

# Genetic variants in *ABCB1* and *CYP2C19* and cardiovascular outcomes after treatment with clopidogrel and prasugrel in the TRITON–TIMI 38 trial: a pharmacogenetic analysis



Jessica L Mega\*, Sandra L Close\*, Stephen D Wiviott, Lei Shen, Joseph R Walker, Tabassome Simon, Elliott M Antman, Eugene Braunwald, Marc S Sabatine

## Summary

**Background** Clopidogrel and prasugrel are subject to efflux via P-glycoprotein (encoded by *ABCB1*, also known as *MDR1*). *ABCB1* polymorphisms, particularly 3435C→T, may affect drug transport and efficacy. We aimed to assess the effect of this polymorphism by itself and alongside variants in *CYP2C19* on cardiovascular outcomes in patients treated with clopidogrel or prasugrel in TRITON–TIMI 38. We also assessed the effect of genotype on the pharmacodynamic and pharmacokinetic properties of these drugs in healthy individuals.

**Methods** We genotyped *ABCB1* in 2932 patients with acute coronary syndromes undergoing percutaneous intervention who were treated with clopidogrel (n=1471) or prasugrel (n=1461) in the TRITON–TIMI 38 trial. We evaluated the association between *ABCB1* 3435C→T and rates of the primary efficacy endpoint (cardiovascular death, myocardial infarction, or stroke) until 15 months. We then assessed the combined effect of *ABCB1* 3435C→T genotype and reduced-function alleles of *CYP2C19*. 321 healthy individuals were also genotyped, and we tested the association of genetic variants with reduction in maximum platelet aggregation and plasma concentrations of active drug metabolites.

**Findings** In patients treated with clopidogrel, *ABCB1* 3435C→T genotype was significantly associated with the risk of cardiovascular death, myocardial infarction, or stroke (p=0.0064). TT homozygotes had a 72% increased risk of the primary endpoint compared with CT/CC individuals (Kaplan–Meier event rates 12.9% [52 of 414] vs 7.8% [80 of 1057 participants]; HR 1.72, 95% CI 1.22–2.44, p=0.002). *ABCB1* 3435C→T and *CYP2C19* genotypes were significant, independent predictors of the primary endpoint, and 681 (47%) of the 1454 genotyped patients taking clopidogrel who were either *CYP2C19* reduced-function allele carriers, *ABCB1* 3435 TT homozygotes, or both were at increased risk of the primary endpoint (HR 1.97, 95% CI 1.38–2.82, p=0.0002). In healthy participants, 3435 TT homozygotes had an absolute reduction in maximum platelet aggregation with clopidogrel that was 7.3 percentage points less than for CT/CC individuals (p=0.0127). *ABCB1* genotypes were not significantly associated with clinical or pharmacological outcomes in patients with an acute coronary syndrome or healthy individuals treated with prasugrel, respectively.

**Interpretation** Individuals with the *ABCB1* 3435 TT genotype have reduced platelet inhibition and are at increased risk of recurrent ischaemic events during clopidogrel treatment. In patients with acute coronary syndromes who have undergone percutaneous intervention, when both *ABCB1* and *CYP2C19* are taken into account, nearly half of the population carries a genotype associated with increased risk of major adverse cardiovascular events while on standard doses of clopidogrel.

**Funding** Daiichi Sankyo Company Ltd and Eli Lilly and Company.

## Introduction

In patients presenting with acute coronary syndromes and in those undergoing percutaneous coronary interventions with stenting, dual antiplatelet treatment with aspirin and the thienopyridine clopidogrel is the guideline-approved standard of care.<sup>1,2</sup> As such, clopidogrel is one of the most frequently prescribed drugs worldwide. However, the pharmacodynamic response to clopidogrel varies substantially between patients,<sup>3</sup> and individuals with low platelet inhibition during treatment with clopidogrel are at increased risk of cardiovascular events.<sup>4</sup> Prasugrel is a third-generation thienopyridine that achieves greater platelet inhibition with less variability between patients than does clopidogrel.<sup>5</sup> In the Trial to Assess Improvement in

Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis in Myocardial Infarction (TRITON–TIMI) 38, treatment with prasugrel compared with clopidogrel resulted in a significantly lower rate of ischaemic events and more bleeding.<sup>6</sup>

Both clopidogrel and prasugrel are prodrugs that need intestinal absorption and subsequent biotransformation to active metabolites by cytochrome P450 enzymes. In several studies, reduced-function genetic variants in *CYP2C19* (located on chromosome 10) have been associated with reduced concentrations of active drug metabolite, diminished platelet inhibition, and higher rates of adverse cardiovascular events in the setting of treatment with clopidogrel, but not prasugrel.<sup>7–15</sup> To that end, the US Food and Drug Administration has

Published Online  
August 29, 2010  
DOI:10.1016/S0140-6736(10)61273-1

See Online/Articles  
DOI:10.1016/S0140-6736(10)61274-3

See Online/Comment  
DOI:10.1016/S0140-6736(10)61313-X

\*Authors contributed equally

TIMI Study Group, Cardiovascular Division, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA (J L Mega MD, S D Wiviott MD, Prof E M Antman MD, Prof E Braunwald MD, M S Sabatine MD); Indiana University, Indianapolis, IN, USA (S L Close PhD); Eli Lilly and Company, Indianapolis, IN, USA (L Shen PhD, S L Close PhD); Daiichi Sankyo Inc, Edison, NJ, USA (J R Walker PharmD); and Assistance Publique–Hôpitaux de Paris, UPMC-Paris06, France (Prof T Simon MD)

Correspondence to:  
Dr Jessica L Mega or  
Dr Marc S Sabatine, Brigham and Women's Hospital, TIMI Study Group, Cardiovascular Division, 350 Longwood Ave, Boston, MA 02115, USA  
jmega@partners.org  
msabatine@partners.org

incorporated *CYP2C19* genetic information into the updated clopidogrel label in the form of a boxed warning noting that carriers of two reduced-function *CYP2C19* alleles have a diminished response to standard doses of clopidogrel.

