Relation of cytochrome P450 2C19 loss-of-function polymorphism to occurrence of drug-eluting coronary stent thrombosis

Relation of Cytochrome P450 2C19 Loss-of-Function Polymorphism to Occurrence of Drug-Eluting Coronary Stent Thrombosis

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Residual platelet reactivity (RPR) to adenosine 5′ diphosphate (ADP) was an independent predictor of stent thrombosis (ST) in patients receiving drug-eluting stents on dual-antiplatelet treatment and was associated with the cytochrome P450 (CYP)2C19*2 polymorphism. The aim was to evaluate the role of the CYP2C19*2 polymorphism in the occurrence of ST or the composite end point of ST and cardiac mortality within a 6-month follow-up in patients undergoing percutaneous coronary interventions with drug-eluting stent implantation on dual-antiplatelet treatment enrolled in the RECLOSE trial. Seven hundred seventy-two patients were studied for the CYP2C19*2 polymorphism and RPR (using 10-μM ADP-induced platelet aggregation). Patients with ST or the composite of ST and cardiac mortality showed a higher prevalence of carriers of the rare allele (54.1% vs 31.3%; p = 0.025 and 51.7% vs 31.2%; p = 0.020, respectively). At multivariate logistic regression analysis with ST or ST and cardiac mortality as dependent variables and the CYP2C19*2 polymorphism, ADP RPR, and additional previously shown clinical and procedural risk factors for ST as independent variables, the CYP2C19*2 allele (ST odds ratio [OR] 3.43, 95% confidence interval [CI] 1.01 to 12.78, p = 0.047; ST and cardiac mortality OR 2.70, 95% CI 1.00 to 8.42, p = 0.049) and ADP RPR (ST OR 3.08, 95% CI 1.23 to 7.72, p = 0.016; ST and cardiac mortality OR 2.90, 95% CI 1.08 to 12.98, p = 0.019) were independent risk factors. Subjects with the contemporary presence of the CYP2C19*2 allele and ADP RPR showed a strong risk of ST or ST and cardiac mortality (OR 5.79, 95% CI 1.04 to 39.01, p = 0.033 and OR 11.45, 95% CI 1.84 to 71.27, p = 0.009, respectively). In conclusion, the CYP2C19*2 allele was associated with the occurrence of ST or ST and cardiac mortality in high-risk vascular patients on dual-antiplatelet treatment. These findings could impact on the future design of pharmacogenetic antiaggregant strategies. **(Am J Cardiol 2009;103:806–811)**

Several studies investigated the clinical implications of antiplatelet drug resistance in patients with coronary artery disease or stent thrombosis (ST).[1–8] Recently, it was shown that nonresponsiveness to clopidogrel therapy was an independent predictor of ST in patients receiving drug-eluting stents.[9,10] Genetic polymorphisms could have a pivotal role in determining individual susceptibility to antiplatelet drug response. Several polymorphisms in genes coding platelet components (glycoprotein Ia, glycoprotein IIIa, glycoprotein Ib-α, glycoprotein VI, adenosine 5′ diphosphate [ADP] receptor $P2Y_{12}$ [P2Y12], P-selectin, cyclooxygenase-1, and cyclooxygenase-2) or cytochrome P450 (CYP) enzyme isoforms (CYP-3A4 and CYP-3A5) have been proposed and investigated.[11–13] Regarding nonresponsiveness to clopidogrel therapy, the active metabolite of this molecule, which irreversibly blocked the platelet ADP P2Y12 receptor, arose from biochemical reactions[14–16] involving several CYP isoforms.[11,15] Variability in the catalytic activity of these isoforms affected the pharmacodynamic action of clopidogrel. Studies of healthy subjects[17–20] showed that the CYP2C19*2 allelic variant (681A allele) encoding a deficient drug-metabolizing enzyme[21] was associated with impaired platelet inhibition to clopidogrel treatment.

Recently, in 1,419 patients with acute coronary syndromes undergoing percutaneous coronary interventions on dual-antiplatelet treatment, we showed that carriers of the CYP2C19*2 allele had significantly higher platelet aggregation after ADP and arachidonic acid stimuli, and the polymorphism was an independent predictor of antiplatelet treatment variability.[22] These data were confirmed in an additional study of 603 patients with non–ST-elevation acute coronary syndrome.[23] In the frame of the Low Responsiveness to Clopidogrel and Sirolimus- or Paclitaxel-Eluting Stent Thrombosis (RECLOSE) trial,[9,24] we evaluated the role of the CYP2C19*2 polymorphism on the occurrence of drug-eluting ST (primary end point) and the composite feature of cardiac mortality and drug-eluting ST (secondary end point) in a 6-month follow-up of 772 pa-

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tients undergoing percutaneous coronary intervention on dual-antiplatelet treatment.

