No Association of ABCB1 C3435T Genotype With Clopidogrel Response or Risk of Stent Thrombosis in Patients Undergoing Coronary Stenting

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Background—The prodrug clopidogrel requires intestinal absorption by the efflux pump P-glycoprotein MDR1 (multidrug resistant-1), encoded by the ABCB1 gene. Prior studies suggested that a common and functional genetic variant (C3435T, rs1045642) within ABCB1 influences clopidogrel treatment efficacy; however, existing data are highly inconsistent, because other studies failed to replicate this postulated association. Thus, the aim of this study was to assess the association of ABCB1 C3435T genotypes with the antiplatelet efficacy of clopidogrel and the risk of stent thrombosis (ST) in large cohorts of clopidogrel-treated patients undergoing percutaneous coronary intervention.

Methods and Results—DNA samples from 1524 clopidogrel-treated patients undergoing percutaneous coronary intervention were genotyped for ABCB1 C3435T, and ADP-induced platelet aggregation was assessed in whole blood on a Multiplate analyzer. The clinical impact of the genetic variant was investigated by comparison of genotype frequencies in a registry of 66 cases with definite drug-eluting stent ST versus an ST-free control cohort (n=1408). Platelet aggregation values were similar across ABCB1 C3435T genotypes (P=0.73). No significant influence of ABCB1 C3435T genotypes on the occurrence of ST was found when ST case subjects were compared with control subjects (P=0.89).

Conclusions—ABCB1 C3435T genotypes did not influence the antiplatelet response to clopidogrel or the risk of ST in clopidogrel-treated patients undergoing percutaneous coronary intervention. Routine genotyping of ABCB1 C3435T polymorphisms should not be recommended for risk stratification in clopidogrel-treated patients undergoing percutaneous coronary intervention who are similar to those evaluated in the present study. (Circ Cardiovasc Interv. 2012;5:82-88.)

Key Words: ABCB1 protein, human ■ clopidogrel ■ genetics ■ stents ■ thrombosis ■ percutaneous coronary angioplasty ■ antiplatelet agents

For prevention of thrombotic events, including the occurrence of stent thrombosis (ST), dual-antiplatelet treatment with aspirin and a P2Y12 receptor antagonist such as clopidogrel is the recommended therapy in patients with acute coronary syndromes and in those undergoing percutaneous coronary interventions (PCIs). The antiplatelet effect of clopidogrel is characterized by considerable interindividual variability that depends on both genetic and nongenetic factors and by the fact that a significant proportion of patients show a high on-treatment platelet reactivity with a consecutively higher risk for ischemic events.

Clopidogrel, an inactive prodrug, requires intestinal absorption by the P-glycoprotein multidrug resistant-1 (MDR1) efflux transporter, encoded by the ABCB1 gene. After absorption, in vivo bioactivation is mediated via the cytochrome P450 (CYP) system to generate its active thiol metabolite, which targets the P2Y12 receptor on blood platelets.

Numerous studies have provided compelling evidence that genetic variants in genes encoding for CYP isoenzymes (specifically, within the CYP2C19 gene) affect both clopidogrel responsiveness and the clinical outcome of clopidogrel-treated patients undergoing PCI. In contrast to this, the influence of a common and functional genetic variant within the ABCB1 gene (C3435T, rs1045642) on both clopidogrel pharmacodynamics and the clinical outcome of clopidogrel-treated patients undergoing PCI was highly inconsistent across recent studies. Although some studies suggested an impact of the ABCB1 3435TT genotype (associated with low gene expression) on both clopidogrel responsive-
ness\(^3,15\) and the risk for thrombotic events in PCI-treated patients\(^2,15,16\) other studies failed to confirm this association\(^17–20\) or even reported a worse outcome for patients carrying the opposite genotype (\(ABCB1\) 3435CC).\(^21\) Of note, the majority of these studies were limited by the small number of subjects investigated,\(^18,19,22\) a low number of ST case subjects reported,\(^15,22\) or the fact that clinical outcome and platelet aggregation measures with regard to \(ABCB1\) C3435T genotypes were not assessed in parallel.\(^1,13,17–21,23\) Thus, the aim of the present study was to assess in parallel the association of \(ABCB1\) C3435T genotypes with both clopidogrel pharmacodynamics and the risk of ST in large cohorts of clopidogrel-treated patients undergoing PCI.