Additionally, a key protein involved in thienopyridine absorption is the efflux pump P-glycoprotein, which is encoded by *ABCB1* (also known as *MDR1*, located on chromosome 7). P-glycoprotein is an ATP-dependent efflux pump that transports various molecules across extracellular and intracellular membranes. It is expressed, among other places, on intestinal epithelial cells, where increased expression or function can affect bioavailability of drugs that are substrates. Previous research suggests that when treated with clopidogrel, individuals with genetic variants in *ABCB1* (specifically those who are TT homozygotes for the 3435C→T variant) have reduced concentrations of the active drug metabolite<sup>6</sup> and increased rates of adverse clinical outcomes.<sup>10</sup> Further investigation into the effect of this polymorphism on outcomes in patients treated with clopidogrel, the effect in relation to *CYP2C19* reduced-function variants, and the effect in those treated with the third-generation thienopyridine prasugrel is needed.

We genotyped a subset of patients in the TRITON–TIMI 38 trial who provided samples for genetic analysis with the aim of assessing the association between the *ABCB1* 3435C→T polymorphism and adverse cardiovascular outcomes during treatment with clopidogrel or prasugrel. To obtain supporting pharmacological data, *ABCB1* genotyping was also done in healthy individuals in whom platelet inhibition and drug concentrations were measured in response to clopidogrel or prasugrel. We also assessed the contribution of the *ABCB1* 3435C→T polymorphism in the context of *CYP2C19* status to elucidate the independent contribution of variants in these two genes.

## Methods

### Patients

The design and primary results of the TRITON–TIMI 38 trial have been described previously.<sup>6</sup> Patients with acute coronary syndromes undergoing planned percutaneous coronary interventions were randomly allocated to treatment with clopidogrel (300 mg loading dose followed by 75 mg daily) or prasugrel (60 mg loading dose followed by 10 mg daily) for up to 15 months. We undertook this pharmacogenetic analysis in a TRITON–TIMI 38 genetic substudy that included 2932 patients who both provided a genetic sample and had *ABCB1* genotyped (n=1471 for clopidogrel and n=1461 for prasugrel). This study was approved by institutional review boards, and written informed consent was obtained from all participants.

Healthy participants in seven studies (n=321) involving treatment with clopidogrel or prasugrel, or both, were included in the pharmacodynamic and pharmacokinetic analyses (webappendix pp 1 and 4).<sup>8</sup>

These studies were approved by institutional review boards, and written informed consent was obtained from all participants.

### Procedures

In the TRITON–TIMI 38 study, the prespecified primary efficacy endpoint was a composite of cardiovascular death, myocardial infarction, or stroke.<sup>6</sup> A secondary endpoint was definite or probable stent thrombosis as defined by the Academic Research Consortium.<sup>17</sup> Safety endpoints included TIMI major or minor bleeding not related to coronary artery bypass grafting. These outcomes were adjudicated by a clinical events committee unaware of treatment assignment.

Among the healthy participants, pharmacodynamic response was assessed by use of light transmission aggregometry in response to 20 μmol/L ADP, and was expressed as absolute reduction in maximum platelet aggregation from baseline to 4 h. Plasma concentrations of clopidogrel and prasugrel active drug metabolite were measured by liquid chromatography with mass spectrometry.<sup>18</sup> The area under the plasma concentration–time curve was analysed by the log-linear trapezoidal method from time of dose to the 4-h measurable concentration (AUC<sub>0–4</sub>).

Genotyping for *ABCB1* was completed with the Affymetrix Targeted Human DMET 1.0 Assay (Affymetrix, Santa Clara, CA, USA) and Illumina Infinium Beadchip Assay (Illumina, San Diego, CA, USA) to minimise missing data.<sup>19</sup> On the basis of previous studies,<sup>10,16</sup> the main variant of interest was 3435C→T (rs1045642), and participants were classified as homozygous for the C allele (CC), heterozygous (CT), or homozygous for the T allele (TT). Since some in-vitro studies have also assessed a haplotype consisting of 3435C→T and two other *ABCB1* variants, 2677G→T/A (rs2032582) and 1236C→T (rs1128503), we also genotyped these polymorphisms (webappendix p 1).<sup>20</sup> Genotypes were in Hardy-Weinberg equilibrium (webappendix p 5).

Because genetic variation in *CYP2C19* has been associated with pharmacological response and cardiovascular outcomes in patients taking clopidogrel,<sup>8–14,21–24</sup> we assessed the combined effect of genetic variants in *CYP2C19* and *ABCB1* 3435C→T. For *CYP2C19*, participants were genotyped and divided into two groups on the basis of whether they had at least one reduced-function allele (termed carriers) or no reduced-function alleles (termed non-carriers).<sup>8</sup>

### Statistical analysis

Analyses were done with SAS (version 9.1) and S-PLUS (version 8.0). On the basis of previous studies, the primary objective was to investigate the association between *ABCB1* 3435C→T genotypes and rates of the primary efficacy endpoint in patients in the TRITON–TIMI 38 study. For consistency with the main trial analyses, the Gehan-

