

CLINICAL RESEARCH

Interventional Cardiology

# 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

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- Objectives** We investigated whether the loss of function CYP2C19 681G>A \*2 polymorphism is associated with high (>14%) residual platelet aggregation (RPA) on clopidogrel and whether high on-clopidogrel RPA impacts clinical outcome after elective coronary stent placement.
- Background** The cytochrome P450 (CYP)-dependent conversion of clopidogrel to its active metabolite may contribute to the variability in antiplatelet effect of clopidogrel.
- Methods** The study included 797 consecutive patients undergoing percutaneous coronary intervention, who were followed-up for 1 year. Adenosine-diphosphate-induced (5  $\mu$ mol/l) RPA was assessed after a 600-mg loading dose and after the first 75-mg maintenance dose of clopidogrel before discharge. CYP2C19 genotype was analyzed by real-time polymerase chain reaction.
- Results** Of the patients included, 552 (69.3%) were CYP2C19 wild-type homozygotes (\*1/\*1) and 245 (30.7%) carried at least one \*2 allele. Residual platelet aggregation at baseline did not differ significantly between genotypes. On clopidogrel, RPA was significantly ( $p < 0.001$ ) higher in \*2 carriers than in wild-type homozygotes (23.0% [interquartile range (IQR) 8.0% to 38.0%] vs. 11.0% [IQR 3.0% to 28.0%] after loading; 11.0% [IQR 5.0% to 22.0%] vs. 7.0% [IQR 3.0% to 14.0%] at pre-discharge). Between \*2 carriers and wild-type homozygotes, we found significant ( $p < 0.001$ ) differences in the proportion of patients with RPA >14%, both after loading (62.4% vs. 43.4%) and at pre-discharge (41.3% vs. 22.5%). Residual platelet aggregation >14% at pre-discharge incurred a 3.0-fold increase (95% confidence interval 1.4 to 6.8;  $p = 0.004$ ) in the 1-year incidence of death and myocardial infarction.
- Conclusions** Patients carrying at least one CYP2C19\*2 allele are more prone to high-on clopidogrel platelet reactivity, which is associated with poor clinical outcome after coronary stent placement (Effect of Clopidogrel Loading and Risk of PCI [EXCELSIOR]; NCT00457236). (J Am Coll Cardiol 2008;51:1925–34) © 2008 by the American College of Cardiology Foundation

After administration of clopidogrel, high on-treatment platelet reactivity is associated with increased risk of adverse events after percutaneous coronary intervention (PCI) with stent placement. Consistently, this has been shown by both the EXCELSIOR (Impact of Extent of Clopidogrel-

Induced Platelet Inhibition During Elective Stent Implantation on Clinical Event Rate) (1) and the RE-CLOSE (Low REsponsiveness to CLOpidogrel and Sirolimus- or Paclitaxel-Eluting StEnt Thrombosis) trials (2), as well as

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by a number of smaller studies (3–12). Specifically, in the EXCELSIOR trial we demonstrated a more than 6-fold increase in the 30-day risk of death, myocardial infarction (MI), and target vessel reintervention after stent placement, if adenosine diphosphate (ADP)-induced (5  $\mu$ mol/l) resid-

**Abbreviations  
and Acronyms**

<b>ADP</b> = adenosine diphosphate
<b>CI</b> = confidence interval
<b>CYP</b> = cytochrome P450
<b>GP</b> = glycoprotein
<b>MI</b> = myocardial infarction
<b>PCI</b> = percutaneous coronary intervention
<b>PCR</b> = polymerase chain reaction
<b>RPA</b> = residual platelet aggregation

ual platelet aggregation (RPA) after a 600-mg loading dose of clopidogrel was >14% (1). High on-treatment platelet reactivity (RPA >14%) was associated with older age, obesity, and diabetes mellitus. In addition, the propensity for high on-treatment platelet reactivity may be genetically determined, an issue that was not addressed in the initial report on the EXCELSIOR trial.

The prodrug clopidogrel has to be converted into the active metabolite (13,14) that selectively and irreversibly binds to the P2Y<sub>12</sub> receptor

on the platelet membrane (15–18). Conversion is achieved by the highly polymorphic hepatic cytochrome P450 (CYP) system in a 2-step process. In 28 healthy subjects, Hulot et al. (19) investigated polymorphisms of CYP 2C19, 2B6, 1A2, and 3A4/5 with known functional consequences on enzyme activity and a minor allele frequency higher than 5% in Caucasians. Among the polymorphisms investigated, only the loss-of-function *CYP2C19* 681G>A polymorphism (\*2) was associated with blunted antiplatelet responses to clopidogrel. In the gene encoding for *CYP2C19*, the single nucleotide polymorphism 681G>A (rs4244285) in exon 5, mapped to the long arm of chromosome 10 (10q24.1–q24.3) (20), encodes for a cryptic splice variant resulting in no enzyme activity in vivo (21,22). The role of the \*2 polymorphism of *CYP2C19* in healthy volunteers was subsequently confirmed by a second study (23). In a study of 79 patients with coronary artery disease (24), however, the investigators were unable to demonstrate an impact of the *CYP2C19* genotype on the antiplatelet effect of a 600-mg bolus dose. Thus, in the clinical setting, the role of the *CYP2C19*\*2 loss-of-function polymorphism is currently unclear.

Based on these findings, we addressed the question whether in the EXCELSIOR cohort the loss-of-function *CYP2C19*\*2 polymorphism was associated with high on-treatment platelet reactivity (RPA >14%) after administration of clopidogrel. To corroborate the clinical relevance of the EXCELSIOR trial definition of high on-treatment platelet reactivity, we expanded the clinical follow-up to 1 year and assessed the relation between RPA >14% at pre-discharge and 1-year incidence of death and MI. We also analyzed various other platelet function tests, including light transmission aggregometry after stimulation with 20 μmol/l ADP and surface expression of activation-dependent platelet receptors to confirm a potential impact of *CYP2C19*\*2 polymorphism on platelet function after clopidogrel.

**Methods**

**Study selection and interventions.** The current study is a pre-specified analysis of the EXCELSIOR cohort. Details

of the EXCELSIOR study design have been published previously (1). Briefly, patients undergoing elective coronary stent placement after pre-treatment with 600 mg of clopidogrel and aspirin (100 mg per day for at least 5 days) were eligible for this prospective, single-center study conducted in a referral center setting in Germany. We did not include patients with acute MI according to the European Society of Cardiology/American College of Cardiology consensus document (25); patients on chronic oral anticoagulation or thienopyridine treatment within the last 2 weeks before admission; patients with contraindications to aspirin, clopidogrel, or heparin; and patients with cancer or hemodialysis. The study was approved by the ethics committee of the medical faculty of the University of Freiburg, Germany. All patients gave written informed consent.

