tests ■ clopidogrel ■ ticagrelor ■ genetics ■ antiplatelet

Clopidogrel therapy is associated with wide response variability and nonresponsiveness, which mainly have been attributed to variable and insufficient active metabolite generation by hepatic cytochrome P450 (CYP) isoenzymes.¹ In addition, clopidogrel nonresponsiveness and high on-treatment platelet reactivity to ADP have been strongly associated with recurrent ischemic event occurrence in patients treated with percutaneous interventions.² Single-nucleotide polymorphisms (SNPs) in genes encoding CYP isoenzymes, in particular, *2C19*, have been linked to diminished clopidogrel active metabolite generation and pharmacodynamic effects and increased ischemic events in patients treated with coronary artery stents.^{1,3} The relation

of SNP carrier status to clopidogrel effect has been the subject of a recent US Food and Drug Administration “boxed warning” and a clinical alert issued by the American College of Cardiology Foundation/American Heart Association.^{4,5} These limitations provide a strong basis to investigate alternative P2Y₁₂ receptor inhibitors.

Editorial p ●●● Clinical Perspective on p ●●●

Ticagrelor is a reversibly binding, noncompetitive, direct-acting, orally administered P2Y₁₂ receptor antagonist.⁶ Although there is no hypothesis to suggest that ticagrelor pharmacodynamics should be influenced by

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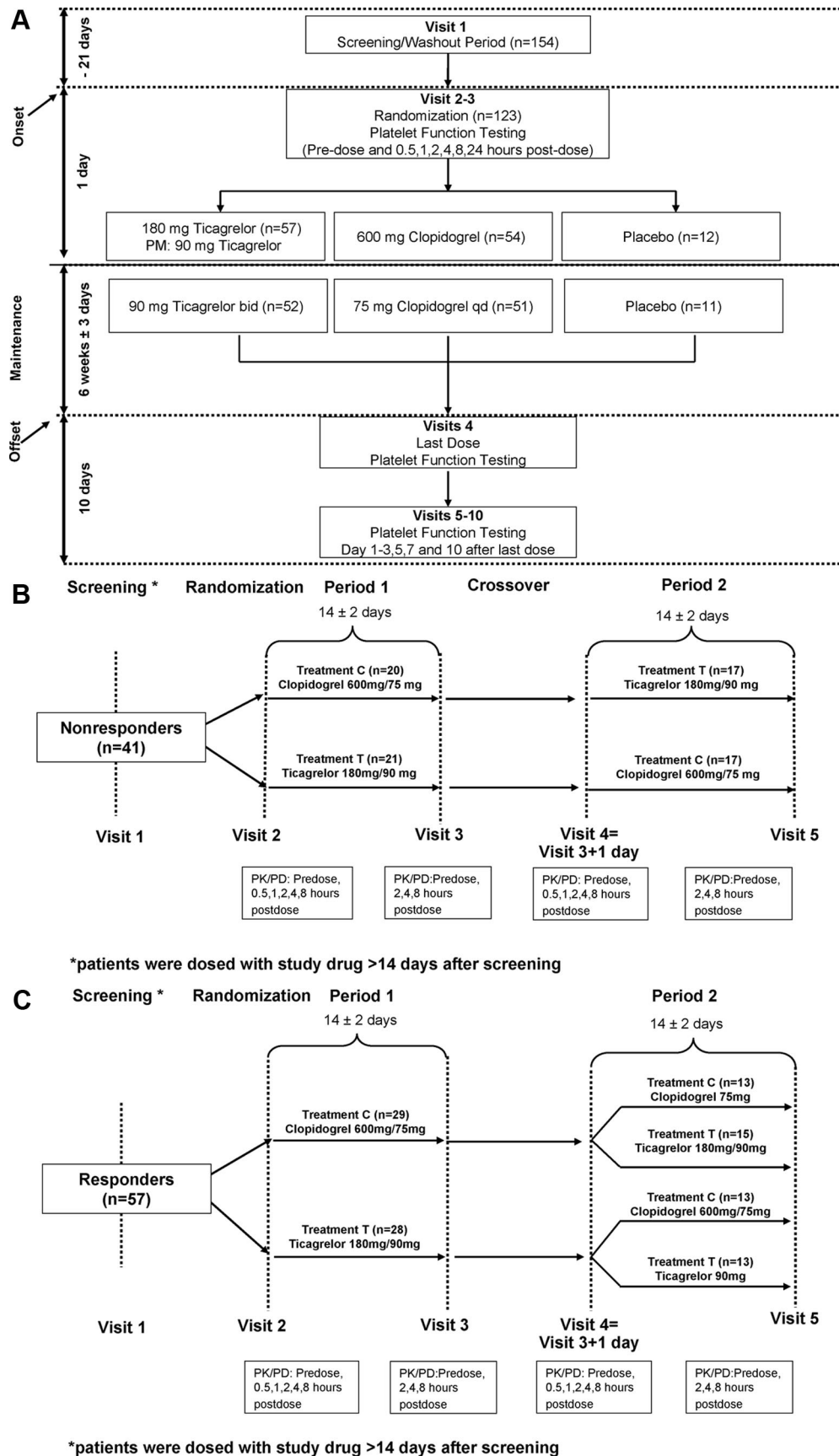


Figure 1. A, Design of the ONSET/OFFSET study. Study flow diagrams demonstrate treatment in nonresponders (B) and responders (C). PK/PD indicates pharmacokinetic/pharmacodynamic.

Table 1. Genotype Classification

| CYP2C19 Genotype/Predicted Phenotype Relationships | |
|--|---|
| Genotype | Phenotype |
| <i>CYP2C19</i> variants | |
| *1/*1 | |
| Wt/Wt | Extensive |
| *1/*2-8 | |
| Wt/LOF | Intermediate |
| *2-8/*2-8 | |
| LOF/LOF | Poor |
| *17/*17 | |
| GOF/GOF | Ultrarapid |
| *1/*17 | |
| Wt/GOF | Rapid heterozygous |
| *2-8/*17 | |
| LOF/GOF | Poor/rapid heterozygous |
| Group I metabolizer status | |
| UM | Ultrarapid+rapid heterozygous |
| EM | Extensive |
| IM | Intermediate+poor/rapid heterozygous |
| PM | Poor |
| Group II LOF carrier status | |
| LOF carriers | Intermediate+poor/rapid heterozygous+poor |
| LOF noncarriers | Extensive+ultrarapid+rapid heterozygous |
| Group III GOF carrier status | |
| GOF carriers | Ultrarapid+rapid heterozygous |
| EM | Extensive |
| LOF carriers | Intermediate+poor/rapid heterozygous+poor |
| <i>ABCB1</i> genotype | |
| C/C | High expression |
| C/T | Intermediate expression |
| T/T | Low expression |

EMs and LOF carriers in groups II and III are the same. LOF indicates loss of function; GOF, gain of function; EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; UM, ultrarapid metabolizer; Wt, wild-type allele.

