Protective effect of the CYP2C19 *17 polymorphism with increased activation of clopidogrel on cardiovascular events

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Background The prodrug clopidogrel requires activation by cytochrome P-450 (CYP) enzymes for its antiplatelet effect. The genes encoding enzymes for clopidogrel activation are polymorphic, leading to reduced or increased function, depending on the respective genotype. Reduced-function alleles have been associated with an increase in cardiovascular events.

Methods We tested the association of the presence of the ABCB1 (C/T) T-allele, CYP2C19*2 (G/A) A-allele, or CYP2C19*17 (C/T) T-allele with the primary end point of the need of clinically-driven target lesion revascularization (TLR) and the secondary end points of major adverse cardiovascular events (MACE; including death, myocardial infarction [MI], and TLR) at 1 year in a high-risk population of 928 patients with acute MI.

Results Carriers of the CYP2C19*17 T-allele, with increased clopidogrel activation, had a 37% relative reduction in the TLR incidence, the primary end point (14.0% vs 22.3%, \(P = .002\)), and a 22% relative reduction of the secondary end point MACE (22.0% vs 28.1%, \(P = .04\)) compared with noncarriers, respectively. The association of the T-allele with TLR remained significant in the multivariate analysis (\(P = .001\)). The ABCB1 (C/T) and the CYP2C19*2 (G/A) polymorphisms were not associated with the incidence of TLR or MACE.

Conclusions Based on the genetic analysis in a high-risk population of acute MI patients with interventional treatment and continuous clopidogrel therapy, our study found a protective effect for carriers of an increased-function CYP2C19*17 T-allele with significantly lower rates of TLR and MACE. T-allele carriers with acute MI and increased clopidogrel activation had significantly reduced clinical event rates. (Am Heart J 2010;160:506-12.)

Percutaneous coronary intervention has become the mainstay of therapy for patients with acute myocardial infarction (MI); and stent implantation efficiently reduces complications and restenosis compared with balloon angioplasty alone, but requires long-term dual-antiplatelet therapy for prevention of atherothrombotic events.1-4

The recommended postinterventional therapy includes dual-antiplatelet inhibition with aspirin and a thienopyridine, usually clopidogrel, an effective inhibitor of the platelet P2Y12 adenosine diphosphate receptor,2-4 with a substantial interpatient variability regarding the pharmacodynamic response.5-6 Clopidogrel is a prodrug that requires active enteric absorption modulated by the intestinal efflux transporter P-glycoprotein encoded by the ABCB1 gene and biotransformation to an active metabolite by cytochrome P-450 (CYP) enzymes. The genes encoding these enzymes are polymorphic, leading to increased or decreased availability of the active metabolite.5-11 Patients with coronary disease or previous MI with reduced platelet inhibition following clopidogrel were associated with an increased risk for atherothrombotic cardiovascular events.5,6,9,10 Acute MI patients with 2 variant alleles of ABCB1 (TT at nucleotide 3435) had a higher rate of cardiovascular events at 1 year than those with the ABCB1 wild-type genotype (CC at nucleotide 3435) (15.5% vs 10.7%, adjusted hazard ratio 1.72, 95% CI 1.20-2.47, \(P = .04\)).6 Approximately 30% of healthy subjects are carriers of at least 1 CYP2C19 reduced-function allele (especially CYP2C19*2), leading to a relative reduction of 32.4% in plasma exposure to the active metabolite of clopidogrel after treatment with clopidogrel, as compared with noncarriers (\(P < .001\)).5 Carriers also had an absolute reduction in maximal platelet aggregation in response to clopidogrel that was 9 percentage points less than that seen in noncarriers (\(P < .001\)). Among clopidogrel-treated subjects in
TRITON–TIMI 38 study, carriers had a relative increase of 53% in the composite primary efficacy outcome of the risk of death from cardiovascular causes, MI, or stroke as compared with noncarriers (12.1% vs 8.0%, hazard ratio for carriers 1.53, 95% CI 1.07-2.19, \( P = .01 \)) and an increase by a factor of 3 in the risk of stent thrombosis (2.6% vs 0.8%, hazard ratio 3.09, 95% CI 1.19-8.00, \( P = .02 \)). Among 1,535 patients with acute MI treated with percutaneous coronary intervention, the rate of cardiovascular events among patients with 2 CYP2C19 loss-of-function alleles was 3.58 times the rate among those with none (95% CI, 1.71-7.51). The CYP2C19*17 (C/T) T-allele was associated with an increased activation of clopidogrel in vitro and in vivo. 