Before PCI, all patients received a loading dose of 600 mg of clopidogrel. Percutaneous coronary intervention with stent placement was performed as described previously (1,26). The choice of stent type, bare-metal stent or paclitaxel- or sirolimus-eluting stent, was left to the operator's discretion. All patients received an intra-arterial dose of 100 to 140 U/kg heparin; glycoprotein (GP) IIb/IIIa inhibitors were not allowed except for bail-out. After PCI, all patients received aspirin (≥100 mg per day) lifelong and clopidogrel (75 mg per day) for 30 days after placement of bare-metal stents or for 6 months after placement of at least 1 drug-eluting stent.

Baseline blood samples were drawn before administration of clopidogrel for platelet function assays using tubes containing 3.8% sodium-citrate (Sarstedt AG, Nuembrecht, Germany). Blood for genomic deoxyribonucleic acid (DNA) extraction was sampled using tubes containing 1.2 to 2 mg potassium ethylenediaminetetraacetic acid per milliliter of blood (Sarstedt AG). We obtained the second blood sample at the time of catheterization before administration of heparin or contrast medium and a third pre-discharge sample at day 1 after loading 2 to 4 h after intake of the first maintenance dose of clopidogrel. Samples were processed within 1 h after blood drawing.

We performed a phone interview at 30 days and after 12 months. Thirty-day follow-up was complete in all patients and 12-month follow-up in all but 7 patients (99.1%). For patients reporting cardiac symptoms, at least 1 clinical and electrocardiographic examination was performed in the outpatient clinic or by the referring physician. The information derived from contingent hospital readmission records or provided by the referring physician or by the outpatient clinic was entered into the computer database.

**Platelet function assays.** Platelet aggregation was assessed by light transmission aggregometry using a 4-channel Bio/Data PAP4 aggregometer (Mölab, Langenfeld, Germany), as described previously (26). Blood for platelet aggregation was available from all patients at baseline and at cardiac catheterization. Platelet function could not be determined in 37 patients pre-discharge. The reasons were: discharge earlier than 24 h after PCI in 32 patients, peri-interventional admin-

istration of abciximab in 2 patients, and transfer to the intensive care unit for large MI in 3 patients. Platelet-rich plasma was prepared by centrifugation of citrated venous blood at 750 g for 2 min and adjusted to  $275$  to  $325 \times 10^9$  thrombocytes/l by dilution with autologous platelet-poor plasma. Residual platelet aggregation was determined as light transmission in platelet-rich plasma 5 min after addition of ADP (Sigma, Munich, Germany) at final concentrations of 5 and 20  $\mu\text{mol/l}$ . Percentage of maximal light transmission was calculated using platelet-poor plasma from the same patient as reference (100% aggregation). The coefficient of variation of our optical aggregometry assay is 6.1% (26).

Adenosine diphosphate-induced surface expression of P-selectin (CD62P), activated GP IIb/IIIa (PAC-1), CD40L, CD41, and CD63 was determined by triple color flow cytometry as previously described (26). Platelets in whole blood were stained with 2 antibody mixtures containing either fluorescein-isothiocyanate-tagged PAC-1 (activated GP IIb/IIIa receptors), phycoerythrin-tagged anti-CD62P (P-selectin), and phycoerythrin-cyanin 5.1-tagged anti-CD41 (total GP IIb/IIIa receptors) monoclonal antibodies or phycoerythrin-tagged anti-CD40L, fluorescein-isothiocyanate-tagged anti-CD63, and anti-CD41 monoclonal antibodies (PAC-1 by Becton-Dickinson, Heidelberg, Germany, all other antibodies by Beckman Coulter, Krefeld, Germany). Platelets were incubated with the antibodies and ADP at a final concentration of 20  $\mu\text{mol/l}$  for 30 min. Thereafter, 300  $\mu\text{l}$  of para-formaldehyde 1% was added for fixation. A 4-channel flow cytometer equipped with a 488-nm argon laser (FACSCalibur, Becton Dickinson, Heidelberg, Germany) was used. Platelets were identified in whole blood by size, and a platelet-specific monoclonal antibody (CD41), and 10,000 events from each sample were analyzed. The mean channel of fluorescence intensity was taken as a measure for antibody binding, and thus antigen surface exposure.

**Genotyping by TaqMan polymerase chain reaction (PCR).** Genomic DNA was extracted from 200  $\mu\text{l}$  peripheral potassium ethylenediaminetetraacetic acid-anticoagulated blood with the Flexigene Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instruction. After extraction, the concentration of DNA was measured photometrically and DNA was diluted to a concentration of 5 mg/l. *CYP2C19\*2* (681G>A; rs4244285) was genotyped using a commercially available validated Drug Metabolism Genotyping Assay (Applied Biosystems, Frankfurt, Germany; part no. C\_25986767\_70). Amplification was performed in a final volume of 5  $\mu\text{l}$  containing 1  $\mu\text{l}$  of DNA solution of a concentration of 5 mg/l, 0.25  $\mu\text{l}$  primer mix, 2.5  $\mu\text{l}$  QPCR Master Mix (Abgene, Hamburg, Germany), and 3.75  $\mu\text{l}$  of distilled water. Reaction mixtures were loaded into 384 well plates and placed in an ABI Prism Sequence Detector 7900 (Applied Biosystems). Polymerase chain reaction conditions were as follows: initial denaturation at 95°C for 10 min, followed by 45 cycles of denaturation (92°C for 30 s), annealing and extension in 1 step (60°C for 60 s). After PCR, fluorescence yield for the 2 different dyes was measured and presented in a 2-dimensional graph.

**Outcomes.** In the analysis of the impact of the *CYP2C19\*2* polymorphism on platelet reactivity after administration of clopidogrel, the proportion of patients achieving an RPA >14% after stimulation with 5  $\mu\text{mol/l}$  ADP was our primary outcome measure. The primary analysis of the EXCELSIOR trial had revealed that this threshold delineates subsets of patients at increased 30-day risk of major adverse cardiac events. Compared with maximal light transmission used in some of the previous studies (27–29), RPA is considered to more closely resemble P2Y<sub>12</sub>-dependent responses and comprises both formation and stability of aggregates.

