The relationship between CYP2C19 polymorphisms and ischaemic and bleeding outcomes in stable outpatients: the CHARISMA genetics study

Deepak L. Bhatt 1*, Guillaume Paré 2, John W. Eikelboom 2, Katy L. Simonsen 3, 4, Eileen S. Emison 5, Keith A.A. Fox 6, Ph. Gabriel Steg 7, 8, 9, Gilles Montalescot 10, 11, Nihar Bhakta 12, Werner Hacke 14, Marcus D. Flather 14, Koon-Hou Mak 15, Patrice Cacoub 16, Mark A. Creager 17, Peter B. Berger 18, Steven R. Steinhubl 18, Gurunathan Murugesan 19, Shamir R. Mehta 2, Kandice Kotkje-Marchant 19, A. Michael Lincoff 19, and Eric J. Topol 20, on behalf of the CHARISMA Investigators

1VA Boston Healthcare System, Brigham and Women’s Hospital, and Harvard Medical School, 1400 VFW Parkway, Boston, MA 02132, USA; 2McMaster University, Hamilton, Canada; 3Department of Statistics, Purdue University, West Lafayette, IN, USA; 4Global Biomarker Sciences, Bristol-Myers Squibb, Princeton, NJ, USA; 5Clinical Biomarkers, Pharmacogenetics, Bristol-Myers Squibb, Princeton, NJ, USA; 6University and Royal Infirmary of Edinburgh, Edinburgh, UK; 7INSERM U-698, Paris, France; 8Université Paris 7, Paris, France; 9Assistance Publique-Hôpitaux de Paris, Paris, France; 10Institut de Cardiologie (AP-HP)—INSERM U-937, Centre Hospitalier Universitaire Pitié-Salpêtrière, Paris, France; 11Université Paris 6, Paris, France; 12Bristol-Myers Squibb, Princeton, NJ, USA; 13Department of Neurology, University of Heidelberg, Heidelberg, Germany; 14Clinical Trials and Evaluation Unit, Royal Brompton Hospital and Imperial College, London, UK; 15Mak Heart Clinic, Singapore, Singapore; 16AP HP Hôpital La Pitié Salpêtrière, Department of Internal Medicine and UMR 7211 (UPMC/CNRS), H 959 (INSERM) Université Pierre Marie Curie, Paris, France; 17Division of Cardiovascular Medicine, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA, USA; 18Division of Cardiology, Geisinger Medical Center, Danville, PA, USA; 19Division of Cardiology, Gesinger Medical Center, Danville, PA, USA; 20Scripps Translational Science Institute and Scripps Clinic, La Jolla, CA, USA

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Aims
Clinical trials have established the value of clopidogrel therapy in a wide spectrum of patients with cardiovascular diseases. Both loss- and gain-of-function single nucleotide variants of CYP2C19 genes have been identified that affect clopidogrel metabolism and anti-platelet response. We sought to determine the impact of CYP2C19 polymorphisms on ischaemic and bleeding events.

Methods and results
A subset of patients from the Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance (CHARISMA) trial who consented to genotyping was analysed. Patients with clinically evident cardiovascular disease or multiple risk factors were enrolled in the trial. The rates of ischaemic and bleeding events were compared between carriers and non-carriers of loss-of-function and gain-of-function alleles in patients randomized to clopidogrel vs. placebo. A total of 4819 patients were genotyped and available for the analysis. Carriers of CYP2C19 loss-of-function alleles did not have an increased rate of ischaemic events. However, clopidogrel-treated patients did have a significantly lower rate of any bleeding in carriers: 36.1% (240/665) vs. 42.5% (681/1601) in non-carriers, HR: 0.80, 95% CI: 0.69–0.93, P = 0.003 (genotype/treatment interaction, P-value = 0.023). The CYP2C19 gain-of-function alleles did not affect ischaemic or bleeding endpoints.

Conclusion
No relationship was seen between CYP2C19 status and ischaemic outcomes in stable patients treated with clopidogrel. There was, however, significantly less bleeding with clopidogrel in carriers of the loss-of-function allele, suggesting less anti-platelet response. Although several prior studies, including mainly stented patients, have emphasized the relationship between CYP2C19 loss-of-function alleles and efficacy of clopidogrel, this study of stable patients establishes a potential link with reduced bleeding complications.