CYP2C19 genotype, the comparative pharmacodynamic effects of clopidogrel and ticagrelor in relation to *2C19* genotype are unknown. Even more important is whether patients treated with ticagrelor have greater platelet inhibition than patients treated with clopidogrel who possess a genotype associated with efficient drug metabolism (ie, a “clopidogrel responder” genotype). In the Platelet Inhibition and Patient Outcomes (PLATO) trial, ticagrelor significantly reduced the rate of the combined end point of cardiovascular death, myocardial infarction, or stroke in patients with acute coronary syndrome (ACS) compared with clopidogrel (1.87% absolute and 16% relative risk reduction; $P=0.0003$).⁷ In the Multi-Centre Randomized, Double-Blind, Double-Dummy Parallel Group Study of the Onset and Offset of Antiplatelet Effects of AZD6140

Table 2. Patient Demographics

| | Ticagrelor (n=92) | Clopidogrel (n=82) | P |
|---|-------------------|--------------------|------|
| Age, y | 63±9 | 65±8 | 0.12 |
| Male sex | 67 (73) | 62 (76) | 0.67 |
| Race | | | |
| White | 79 (86) | 74 (90) | 0.42 |
| Black | 9 (10) | 6 (7) | 0.48 |
| Weight, kg | 88±20 | 86±14 | 0.45 |
| Body mass index, kg/m ² | 30±5 | 29±4 | 0.15 |
| Medical history | | | |
| Hypertension | 77 (84) | 60 (73) | 0.08 |
| Dyslipidemia including hypercholesterolemia | 85 (92) | 79 (96) | 0.27 |
| Current-smoker | 7 (8) | 8 (10) | 0.64 |
| Diabetes mellitus | 22 (24) | 17 (21) | 0.63 |
| Myocardial infarction | 43 (47) | 41 (50) | 0.69 |
| Coronary artery bypass graft | 37 (40) | 32 (39) | 0.89 |
| Baseline medications | | | |
| Statins | 80 (87) | 76 (93) | 0.19 |
| β-blockers | 63 (69) | 57 (70) | 0.89 |
| Diuretics | 36 (39) | 25 (31) | 0.27 |
| Angiotensin-converting enzyme inhibitors | 20 (22) | 12 (15) | 0.24 |
| Organic nitrates | 11 (12) | 13 (16) | 0.45 |
| Dihydropyridine derivatives | 27 (29) | 15 (18) | 0.09 |
| Proton pump inhibitors | 22 (24) | 17 (21) | 0.64 |
| Calcium channel blockers | 27 (29) | 18 (22) | 0.29 |

Data are presented as mean±SD or no. (%).

Compared With Clopidogrel and Placebo With Aspirin as Background Therapy in Patients With Stable Coronary Artery Disease (ONSET/OFFSET) study, ticagrelor therapy was associated with a rapid onset of action, a greater level of inhibition that persisted during maintenance therapy, and a more rapid offset of pharmacodynamic action compared with clopidogrel.⁸ In the Randomized, Double-Blind, Outpatient, Crossover Study of the Anti-Platelet Effects of AZD6140 Compared With Clopidogrel in Patients With Stable Coronary Artery Disease Previously Identified as Clopidogrel Non-Responders or Responders (RESPOND) study, ticagrelor therapy was associated with greater platelet inhibition compared with clopidogrel in both clopidogrel responders and nonresponders.⁹ Ticagrelor was extremely effective in reducing the prevalence of high platelet reactivity within 30 minutes of therapy. These pharmacodynamic results observed in the ONSET/OFFSET and RESPOND studies provide a mechanism for the outcomes observed in the PLATO trial.⁷⁻⁹ The primary aim of the current study was to determine the effect of genotypically predicted *CYP2C19* metabolizer status on platelet reactivity in patients taking either ticagrelor or clopidogrel in the ONSET/OFFSET and RESPOND stud-

ies and to compare platelet reactivity between treatments within specific genotypes.

Methods

Study Design

A total of 174 patients (ticagrelor, n=92; clopidogrel, n=82) enrolled in the ONSET/OFFSET and RESPOND studies underwent genotyping. The detailed study design and patient inclusion and exclusion criteria were previously described.^{8,9} In brief, the studies were performed in accordance with standard ethical principles; written consent was obtained from all patients. Patients aged ≥ 18 years with documented stable coronary artery disease on aspirin therapy (75 to 100 mg/d) were included. Key exclusion criteria were ACS within 12 months of screening; a history of bleeding diathesis or severe pulmonary disease; pregnancy; concomitant therapy with moderate or strong CYP3A inhibitors or strong inducers; atrial fibrillation, mitral stenosis, or prosthetic heart valve requiring antithrombotic treatment; platelet count $< 100\,000/\text{mm}^3$ or hemoglobin $< 10\text{ g/dL}$; any smoking (ONSET/OFFSET); and current smoking > 1 pack/d (RESPOND).^{8,9}

ONSET/OFFSET was a randomized, double-blind, double-dummy, parallel-group, multicenter study that evaluated the onset and offset of ticagrelor antiplatelet effect versus clopidogrel or placebo for 6 weeks. An initial loading dose of ticagrelor (180 mg), clopidogrel (600 mg), or placebo was given after randomization followed by a maintenance administration (90 mg of ticagrelor or placebo) in the evening with a 12-hour interval between dosing. Patients then received maintenance treatment for 6 weeks (ticagrelor 90 mg BID, clopidogrel 75 mg/d, or placebo) followed by a 10-day drug-offset period during which patients received a final dose of the study drug on the first day of the offset period (Figure 1A).⁸