Although less dramatic, restenosis secondary to neointimal hyperplasia is more frequent compared with atherothrombotic events and often requires subsequent reintervention. Angiographic restenosis is present in around 30% of cases using bare metal stents and 10% to 15% for drug-eluting stents (DESs), with higher rates for complex lesions and for diabetic patients. Previous studies have shown an association of genetic factors with the development of stent restenosis and thrombotic events. Recent data from large registries and randomized trials support the use of DES in acute MI based on similar safety profiles with reduced rates of restenosis. The presence and the extent of thrombotic material in patients with acute MI, depending on platelet activation status, may lead to undersizing of stents and incomplete stent expansion due to interposition of thrombotic material between stent struts and vessel wall, with subsequent stent malapposition and smaller minimum stent area, factors that are all associated with higher rates of restenosis.

The aim of this study was to assess the effect of genetic variants in the activation pathway of the prodrug clopidogrel on the efficacy of stenting, expressed by the need for repeated target vessel revascularization in a large population of high-risk patients with acute MI.

### Methods

#### Patients

The significance of the studied polymorphisms was evaluated in a cohort study that comprised a high-risk population of 928 consecutive patients with acute MI between 2005 and 2008 who underwent coronary angiography and successful recanalization with a final Thrombolysis in Myocardial Infarction (TIMI) flow ≥2 at Deutsches Herzzentrum München and 1. Medizinische Klinik rechts der Isar, Technische Universität München. Coronary stent implantation was performed as previously described, and DES implantation was performed in >90% of cases. Blood was available for all patients included in this study. All patients received a loading dose of 600 mg clopidogrel. Postprocedural therapy consisted of aspirin (100 mg twice daily, indefinitely) and clopidogrel (75 mg once daily for at least 6 months). Follow-up angiography was routinely scheduled at 6 months poststenting or whenever the patient complained of anginal symptoms and was available for all patients included in this study. Creatine kinase levels and electrocardiographic changes were assessed systematically over 48 hours after the procedure. Clinical events were monitored throughout the 1-year period following the intervention. The data regarding cardiovascular risk factors were obtained during the actual hospitalization or from the patient's chart. Exclusion criteria included a target lesion in the left main trunk; any contraindication to the use of aspirin, clopidogrel, heparin, bivalirudin, stainless steel, and cobalt-chromium; or lack of consent to participate in the study. The local ethics committee approved the study; and written informed consent was obtained from all patients for the procedure, the release of patient information, and the genetic analysis.

#### Definitions

The diagnosis of MI was based on the presence of new pathologic Q waves or a value of creatine kinase or its MB isoenzyme at least 3 times the upper limit. Stent thrombosis was defined as definite or probable stent thrombosis with presence of angiographic thrombus at the site of the stented lesion or acute ST-elevation MI of the target vessel according to the Academic Research Consortium definition. Defect final denotes the final myocardial size in relation to the entire myocardium assessed by scintigraphic analysis 5 to 7 days after the acute MI.

#### Genotyping

Genomic DNA was extracted from 200 μL of peripheral blood using commercially available kits (Qiagen, Hilden, Germany, and Roche Molecular Biochemicals, Mannheim, Germany). Genotype analyses of the ABCB1 C/T (rs1045642) polymorphism and the CYP2C19*17 C/T (rs12248560) polymorphism, the CYP2C19*2 C/T (rs4244285) polymorphism, were based on polymerase chain reaction and the TaqMan assay, and involved fluorogenic oligonucleotide probes. TaqMan assays were performed with the ABI Prism Sequence Detection System 7700 (Applied Biosystems, Darmstadt, Germany). Primers and fluorescent dye probes were selected based on previously reported sequences (GenBank accession no. FJ158815.1 for ABCB1 C/T [rs1045642], NC_000010 for the CYP2C19*2 C/T [rs4244285] polymorphism, and NG_008384.1 for CYP2C19*17 C/T [rs12248560]) in the proximity of the polymorphic sites.