**WHAT IS KNOWN**

- Clopidogrel antiplatelet efficacy is subject to genetic variants in genes associated with absorption and bioactivation of the drug.
- The \(CYP2C19\)*/2 loss-of-function polymorphism is a major determinant of clopidogrel bioactivation.
- Conflicting data exist on \(ABCB1\) C3435T genotypes and their influence on clopidogrel antiplatelet efficacy.

**WHAT THE STUDY ADDS**

- \(ABCB1\) C3435T genotypes do not show an influence on the antiplatelet response to clopidogrel as determined by platelet function testing.
- \(ABCB1\) C3435T genotypes do not show an influence on the risk for early drug-eluting stent thrombosis after coronary stenting.

## Methods

### Study Cohorts and Study Design

For the present analysis, 2 cohorts of PCI-treated patients (all of Caucasian ethnicity) were enrolled; a dual-antiplatelet regimen consisting of aspirin and clopidogrel was used. Details of these study cohorts and results in relation to other genetic variants (\(CYP2C19\)*/2 and paraoxonase-1 Q192R genotypes) have been reported previously.\(^24\) In brief, a consecutively recruited PCI cohort of 1524 patients enabled us to assess the impact of the \(ABCB1\) C3435T genotypes on ADP-induced platelet aggregation after loading with 600 mg of clopidogrel. In addition, a registry of case subjects with definite ST was also included for the present analysis. A total of 127 definite ST cases that occurred within 30 days after the PCI procedure constituted this registry, and case subjects included were without apparent discontinuation of antiplatelet therapy before the event. For the present analysis, only patients (\(n=66\)) with drug-eluting stent thrombosis were included. Drug-eluting stent case subjects were recruited consecutively between 2004 and 2008. The mean age (±SD) of case subjects was 68.6±11.2 years, and the proportion of women was 22.7%. Definite ST was defined according to the Academic Research Consortium criteria.\(^27\) For comparisons of genotype distributions between ST case subjects and event-free control subjects, the respective control group was taken from the prospective PCI cohort (see above) after exclusion of patients who received only plain balloon angioplasty without stent (\(n=75\)) and those who received a bare-metal stent (\(n=31\)), as well as those who incurred early definite ST (\(n=10\)). Therefore, a total of 1408 patients were included in this control group.

### Blood Sampling and Genotyping

Blood for DNA extraction and subsequent genotyping was taken from the arterial sheath immediately before PCI. Genomic DNA was extracted from 200 µL of blood with commercially available kits (Nucleo Spin Blood Quick Pure, Macherey-Nagel, Duren, Germany). Genotypes were determined with TaqMan assay with an ABI Prism Sequence Detection System 7000. Primers and fluorescent dye probes were selected on the basis of previously reported sequences (GeneBank accession No. F1158881.5 for \(ABCB1\) C3435T [rs1045642]) in the proximity of polymorphic sites. The sequences of the \(ABCB1\) primers and probes can be found in the online Data Supplement. \(CYP2C19\)*/2 genotyping data\(^24\) were included to test for an interaction with \(ABCB1\) or an additive effect of \(ABCB1\) TT genotype and \(CYP2C19\)*/2 allele carriage on the risk of early ST. The \(CYP2C19\)*/2 primers and probes used have been reported previously.\(^24\) Genotyping was performed by laboratory personnel who were unaware of outcome and platelet aggregation results. To control for correct sample handling, genotyping was repeated in 20% of the patients. Repeated genotyping revealed identical results.