RESPOND was a multicenter, randomized, double-blind, double-dummy, crossover study that compared the antiplatelet effects of ticagrelor with clopidogrel in patients previously identified as either responsive or nonresponsive to clopidogrel (Figure 1B and 1C). Nonresponsiveness to clopidogrel was defined as $\leq 10\%$ absolute change in maximum extent platelet aggregation induced by $20\text{ }\mu\text{mol/L}$ ADP between predose and 6 to 8 hours postdosing with 300 mg of clopidogrel at screening.¹⁰ Nonresponders and responders were randomly treated with either a 600-mg clopidogrel load followed by 14 ± 2 days of 75-mg/d maintenance therapy or a 180-mg ticagrelor load followed by 14 ± 2 days of 90-mg BID maintenance therapy. The last dose of study drug in period 1 was administered in the morning. In period 2, all nonresponders switched treatment, whereas half of the responders continued the same treatment, and the other half of the responders switched to the other treatment. Patients received treatments again for 14 ± 2 days.⁹

Participation in the genetic component (substudy) of both studies was voluntary for patients and required provision of informed consent separate from that of the main studies. Protocols were approved by local ethics committees.

Blood Sampling and Platelet Function Measurements

Blood was collected from the antecubital vein into Vacutainer tubes (Becton-Dickinson; Franklin Lakes, NJ) containing 3.2% trisodium citrate for light transmittance aggregometry and flow cytometry analyses. In addition, 1 tube containing 3.2% sodium citrate (Greiner Bio-One Vacurette; Greiner Bio-One North America, Inc; Monroe, NC) was collected for VerifyNow measurements. After discarding the first 2 to 3 mL of free-flowing blood, the tubes were filled to capacity and gently inverted 3 to 5 times to ensure complete mixing of the anticoagulant.

For the pharmacodynamic analysis after loading, platelet function measurements performed at predose, 8 hours after the loading dose on day 1, and 8 hours after the last maintenance dose were

Table 3. Genotype Frequencies

| | Ticagrelor (n=92) | Clopidogrel (n=82) | P | Total (n=174) |
|------------------|----------------------|-----------------------|------|------------------|
| Group I | | | | |
| UM | 27 (29) | 28 (34) | 0.37 | 55 (32) |
| EM | 28 (30) | 31 (38) | 0.27 | 59 (34) |
| IM | 35 (38) | 20 (24) | 0.05 | 55 (32) |
| PM | 2 (2) | 3 (4) | 0.43 | 5 (3) |
| Group II | | | | |
| LOF carrier | 37 (40) | 23 (28) | 0.10 | 60 (35) |
| Noncarrier | 55 (60) | 59 (72) | 0.10 | 114 (66) |
| Group III | | | | |
| GOF carrier | 27 (29) | 28 (34) | 0.48 | 55 (32) |
| EM | 28 (30) | 31 (38) | 0.27 | 59 (34) |
| LOF carrier | 37 (40) | 23 (28) | 0.10 | 60 (35) |
| CYP2C19 allele | | | | |
| *1/*1 | 28 (30) | 31 (38) | 0.27 | 59 (34) |
| *1/*2 | 20 (22) | 13 (16) | 0.32 | 33 (19) |
| *1/*3 | 1 (1) | 1 (1) | 1.0 | 2 (1) |
| *1/*17 | 22 (24) | 28 (34) | 0.15 | 50 (29) |
| *2/*2 | 2 (2) | 3 (4) | 0.43 | 5 (3) |
| *2/*17 | 13 (14) | 6 (7) | 0.14 | 19 (11) |
| *8/*17 | 1 (1) | 0 (0) | 0.37 | 1 (1) |
| *17/*17 | 5 (5) | 0 (0) | 0.04 | 5 (3) |
| ABCB1 expression | | | | |
| C/C | 30 (33) | 21 (26) | 0.31 | 51 (29) |
| C/T | 43 (47) | 40 (49) | 0.79 | 83 (48) |
| T/T | 19 (21) | 21 (26) | 0.44 | 40 (23) |

Data are presented as no. (%). EMs and LOF carriers in groups III and II are the same. Abbreviations as in Table 1.

used. For the pharmacodynamic analysis during maintenance, platelet function measurements performed 8 hours after the last dose at 2 weeks in RESPOND and at 6 weeks in ONSET/OFFSET were used.^{8,9}

Maximum platelet aggregation (5 and $20\text{ }\mu\text{mol/L}$ ADP) was assessed using a Chronolog Optical Aggregometer (Model 490-4D) as previously described.⁸ P2Y₁₂ reaction units (PRU) were determined using VerifyNow.¹¹ The vasodilator-stimulated phosphoprotein (VASP)-phosphorylation levels reflecting the degree of P2Y₁₂ receptor blockade were determined with monoclonal antibodies by flow cytometry using the platelet VASP-FCM kit (Biocytex, Inc; Marseille, France). The platelet reactivity index (PRI) is calculated after measuring the VASP-phosphorylation levels following stimulation (mean fluorescence intensity [MFI]) with prostaglandin E₁ (PGE₁) (MFI_{PGE1}) and PGE₁+ADP (MFI_{PGE1+ADP}). The PRI (%) = $[(\text{MFI}_{\text{PGE1}}) - (\text{MFI}_{\text{PGE1+ADP}})] / (\text{MFI}_{\text{PGE1}}) \times 100$.¹⁰

Genotyping

Genotyping of the known common loss-of-function (LOF) *CYP2C19**2 variant (rs4244285) as well as of other functional variants of *CYP2C19* (*3 [rs4986893], *4 [rs28399504], *5 [rs56337013], *6 [rs72558184], *7 [rs72558186], *8 [rs41291556], *17 [rs12248560]) and *ABCB1* (rs1045642) was performed with TaqMan Assays (Life Technologies; Pleasanton, Calif). Platelet function data were categorized based on metabolizer status (group I), LOF (group II) and gain-of-function (GOF) carrier status (group III),