Clinical Trial Registration: This study is registered with ClinicalTrials.gov number, NCT00050817.
Introduction

Several studies have established the value of clopidogrel as an anti-platelet agent. Variability in platelet response to clopidogrel has been extensively reported. Polymorphisms of genes encoding CYP450 enzymes have been described that may explain some of this variability. Numerous candidate gene studies and one genome-wide association study have demonstrated a relationship between CYP2C19 polymorphisms and anti-platelet response to clopidogrel, including clinical outcomes.

These studies have been limited by lack of a placebo arm. Therefore, they have been unable to assess if any polymorphism associated with risk is specific to clopidogrel, or potentially due to other factors such as metabolism of different drugs and endogenous substrates metabolized by the CYP450 system. Furthermore, the studies have differed as to whether any potential risk associated with carriage of a CYP2C19 loss-of-function allele is confined to homozygotes or whether it also affects heterozygotes.

The Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance (CHARISMA) trial randomized 15,603 stable patients, who had either manifest atherothrombotic disease (coronary, cerebrovascular, peripheral artery disease) or exhibited multiple risk factors for developing atherothrombotic disease, to clopidogrel plus aspirin or placebo plus aspirin. The overall trial did not find a significant benefit of dual anti-platelet therapy over aspirin alone, though patients enrolled with symptomatic atherothrombosis and, particularly, those with prior ischaemic events did seem to derive benefit.

Methods

A subset of patients from the overall CHARISMA trial were enrolled into this genetics substudy in particular countries (Australia, Belgium, Canada, Chile, Czech Republic, Greece, Hungary, Italy, Mexico, Netherlands, Poland, Portugal, Russia, Singapore, South Africa, Spain, Switzerland, UK, USA) where the substudy was approved. All patients provided additional written informed consent to be included in this substudy. Genotypes for CYP2C19 alleles *2 (rs4244285), and *17 (rs11188072 and rs12248560) were determined by a restriction fragment length polymorphism. The *2, *3, and *17 polymorphisms were observed in complete linkage disequilibrium throughout the genotyped population (D’ = 1 for all pairwise comparisons). Because the available patient population was largely of Caucasian descent, the major reduced function allele at CYP2C19 was expected to be *2. Genotypes at *3 (rs4986893) were determined by the Taqman allelic discrimination. Of the samples, 11% were genotyped in duplicate; no errors were identified in the replicates. Missingness was <2% for all single nucleotide polymorphisms and none showed evidence of deviation from the Hardy–Weinberg equilibrium (P > 0.05). The main analyses were performed on patients of European ancestry (defined as Caucasian).

The primary efficacy endpoint for this analysis was the same as for the overall CHARISMA trial: first occurrence of non-fatal or fatal myocardial infarction, non-fatal or fatal stroke, or cardiovascular death. These events were validated by the Cleveland Clinic Clinical Events Adjudication Committee. The secondary efficacy endpoint additionally included hospitalization for unstable angina, transient ischaemic attack, or revascularization procedure. The safety endpoint for this analysis was the incidence of severe or moderate bleeding events using the GUSTO definition (the primary safety endpoint for the initial CHARISMA trial was GUSTO severe bleeding). An additional safety endpoint used the composite of all GUSTO bleeding.

Additional analyses were performed in the patients categorized as having symptomatic atherothrombosis on entry into the trial and in patients in the ‘CAPRIE-like’ cohort, with prior myocardial infarction, ischaemic stroke, or symptomatic peripheral artery disease, subgroups with apparent benefit from clopidogrel vs. placebo.

The efficacy analysis focused on the difference in the effect of clopidogrel vs. placebo on the risk of a primary event for carriers of a CYP2C19 loss-of-function allele (either *2 or *3) relative to non-carriers. Similar analyses were also performed for the gain-of-function allele *17. Cox proportional hazards models and Kaplan–Meier survival analysis were used to evaluate the effect of CYP2C19 genotype on the time to primary or secondary efficacy events. The main analyses were sex and age adjusted. Additional analyses were performed that included treatment, genotype, and the interaction of treatment and genotype, as well as further covariates in the Cox models. Covariates used were: inclusion criteria (symptomatic vs. asymptomatic), prior use of statins, prior use of calcium channel blockers, and cigarette usage (none, light (1–9 per day), moderate/heavy (10 or more per day)). Information about proton pump inhibitor use was not available. The safety endpoint of moderate or severe GUSTO bleeding events, as well as all GUSTO bleeding events, was analysed using a Cox proportional hazards model including the above covariates. Based on genotype, patients were categorized as poor (*2/*2 or *2/*3), intermediate (wt/*2 or wt/*3), extensive (wt/wt), ultra (wt/*17 or *17/*17), or unknown (*2/*17 or *3/*17) metabolizers, and ischaemic and bleeding outcomes were also examined. Statistical analysis was performed using R 2.10.0 GUI 1.30 Leopard build 64-bit. The significance level was two-sided P < 0.05.