To further corroborate the clinical impact of an RPA >14%, we tested the hypothesis that an RPA >14% after the first maintenance dose at pre-discharge was associated with an increased risk of death and MI during 1-year follow-up as compared with an RPA  $\leq$ 14%. We therefore analyzed the cumulative incidence of death from any cause and nonfatal MI from the first maintenance dose to 12 months after coronary intervention, thus excluding acute procedure-related events that had been addressed in the primary analysis. The diagnosis of MI was made according to the European Society of Cardiology/American College of Cardiology consensus document and based on new rise in troponin T  $\geq$ 0.03 mg/l associated with typical symptoms and/or typical electrocardiogram changes and/or typical angiographic findings (25). We also assessed the incidence of stent thrombosis according to the Academic Research Consortium criteria (30). All events were classified and adjudicated by 2 physicians not involved in the follow-up process and blinded to the results of the platelet function data.

**Statistical analysis.** To compare the proportion of patients with RPA >14% between carriers of the *CYP2C19\*2* allele and wild-type homozygotes, we used the chi-square test and calculated adjusted and unadjusted odds ratios from logistic regression models. In the adjusted models, we included baseline variables (Table 1) with a difference between carriers of the *CYP2C19\*2* allele and wild-type homozygotes at a value of  $p \leq 0.20$ . The Komolgorov-Smirnov test revealed that the platelet function variables had a non-normal distribution. Therefore, we report platelet function variables as median (interquartile range) and tested differences between *CYP2C19* genotypes by the Mann-Whitney *U* test for 2 groups or the Kruskal-Wallis-H test for 3 groups. To corroborate these analyses, we also performed 1-way analysis of variance (ANOVA), which always confirmed significant results of non-parametric testing.

For our main analysis with regard to the impact of pre-discharge RPA on outcome, we assessed the difference in the incidence of the primary end point between the strata defined by pre-discharge RPA by 2-sided testing based on the log-rank statistic and a significance level of  $\alpha = 0.05$ . Cumulative event rates were calculated and graphically described according to the Kaplan-Meier method. To summarize differences in the primary end point between the

**Table 1** Baseline Demographic and Clinical Characteristics of the Study Cohort According to RPA Respective CYP2C19 Genotype

	Entire Cohort (n = 802)	According to RPA at Pre-Discharge			According to CYP2C19 Genotype		
		RPA ≤14% (n = 548)	RPA >14% (n = 217)	p Value	*1/*1 (n = 552)	*1/*2 or *2/*2 (n = 245)	p Value
Age (yrs)	66.4 ± 9.1	65.7 ± 9.2	68.1 ± 8.9	0.001	66.1 ± 9.2	66.9 ± 8.9	0.245
Women	175 (21.8)	118 (21.5)	47 (21.7)	0.969	123 (22.3)	52 (21.2)	0.739
Time from loading dose to PCI (h)	6.3 ± 11.2	6.1 ± 11.0	11.4 ± 0.8	0.510	5.9 ± 10.1	6.9 ± 13.1	0.275
Active smoker	87 (10.8)	62 (11.3)	20 (9.2)	0.398	52 (9.4)	35 (14.3)	0.042
Arterial hypertension	660 (82.3)	440 (80.3)	188 (86.6)	0.039	459 (83.2)	197 (80.4)	0.349
Diabetes mellitus	199 (24.8)	116 (21.2)	70 (32.3)	0.001	139 (25.2)	59 (24.1)	0.740
Total cholesterol (mmol/l)	5.1 ± 1.2	5.2 ± 1.2	5.1 ± 1.1	0.387	5.1 ± 1.2	5.1 ± 1.1	0.828
Body mass index (kg/m <sup>2</sup> )	27.7 ± 3.9	27.4 ± 3.8	28.3 ± 3.9	0.004	27.8 ± 4.0	27.4 ± 3.4	0.179
Platelets (×10 <sup>9</sup> /l)	216 ± 56	219 ± 55	211 ± 53	0.074	214 ± 53	220 ± 58	0.203
Aspirin	802 (100.0)	548 (100.0)	217 (100.0)	1.000	552 (100.0)	245 (100.0)	1.000
Beta-blockers	555 (69.2)	381 (69.5)	147 (67.7)	0.631	381 (69.0)	170 (69.4)	0.918
ACE inhibitors	342 (42.6)	223 (40.7)	101 (46.5)	0.140	236 (42.8)	102 (41.6)	0.768
AT1 antagonists	123 (15.3)	87 (15.9)	31 (14.3)	0.583	76 (13.8)	47 (19.2)	0.051
Diuretics	270 (33.7)	188 (34.3)	69 (31.8)	0.508	177 (32.1)	90 (36.7)	0.197
Nitrates	239 (29.8)	156 (28.5)	74 (34.1)	0.126	164 (29.7)	74 (30.2)	0.888
Any statin	455 (56.7)	305 (55.7)	128 (59.0)	0.402	310 (56.2)	142 (58.0)	0.636
CYP3A4-metabolized statin	377 (47.0)	250 (45.6)	108 (49.8)	0.300	257 (46.6)	118 (48.2)	0.675
Verapamil/diltiazem	34 (4.2)	15 (2.7)	17 (7.8)	0.002	21 (3.8)	12 (4.9)	0.475
Insulin	56 (7.0)	30 (5.5)	21 (9.7)	0.036	39 (7.1)	17 (6.9)	0.949
Oral antidiabetics	115 (14.3)	64 (11.7)	44 (20.3)	0.002	71 (12.9)	43 (17.6)	0.081
Previous balloon angioplasty	274 (34.2)	176 (32.1)	87 (40.1)	0.036	199 (36.1)	75 (30.6)	0.136
Previous CABG	112 (14.0)	71 (13.0)	36 (16.6)	0.192	81 (14.7)	29 (11.8)	0.284
Previous MI	184 (22.9)	126 (23.0)	51 (23.5)	0.880	126 (22.8)	56 (22.9)	0.992
Impaired LV function*	286 (35.8)	186 (33.9)	86 (39.6)	0.138	192 (34.8)	89 (36.3)	0.674
CCS angina class III or IV	199 (24.8)	141 (25.7)	46 (21.2)	0.189	141 (25.5)	56 (22.9)	0.417
Multivessel PCI	178 (22.2)	132 (24.1)	42 (19.4)	0.159	117 (21.2)	61 (24.9)	0.247
Number of stents implanted	1.8 ± 1.0	1.8 ± 1.0	1.8 ± 1.1	0.736	1.8 ± 1.0	1.9 ± 1.1	0.296
Drug-eluting stent implanted	292 (36.4)	211 (38.5)	70 (32.3)	0.106	197 (35.7)	94 (38.4)	0.469
Number of drug-eluting stents implanted	0.4 ± 0.6	0.4 ± 0.6	0.4 ± 0.7	0.382	0.4 ± 0.6	0.4 ± 0.6	0.554
AHA/ACC lesion type B2 or C	528 (65.8)	351 (64.6)	148 (68.8)	0.234	380 (69.0)	149 (60.8)	0.025
Stented segment							
Left main coronary artery	18 (2.2)	13 (2.4)	4 (1.8)	0.655	10 (1.8)	8 (3.3)	0.202
Left anterior descending artery	367 (45.8)	262 (47.8)	92 (42.4)	0.176	255 (46.2)	112 (45.7)	0.900
Circumflex artery	295 (36.8)	199 (36.3)	81 (37.3)	0.793	193 (35.0)	98 (40.0)	0.173
Right coronary artery	281 (35.0)	191 (34.9)	77 (35.5)	0.869	192 (34.8)	89 (36.3)	0.674
Bypass graft	25 (3.1)	18 (3.3)	7 (3.2)	0.967	19 (3.4)	5 (2.0)	0.285
QCA analysis							
Vessel size (mm)	2.85 ± 0.57	2.84 ± 0.57	2.86 ± 0.58	0.720	2.83 ± 0.56	2.91 ± 0.58	0.053
Before PCI							
Minimal luminal diameter (mm)	0.72 ± 0.50	0.73 ± 0.50	0.72 ± 0.50	0.841	0.72 ± 0.50	0.74 ± 0.49	0.639
Diameter stenosis (%)	74.8 ± 15.9	74.7 ± 15.8	75.0 ± 16.2	0.775	74.7 ± 16.2	75.0 ± 15.2	0.819
Stenosis length (mm)	10.2 ± 5.2	10.4 ± 5.1	9.7 ± 5.0	0.112	10.2 ± 5.3	10.1 ± 4.8	0.719
Balloon size (mm)	3.2 ± 0.5	3.2 ± 0.5	3.2 ± 0.5	0.624	3.2 ± 0.5	3.2 ± 0.4	0.148
Inflation pressure (atm)	13.8 ± 2.8	13.8 ± 2.7	13.8 ± 2.6	0.979	13.8 ± 2.8	13.9 ± 2.7	0.789
After PCI							
Minimal luminal diameter (mm)	2.76 ± 0.46	2.77 ± 0.46	2.73 ± 0.45	0.308	2.74 ± 0.46	2.80 ± 0.46	0.107
Diameter stenosis (%)	9.3 ± 6.6	8.8 ± 6.5	10.1 ± 6.6	0.014	9.3 ± 6.56	9.1 ± 6.6	0.723
Stented length (mm)	21.1 ± 11.8	21.1 ± 11.3	20.8 ± 12.1	0.781	21.5 ± 11.8	20.3 ± 11.9	0.176