### Platelet Function Testing

For assessment of on-clopidogrel-treatment platelet reactivity, ADP-induced platelet aggregation was measured in whole blood obtained from the arterial sheath immediately before PCI with multiple-electrode platelet aggregometry on a Multiplate analyzer.\(^25,28\) Blood for platelet function testing was obtained before the administration of any anticoagulant/antithrombotic treatment in the catheterization laboratory and was placed in 4.5-mL plastic tubes that contained the anticoagulant lepirudin (25 mg/mL; Refludan; Dynabyte, Munich, Germany). Platelet aggregation was measured by multiple-electrode platelet aggregometry in response to 6.4 µmol/L ADP, and values with the Declaration of Helsinki, and all patients gave written informed consent before participation.

### Prospective PCI Cohort

The present study cohort was consecutively recruited between February 2007 and April 2008 at the Deutsches Herzzentrum München (Technische Universität München, Munich, Germany) in the setting of a prospective trial that enrolled 1608 patients with platelet function testing at the time point of the coronary intervention after loading with 600 mg of clopidogrel.\(^23\) Blood for DNA extraction and subsequent genotyping was available for 1524 patients (95%) included in this cohort.\(^26\) The mean age (±SD) of patients was 67.4±10.5 years, and the proportion of women was 23.0%. The recommended pretreatment interval for clopidogrel was ≥2 hours. Exclusion criteria included contraindications to aspirin or clopidogrel treatment and prior treatment with glycoprotein IIb/IIIa inhibitors during the 10 days before the PCI.

### ST Registry and Control Subjects

A registry\(^24\) of case subjects with definite ST was also included for the present analysis. A total of 127 definite ST cases that occurred within 30 days after the PCI procedure constitute this registry, and case subjects included were without apparent discontinuation of antiplatelet therapy before the event. For the present analysis, only patients (\(n=66\)) with drug-eluting stent thrombosis were included. Drug-eluting stent case subjects were recruited consecutively between 2004 and 2008. The mean age (±SD) of case subjects was 68.6±11.2 years, and the proportion of women was 22.7%. Definite ST was defined according to the Academic Research Consortium criteria.\(^27\) For comparisons of genotype distributions between ST case subjects and event-free control subjects, the respective control group was taken from the prospective PCI cohort (see above) after exclusion of patients who received only plain balloon angioplasty without stent (\(n=75\)) and those who received a bare-metal stent (\(n=31\)), as well as those who incurred early definite ST (\(n=10\)). Therefore, a total of 1408 patients were included in this control group.

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are expressed as area under the curve in aggregation units (AU/min). All platelet aggregation measurements were undertaken by laboratory personnel who were unaware of patients’ outcome and ABCB1 genotyping results.

**Statistical Analysis**

Variables are presented as mean±SD, counts (percentages), or median with interquartile range. Categorical variables were compared by use of the χ² test. To test for a normal distribution of continuous data, the Kolmogorov-Smirnov test was used. Normally distributed continuous data were compared between genotype groups with the 1-way ANOVA test and across 2 groups with a 2-sided unpaired t test. Nonnormally distributed continuous data, including platelet aggregation measurements, were compared between genotype groups with the Kruskal-Wallis test and across 2 groups with a 2-sided unpaired Wilcoxon test. A multiple logistic regression model was used to test whether ABCB1 3435TT genotype was an independent predictor of ST. In addition to ABCB1 3435TT, CYP2C19*2 allele carriage and all variables that differed (P<0.10) between ST case and control subjects were also entered into the multivariable model. Moreover, we tested for an interaction in a multivariable model and for an additive effect of ABCB1 TT genotype and CYP2C19*2 allele carriage in univariate analysis. All analyses were performed with the S-PLUS software package (TIBCO Software Inc, Palo Alto, CA). For all statistical analyses, P<0.05 was considered statistically significant.

**Results**

**ABCB1 C3435T Genotypes and Platelet Aggregation**

Baseline characteristics of the PCI cohort according to ABCB1 C3435T genotypes are shown in Table 1. Variables were well balanced between the 3 genotype groups. Of the 1524 patients included in the present analysis, 340 (22.3%) were carriers of the ABCB1 wild-type genotype (ABCB1 3435CC), 740 (48.6%) were heterozygous T-allele carriers (ABCB1 3435CT), and 444 (29.1%) were carriers of the ABCB1 3435TT genotype. On the basis of this genotype distribution, 1184 patients (77.7%) were carriers of at least 1 T allele. The genotype distribution did not deviate significantly from Hardy-Weinberg equilibrium (P=0.34, calculated with Pearson goodness-of-fit test). The median (interquartile range) ADP-induced platelet aggregation value in the study population (n=1524) was 226 (141–364) AU/min.