Results

A total of 4924 samples of whole blood were collected. Of these, 4862 had adequate DNA recovered and were available for analysis. Because the number of genetic samples available for the analysis represented only one-third of the clinical trial population, the time to the primary event was compared for the genotyped and non-genotyped subjects. Within the placebo arm, a reduced risk for the primary endpoint was observed in the genotyped subjects (5.67%) relative to the non-genotyped subjects (8.11%), P = 0.001.
The baseline characteristics of all subjects based on genotype status are shown in Supplementary material online, Table S1. The minor allele frequencies of the *2, *3, and *17 alleles were 15.4, 0.01, and 21.4%, respectively. Most baseline characteristics were not significantly (P > 0.05) different according to genotypes.

Of the 4862 subjects with adequate DNA for analysis, 43 patients had undefined ancestry, leaving 4819 patients. Of these, 4.1% were of African ancestry and 1.7% were of Asian ancestry. The remaining 4537 patients who were of European ancestry comprised the main analysis group presented herein. The baseline characteristics and metabolizer phenotypes are listed in Table 1.

The analyses for the patients of European ancestry are shown in Figures 1 and 2. Analyses shown are age and sex adjusted. The analyses for the entire genotyped population adjusted for multiple covariates are shown in Supplementary material online, Figures S1 and S2 and are very similar to the European ancestry analyses.

The results were also very similar in the CAPRIE-like cohort (see Supplementary material online, Figures S3 and S4). The results were also similar in patients with a prior history of percutaneous coronary intervention (see Supplementary material online, Figure S5).

Figure 1A shows that there is no difference in the primary efficacy endpoint associated with loss-of-function allele carrier status in the clopidogrel or placebo arms; Figure 1B shows the same for the secondary efficacy endpoint. Figure 1C shows no difference in severe or moderate GUSTO bleeding associated with loss-of-function allele carrier status; however, Figure 1D shows that, for the endpoint of all GUSTO bleeding, loss-of-function allele carrier status is associated with significantly less bleeding in clopidogrel-treated patients (HR: 0.80, 95% CI: 0.69–0.93, P = 0.003), though not in placebo patients. In clopidogrel-treated patients, the rate of any bleeding was 36.1% (240/665) in carriers vs. 42.5% (681/1601) in non-carriers. The interaction term for loss-of-function carrier status and treatment with clopidogrel resulting in less bleeding was significant (P = 0.023).

Figure 2A shows that there is no difference in the primary efficacy endpoint associated with gain-of-function allele carrier status in the clopidogrel or placebo arms; Figure 2B shows the same for the secondary efficacy endpoint. Figure 2C shows no difference in severe or moderate GUSTO bleeding associated with gain-of-function allele carrier status, though, of course, there is more bleeding noted in the clopidogrel vs. placebo patients; Figure 2D shows the same for all GUSTO bleeding.

Figure 3 shows no statistically significant interaction of metabolizer phenotype on either the primary or secondary efficacy or bleeding endpoints for clopidogrel vs. placebo. When examining the clopidogrel arm alone, the poor metabolizers had a higher rate of the primary efficacy endpoint compared with all other phenotypes (HR: 2.27, 95% CI: 1.06–4.85, P = 0.035), though the number of events in the poor metabolizers was low (seven events). When examining the placebo arm alone, the poor metabolizers also had a higher rate of the primary efficacy endpoint compared with all other phenotypes (HR: 1.59, 95% CI: 0.59–4.29, P = 0.36), though the number of events in the poor metabolizers was again low (four events). Importantly, the interaction term for the clopidogrel vs. placebo effect on the primary efficacy endpoint as a function of metabolizer phenotype was not statistically significant, P = 0.21. Similarly, the interaction term for the secondary efficacy endpoint was not significant, P = 0.55.