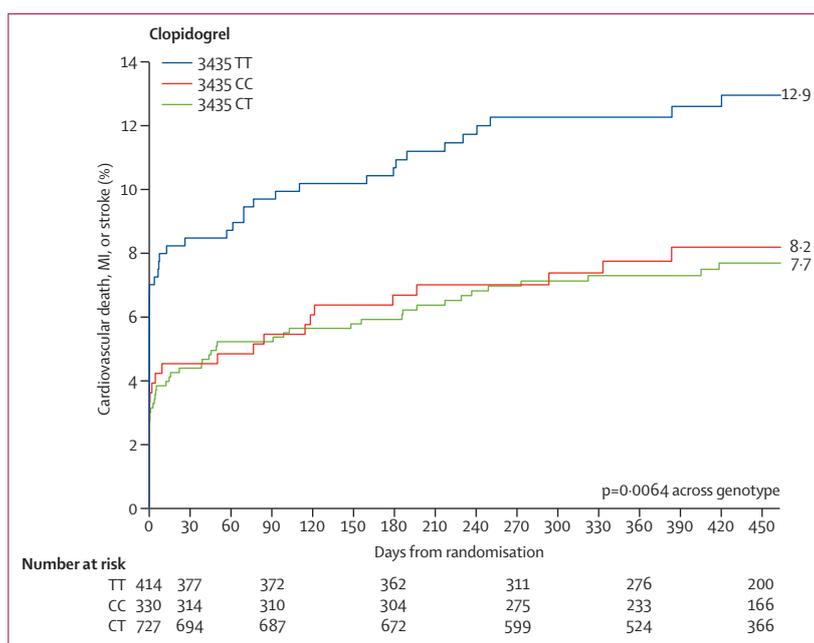
Wilcoxon test was used for the primary efficacy endpoint and log-rank for other endpoints. Event rates were expressed as Kaplan-Meier estimates at 15 months.<sup>25</sup> Hazard ratios and 95% CIs were calculated on the basis of Cox proportional hazards regression models with clinical syndrome (non-ST-elevation vs ST-elevation acute coronary syndromes) as a stratification factor. Two-sided p values were calculated to test for differences in cardiovascular event rates between patients stratified by genotype. If a significant association for the primary efficacy evaluation was identified in patients treated with clopidogrel, additional efficacy endpoints were also tested, including the hazards for the components of the composite primary endpoint, the primary endpoint at 30 days, and stent thrombosis. In terms of safety endpoints, TIMI major or minor bleeding not related to coronary artery bypass grafting was assessed until 15 months. Parallel analyses were done for patients allocated treatment with prasugrel.

To elucidate further the contribution of *ABCB1* variants, the associations between additional *ABCB1* genotypes (2677G→T/A, 1236C→T, and the haplotype that included 1236C→T, 2677G→T/A, and 3435C→T; webappendix p 1) and cardiovascular outcomes were tested in each treatment group, with the same methods. We then evaluated 3435C→T in the context of *CYP2C19*. We created Cox proportional hazards regression models examining 3435C→T that were adjusted for *CYP2C19* reduced-function allele status as well as models that stratified patients into four groups on the basis of 3435C→T genotype and *CYP2C19* reduced-function allele status. We did a meta-analysis that included results from FAST-MI<sup>10</sup> by combining HRs for each study using a fixed-effects model with weighting based on inverse variance.

We tested the associations between genetic variation and pharmacodynamic and pharmacokinetic parameters using likelihood ratio tests based on linear regression or mixed-effects models. The primary outcomes were platelet inhibition (change in maximum platelet aggregation) and exposure to active drug metabolite [ $\log(\text{AUC}_{0-\infty})$ ]. The models contained subject as a random effect when repeated measures were present, genotype as the predictor of main interest, and other fixed effects including study, dose, and ethnic origin, and for pharmacodynamics, maximum platelet aggregation at baseline. Other demographic variables, including bodyweight, age, sex, and smoking, were included as judged to be appropriate for each drug, as has been done previously.<sup>8</sup> Additional models were also created with adjustment for *CYP2C19*.

### Role of the funding source

The TRITON-TIMI 38 genetic study was designed and undertaken in collaboration between the TIMI Study Group and the sponsors. The academic authors directed and had access to all the analyses and the full clinical database, wrote all drafts of the report, decided to publish



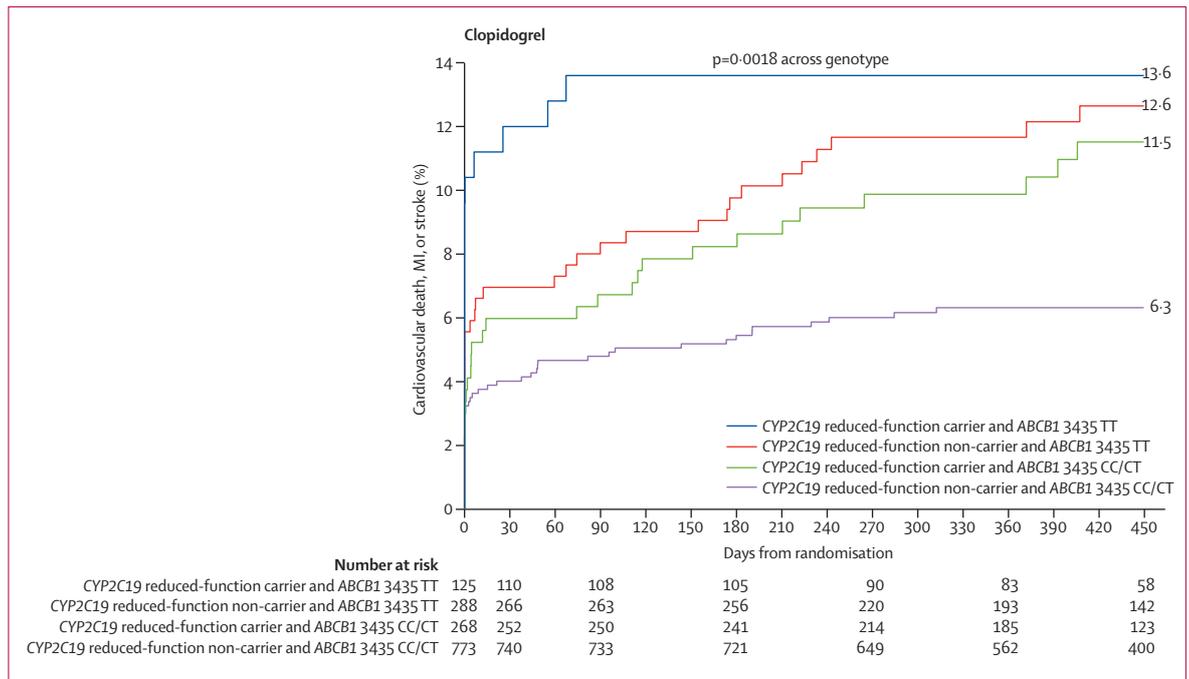
**Figure 1: *ABCB1* 3435C→T and cardiovascular outcomes in patients treated with clopidogrel**  
Cumulative risk of cardiovascular death, myocardial infarction (MI), or stroke for each genotype, with a p value across genotype.

the results, and vouch for the accuracy and completeness of the data.