Sequences of the primers and probes are available upon request and have been published in part previously. DNA was amplified during 40 cycles of denaturation at 95°C for 15 seconds and primer annealing as well as extension at 60°C for 1 minute. To control for correct sample handling, genotyping was repeated for 20% of the population for each polymorphism. All control experiments revealed identical results compared with the first genotyping. Two independent operators assessed all TaqMan results. Genotyping for 6 patients was not possible with the TaqMan assay (2 for the ABCB1 and 4 for the CYP2C19*17 polymorphisms) and was successfully performed by sequencing the respective DNAs using the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) capillary sequencing system and BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, patent no. 4356776) using the same primers as for the TaqMan assay.
Angiographic assessment

Quantitative angiographic analysis was performed off-line with the use of the automated edge detection system CMS (Medis Medical Imaging Systems, Nuenen, the Netherlands) assessing matched views of the target lesions. The operators were blinded to the patients' respective genotypes or patients' clinically data. The lesion morphology was assessed according to the modified American Heart Association/American College of Cardiology and classified as type A, B1, B2, or C. Lesions of types B2 and C were classified as type A, B1, B2, or C. Lesions of types B2 and C were considered complex lesions.27 Lesion length, reference diameter, minimal lumen diameter (MLD), and diameter stenosis were measured and assessed for each patient. Angiographic parameters were recorded before and immediately following the intervention, as well as at follow-up angiography.

Study end points

The primary end point of the study was clinically driven target lesion revascularization (TLR). Clinically driven TLR was defined as the necessity for TLR with percutaneous transluminal coronary angioplasty or aortocoronary bypass grafting due to symptoms or signs of ischemia in the presence of angiographic restenosis during the first year following stenting.

Angiographic restenosis was defined as a diameter stenosis of ≥50% at 6-month follow-up angiography. Secondary end points were the incidence of death from any cause, the incidence of nonfatal MI during a 1-year follow-up period, the incidence of stroke, final infarct size assessed by scintigraphy, and the combined end point of major adverse cardiovascular events (MACE) including death, MI, and clinically driven TLR.

Statistical analysis

Continuous variables were expressed as mean ± SD and were compared by means of the unpaired, 2-sided t test or analysis of variance for >2 groups. Discrete variables were expressed as counts or percentages and compared with the χ² or Fisher exact test, as appropriate. Pearson goodness-of-fit χ² method was used to test for deviation from the Hardy-Weinberg equilibrium. The multivariate logistic regression analysis was performed to control for potentially confounding variables, including pre-specified variables like the genotype distributions, as well as all variables significantly associated (P < .05) with the primary end point in the univariate analysis. The sample size included had an 90%, 80%, and 80% power to detect a significant 25% increase in target vessel revascularization among carriers of the ABCB1 T-allele, CYP2C19*2 allele, and the CYP2C19*17 allele, respectively, equivalent to absolute differences of 4% to 6%. Statistical analyses were performed using S-Plus software (Insightful Corp, Seattle, WA). A 2-sided P value < .05 was considered statistically significant.

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Results

The genotype distribution was 21.9% CC, 49.2% CT, and 28.9% TT for the ABCB1 C/T polymorphism; 73.3% GG, 24.5% GA, and 2.2% AA for the CYP2C19*2 polymorphism; and 60.9% CC, 33.3% CT, and 5.8% TT for the CYP2C19*17 C/T polymorphism, respectively, corresponding to the Hardy-Weinberg equilibrium (P = .76 for the ABCB1 genotypes, P = .14 for the CYP2C19*2 genotypes, and P = .18 for the CYP2C19*17 genotypes). The baseline and procedural characteristics of the patients and the discharge medications were evenly distributed between the studied genotype groups (Tables I and II). The mean age was 64.8 years; 75% were male; and diabetes mellitus was present in 24% of patients, with 6.5% of all patients requiring insulin. Hypertension, hypercholesterolemia, and previous or current smoking were present in 74%, 52%, 55%, and 37% of patients, respectively. The average time from symptom onset to the coronary intervention procedure was 210 minutes on average; and the average time from clopidogrel loading to percutaneous coronary intervention was 73.2 minutes, without significant differences between the studies genotype groups (P > .11).