### Discussion

The results of this analysis do not show an effect of CYP2C19 genotype on ischaemic outcomes in clopidogrel-treated vs. placebo-treated patients in a population with either clinically evident cardiovascular disease or multiple risk factors. Loss-of-function allele carriers did not have higher rates of ischaemic outcomes, nor did gain-of-function allele carriers have a lower rate of ischaemic outcomes. The overall CHARISMA trial did not find a benefit of dual anti-platelet therapy in a very stable patient population and, similarly, there was no benefit as a function of genotype in the present analysis. These results differ from several previous studies of stented patients showing an adverse association with

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<th>Table 1 Baseline characteristics and metabolizer phenotypes of patients of European ancestry who were genotyped</th>
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Figure 1 Kaplan–Meier curves for the primary (A) and secondary (B) efficacy endpoints for loss-of-function allele carriers vs. non-carriers in the clopidogrel- and placebo-treated patients and Kaplan–Meier curves for GUSTO severe and moderate bleeding (C) and all GUSTO bleeding (D) for loss-of-function allele carriers vs. non-carriers in the clopidogrel- and placebo-treated patients. Analyses are for patients of European ancestry.
Figure 2: Kaplan–Meier curves for the primary (A) and secondary (B) efficacy endpoints for gain-of-function allele carriers vs. non-carriers in the clopidogrel- and placebo-treated patients and Kaplan-Meier curves for GUSTO severe and moderate bleeding (C) and all GUSTO bleeding (D) for gain-of-function allele carriers vs. non-carriers in the clopidogrel- and placebo-treated patients. Analyses are for patients of European ancestry.
loss-of-function alleles. In the present analysis, gain-of-function carriers did not have more bleeding than non-carriers with clopidogrel treatment. However, loss-of-function carriers did have significantly less overall bleeding than non-carriers with clopidogrel therapy.

Loss-of-function carriers would be expected to have lower levels of clopidogrel active metabolite and gain-of-function carriers would be expected to have higher levels of active metabolite. However, data suggest that there is not necessarily a linear correlation between active drug levels and measured anti-platelet response, nor between measured anti-platelet response and anti-thrombotic efficacy or bleeding propensity.\(^{19,20}\) Additionally, CYP2C19 is not the only pathway involved with clopidogrel activation, as demonstrated by the fact that poor metabolizers are still able to produce clopidogrel active metabolite, albeit at much reduced levels. Furthermore, although variants in CYP2C19 represent the only genome-wide significant marker of clopidogrel’s platelet response that has been identified to date, further studies are needed to determine whether other gene variants might interfere with this drug’s anti-platelet action.\(^{15,16,21}\) Clinical factors, such as whether a patient has activated platelets due to an acute coronary syndrome, a newly placed stent, diabetes mellitus, smoking, or obesity, for example, may influence the response to anti-platelet therapy to a significant extent.\(^{22}\) Also, the pharmacodynamic effect of clopidogrel is influenced by other drugs that are metabolized by the CYP450 system. In the current study, the potential influence of drug–drug interactions (such as due to proton pump inhibitors) in carriers of loss-of-function or gain-of-function alleles on the clinical outcome was not analysed.

It is difficult to fully reconcile the relative protection from bleeding complications with lack of increase in ischaemic outcomes among the patients with loss-of-function CYP2C19 alleles. One would have expected these two effects to track together, as the reduction in bleeding strongly suggests less anti-platelet effect of the drug. There are multiple possible explanations for the unanticipated divergence, likely tied to the clinical features of the cohort under study. Notably, the results regarding lack of effect of CYP2C19 loss-of-function alleles seen here are concordant with the genetic analyses from the CURE and ACTIVE studies, and all three of these studies differ with respect to patient demographics (particularly with respect to coronary stenting) compared with the earlier reports that demonstrated a relationship to the ischaemic outcome.\(^{2,3,12–14,23–25}\)

One important limitation to this analysis is that genotyped patients differed from non-genotyped patients with respect to their baseline demographics, such that the subgroup reported on here is not likely to be representative of the overall cohort. However, there is no reason to expect that the distribution of

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**Figure 3** Hazard ratios for clopidogrel vs. placebo for efficacy and bleeding endpoints as a function of metabolizer phenotype. Analyses are for patients of European ancestry and are adjusted for age and sex.
Supplementary material

Supplementary material is available at European Heart Journal online.

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