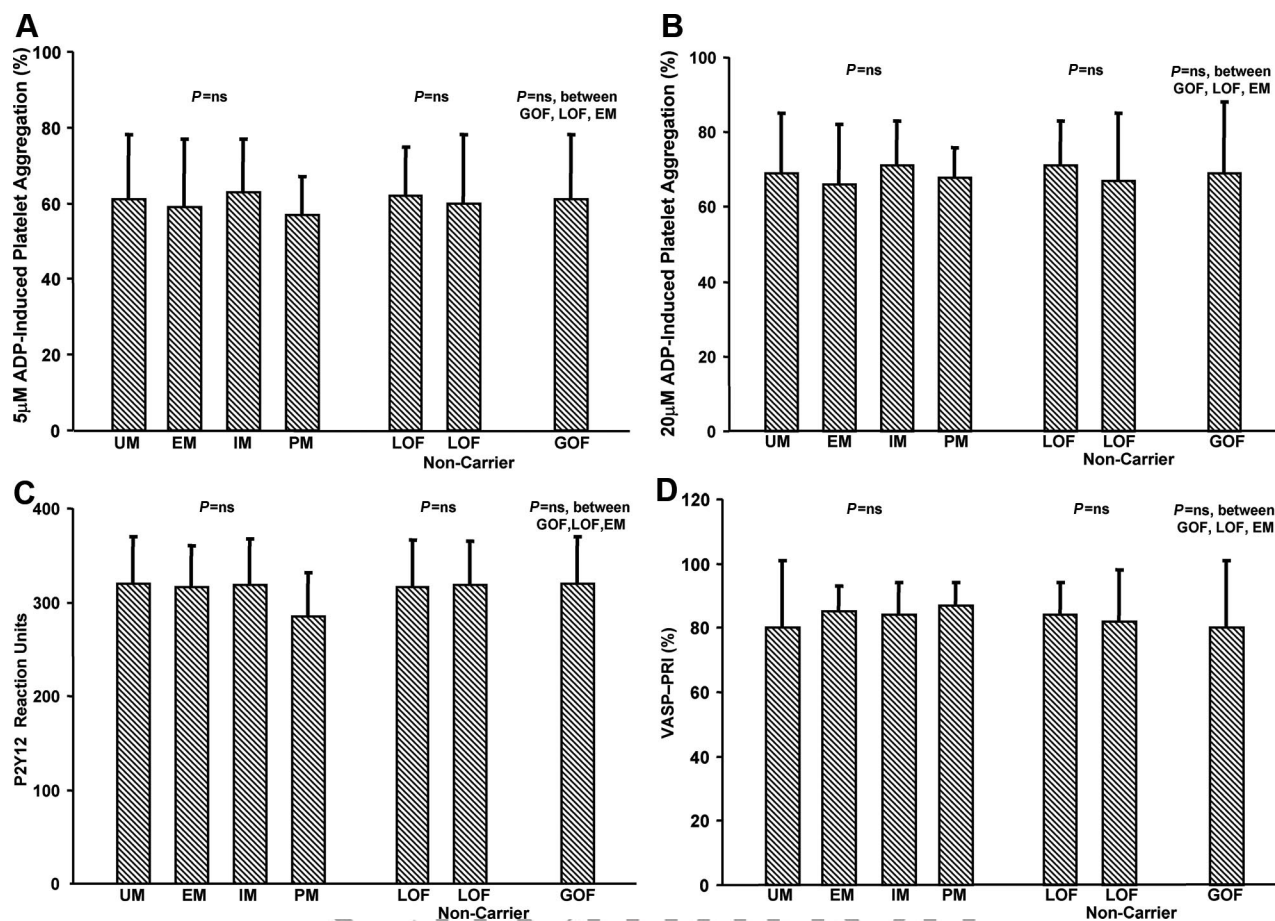


Figure 2. Platelet function measured by 5 and 20 $\mu\text{mol/L}$ ADP-induced platelet aggregation (maximum extent) (A and B), VerifyNow P2Y12 assay (C) and VASP-phosphorylation assay (D) in aspirin-alone-treated patients. EM, indicates extensive metabolizer; IM, intermediate metabolizer; ns, nonsignificant; PM, poor metabolizer; UM, ultrametabolizer; LOF, loss of function; GOF, gain of function; PRI, platelet reactivity.

and *ABCBI* genotype (Table 1). The latter classification is for the predicted phenotype based on a recently published PLATO genetic substudy.¹²

Statistical Methods

Pharmacodynamic data from the ONSET/OFFSET and RESPOND studies were pooled (excluding the placebo control group from ONSET/OFFSET and period 2 from RESPOND), and statistical analysis of the platelet function data were carried out by AstraZeneca Biostatistics and programming using SAS version 8.2+ (SAS Institute Inc; Cary, NC). Demographic data and genotype frequencies were compared between the 2 treatment groups using the *t* test for numeric data or the Fisher exact test for categorical data by Statistica (StatSoft, Inc; Tulsa, Okla). Kruskal-Wallis test was used to compare platelet function among the genotype categories for each treatment, and Wilcoxon rank sum test was used to compare platelet function between the clopidogrel and the ticagrelor groups for genotype category. No adjustment for multiple comparisons was made because of the high correlation among the pharmacodynamic assays. $P < 0.05$ was considered statistically significant.

Results

Patient Demographics

Both treatment groups exhibited similar demographics and baseline medications, except more patients with hypertension

and more treated with dihydropyridine derivatives were in the ticagrelor group (Table 2).

Genotype Frequencies

Both treatment groups were equally balanced with regard to genotype frequencies, except the ticagrelor group had more intermediate metabolizers ($P = 0.05$), and all patients with the $*17/*17$ genotype ($n = 5$) were in the ticagrelor group ($P = 0.04$) (Table 3).