![Figure 1](http://circinterventions.ahajournals.org/)

Figure 1. ABCB1 genotypes and ADP-induced platelet aggregation. Box-plot analyses showing the ADP-induced platelet aggregation (in aggregation units per minute [AU/min]) in relation to ABCB1 genotypes (ABCB1 3435CC, ABCB1 3435CT, and ABCB1 3435TT).
The ADP-induced platelet aggregation values for the 3 genotypes were as follows: 238 (140–380) AU×min for ABCB1 3435CC patients, 224 (143–357) AU×min for ABCB1 3435CT patients, and 220 (136–362) AU×min for ABCB1 3435TT patients. As shown in Figure 1, ADP-induced platelet aggregation did not differ across genotype groups (P=0.73). In addition, platelet aggregation values were similar and numerically lower when we compared homozygous T-allele carriers (n=444) with the remaining (n=1080) patients (220 [136–362] versus 228 [142–367] AU×min, respectively; P=0.45).

**ABCB1 C3435T Genotypes and ST**

ABCB1 genotypes were determined in 66 ST case subjects and 1408 ST-free control subjects. Table 2 shows the baseline characteristics of these 2 groups. The registry of the 66 patients with drug-eluting stent thrombosis also included the 10 ST patients from the PCI cohort (n=1524). We tested for significant differences between these 10 ST case subjects and the remaining 56 ST case subjects in the registry with regard to all variables outlined in Table 2. Baseline characteristics of the 10 ST case subjects recruited within the PCI cohort did not differ (P>0.17) from the remaining 56 ST case subjects except for the proportion of women (P=0.01). Both subsets of ST case subjects were comparable with regard to genotype distribution for the genetic markers under investigation (P=0.44 for CYP2C19*2 genotypes and P=0.69 for ABCB1 3435TT genotypes).

Figure 2 shows the ABCB1 C3435T genotype distribution in case subjects versus control subjects. The genotype distribution did not differ between case subjects and control subjects (P=0.89). Among the 66 ST case subjects, 19 patients (28.8%) were carriers of the ABCB1 3435TT genotype. This was not significantly different (P=0.91) from the rate of TT carriers in the control group (n=1414, 29.4%). Among the 66 ST case subjects, 25 patients (37.9%) were CYP2C19*2 allele carriers. This proportion was significantly higher than the rate of *2 carriers in the control group (n=353, 25.1%; P=0.02).

The results of a multivariable logistic regression model that adjusted for all variables (Table 2) that differed between ST case subjects and control subjects (P<0.10) demonstrated that the ABCB1 3435TT genotype did not significantly correlate with ST (odds ratio 0.85, 95% confidence interval 0.46–1.58, P=0.61; calculated for 3435TT patients versus the remaining patients), whereas CYP2C19*2 carriage was found to be an independent predictor of drug-eluting stent thrombosis (odds ratio 1.86, 95% confidence interval 1.05–3.51, P=0.03; calculated for CYP2C19*2 carriers versus the remaining patients). In multivariate analysis, no interaction of ABCB1 3435TT genotype with CYP2C19*2 allele carriage was observed (P for interaction=0.51). In the entire cohort of case subjects and control subjects (n=1474), a total of 259 (17.6%) of the patients were CYP2C19*2 carriers and without the ABCB1 TT genotype, whereas 119 patients (8.1%) carried...
both *2 and ABCB1 TT genotype. Sixteen ST patients (6.2%) were carriers of CYP2C19*2 only, and 9 ST patients (7.6%) were combined *2 and TT genotype carriers. This proportion was similar across the 2 groups (P=0.61).