Data are expressed as mean ± SD or number of patients (percentage). The p values were determined by 1-way analysis of variance or chi-square between patients with residual platelet aggregation (RPA) ≤14% and RPA >14% or between CYP2C19 genotypes. CYP3A4-metabolized statin summarizes patients treated with atorvastatin, lovastatin, or simvastatin. \*Left ventricular ejection fraction <55%.

ACC = American College of Cardiology; ACE = angiotensin-converting enzyme; AHA = American Heart Association; AT = angiotensin; CABG = coronary artery bypass grafting; CCS = Canadian Cardiovascular Society; LV = left ventricular; MI = myocardial infarction; PCI = percutaneous coronary intervention; QCA = quantitative coronary angiography; RPA = residual platelet aggregation.

strata defined by pre-discharge RPA, we derived hazard ratios with associated 95% confidence intervals (CIs) from the Cox proportional hazards model. To assess the interac-

tion of drug-eluting stents with pre-discharge RPA and to adjust for potential confounders, we performed Cox regression analyses. In addition to the interaction term, the



multivariable models included demographic, clinical, and angiographic variables (Table 1) with a difference between the 2 strata defined by pre-discharge RPA at a value of  $p \leq 0.20$ .

If not stated otherwise, discrete variables are reported as counts (percentages) and continuous variables as mean  $\pm$  standard deviation. For discrete variables, we tested differences between groups with the chi-square test or Fisher exact test when expected cell sizes were less than 5. To compare continuous variables, we used 1-way ANOVA. In the 2-sided test, a  $p$  value  $< 0.05$  was regarded as significant. We used the SPSS software package, version 14 (SPSS Inc., Chicago, Illinois) for all statistical analyses.

The sample size of the EXCELSIOR study was based on the power calculation for the 30-day primary end point as published previously (1).

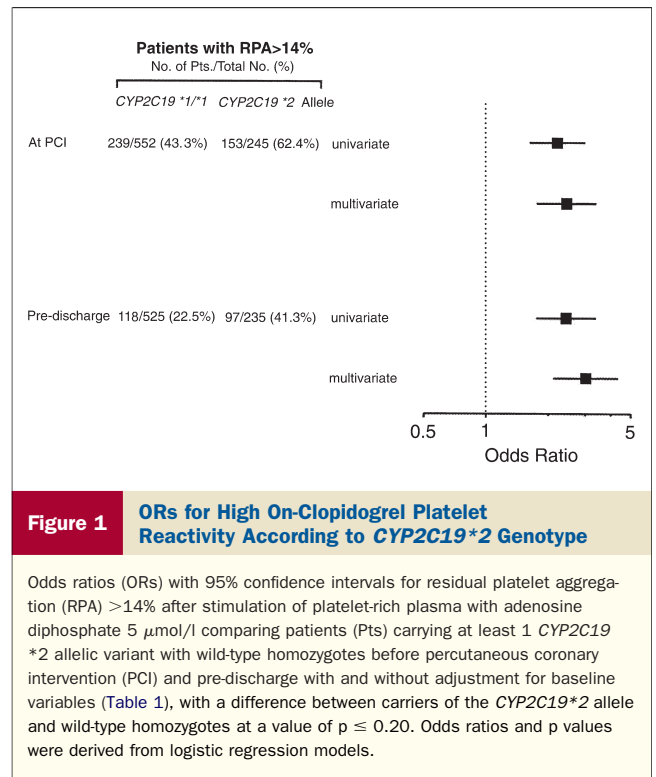
## Results

**CYP2C19 genotyping results.** *CYP2C19* genotype could be determined in 797 patients out of the whole cohort of 802 patients enrolled in the EXCELSIOR study. Five patients refused to consent to blood sampling for genetic investigations.