Influence of Metabolizer and Carrier Status on Platelet Function Measurements

In patients treated with aspirin alone, there was no statistically significant influence of genotypes on platelet function as measured by ADP-induced platelet aggregation, VerifyNow P2Y12 assay, and VASP-phosphorylation assay (Figure 2A through 2D). *ABCBI* genotype did not influence platelet function before or during ticagrelor or clopidogrel therapy (data not shown). Patients treated with ticagrelor had significantly lower platelet function ($P \leq 0.0016$) as measured by all assays than patients treated with clopidogrel among all *2C19* genotypes studied, except for poor metabolizers because of the low number of patients ($n = 5$) exhibiting wide CIs (Figures 3 through 6). Within treatment groups, there was no influence of genotype on

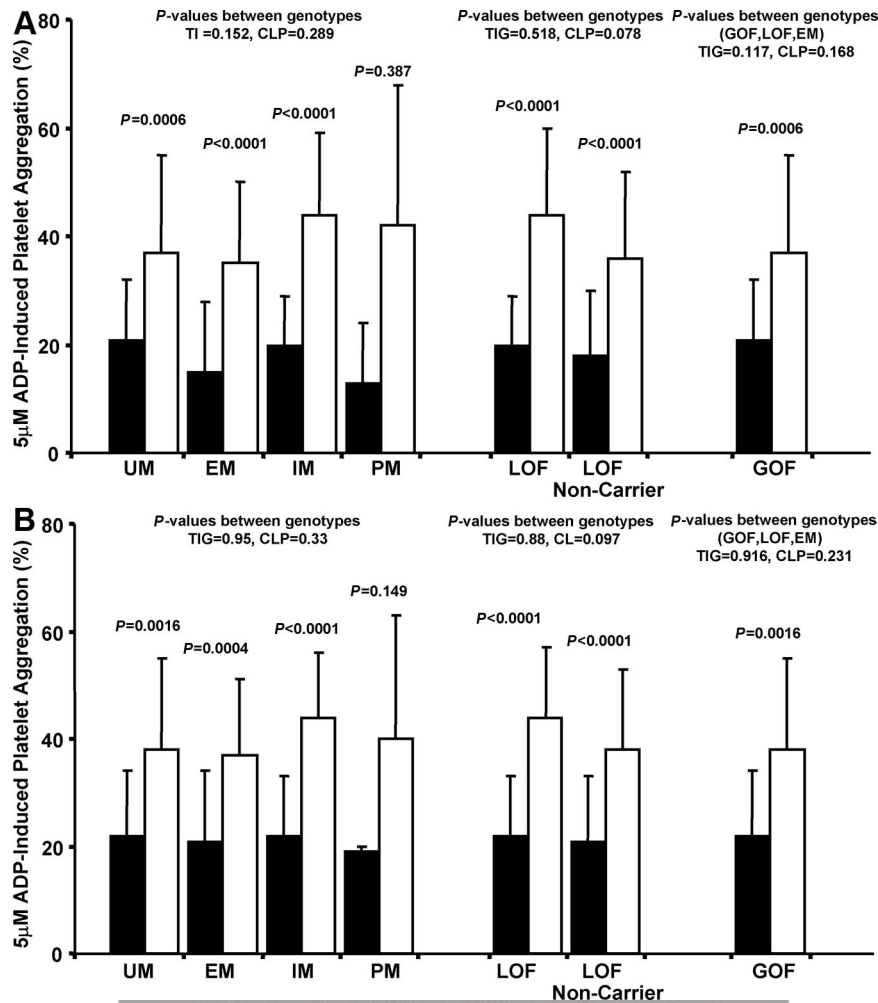


Figure 3. Platelet function measured by 5 $\mu\text{mol/L}$ ADP-induced platelet aggregation (maximum extent) in patients treated with ticagrelor (solid bars) and clopidogrel (open bars) at 8 hours postloading (A) and during maintenance phases (2 to 6 weeks, 8 hours after the last dose) (B). CLP indicates clopidogrel; TIG, ticagrelor. Other abbreviations as in Figure 2.

platelet function in ticagrelor-treated patients either postloading or during maintenance therapy. Among clopidogrel-treated patients, the influence of genotype on platelet function was more evident as measured by VerifyNow P2Y12 assay postloading ($P=0.019$ among different metabolizers; $P=0.01$ between LOF carriers and LOF noncarriers; $P=0.028$ among GOF, LOF, and extensive metabolizers). The influence was more pronounced during clopidogrel maintenance therapy as measured by VerifyNow P2Y12 assay ($P=0.006$ among different metabolizers; $P=0.002$ between LOF carriers and LOF noncarriers; $P=0.007$ among GOF, LOF, and extensive metabolizers) (Figure 5A and 5B).

Influence of *CYP2C19* Diplotype Status on Platelet Function Measurements

There was no statistically significant effect of diplotype status on platelet function during aspirin therapy (data not shown). During the maintenance phase, ticagrelor was associated with significantly lower platelet function as measured by all assays in $*1/*1$, $*1/*2$, $*1/*17$, and $*2/*17$ diplotypes ($P\leq 0.009$) (Figure 7A through 7D). The comparison of the 2 treatments was precluded in patients with

the most rare diplotypes ($*1/*3$, $*2/*2$, $*8/*17$, and $*17/*17$). Within the clopidogrel-treated group, there was a significant influence of diplotype status on platelet function as measured by VerifyNow P2Y12 assay ($P\leq 0.006$), and a trend was observed as measured by 20 $\mu\text{mol/L}$ ADP-induced aggregation and VASP-phosphorylation assay ($P=0.0995$ and $P=0.0996$, respectively).

Discussion

To our knowledge, this study is the first to evaluate the influence of *CYP2C19* genotype on the antiplatelet effects of ticagrelor compared with clopidogrel as well as the first study to examine the influence of genotype on platelet function during both postloading and maintenance therapy phases. Three widely used platelet function assays were used. The present study has 6 important observations: (1) irrespective of *CYP2C19* genotype, platelet reactivity in ticagrelor-treated patients was consistently lower than that in clopidogrel-treated patients; (2) there was no statistically significant influence of *ABCB1* genotype on platelet reactivity to ADP in aspirin alone-treated, clopidogrel-treated, or ticagrelor-treated patients; (3) *CYP2C19* geno-