| Table I. Distribution of the risk alleles for the respective baseline characteristics (N = 928) |
|---|---|---|---|---|---|---|---|
| ABCB1 | CYP2C19*2 | CYP2C19*17 |
| CC | T allele | P value | GG | A allele | P value | CC | T allele | P value |
| n = 203 | n = 723 | | n = 680 | n = 248 | | n = 565 | n = 363 | |
| Age | 64.5 ± 13.8 | 64.9 ± 12.7 | .658 | 64.5 ± 13.1 | 65.8 ± 12.5 | .160 | 64.6 ± 13.1 | 65.2 ± 12.7 | .458 |
| Women | 51 (25.1) | 183 (25.2) | .972 | 166 (24.4) | 68 (27.4) | .350 | 141 (25.0) | 93 (25.6) | .820 |
| Hypertension | 153 (75.4) | 538 (74.2) | .737 | 510 (75.0) | 181 (73.0) | .533 | 416 (73.6) | 275 (75.8) | .468 |
| Hypercholesterolemia | 104 (51.2) | 378 (52.1) | .819 | 351 (51.6) | 131 (54.6) | .745 | 292 (51.7) | 190 (52.3) | .844 |
| Current smoker | 74 (36.5) | 265 (36.6) | .979 | 259 (38.1) | 80 (32.3) | .103 | 207 (36.6) | 132 (36.4) | .933 |
| Diabetes mellitus | 43 (21.2) | 181 (25.0) | .266 | 170 (25.0) | 54 (21.8) | .391 | 122 (21.6) | 102 (28.1) | .024 |
| Multivessel CAD | 146 (71.9) | 523 (72.1) | .951 | 493 (72.5) | 176 (71.0) | .645 | 390 (69.0) | 279 (76.9) | .009 |
| Previous CAGB | 9 (4.4) | 48 (6.6) | .251 | 44 (6.5) | 13 (5.2) | .490 | 29 (5.1) | 28 (7.7) | .110 |
| Previous MI | 30 (14.8) | 99 (13.7) | .683 | 105 (15.4) | 24 (9.7) | .025 | 68 (12.0) | 61 (16.8) | .040 |
| BMI | 27.3 ± 4.3 | 27.0 ± 3.9 | .367 | 27.0 ± 4.0 | 27.2 ± 4.1 | .371 | 26.9 ± 4.0 | 27.2 ± 4.0 | .429 |
| EF in % | 47.6 ± 12.1 | 48.0 ± 11.5 | .657 | 47.6 ± 11.8 | 48.6 ± 10.9 | .263 | 47.7 ± 11.2 | 48.2 ± 12.2 | .533 |

Data are number of patients (percentage) or mean ± SD followed by respective abbreviations. CABG, Coronary arterial bypass grafting; BMI, body mass index; EF, ejection fraction.
In this high-risk population, 72% of patients had multivessel disease with a mean ejection fraction of 48%. The leading MI localization was anterior with 39%, followed by inferior MI (36%), lateral MI (24%), and multiple culprit vessels in the remaining 1% of cases. The mean reference diameter of the treated vessel was 2.87 mm at the site of the lesion. Drug-eluting stents followed by inferior MI (36%), lateral MI (24%), and multiple culprit vessels in the remaining 1% of cases. The mean reference diameter of the treated vessel was 2.87 mm at the site of the lesion. Drug-eluting stents were used in >90% of cases, and the stent type had no significant impact on the incidence of TLR or MACE (P > .24). The information regarding the discharge therapy is shown in Table II. Significant differences between patients with and without MACE were observed in the univariate analysis for the distribution of the ABCB1, LAD, LCA, LCx, RCA, female gender, diabetes mellitus, and extent of coronary artery disease (CAD) reflected by number of diseased vessels (Table III). These variables were included in the final multivariate logistic regression analysis testing their independent association with the primary end point.

There was a highly significant reduction in the incidence of the primary end point TLR for patients with the CYP2C19*17 T-allele compared with noncarriers in the univariate analysis (P = .002, odds ratio [OR] 0.57, 95% CI 0.39-0.82) (Table IV, Figure 1). The significant difference was also present for the secondary end point MACE (P = .04, OR 0.72, 95% CI 0.53-0.98) (Table IV), whereas no significant differences were observed for the incidence of death, subsequent MI, stroke, final scintigraphic defect size, or stent thrombosis. There were no significant differences in the distribution of the ABCB1
and CYP2C19 risk allele carriers regarding primary or secondary end points (Table IV). In the multivariate logistic regression analysis including the significantly associated risk factors from the univariate analysis like presence of polymorphic risk alleles, age, gender, diabetes mellitus, and extent of CAD, only the presence of the protective T-allele of the CYP2C19*17 polymorphism was associated with a reduction for the need of clinically driven TLR ($P = .001$, OR 0.55, 95% CI 0.39-0.79). The angiographic restenosis rate was significantly reduced for CYP2C19*17 T-allele carriers in the univariate and multivariate analysis ($P < .01$). The association of the CYP2C19*17 T-allele with MACE observed in the univariate analysis was not significant in the multivariate analysis ($P = .46$). All other studied variables were not significantly associated with the primary end point in the multivariate analysis.