### Results

For the 2932 patients in the TRITON-TIMI 38 genetic substudy, the average age was 60.2 (SD 10.9) years, 831 (28%) were women, 2064 (70%) presented with non-ST-elevation acute coronary syndromes, and 868 (30%) presented with ST-elevation myocardial infarction. For *ABCB1* 3435C→T, 804 (27%) participants in the genetic study population were TT homozygotes, 1459 (50%) CT heterozygotes, and 669 (23%) CC homozygotes. Baseline characteristics in the TRITON-TIMI 38 trial by 3435C→T genotype are shown in the webappendix p 6.

In patients in the TRITON-TIMI 38 genetic substudy who were allocated to treatment with clopidogrel (n=1471), 3435C→T genotype was significantly associated with risk of the primary endpoint of cardiovascular death, myocardial infarction, or stroke (p=0.0064; figure 1). TT homozygotes were at significantly increased risk compared with CC individuals (HR 1.69, 95% CI 1.05–2.72); CT heterozygotes were at similar risk to CC individuals (HR 0.94, 0.58–1.51). Thus, TT homozygotes for 3435C→T had a 72% increased risk of the primary endpoint compared with CT/CC individuals (Kaplan-Meier event rates 12.9% [52 of 414] vs 7.8% [80 of 1057 participants]; HR 1.72, 95% CI 1.22–2.44, p=0.002) when assessed until 15 months. Among 3435 TT versus CT/CC patients, the HR for cardiovascular death was 1.63 (Kaplan-Meier event rates 1.3% [five of 414] vs 0.9% [eight of 1057]; 95% CI 0.53–4.98, p=0.388), that for non-fatal myocardial infarction was 1.82 (12.0% [48 of 414] vs 6.8% [70 of 1057]; 1.26–2.62, p=0.0013), and that



**Figure 2: ABCB1 3435C→T, CYP2C19, and cardiovascular outcomes in patients treated with clopidogrel**  
Cumulative risk of cardiovascular death, myocardial infarction (MI), or stroke for the four genotype categories, with the p value across genotype category.

for non-fatal stroke was 1.66 (0.6% [two of 414] vs 0.3% [three of 1057 participants]; 0.28–9.93, p=0.575). Moreover, the increased risk was evident by 30 days, by which time the risk of the primary endpoint for the 3435 TT homozygotes was roughly twice as high as that for CT/CC individuals (Kaplan-Meier event rates 8.5% [35 of 414] vs 4.5% [47 of 1057 participants]; HR 1.96, 95% CI 1.26–3.03, p=0.0022).

Rates of stent thrombosis did not differ significantly between 3435 TT and CT/CC individuals (Kaplan-Meier event rates 1.3% [five of 396] vs 1.3% [12 of 1004 participants]; HR 1.07, 95% CI 0.38–3.04, p=0.9). Rates of TIMI major or minor bleeding not related to coronary artery bypass grafting did not differ significantly by genotype (Kaplan-Meier event rates 3.6% [15 of 414] TT homozygotes vs 2.5% [26 of 1052] CT/CC individuals; HR 1.49, 95% CI 0.79–2.82, p=0.214).

To elucidate further the contribution of ABCB1 variants in the setting of treatment with clopidogrel, two other ABCB1 polymorphisms were explored: 2677G→T/A and 1236C→T. Neither variant was significantly associated with risk of cardiovascular death, myocardial infarction, or stroke (webappendix pp 2, 7, and 8). We constructed haplotypes using the three loci (1236C→T, 2677G→T/A, and 3435C→T) and did not identify associations beyond what was seen for 3435C→T alone (webappendix p 2).

In a model containing both ABCB1 3435C→T genotype and CYP2C19 reduced-function allele carrier status in patients in the TRITON-TIMI 38 genetic substudy treated with clopidogrel, both variants were significant, independent predictors of cardiovascular death, myocardial

infarction, or stroke (ABCB1 3435 TT vs CT/CC, HR 2.01, 95% CI 1.30–3.11, p=0.0017; CYP2C19 reduced-function allele carrier vs non-carrier, HR 1.77, 1.11–2.80, p=0.0155). When the participants were divided into four groups on the basis of ABCB1 3435C→T genotype and CYP2C19 status (figure 2), the 773 patients (53% of 1454 genotyped) who did not carry at-risk genotypes in either gene had a low rate of cardiovascular death, myocardial infarction, or stroke at 15 months (Kaplan-Meier event rate 6.3%, 48 of 773 participants). By contrast, event rates were significantly higher in the 681 patients (47% of 1454 genotyped) who were either carriers of a CYP2C19 reduced-function allele only (Kaplan-Meier event rate 11.5%, 29 of 268 participants), ABCB1 3435 TT homozygotes only (Kaplan-Meier event rate 12.6%, 35 of 288 participants), or both (Kaplan-Meier event rate 13.6%, 17 of 125 participants) (pooled HR 1.97, 95% CI 1.38–2.82, p=0.0002).