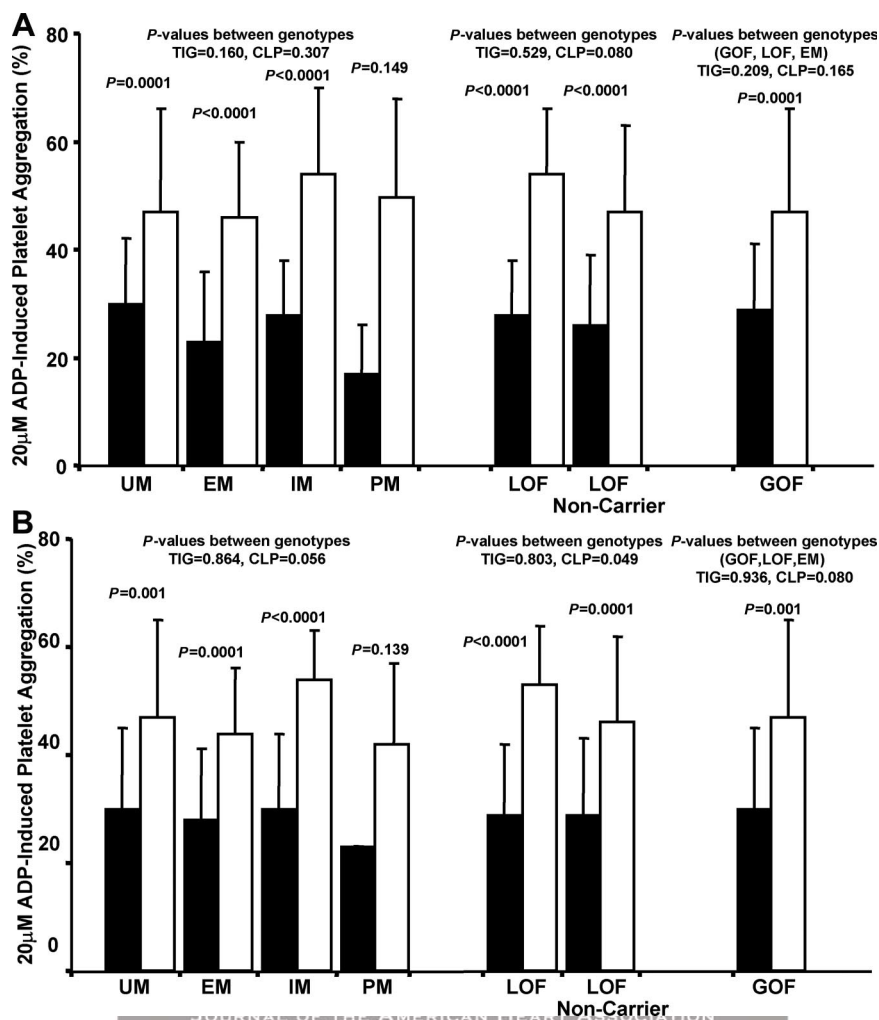


Figure 4. Platelet function measured by 20 μ mol/L ADP-induced platelet aggregation (maximum extent) in patients treated with ticagrelor (solid bars) and clopidogrel (open bars) at 8 hours postloading (A) and during maintenance phases (2 to 6 weeks, 8 hours after the last dose) (B). Abbreviations as in Figures 2 and 3.

type had no influence on the antiplatelet effect of ticagrelor; (4) *CYP2C19* genotype influenced the antiplatelet effect of clopidogrel, and LOF carriers and intermediate metabolizers had higher platelet function during clopidogrel therapy; (5) the influence of *CYP2C19* genotype in clopidogrel-treated patients was most evident during maintenance therapy compared with postloading and was most effectively demonstrated by the VerifyNow P2Y₁₂ assay; and (6) platelet reactivity during clopidogrel therapy was similar in the *1/*17 and *1/*1 genotypes.

The absence of any influence of SNPs of genes encoding P2Y₁, P2Y₁₂, and integrin β_3 receptors was previously demonstrated in patients with cardiovascular disease treated with ticagrelor.¹³ The present study findings in the ticagrelor group may be self-evident because ticagrelor is a direct P2Y₁₂ receptor blocker metabolized by CYP3A4/5 isoforms to an equipotent active metabolite (data on file, AstraZeneca). However, the interesting observation is that platelet reactivity in ticagrelor-treated patients is lower than in clopidogrel-treated patients who are genotypically defined as clopidogrel responders, that is, ultrarapid metabolizers

(GOF allele carriers) and extensive metabolizers (homozygous for the wild-type allele). In the latter group of patients, clopidogrel active metabolite generation would be expected to be optimal and result in low platelet reactivity. However, the data from the current study support a superior pharmacodynamic effect of ticagrelor in all patients, even those predicted to be clopidogrel responders who do not carry an LOF allele. These findings may explain, in part, the superior clinical outcomes observed in the PLATO trial in the ticagrelor arm.⁷ The effect of LOF genotype carrier status on the antiplatelet properties of clopidogrel is consistent with previous studies.^{14–17} However, our data contrast with those of Sibbing et al,¹⁸ who reported an effect of *CYP2C19**17 carrier status on platelet function in patients on clopidogrel therapy. Importantly, Sibbing et al studied a larger number of patients, and there were no *CYP2C19**17 homozygotes in our study population treated with clopidogrel. Nevertheless, platelet function as measured by all assays in our study did not differ between GOF carriers and extensive metabolizers, whereas LOF carriers had greater platelet function than extensive