Of the 797 patients, 552 (69.3%) were *CYP2C19* wild-type homozygotes (\*1/\*1), 228 (28.6%) were *CYP2C19* (\*1/\*2) heterozygote, and 17 (2.1%) were homozygous with respect to the \*2 allelic variant. This corresponds to allele frequencies of 83.6% for *CYP2C19*\*1 (G) and 16.4% for *CYP2C19*\*2 (A), which is consistent with the known allele frequency in Caucasians. The distribution of the genetic variants did not deviate significantly from the Hardy-Weinberg equilibrium ( $p > 0.20$ ). Due to the low numbers of \*2 homozygotes, we based most of our analyses on the comparison between carriers of the *CYP2C19*\*2 allele and wild-type homozygotes.

Baseline demographic and clinical characteristics of the study population according to their *CYP2C19* genotype are summarized in Table 1. There were no significant differences between wild-type homozygous patients and patients with the allelic variant *CYP2C19*\*2 except a slightly higher proportion of active smokers and a slightly lower proportion of patients with type B2 or C coronary lesions according to the American Heart Association/American College of Cardiology definitions in the cohort carrying the \*2 allelic variant of *CYP2C19*.

**CYP2C19 polymorphism and on-clopidogrel platelet reactivity.** Both at the time of PCI after the 600-mg loading dose of clopidogrel and at pre-discharge after the first maintenance dose, carriers of the *CYP2C19*\*2 allele were significantly more likely than wild-type homozygotes to miss the level of RPA  $> 14\%$  after stimulation with 5  $\mu\text{mol/l}$  ADP (Fig. 1). At pre-discharge, 41.3% of the carriers of the *CYP2C19*\*2 allele, but only 22.5% of wild-type homozygotes had an RPA  $\geq 14\%$  ( $p < 0.001$ ); at the time of PCI the respective proportions were 62.4% and



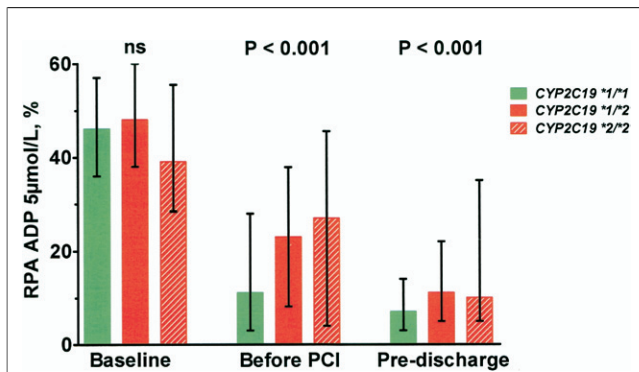
**Figure 1** ORs for High On-Clopidogrel Platelet Reactivity According to *CYP2C19*\*2 Genotype

Odds ratios (ORs) with 95% confidence intervals for residual platelet aggregation (RPA)  $> 14\%$  after stimulation of platelet-rich plasma with adenosine diphosphate 5  $\mu\text{mol/l}$  comparing patients (Pts) carrying at least 1 *CYP2C19*\*2 allelic variant with wild-type homozygotes before percutaneous coronary intervention (PCI) and pre-discharge with and without adjustment for baseline variables (Table 1), with a difference between carriers of the *CYP2C19*\*2 allele and wild-type homozygotes at a value of  $p \leq 0.20$ . Odds ratios and  $p$  values were derived from logistic regression models.

43.3% ( $p < 0.001$ ). Thus, at pre-discharge the unadjusted odds ratio for RPA  $\geq 14\%$  was 2.43 (95% CI 1.74 to 3.38;  $p < 0.001$ ) in carriers of the *CYP2C19*\*2 allele as compared with wild-type homozygotes and it was 2.18 (95% CI 1.60 to 2.97;  $p < 0.001$ ) at the time of PCI (Fig. 1). Similar results were obtained after adjustment for pertinent covariables, including clinical characteristics (active smoker, body mass index, hypertension, previous PCI), drug therapy (angiotensin-1 blockers, diuretics, oral antidiabetics), or angiographic parameters (American Heart Association/American College of Cardiology coronary lesion type B2 or C, stenting in circumflex artery, vessel size, balloon size, minimal lumen diameter after PCI, stented length) (Fig. 1).

These findings could be attributed to a significantly attenuated platelet inhibition by clopidogrel in carriers of the *CYP2C19*\*2 allele as compared with wild-type homozygotes (Fig. 2, Table 2). While baseline RPA was similar irrespective of *CYP2C19* genotype, RPA induced by 5  $\mu\text{mol/l}$  ADP differed significantly between *CYP2C19* genotypes, both after the 600-mg loading dose of clopidogrel at the time of PCI and after the first maintenance dose at pre-discharge. At both time points, carriers of the *CYP2C19*\*2 allele had a significantly higher RPA than wild-type homozygotes (Table 2).

As shown in Table 2, we obtained similar results when platelet aggregation was induced by 20  $\mu\text{mol/l}$  ADP. The association between the allelic variant *CYP2C19*\*2 and higher on-clopidogrel platelet reactivity was also confirmed by flow cytometry of the surface expression of activation-dependent proteins after stimulation with ADP 20  $\mu\text{mol/l}$ ,



**Figure 2 Platelet Aggregation According to CYP2C19\*2 Genotype**

Residual platelet aggregation in response to adenosine diphosphate (ADP) 5 µmol/in 3 CYP2C19 genotypes at various time points, as indicated below the columns. The columns represent median with interquartile range. The p value was determined by Kruskal-Wallis-H test. ns = not significant; other abbreviations as in Figure 1.

which could be analyzed in 699 patients. No significant differences in the expression of any of the surface proteins analyzed were found at baseline (Table 2). During PCI, however, surface expression of P-selectin, activated GP IIb/IIIa, CD63, as well as CD40L was significantly higher in patients with the \*2 allele than in wild-type homozygous patients indicating an attenuated antiplatelet effect of clopidogrel in carriers of the CYP2C19\*2 allele as compared with CYP2C19 \*1/\*1 patients (Table 2). We obtained consistent results at pre-discharge (Table 2).

**Pre-discharge platelet aggregation and 1-year clinical outcome.** Of the 802 patients of the EXCELSIOR cohort, pre-discharge RPA could be obtained in 765 patients. Pre-discharge RPA was >14% in 217 patients and ≤14% in 548 patients. Table 1 shows the demographic and baseline clinical characteristics as well as the procedural and angiographic variables in these cohorts stratified with regard to this cut point in RPA. Compared with the patients with RPA ≤14%, patients with RPA >14% were significantly older, had a significantly higher body mass index, and had a significantly higher residual stenosis after PCI. Likewise, the proportion of patients with diabetes mellitus, arterial hypertension, and previous PCI as well as the proportion of patients being treated with oral antidiabetics, insulin, or verapamil/diltiazem was significantly increased in patients with RPA >14%, as compared with the patients with RPA ≤14%.