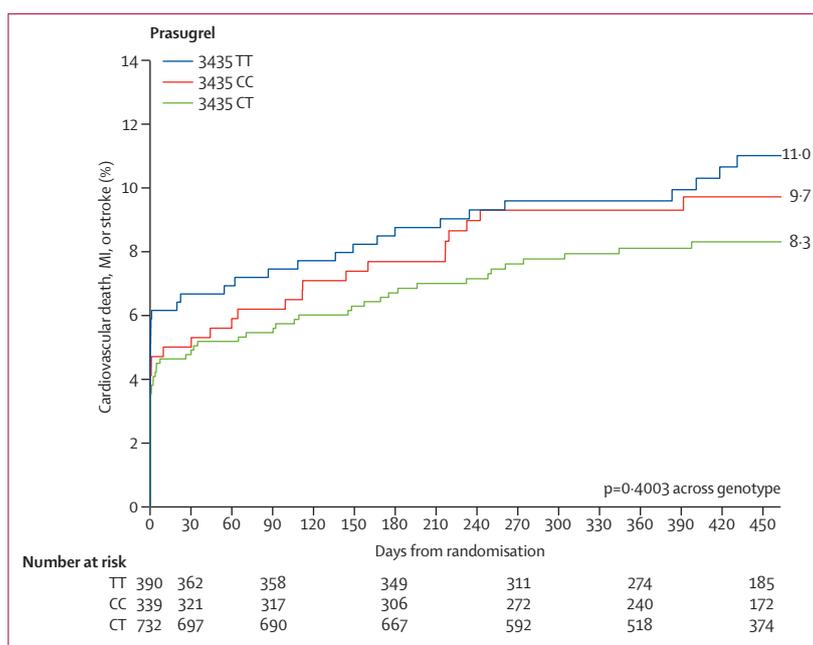
When we examined the early timepoint of 30 days, individuals who did not carry either at-risk variant were at low risk (Kaplan-Meier event rate 4.0%, 31 of 773 participants), those who were either ABCB1 3435 TT homozygotes or carriers of a CYP2C19 reduced-function allele were at intermediate risk (Kaplan-Meier event rate 7.0% [20 of 288 participants] for TT homozygotes and 6.0% [16 of 268 participants] for carriers of a reduced-function allele; pooled HR 1.64, 95% CI 1.01–2.65, p=0.0441 vs carriers of neither), and individuals who were both CYP2C19 reduced-function allele carriers and ABCB1 3435 TT homozygotes were at high risk (Kaplan-Meier event rate 12.0%, 15 of 125 participants; HR 3.16, 95% CI 1.71–5.85, p=0.0003 vs carriers of neither).

There was no significant association between *ABCB1* 3435C→T genotype and risk of cardiovascular death, myocardial infarction, or stroke among patients in the TRITON-TIMI 38 genetic substudy who had been allocated to prasugrel ( $n=1461$ ; figure 3 and webappendix p 2). Specifically, TT homozygotes did not have a significantly higher risk of the primary efficacy endpoint of cardiovascular death, myocardial infarction, or stroke than did CT/CC carriers (Kaplan-Meier event rates 11·0% [41 of 390] vs 8·7% [91 of 1071 participants]; HR 1·25, 95% CI 0·86–1·81,  $p=0\cdot235$ ) when assessed until 15 months. Rates of TIMI major or minor bleeding not related to coronary artery bypass grafting did not differ significantly by *ABCB1* 3435C→T genotype (webappendix p 2). In terms of other *ABCB1* variants, the 2677G→T/A and 1236C→T genotypes overall were not significantly associated with risk of the primary efficacy endpoint in patients treated with prasugrel, although there was a non-significant trend for 2677 TT homozygotes versus CT/CC individuals to be at increased risk (Kaplan-Meier event rates 11·8% [29 of 253] vs 8·8% [94 of 1104 participants]; HR 1·38, 95% CI 0·91–2·09,  $p=0\cdot1290$ ; webappendix pp 3, 9, and 10). When we divided patients on the basis of *ABCB1* 3435C→T genotypes and *CYP2C19* status, rates of cardiovascular death, myocardial infarction, or stroke at 15 months were similar in the four groups ( $p=0\cdot4851$ , figure 4).

In healthy participants treated with clopidogrel, *ABCB1* 3435 TT homozygotes had a diminished pharmacodynamic effect, with an absolute reduction in maximum platelet aggregation in response to a clopidogrel loading dose that was 7·3 percentage points lower (ie, less platelet inhibition) than that seen in CT/CC individuals ( $p=0\cdot0127$ ). After adjustment for *CYP2C19* genotype, the response was 6·6 percentage points lower ( $p=0\cdot022$ ). The pharmacodynamic effects of clopidogrel on TT carriers were discernible only after a loading dose (for both 300 mg and 600 mg); no significant association was identified during maintenance dosing. There was no significant association between 3435C→T genotype and exposure to clopidogrel active metabolite concentrations. In prasugrel-treated individuals, 3435C→T genotype was not significantly associated with platelet response (1·3 percentage points higher;  $p=0\cdot4345$ ) or exposure to prasugrel's active metabolite. There was no relation between genotype status for either 2677G→T/A or 1236C→T and pharmacodynamic outcomes in patients treated with clopidogrel or prasugrel.

## Discussion

The pharmacological and clinical response to clopidogrel varies widely between patients, and genetic variants in *CYP2C19* have been shown to affect the response. P-glycoprotein is important in drug transport, and pharmacogenetic interactions with various classes of drugs have been suggested.<sup>26</sup> Our findings show that TT