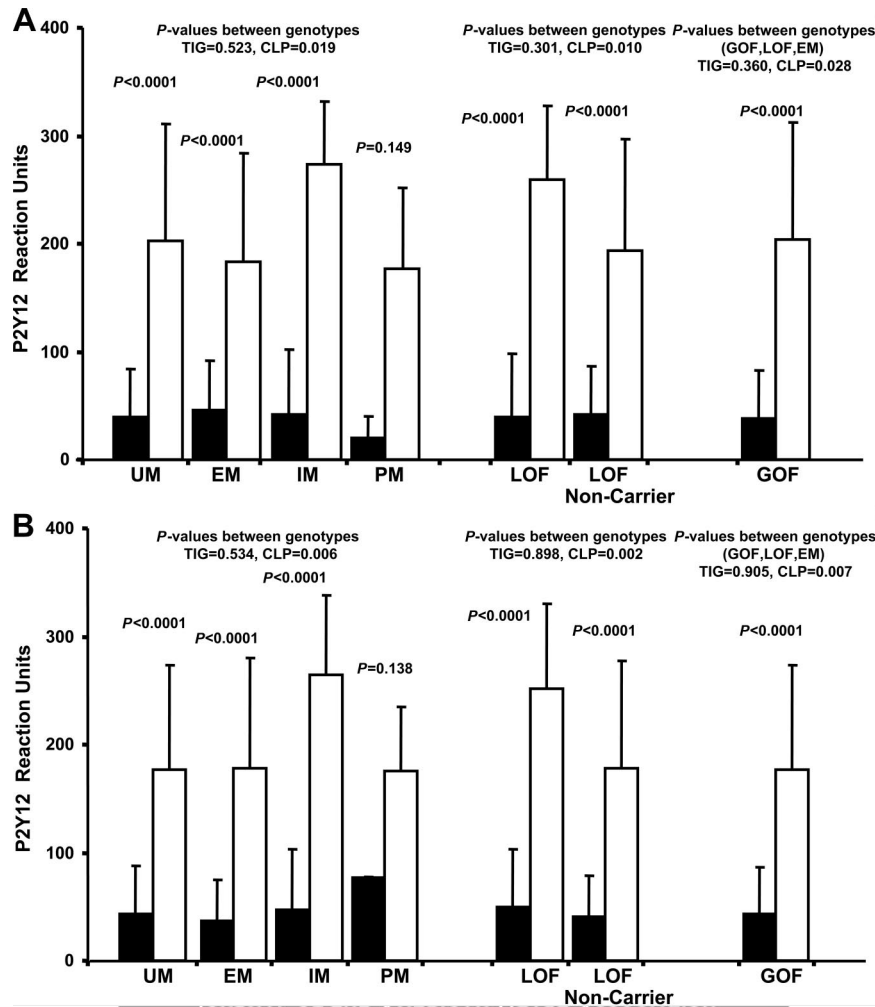


Figure 5. Platelet function measured by VerifyNow P2Y12 assay in patients treated with ticagrelor (solid bars) and clopidogrel (open bars) at 8 hours postloading (A) and during maintenance phases (2 to 6 weeks, 8 hours after the last dose) (B). Abbreviations as in Figures 2 and 3.

metabolizers. Taken together, these data suggest that LOF alleles play a dominant role compared with GOF alleles in determining platelet reactivity during clopidogrel therapy.

Another interesting observation is the demonstration of a more-pronounced influence of *CYP2C19* genotype on platelet reactivity during maintenance dose clopidogrel compared with postloading. Previous studies examined the influence of *CYP2C19* genotype mostly during postloading; few studies were performed during clopidogrel maintenance therapy, and unlike our study, none evaluated the influence during both phases.^{14–17,19} The present study data suggest that VerifyNow P2Y12 assay may be the most effective method to demonstrate the influence of *CYP2C19* genotype on clopidogrel response.

Multiple lines of evidence suggest that the *CYP2C19* isoenzyme is the predominant, but not exclusive isoenzyme responsible for the generation of the active metabolite. In vitro studies using cDNA-expressed human CYP isoenzymes demonstrated that *CYP2C19* contributes 45% in the generation of 2-oxo-clopidogrel in the first step and 29% in the generation of thiol active metabolite in the second step. However, other isoenzymes such as *CYP1A2* and *CYP2B6* in

the first step and *CYP2B6*, *CYP2C9*, and *CYP3A4* in the second step also are involved.³ In a genome-wide association study involving healthy Amish subjects, it was shown that 70% of clopidogrel antiplatelet response (measured by inhibition of ADP-induced platelet aggregation) was accounted for by genetic components. Moreover, the *CYP2C19* locus was the major contributor, but it accounted for only 12% of the clopidogrel response variability.¹⁴ Taken together, these results suggest that SNPs of genes encoding isoenzymes other than *CYP2C19*, and indeed genes outside of the drug metabolism area, also may play an important role in explaining clopidogrel response variability and the occurrence of clinical events in clopidogrel-treated patients. Similar to previous studies, the present study showed that platelet function was lower than baseline (≈ 300 PRU on aspirin therapy versus 170 PRU on aspirin+clopidogrel therapy) in *CYP2C19**2 homozygotes (poor metabolizers and presumably other genetically determined poor metabolizers) where expression of active enzymes is absent.^{14–17} Our results indicate that the contribution of other isoenzymes during active metabolite generation and drug response gain importance in patients predicted to be poor metabolizers based on *CYP2C19* geno-