Figure 3 shows the cumulative incidence of death and MI during 1-year follow-up. This composite end point was reached by 13 of 217 patients (6.0%) with pre-discharge RPA >14% and by 11 of 548 patients (2.0%) with RPA ≤14%. Thus, pre-discharge RPA >14% was associated with a hazard ratio for death and MI of 3.0 (95% CI 1.4 to 6.8; p = 0.004) compared with RPA ≤14%. The excess risk associated with a pre-discharge RPA >14% prevailed after adjustment for baseline and procedural variables, with a difference between the 2 strata defined by pre-discharge

RPA at a value of p ≤ 0.20. The corresponding adjusted hazard ratio was 3.7 (95% CI 1.5 to 8.8; p = 0.004).

Table 3 shows the incidence of individual cardiac events in the 2 strata defined by pre-discharge RPA. The difference between the 2 strata defined by pre-discharge RPA was driven by death and large MIs. With the exception of 2 infarctions in patients without a drug-eluting stent of the stratum with RPA ≤14%, all infarctions occurred in areas subtended by a treated vessel. Most of the events constituting the primary end point could be classified as definite, probable, or possible stent thrombosis (Table 3).

Figure 4 shows the cumulative incidence of the primary end point in the 2 subsets defined by stent type stratified by pre-discharge RPA, which was significantly different between the 4 strata (log-rank p = 0.020 for the overall comparison). In patients who received at least 1 drug-eluting stent, the 1-year incidence of death and MI was 7.1% in patients with pre-discharge RPA >14% and 0.9% in patients with pre-discharge RPA ≤14%, corresponding to a hazard ratio for death and MI of 7.8 (95% CI 1.5 to 40.3; p = 0.004). Among patients who did not receive a drug-eluting stent (Fig. 4), the difference in 1-year outcome between the 2 strata with respect to death and MI was smaller (5.4% for RPA >14% vs. 2.7% for RPA ≤14%) and did not reach statistical significance (p = 0.13). On formal testing of the interaction of pre-discharge RPA with stent type regarding the primary end point, the p for interaction was 0.10 when added to the Cox regression model including baseline and procedural variables, with a difference between the 2 strata defined by pre-discharge RPA at a value of p ≤ 0.20. In this model, the hazard ratio of RPA >14% for the primary end point was 11.4 (95% CI 2.1 to 61.4; p = 0.005).

When we analyzed the 1-year incidence of death and MI with respect to the CYP2C19\*2 polymorphism, we found 5 (2.0%) patients with an event among carriers of the CYP2C19\*2 allele and 19 (3.4%) patients with an event among wild-type homozygotes (p = 0.371). Within the group with at least 1 drug-eluting stent, the percentage of patients with an event was 3.3% (3 of 90) among carriers of the CYP2C19\*2 allele versus 2.1% (4 of 190) among wild-type homozygotes (p = 0.684).

## Discussion

Our current study in a large cohort of patients undergoing elective PCI conveys 2 main messages: 1) due to a substantially attenuated antiplatelet effect of clopidogrel in carriers of the loss-of-function \*2 allelic variant of CYP2C19 as compared with wild-type homozygotes, the \*2 allelic variant of CYP2C19 is associated with an almost 2-fold risk of high on-clopidogrel platelet reactivity; and 2) such high on-clopidogrel platelet reactivity increases risk of death and MI during the first year after elective stent placement by about 3-fold. Based on the previously published data from the EXCELSIOR trial, we defined high on-treatment platelet reactivity as RPA after stimulation with 5 µmol/l ADP >14%,

**Table 2 Platelet Aggregation and Expression of Surface Proteins According to CYP2C19 Genotype**

	CYP2C19 *1/*1 (n = 552)		CYP2C19 *1/*2 or *2/*2 (n = 245)		p Value
	Median	Interquartile Range	Median	Interquartile Range	
<b>Light transmission aggregometry</b>					
<b>RPA ADP 5 μmol/l (%)</b>					
Baseline	46.0	(36.0–57.0)	48.0	(38.0–60.0)	0.106
At cath lab	11.0	(3.0–28.0)	23.0	(8.0–38.0)	<0.001
Pre-discharge*	7.0	(3.0–14.0)	11.0	(5.0–22.0)	<0.001
<b>% inhibition of RPA ADP 5 μmol/l</b>					
At cath lab	74.2	(36.1–92.1)	51.6	(20.0–81.6)	<0.001
Pre-discharge*	83.7	(67.4–94.3)	74.5	(56.9–88.5)	<0.001
<b>RPA ADP 20 μmol/l (%)</b>					
Baseline	73.0	(65.0–80.0)	74.0	(66.0–82.5)	0.125
At cath lab	35.0	(11.3–56.0)	50.0	(28.0–64.0)	<0.001
Pre-discharge*	20.0	(8.0–38.5)	31.0	(15.0–50.0)	<0.001
<b>% inhibition of RPA ADP 20 μmol/l</b>					
At cath lab	51.4	(24.2–83.9)	32.9	(14.2–64.1)	<0.001
Pre-discharge*	73.6	(48.5–89.3)	58.1	(32.5–80.9)	<0.001
<b>Surface protein expression after stimulation with ADP 20 μmol/l (au)</b>					
<b>P-selectin (CD62P)</b>					
Baseline	109.0	(77.0–147.0)	109.0	(84.3–149.0)	0.600
At cath lab	26.0	(13.0–54.0)	44.0	(21.3–77.8)	<0.001
Pre-discharge*	20.0	(11.0–36.0)	35.0	(17.0–59.0)	<0.001
<b>Activated GP IIa/IIIb (PAC-1)</b>					
Baseline	88.0	(78.0–100.0)	90.0	(78.0–101.0)	0.669
At cath lab	59.0	(42.0–79.0)	75.5	(54.0–90.0)	<0.001
Pre-discharge*	51.0	(36.0–66.5)	63.0	(46.0–79.5)	<0.001
<b>CD63</b>					
Baseline	19.0	(16.0–23.0)	20.0	(16.3–23.0)	0.378
At cath lab	12.0	(10.0–15.0)	14.0	(12.0–17.0)	<0.001
Pre-discharge*	11.0	(10.0–14.0)	13.0	(11.0–16.0)	<0.001
<b>CD40L</b>					
Baseline	3.5	(3.0–4.3)	3.5	(3.0–4.2)	0.845
At cath lab	3.1	(2.7–3.6)	3.2	(2.8–3.8)	0.013
Pre-discharge*	3.0	(2.7–3.5)	3.2	(2.8–3.8)	0.010
<b>CD41 (GP IIb)</b>					
Baseline	655.0	(581.0–736.0)	665.5	(571.3–758.5)	0.408
At cath lab	563.5	(484.0–639.5)	608.5	(520.5–688.3)	<0.001
Pre-discharge*	554.0	(475.5–633.0)	581.0	(508.3–659.0)	0.003