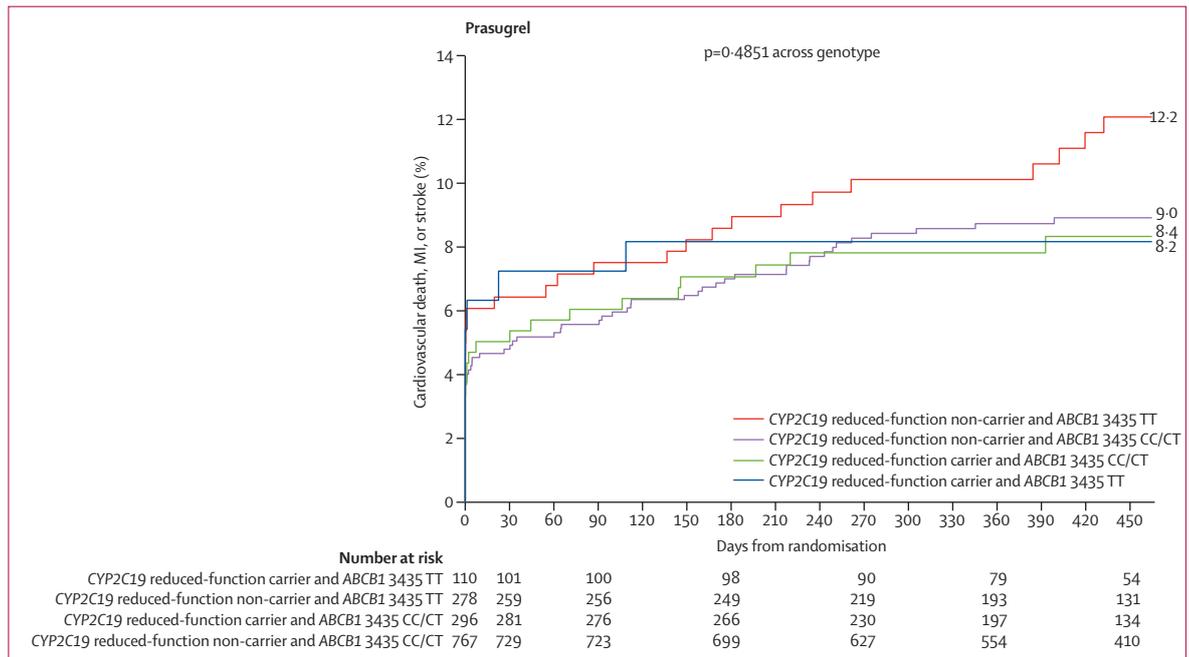


**Figure 3:** *ABCB1* 3435C→T and cardiovascular outcomes in patients treated with prasugrel

Cumulative risk of cardiovascular death, myocardial infarction (MI), or stroke for each genotype, with a p value across genotype.

homozygotes for the 3435C→T variant in *ABCB1* (27% of the study population), as compared with CT/CC individuals, had reduced platelet inhibition with a clopidogrel loading dose in a healthy study population and a significantly increased risk of adverse cardiovascular events during treatment with clopidogrel in patients with acute coronary syndromes undergoing percutaneous coronary intervention. When we considered *ABCB1* 3435C→T genotype in the context of *CYP2C19* reduced-function allele status in patients treated with clopidogrel, we showed that variants in the two genes offered significant, independent information about the risk of cardiovascular death, myocardial infarction, or stroke. Conversely, there were no significant associations between the *ABCB1* variants tested and the response to prasugrel.

*ABCB1* encodes the P-glycoprotein efflux transporter. Clopidogrel is a P-glycoprotein substrate, and inhibition of P-glycoprotein affects the bioavailability of clopidogrel.<sup>16</sup> The 3435C→T variant in *ABCB1* is one of the most studied polymorphisms in pharmacogenetic research, and has been associated with altered disposition of several drugs.<sup>26</sup> Although a genome-wide association study identified only *CYP2C19* as being associated with the pharmacodynamic response to clopidogrel, that study showed that platelet response to clopidogrel was highly heritable and was not entirely accounted for by *CYP2C19* status, suggesting that additional genetic variants might be relevant. In a study of patients treated with clopidogrel after elective percutaneous coronary intervention, 3435 TT homozygotes had significantly lower active clopidogrel metabolite concentrations than did CT/CC



**Figure 4: ABCB1 3435C→T, CYP2C19, and cardiovascular outcomes in patients treated with prasugrel**  
 Cumulative risk of cardiovascular death, myocardial infarction (MI), or stroke for the four genotype categories, with the p value across genotype category.

individuals, suggesting increased intestinal efflux possibly mediated by higher P-glycoprotein expression associated with the 3435 TT genotype.<sup>16</sup> Although evidence on P-glycoprotein expression and activity is inconsistent, mRNA expression in duodenal enterocytes has been reported to be two-to-three-times higher for the ABCB1 3435 TT genotype than for either the CC or CT genotype.<sup>27–29</sup> In the healthy participants in our analysis, TT homozygotes had an absolute reduction in maximum platelet aggregation after a loading dose of clopidogrel that was 7.3 percentage points lower (ie, decreased platelet inhibition) than in CC or CT individuals. Although we did not record a significant association between 3435C→T genotype and pharmacokinetic data, other researchers have shown this relation, and the differences in results could be related to patients, methods, and single-centre versus multicentre study design.

In terms of clinical outcomes, we showed that ABCB1 3435 TT homozygotes had a 72% increased risk of adverse cardiovascular events compared with CT/CC individuals in the setting of treatment with clopidogrel in TRITON-TIMI 38. Likewise, in a previous study in patients receiving clopidogrel after an acute myocardial infarction, those who were 3435 TT homozygotes had an increase of about 70% in cardiovascular events during follow-up.<sup>10</sup> In that previous study, however, 3435 CT heterozygotes were also at increased risk of adverse cardiovascular events, albeit less so than were TT homozygotes; the differences in the findings could be attributable to the patient populations. Combination of the results of the previous study and our findings yielded an apparent graded allele-dose response

with an HR for adverse cardiovascular events of 1.29 (95% CI 0.99–1.69) for 3435 CT versus CC individuals and a HR of 1.70 (1.28–2.26) for 3435 TT versus CC individuals. Incorporation of clinical data from other studies will be helpful to further refine the risk estimates. In our analysis, 2677G→T/A and 1236C→T genotypes did not add additional significant information. Nonetheless, further basic genetic pharmacology studies could be helpful to further define the actual functional ABCB1 variants and the most appropriate genetic model with respect to response to clopidogrel.

In our study, assessment of the contribution of ABCB1 variants in the context of CYP2C19 showed that variants in the two genes offered complementary information about cardiovascular risk. When we divided patients into four groups on the basis of ABCB1 3435C→T and CYP2C19 reduced-function allele status, rates of cardiovascular death, myocardial infarction, or stroke until 15 months were nearly twice as high in the study population who were either carriers of a CYP2C19 reduced-function allele, 3435 TT homozygotes, or both, compared with individuals who did not carry either. Moreover, when both ABCB1 and CYP2C19 were taken into account, in this population of patients with an acute coronary syndrome undergoing percutaneous coronary intervention, nearly half of the population carried a genotype associated with increased risk of major adverse cardiovascular events during treatment with standard doses of clopidogrel.