**Discussion**

This study showed a highly significant association of the T-allele of the CYP2C19*17 polymorphism with the need of clinically driven TLR, the primary end point of our analysis, in the univariate as well as in the multivariate analysis in patients with acute MI. The presence of the CYP2C19*17 (C/T) T-allele was previously shown to be associated with an increased function of the CYP2C19 enzyme, which plays a pivotal role in the activation process of clopidogrel, with increased conversion rates of the prodrug clopidogrel to an active compound in carriers of the T-allele, leading to a higher degree of platelet inhibition assessed by aggregometry.6,11,20 In patients with acute MI, the presence and extent of thrombotic material may lead to undersizing of stents because of underestimation of the true lumen size and to incomplete stent expansion due to interposition of thrombotic material between stent struts and vessel wall, with subsequent stent malapposition and smaller minimum stent area, factors that are all associated with higher rates of restenosis.17,18 Previous studies evaluating the impact of different polymorphisms located in the genes encoding proteins involved in the activation of clopidogrel focused on acute thrombotic events as the primary end point.5,6,9,11,13,21 A large study from our institution found a numerically lower restenosis rate with the use of the potent GPIIb/IIIa inhibitor abciximab in patients with acute MI, but this observation was not statistically significant ($P = .27$).22 The implantation of a DES at a site of a thin-cap atheroma or at the site of a thrombotic lesion is associated with increased inflammation and subsequent higher risk of stent thrombosis, atherosclerosis, and therefore restenosis.23 We defined TLR as the primary end point of this study and calculated the power analysis accordingly. The incidence of death,
repeat MI, stroke, and stent thrombosis and the composite of these end points were defined as secondary end points.

We found a highly significant association of the primary end point TLR with the presence of the CYP2C19*17 T-allele in the univariate and multivariate analyses. We found no significant association of the ABCB1 T-allele and the CYP2C19*2 A-allele with the studied end points, despite the previously described highly significant association of the CYP2C19*2 A-allele with adverse cardiac events and stent thrombosis. Target lesion revascularization, our primary end point, was not evaluated in these studies at all. The exact mechanisms influenced by the CYP2C19*17 T-allele leading to such a significant reduction of TLR deserve further exploration. The average time from clopidogrel loading to the coronary intervention procedure was quite long with 73.2 minutes. This is due to the higher number of rural hospitals referring patients with acute MI to our centers. Carriers of the CYP2C19*17 T-allele would have not only a higher overall percentage of clopidogrel activation, but also a genetically determined faster activation of clopidogrel, and would therefore benefit more because of decreased thrombus burden before mechanical reperfusion. In addition, the CYP2C19*17 T-allele might further be protective given the very high number of patients on chronic clopidogrel therapy at discharge.

Given the 90% power calculation for the ABCB1 T-allele for the primary end point, we can be quite confident regarding the lack of significant clinical association of this polymorphism with adverse cardiac events, thereby confirming the recently published negative findings for this polymorphism. No association was observed regarding the final infarct size assessed by scintigraphic uptake of the viable myocardium at 5 to 7 days of follow-up.

Strengths of our study were the series of 928 consecutive patients in the current DES era with completeness of available angiographic follow-up, and the complete genotyping and angiographic assessment. The inclusion of successful cases with postinterventional TIMI flow $\geq 2$ enabled the evaluation of TLR as the primary end point. The angiographic and clinical restenosis rates in this study were comparable to recently reported studies. One limitation of this study might be related to the exclusion of patients with unsuccessful attempt to reestablish adequate epicardial flow in the target vessel at the index procedure, which was necessary because of the definition of the primary end point. The inclusion of unsuccessfully treated patients with higher mortality and morbidity rates might have provided important information regarding the impact of the studied polymorphisms on other adverse cardiac events. Another limitation might be related to the allowance of using both bare metal stents and DESs; but this reflects common clinical practice, given the ongoing debate regarding the safety of DESs in acute MI with hypercoagulable state, increased platelet activation, and compliance to long-term dual-antiplatelet therapy. The population can still be viewed as homogeneous with >90% DES use in an acute MI population.

In conclusion, this study showed for the first time a highly significant association of the T-allele of the CYP2C19*17 polymorphism associated with increased clopidogrel activation with the need of clinically driven TLR, the primary end point of our analysis, in the univariate as well as in the multivariate analysis in patients with acute MI and does not support an association of the ABCB1 and CYP2C19*2 polymorphism with TLR. Overall, CYP2C19*17 T-allele carriers with acute MI and increased clopidogrel activation have significantly reduced clinical event rates.

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References