**Discussion**

The exploration of novel genetic markers that may alter the response to clopidogrel treatment in patients undergoing coronary stenting has been the focus of numerous research projects in recent years. However, with a growing body of study results reported, for some genetic markers, including the ABCB1 C3435T polymorphism, overall results for the association of the genetic marker with clopidogrel pharmacodynamics and clinical outcome have been contradictory. With the present study, we aimed to clarify the inconsistent results with regard to ABCB1 C3435T genotypes and clopidogrel treatment efficacy. The key messages of the present study are that this genetic variant is not associated with the antiplatelet response to clopidogrel treatment or with the risk of ST in patients undergoing PCI. Platelet aggregation measurements obtained in a large cohort of PCI-treated patients were virtually identical across ABCB1 C3435T genotypes (and even numerically lower in ABCB1 3435TT patients). With regard to ST risk, the same circumstance applies for ABCB1 C3435T genotype distribution across ST case subjects versus ST-free control subjects. Moreover, we did not find any evidence for an interaction or an additive impact of ABCB1 TT genotype in combination with CYP2C19*2 allele carriage.

Strengths of the present study include the ability to assess in parallel both clopidogrel pharmacodynamics and clinical outcome in PCI-treated patients with regard to ABCB1 C3435T genotypes. A comparatively large cohort of >1500 PCI-treated patients with platelet aggregation measurements available was studied here, as well as a large registry of >60 cases of definite and early ST (defined according to Academic Research Consortium criteria). Of note, we were able to confirm the prominent association of CYP2C19*2 with clopidogrel treatment efficacy in our case-control study. CYP2C19*2 genotyping results here and in 1 of our prior studies may be considered as a positive control for platelet aggregation and clinical outcome measures with regard to the ABCB1 polymorphism assessed here. On the basis of prior mechanistic studies, the ABCB1 C3435T polymorphism may affect P-glycoprotein efflux transporter expression, although the data on this association are ambiguous. Indeed, studies reporting on the impact of ABCB1 C3435T genotypes on clopidogrel treatment efficacy also provided inconsistent results. Taubert et al for the first time reported a significant influence of this genetic variant on clopidogrel pharmacodynamics in a small cohort of 60 patients with coronary artery disease. With regard to the clinical outcome of patients, Simon et al for the first time reported a significant effect of the ABCB1 3435TT genotype on adverse cardiovascular events in patients who had an acute myocardial infarction and were receiving clopidogrel therapy. This association, however, was not confirmed in another acute myocardial infarction cohort of clopidogrel-treated patients. In addition, numerous, mostly smaller studies have provided inconsistent data, with some of the studies suggesting an impact of ABCB1 C3435T genotypes on clopidogrel responsiveness or on the risk of adverse events in clopidogrel-treated patients undergoing PCI and other studies being unable to confirm this postulated association in regard to clopidogrel pharmacodynamics and clinical outcome measures. Recently, Campo et al reported on the evolving pattern over time of the antiplatelet action of clopidogrel and its relationship with different genetic variants (including ABCB1 C3435T genotypes). Using a different platelet function assay (VerifyNow P2Y12) than was used in the present study, the authors reported an influence of CYP2C19*2 and *17 that was consistent over time, whereas that of ABCB1 appeared to be of greater impact at baseline.

The inconsistency of results with regard to this polymorphism is perhaps most clearly demonstrated in the genetic substudies from the clopidogrel arms of the randomized TRITON-TIMI 38 (Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis in Myocardial Infarction) and PLATO (Platelet Inhibition and Patient Outcomes) trials. Whereas within the TRITON-TIMI 38 population, the ABCB1 3435TT genotype was associated with a higher risk of cardiovascular death, myocardial infarction, and stroke, in the PLATO trial, the highest event rates in the clopidogrel arm of the study cohort were observed in carriers of the opposite (3435CC) ABCB1 genotype; the reason for this remains unclear. Mechanistic and clinical outcome results provided here, however, do not suggest a relevant impact for any of the ABCB1 C3435T genotypes in clopidogrel-treated patients undergoing PCI.