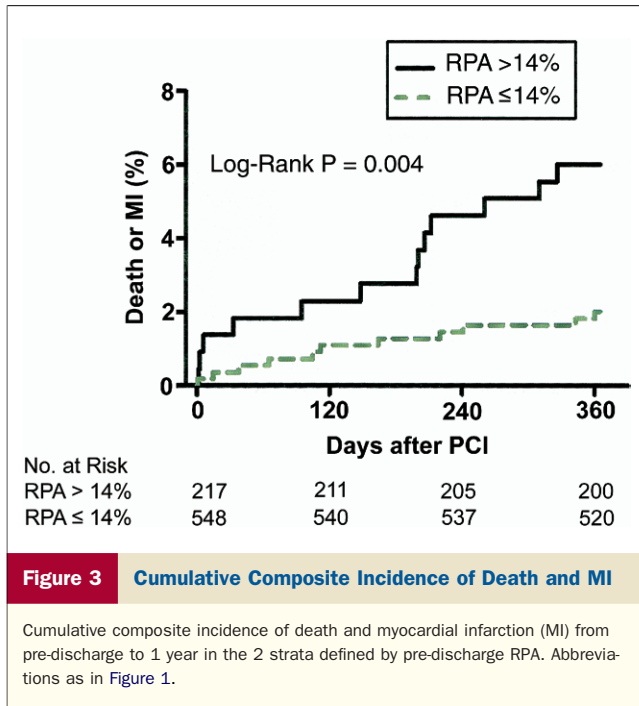
\*Platelet function tests were available pre-discharge in 760 patients (n = 525 wild-type homozygotes and n = 235 patients carrying at least 1 CYP2C19\*2 allelic variant) and surface protein expression in 666 patients (n = 465/n = 201); data are expressed as median (interquartile range). p value by Mann-Whitney U test between CYP2C19\*1/\*1 and CYP2C19\*2 genotype.

ADP = adenosine diphosphate; au = arbitrary units; GP = glycoprotein; RPA = residual platelet aggregation.

which was associated with a more than 6-fold increase in the 30-day risk of death, MI, and urgent target lesion revascularization after elective stent placement. Taken together, the original EXCELSIOR trial data and the current analysis present robust evidence that high-on treatment platelet reactivity as defined by RPA >14% exposes patients with elective PCI to a substantially increased risk of cardiac complications, both peri-interventionally and during 1-year follow-up.

**Impact of CYP2C19 681G>A polymorphism on clopidogrel effect.** At the time of PCI after loading with 600 mg clopidogrel, the proportion of patients with RPA >14% in carriers of the \*2 allele was 19.1 percentage points higher than in wild-type homozygotes, and the majority (62.4 %) of carriers of the \*2 allele failed to reach an RPA ≤14%. Although the proportion of patients with RPA ≤14% had

increased in both genotypes after the first maintenance dose at pre-discharge, a difference of 18.8 percentage points in the proportion of patients with RPA >14% persisted between carriers of the \*2 allele and wild-type homozygotes. The higher on-treatment platelet reactivity in carriers of the \*2 allele as compared with wild-type homozygotes was caused by an attenuated platelet inhibition by clopidogrel, but not by differences in baseline platelet reactivity. This was shown by light transmittance aggregometry after stimulation with both 5 and 20 μmol/l ADP. Flow-cytometry analysis of surface expression of platelet proteins after stimulation with ADP confirmed the results of light transmittance aggregometry. In patients with at least 1 CYP2C19\*2 allele, surface expressions of P-selectin (CD62P), activated GP IIb/IIIa (PAC-1), CD40L, CD41,



**Figure 3** Cumulative Composite Incidence of Death and MI

Cumulative composite incidence of death and myocardial infarction (MI) from pre-discharge to 1 year in the 2 strata defined by pre-discharge RPA. Abbreviations as in Figure 1.

and CD63 after loading with clopidogrel were significantly higher than in wild-type homozygotes, both during PCI and at pre-discharge.

We did not determine plasma concentrations of the active metabolite of clopidogrel (R130,964), which constitutes a limitation of our study. Thus, we cannot provide direct evidence for the relationship between a diminished systemic availability of the active metabolite and the blunted antiplatelet effect of clopidogrel in patients with nonfunctioning CYP2C19. The chemical instability of the metabolite requires extensive pre-analytical precautions and rapid chemical derivatization after blood withdrawal, which cannot be warranted in a study on large patient cohorts under clinical conditions. Data from a case report describing a nonresponder to clopidogrel treatment (31); the results of a

dose-response study investigating the antiplatelet effects of increasing bolus doses of clopidogrel 300, 600, and 900 mg (32); and a recent study investigating antiplatelet effects of a 300-mg bolus dose of clopidogrel in healthy subjects (33) provide robust evidence that the antiplatelet effects of clopidogrel in vivo are correlated to the systemic availability of the active metabolite. Thus, it is reasonable to assume that a decrease in systemic exposure to the active compound is the mechanism responsible for the attenuated efficacy of clopidogrel in CYP2C19\*2 patients.

Our findings expand the reports on attenuated antiplatelet effects of clopidogrel in healthy volunteers carrying the loss-of-function CYP2C19\*2 allele (19,23) and, thus, add substantial evidence for the involvement of CYP2C19 in the formation of the active metabolite of clopidogrel. Although the pharmacologic activity of clopidogrel is significantly attenuated in patients carrying one CYP2C19\*2 allele, these patients and even CYP2C19\*2 homozygous patients exhibit significant antiplatelet effects of clopidogrel underlining the contribution of alternative metabolic pathways via different members of the CYP system for the formation of the active metabolite.

**Potential clinical relevance in the setting of coronary stent placement.** The clinical relevance of the attenuation of the antiplatelet effect of clopidogrel in carriers of the \*2 allele is a key issue. To approach this issue, the principle question has to be answered whether high on-clopidogrel reactivity is, in fact, associated with poor clinical outcome after stent placement. Although we previously showed that high on-clopidogrel reactivity, as defined by RPA >14%, is associated with a substantially increased 30-day risk of major adverse cardiac events after PCI with stent placement (1), we intended to corroborate these findings by extension of the follow-up to 1 year.