In patients taking prasugrel in TRITON-TIMI 38, ABCB1 3435C→T polymorphisms were not significantly associated with cardiovascular outcomes. Likewise, in

healthy participants, no associations between the 3435C→T variant and pharmacokinetic and pharmacodynamic outcomes were seen with prasugrel. The rapid metabolism of prasugrel might mitigate the genetic effect of *ABCB1* 3435C→T polymorphisms, even though the drug is subject to the P-glycoprotein system. Among participants treated with prasugrel, there was a non-significant trend towards 2677 TT homozygotes having higher rates of adverse cardiovascular events compared with the rest of the population. No association was seen with 2677G→T/A and the pharmacological data. Future studies will assist in further examination of these exploratory findings.

There are several limitations to this analysis. First, few non-Caucasian individuals were included in these studies, and future investigations in other populations would be useful. Second, because of the sample handling and the need for repeat measurements for the pharmacokinetic and pharmacodynamic assessments, these investigations were done in healthy individuals, not in the acute clinical trial study population. Third, in our clinical outcomes study, patients treated with clopidogrel received a 300 mg loading dose and 75 mg daily maintenance dose, and patients treated with prasugrel received a 60 mg loading dose and 10 mg daily maintenance dose; we cannot comment on the effect of *ABCB1* genetic variants in patients receiving other doses of these drugs. Fourth, the number of bleeding and stent thrombosis events was small, and our analysis had restricted power to detect an association between the tested *ABCB1* variants and these outcomes. Additional studies that include more such events will be particularly important to further elucidate the relations between *ABCB1* genetic variants and outcomes. Finally, there might be other genetic variants affecting the association between treatment with clopidogrel and cardiovascular outcomes.

In conclusion, we found that *ABCB1* 3435 TT homozygotes had an increased risk of adverse cardiovascular outcomes during treatment with clopidogrel after an acute coronary syndrome and percutaneous coronary intervention. Thus, the association between *ABCB1* polymorphisms and ischaemic risk in patients treated with clopidogrel has been noted now in several pharmacological and clinical outcomes studies. Our analysis also shows that the pharmacogenetic effects of *ABCB1* 3435C→T are independent of and complementary to those of *CYP2C19*. As clinicians, professional societies, and patients integrate information about genetic factors affecting the response to thienopyridines, the roles of both *ABCB1* and *CYP2C19* should be considered.

#### Contributors

JLM and MSS conceived of and designed the research. SDW and EMA acquired the data. JLM, SLC, and MSS analysed and interpreted the data. LS did statistical analyses. JLM and MSS drafted the initial report. JRW and EB participated in funding and supervision. JLM, SLC, SDW, JRW, TS, EMA, EB, and MSS made critical revisions to the report for important intellectual content.

#### Conflicts of interest

The TIMI Study Group receives research grant support from Daiichi Sankyo, Eli Lilly, Sanofi-Aventis, Bristol-Myers Squibb, AstraZeneca, Schering-Plough/Merck, Johnson & Johnson, and Bayer Healthcare. Additionally, JLM is supported in part by grant K99/R00 HL098461-01 from the National Institutes of Health and reports consulting fees from Sanofi-Aventis, Bristol-Myers Squibb, and AstraZeneca. SDW reports consulting fees from Sanofi-Aventis, Bristol-Myers Squibb, AstraZeneca, ARENA, Medco, and Portola, and lecture fees for CME from Schering-Plough, Daiichi Sankyo, Eli Lilly, Novartis, and AstraZeneca. TS reports research grant support from Servier, Pfizer, Daiichi Sankyo, Eli Lilly, Sanofi-Aventis, AstraZeneca, and Caisse d'Assurance Maladie and consulting fees from Eli Lilly, Daiichi Sankyo, Sanofi-Aventis, Bristol-Myers Squibb, and AstraZeneca. EMA has no additional relationships to disclose. EB reports consulting fees from Daiichi Sankyo and lecture fees from Schering-Plough and Merck. MSS reports consulting fees from AstraZeneca, Bristol-Myers Squibb, Sanofi-Aventis, Daiichi Sankyo, and Eli Lilly and lecture fees from Bristol-Myers Squibb, Sanofi-Aventis, and Eli Lilly. JRW is an employee of Daiichi Sankyo and holds equity ownership or stock options therein. SLC is a former employee of Eli Lilly and holds equity ownership or stock options therein. LS is an employee of Eli Lilly and holds equity ownership or stock options therein.