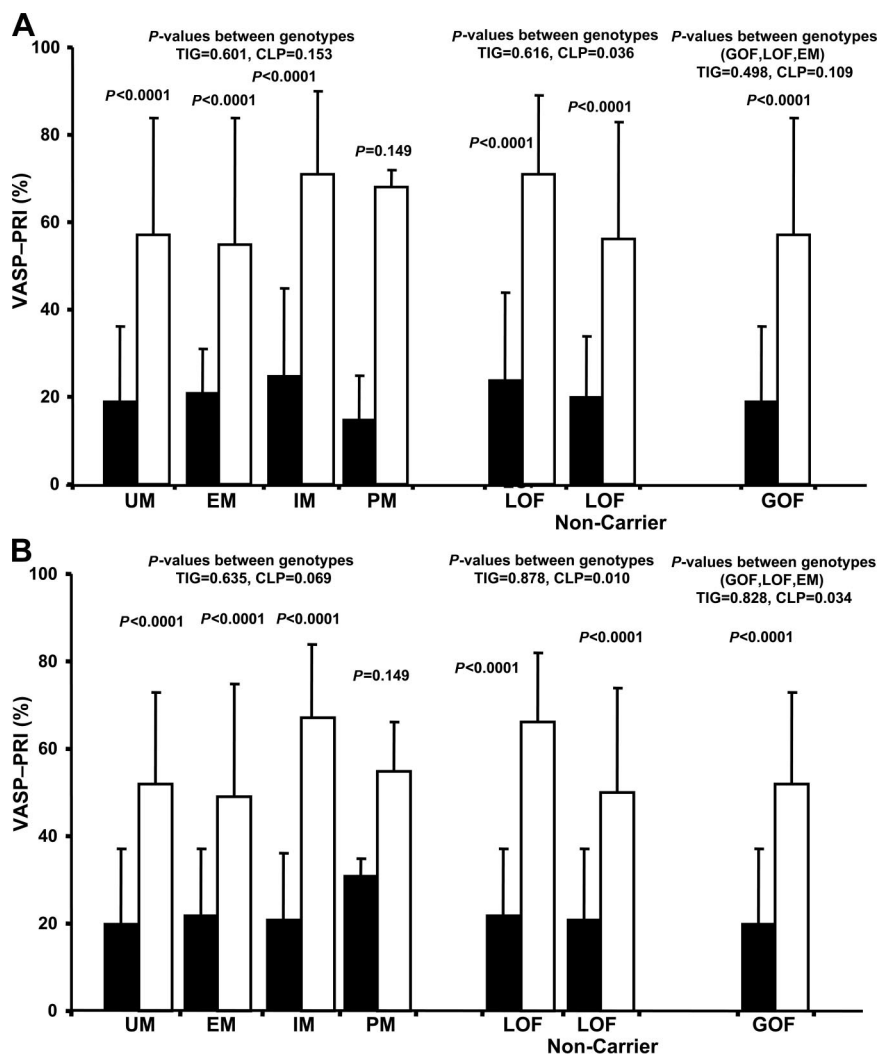


Figure 6. Platelet function measured by VASP-phosphorylation assay in patients treated with ticagrelor (solid bars) and clopidogrel (open bars) at 8 hours postloading (A) and during maintenance phases (2 to 6 weeks, 8 hours after the last dose) (B). Abbreviations as in Figures 2 and 3.

type. Our results are consistent with the findings of the genetic substudy of the PLATO trial, where ticagrelor was a more clinically efficacious treatment for ACS patients than clopidogrel irrespective of *CYP2C19* genotype. In our study, we observed greater pharmacodynamic efficacy with ticagrelor treatment irrespective of genotype.¹²

Limitations

The number of patients in the rare genotype groups limits the ability to make definitive statements regarding their relation to platelet reactivity. There were no GOF homozygotes in the clopidogrel group.

Conclusions

This report is the first to demonstrate the superior antiplatelet effect of ticagrelor compared with clopidogrel irrespective of *CYP2C19* genotype. Whereas *CYP2C19* genotype influenced the antiplatelet effect of clopidogrel, there was no effect during ticagrelor therapy. Further studies with larger numbers

of patients are required to examine the relative influences of the *2 and *17 carriers on the antiplatelet effects of clopidogrel during the maintenance phase of therapy. The results of the current study are consistent with the outcomes observed in the PLATO trial.

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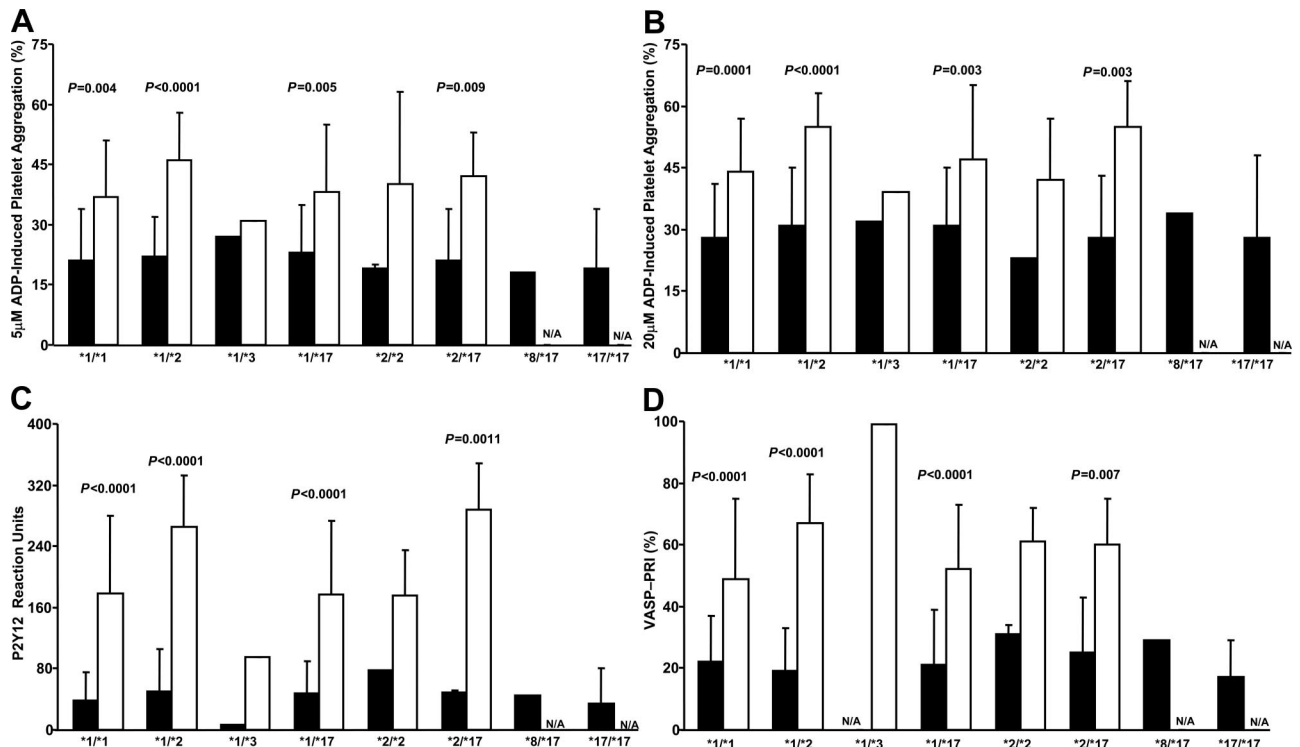


Figure 7. Influence of *CYP2C19* diplotypes on platelet function measured by 5 and 20 μmol/L ADP-induced platelet aggregation (maximum extent) (A and B), VerifyNow P2Y12 assay (C) and VASP-phosphorylation assay (D) during maintenance phases (2 to 6 weeks, 8 hours after the last dose) in patients treated with ticagrelor (solid bars) and clopidogrel (open bars). N/A indicates not applicable.

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CLINICAL PERSPECTIVE

In contrast to clopidogrel, the antiplatelet effect of ticagrelor is not influenced by cytochrome P450 2C19 genotype. Ticagrelor therapy was associated with significantly greater platelet inhibition than clopidogrel irrespective of genotype. Further studies with larger numbers of patients are required to examine the relative influences of *2 and *17 carrier on the antiplatelet effects of clopidogrel during the maintenance phase of therapy. The results of the current study are consistent with the results of the Platelet Inhibition and Patient Outcomes genetics substudy, demonstrating that ticagrelor is a more-effective treatment for acute coronary syndromes than clopidogrel irrespective of cytochrome P450 2C19 polymorphisms.



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