We found that the pre-discharge level of RPA in patients on clopidogrel is a major determinant of the 1-year cardiac outcome after PCI with stent placement. In patients who did not achieve a pre-discharge RPA ≤14% after the first

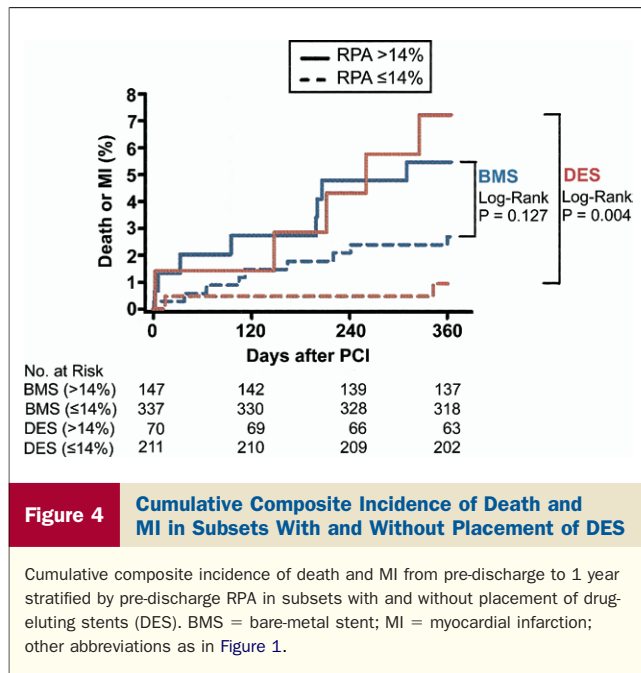
**Table 3** Clinical Outcome According to Pre-Discharge RPA

	RPA ≤14% (n = 548)	RPA >14% (n = 217)	HR (95% CI)	p Value
Death or MI	11 (2.0)	13 (6.0)	3.0 (1.4-6.8)	0.004
Death	3 (0.5)	7 (3.2)	6.0 (1.5-23.2)	0.003
Nonfatal MI				
ST-segment elevation MI	2 (0.4)	2 (0.9)	2.6 (0.4-18.1)	0.33
Non-ST-segment elevation MI	6 (1.1)	4 (1.8)	1.7 (0.5-6.1)	0.40
Target lesion reintervention	60 (10.9)	26 (12.0)	1.1 (0.7-1.8)	0.67
Any stent thrombosis	7 (1.3)	10 (4.6)	3.7 (1.4-9.6)	0.008
Definite or probable	5 (0.9)	8 (3.7)	4.1 (1.3-12.5)	0.01
Definite	2 (0.4)	1 (0.5)	1.3 (0.1-14.1)	0.84
Probable	3 (0.5)	7 (3.2)	5.9 (1.5-23.0)	0.01
Possible	2 (0.4)	2 (0.9)	2.6 (0.4-18.4)	0.34

Stent thrombosis was defined according to Academic Research Consortium criteria. Data are expressed as number of patients (percentage). Hazard ratios (HRs) (95% confidence intervals [CIs]) are based on the Cox proportional hazards model, and the associated p values are based on log-rank statistics.

Abbreviations as in Table 1.





75-mg maintenance dose of clopidogrel after loading with 600 mg the day before, the 1-year incidence of death and MI was increased by 3-fold compared with patients whose RPA fell below the cut point. The excess risk associated with high levels of RPA at pre-discharge was driven by a significantly increased mortality and by a surplus of large MIs. Thus, our findings underscore the clinical relevance of adequate RPA levels in the prevention of subsequent serious cardiac complications. Further studies will clarify whether more intense therapy designed for correction of inadequate suppression of platelet reactivity will improve outcome.

The impact of pre-discharge RPA on outcome differed substantially depending on stent type. In patients having received at least 1 drug-eluting stent who continued on clopidogrel for 6 months, a pre-discharge RPA >14% was particularly deleterious. In this subset, it conferred an almost 8-fold increase in the risk of death and MI during the 1-year follow-up, as compared with a pre-discharge RPA ≤14%. In patients who exclusively received bare-metal stents, we also found an increased risk with pre-discharge RPA >14%, but the association between high pre-discharge RPA and poor outcome was weaker than in the subset with drug-eluting stents and did not reach statistical significance. These findings support the generally accepted view that the delayed vascular healing after drug-eluting stents necessitates a more robust and sustained platelet inhibition than that needed after placement of bare-metal stents (34).

Although we were able to demonstrate that high on-clopidogrel platelet reactivity is associated with poor clinical outcome and that the presence of a \*2 allele of *CYP2C19* increases the propensity for high on-clopidogrel platelet reactivity, we were unable to demonstrate the impact of the \*2 allele of *CYP2C19* on clinical outcome directly. The EXCELSIOR trial was not powered to address this issue

directly. Given that a 100% difference in the proportion of patients with RPA >14% was associated with a 3-fold risk of death and MI during 1-year follow-up, it can be estimated in the given setting that a 20% difference (as that between \*2 allele carriers and non-carriers) would result in a relative risk of 1.26. Thus, a substantially higher number of patients would have been needed to demonstrate a direct relation between the \*2 allele of *CYP2C19* and clinical outcome in clopidogrel-treated patients having undergone stent placement.

**Implications.** The results of our study identify a cohort with a genetic background that predisposes to attenuated antiplatelet effects of clopidogrel, which may expose patients to an increased risk of thrombotic events. It is conceivable that the clinical impact of attenuated antiplatelet effects of clopidogrel may be particularly prominent in patients with higher risk, such as patients with acute coronary syndromes (12). Our study, however, demonstrates that even in patients considered to be at low to intermediate risk of cardiac complications after coronary stent placement, a high residual platelet reactivity after administration of clopidogrel has a major impact on long-term outcome. Patients having received a drug-eluting stent are particularly sensitive to high residual levels of platelet aggregation during treatment with clopidogrel, with an almost 8-fold increased risk of death and MI during the first year after stent placement.

It is unclear so far, if the antiplatelet effect of clopidogrel in patients carrying the \*2 allelic variant of *CYP2C19* can be improved by administration of higher bolus and/or maintenance doses of clopidogrel or if alternative treatment regimens should be preferred. In vitro and in vivo studies investigating the metabolism of the newly developed P2Y<sub>12</sub>-receptor antagonist prasugrel indicate that CYP3A and CYP2B6 provide the greatest contribution to the metabolic activation of this new compound with less contributions from CYP2C9 and CYP2C19 (35). Given the susceptibility of the thienopyridine metabolism to the highly polymorphic CYP system, genetic profiling may become a tool in tailoring the choice of antiplatelet agent to the individual patient's needs. In this respect, our current study may help design a clinical relevant panel of polymorphic genes to be assessed.

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