#### References

- Anderson JL, Adams CD, Antman EM, et al. ACC/AHA 2007 guidelines for the management of patients with unstable angina/non-ST-Elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the 2002 Guidelines for the Management of Patients With Unstable Angina/Non-ST-Elevation Myocardial Infarction) developed in collaboration with the American College of Emergency Physicians, the Society for Cardiovascular Angiography and Interventions, and the Society of Thoracic Surgeons endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation and the Society for Academic Emergency Medicine. *J Am Coll Cardiol* 2007; **50**: e1–157.
- Kushner FG, Hand M, Smith SC Jr, et al. 2009 Focused updates: ACC/AHA Guidelines for the Management of Patients With ST-Elevation Myocardial Infarction (updating the 2004 Guideline and 2007 Focused Update) and ACC/AHA/SCAI Guidelines on Percutaneous Coronary Intervention (updating the 2005 Guideline and 2007 Focused Update): a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation* 2009; **120**: 2271–306.
- Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. *Circulation* 2003; **107**: 2908–13.
- Hochholzer W, Trenk D, Bestehorn HP, et al. Impact of the degree of peri-interventional platelet inhibition after loading with clopidogrel on early clinical outcome of elective coronary stent placement. *J Am Coll Cardiol* 2006; **48**: 1742–50.
- Wiviott SD, Trenk D, Frelinger AL, et al. Prasugrel compared with high loading- and maintenance-dose clopidogrel in patients with planned percutaneous coronary intervention: the Prasugrel in Comparison to Clopidogrel for Inhibition of Platelet Activation and Aggregation-Thrombolysis in Myocardial Infarction 44 trial. *Circulation* 2007; **116**: 2923–32.
- Wiviott SD, Braunwald E, McCabe CH, et al. Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med* 2007; **357**: 2001–15.
- Trenk D, Hochholzer W, Fromm MF, et al. Cytochrome P450 2C19 681G→A polymorphism and high on-clopidogrel platelet reactivity associated with adverse 1-year clinical outcome of elective percutaneous coronary intervention with drug-eluting or bare-metal stents. *J Am Coll Cardiol* 2008; **51**: 1925–34.
- Mega JL, Close SL, Wiviott SD, et al. Cytochrome p-450 polymorphisms and response to clopidogrel. *N Engl J Med* 2009; **360**: 354–62.
- Collet JP, Hulot JS, Pena A, et al. Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. *Lancet* 2009; **373**: 309–17.
- Simon T, Verstuyft C, Mary-Krause M, et al. Genetic determinants of response to clopidogrel and cardiovascular events. *N Engl J Med* 2009; **360**: 363–75.

- 11 Giusti B, Gori AM, Marcucci R, et al. Relation of cytochrome P450 2C19 loss-of-function polymorphism to occurrence of drug-eluting coronary stent thrombosis. *Am J Cardiol* 2009; **103**: 806–11.
- 12 Sibbing D, Stegheer J, Latz W, et al. Cytochrome P450 2C19 loss-of-function polymorphism and stent thrombosis following percutaneous coronary intervention. *Eur Heart J* 2009; **30**: 916–22.
- 13 Shuldiner AR, O'Connell JR, Bliden KP, et al. Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. *JAMA* 2009; **302**: 849–57.
- 14 Anderson JL, Mower CP, Horne BD, et al. Carriage of the *CYP2C19*\*2 allele increases one-year risk of myocardial infarction among recipients of drug-eluting stents treated with clopidogrel. *J Am Coll Cardiol* 2009; **53** (10 suppl A): A27.
- 15 Mega JL, Close SL, Wiviott SD, et al. Cytochrome P450 genetic polymorphisms and the response to prasugrel: relationship to pharmacokinetic, pharmacodynamic, and clinical outcomes. *Circulation* 2009; **119**: 2553–60.
- 16 Taubert D, von Beckerath N, Grimberg G, et al. Impact of P-glycoprotein on clopidogrel absorption. *Clin Pharmacol Ther* 2006; **80**: 486–501.
- 17 Wiviott SD, Braunwald E, McCabe CH, et al, for the TRITON-TIMI 38 Investigators. Intensive oral antiplatelet therapy for reduction of ischaemic events including stent thrombosis in patients with acute coronary syndromes treated with percutaneous coronary intervention and stenting in the TRITON-TIMI 38 trial: a subanalysis of a randomised trial. *Lancet* 2008; **371**: 1353–63.
- 18 Farid NA, McIntosh M, Garofolo F, et al. Determination of the active and inactive metabolites of prasugrel in human plasma by liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom* 2007; **21**: 169–79.
- 19 Dumauld C, Miao X, Daly TM, et al. Comprehensive assessment of metabolic enzyme and transporter genes using the Affymetrix Targeted Genotyping System. *Pharmacogenomics* 2007; **8**: 293–305.
- 20 Kimchi-Sarfaty C, Oh JM, Kim JW, et al. A “silent” polymorphism in the *MDR1* gene changes substrate specificity. *Science* 2007; **315**: 525–28.
- 21 Hulot JS, Bura A, Villard E, et al. Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. *Blood* 2006; **108**: 2244–47.
- 22 Fontana P, Hulot JS, De Moerloose P, Gaussem P. Influence of *CYP2C19* and *CYP3A4* gene polymorphisms on clopidogrel responsiveness in healthy subjects. *J Thromb Haemost* 2007; **5**: 2153–55.
- 23 Brandt JT, Close SL, Iturria SJ, et al. Common polymorphisms of *CYP2C19* and *CYP2C9* affect the pharmacokinetic and pharmacodynamic response to clopidogrel but not prasugrel. *J Thromb Haemost* 2007; **5**: 2429–36.
- 24 Gladding P, Webster M, Zeng I, et al. The pharmacogenetics and pharmacodynamics of clopidogrel response: an analysis from the PRINC (plavix response in coronary intervention) trial. *JACC Cardiovasc Interv* 2008; **1**: 620–27.
- 25 Wiviott SD, Antman EM, Gibson CM, et al. Evaluation of prasugrel compared with clopidogrel in patients with acute coronary syndromes: design and rationale for the TRIal to assess Improvement in Therapeutic Outcomes by optimizing platelet Inhibition with prasugrel Thrombolysis In Myocardial Infarction 38 (TRITON-TIMI 38). *Am Heart J* 2006; **152**: 627–35.
- 26 Zhou SF, Di YM, Chan E, et al. Clinical pharmacogenetics and potential application in personalized medicine. *Curr Drug Metab* 2008; **9**: 738–84.
- 27 Moriya Y, Nakamura T, Horinouchi M, et al. Effects of polymorphisms of *MDR1*, *MRP1*, and *MRP2* genes on their mRNA expression levels in duodenal enterocytes of healthy Japanese subjects. *Biol Pharm Bull* 2002; **25**: 1356–59.
- 28 Nakamura T, Sakaeda T, Horinouchi M, et al. Effect of the mutation (C3435T) at exon 26 of the *MDR1* gene on expression level of *MDR1* messenger ribonucleic acid in duodenal enterocytes of healthy Japanese subjects. *Clin Pharmacol Ther* 2002; **71**: 297–303.
- 29 Hoffmeyer S, Burk O, von Richter O, et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci USA* 2000; **97**: 3473–78.