Newly developed and more potent P2Y12 receptor blockers such as prasugrel or ticagrelor are currently available. In recent years, efforts have been undertaken to gain experience with more individualized approaches to antiplatelet treatment regimens, leaving behind the “one-size-fits-all” strategy of drug choice and dosing. Indeed, such an approach emphasizes the importance in clinical judgment of the attending physician regarding choice of antiplatelet therapy by balancing the risk of thrombotic and bleeding events. With a growing armamentarium of drugs, more guidance to tailor antiplatelet treatment will be necessary. However, debates on to what degree platelet function testing (phenotyping) genotyping alone, or a combined approach of phenotyping and genotyping will be a useful approach in a clinical setting are still ongoing. Inconsistencies regarding genetic markers in the setting of clopidogrel treatment not only with regard to ABCB1 polymorphisms but also with regard to the recently explored PON1 Q192 genotype or CYP2C19*2 hamper the evolution toward more individualized treatment approaches. There are still questions regarding which genetic markers to add or not add to a panel of markers that may be genotyped in the setting of clopidogrel treatment. Thus, further studies exploring the genetic background of antiplatelet treatment and the value of individualized treatment regimens are urgently needed to allow for the widespread adoption of such strategies, which surely will be beneficial for the individual patient.
Study Limitations
The present study has limitations that merit mention. Here, we only assessed the impact of 1 single-nucleotide polymorphism within the ABCB1 gene on clopidogrel responsiveness and clinical outcome. In addition, we collected only platelet aggregation measurements and did not analyze plasma levels of absorbed clopidogrel or of the active metabolite of clopidogrel, which would have provided further mechanistic insights into the observed platelet response. A further limitation is that ST cases stemmed from a registry of ST, whereas the control patients were part of a clinical trial cohort. Although both of these groups represented series of consecutive patients, we cannot exclude a possible bias introduced by the slightly different time periods during which case and control patients were recruited. Finally, a further limitation is that the present analysis was a post hoc analysis of a study population that stemmed from a prospective trial; therefore, it is subject to the limitations inherent to all such analyses.

Conclusions
The ABCB1 C3435T genotype did not influence the antiplatelet response to clopidogrel or the risk of ST in clopidogrel-treated patients undergoing PCI. Routine genotyping of ABCB1 C3435T polymorphisms should not be recommended for risk stratification in clopidogrel-treated patients undergoing PCI who are similar to those evaluated in the present study.

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Disclosures
Dr Kastrati has received speaker fees from Eli Lilly, Daiichi Sankyo, Bristol-Myers Squibb, and Astra Zeneca. Dr Sibbing has received consulting fees from Dynabyte and The Medicines Company and fees for advisory board activities from Eli Lilly and Astra Zeneca. The remaining authors report no conflicts.

References


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SUPPLEMENTAL MATERIAL

No Association of ABCB1 C3435T Genotype with Clopidogrel Response or Risk of Stent Thrombosis in Patients Undergoing Coronary Stenting

Jaitner et al.: ABCB1 genotype, clopidogrel response and coronary stenting

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Supplemental Methods

ABCB1 C3435T primers and probes

Primers 5'-TGACTGCAGCATTGCTGAGAA-3' and 5'-CTTACATTAGGCAGTGACTCGATGAA-3' were used to amplify the sequence of the ABCB1 gene containing the single nucleotide polymorphism C3435T (rs1045642). The sequence of the A-allele-specific probe was 5'-FAM-CCTCACAAATCTCTT-3', and the sequence of the G-allele-specific probe was 5'-VIC-CCTCAGATCTCTT-3'. All genotypes were determined by TaqMan assays which combine the standard PCR and the 5' nuclease reaction. The sequences of primers and probes were designed in house using Primer Express (version 2.0) software (Applied Biosystems). Primers and probes were synthesized by Applied Biosystems. Probes were allele-specifically labelled with one of the fluorescent dyes FAM (6-carboxy-fluorescein) and VIC (proprietary dye of Applied Biosystems) and contained a minor groove binder group and a dark quencher. More detailed information on PCR conditions is available upon request.