Methods

The study population included 804 consecutive patients enrolled in the RECLOSE trial admitted to the Department of Cardiology of the Careggi Hospital, Florence, Italy, who underwent successful sirolimus- or paclitaxel-eluting stent implantation and for whom platelet reactivity after dual-antiplatelet (clopidogrel and aspirin) treatment was prospectively assessed in previous studies[9,24] and partially (n = 510) included in an additional report.[22] All patients gave informed consent for the clinical study, and 772 of 804 patients gave informed consent for the genetic study. The study was approved by the local ethical review board. Patients with acute coronary syndromes and ST-segment elevation acute myocardial infarction were included, as well as patients with left main disease, chronic total occlusions, bifurcation lesions, or diffuse disease. Exclusion criteria were (1) in-hospital death not caused by ST, (2) anticipated noncompliance with dual-antiplatelet treatment for ≥6 months, and (3) premature discontinuation of clopidogrel therapy. Exclusion criteria included a history of bleeding diathesis, platelet count ≤100,000/μL, hematocrit ≤30%, and serum creatinine ≥4.0 mg/dl. All interventions were performed according to current standard guidelines, and type of stent implanted and use of glycoprotein IIb/IIIa inhibitors were at the discretion of the operator. All patients received aspirin (325 mg) and a loading dose of clopidogrel 600 mg before the procedure, followed by a maintenance dose of clopidogrel 75 mg and aspirin 325 mg/day. Unfractionated heparin 70 IU/kg was used during the procedure as anticoagulant. In-hospital compliance with clopidogrel and aspirin therapy was verified by nursing staff, and compliance during follow-up was ensured through a structured interview. Patients were considered to have hypertension if hypertension had been diagnosed according to the European Society of Hypertension/European Society of Cardiology guidelines or they were using antihypertensive drugs. Dyslipidemia was defined according to the Third Report of the National Cholesterol Education Program, and diabetes, according to the American Diabetes Association.

Venous blood samples were obtained 12 to 18 hours from clopidogrel loading in tubes containing 3.2% trisodium citrate. For patients receiving both the loading dose of clopidogrel and the glycoprotein IIb/IIIa inhibitor in the catheterization laboratory, blood samples were obtained after 6 days.

Platelet aggregation was assessed using platelet-rich plasma with the turbidimetric method in a 4-channel aggregometer (APACT4; Helena Laboratories, Milan, Italy) according to our previous reports.[9,22,24] For antiplatelet response, the definition used was residual platelet reactivity (RPR) evaluated using 10 μM of ADP-induced platelet aggregation (ADP-RPR) = ADP-induced platelet aggregation ≥70%.[9,25]

Genomic DNA from 772 patients was isolated from whole blood using the FlexiGene DNA kit (Qiagen, Hilden, Germany).

The CYP2C19 gene sequence was obtained from GeneBank (accession no. NM_000769). The presence of the CYP2C19*2 (rs4244285) polymorphism was determined using polymerase chain reaction restriction fragment length polymorphism analysis.[22] The polymerase chain reaction primers used were forward 5′-CAGAGCTTGGCATATTGTATC-3′ and reverse 5′-GTAAACACAAAACTAGTCAATG-3′. Polymerase chain reaction products (321 base pairs) were subjected to digestion with the *Sma*I restriction enzyme (Promega Italia, Milan, Italy).

Briefly, the primary end point of the study was definite or probable ST during a 6-month follow-up. Definite ST was defined as acute coronary syndrome and either angiographic or pathologic confirmation of thrombosis. Probable ST was defined as unexplained death or myocardial infarction in the territory supplied by a stented vessel without angiographic confirmation. The secondary end point was the composite of cardiac mortality and definite or probable ST. All events were adjudicated by 3 observers who were blinded to laboratory results and not involved in the follow-up process. Follow-up details were reported in our previous article.[9]

Statistical analysis was performed using the SPSS statistical package, version 11.5 (SPSS Inc., Chicago, Illinois).

Table 1
Demographic and clinical characteristics of the 772 enrolled patients

Variable	Patients (n = 772)
Men	576 (74.6%)
Smokers	266 (34.4%)
Hypertension	505 (65.4%)
Diabetes mellitus	171 (22.2%)
Dyslipidemia	461 (59.7%)
Previous myocardial infarction	191 (24.7%)
Previous percutaneous coronary intervention	161 (20.9%)
Previous coronary bypass	58 (7.5%)
Stable angina pectoris	262 (33.9%)
Unstable angina pectoris	310 (40.2%)
Acute myocardial infarction	197 (25.5%)
Multivessel coronary disease	439 (56.8%)
Left ventricle ejection fraction (%)	47 ± 12
Long-term total occlusion	90 (11.6%)
Bifurcation lesion	349 (45.2%)
Total stent length (mm)	37 ± 28
Drugs	
β Blockers	467 (60.5%)
Calcium antagonists	127 (16.4%)
Statins	690 (89.4%)
Angiotensin-converting enzyme inhibitors	636 (82.4%)
Glycoprotein IIb/IIIa inhibitors	334 (43.3%)
Proton pump inhibitors	732 (94.8%)

Table 2
Clinical outcomes

Clinical Outcome	Overall (n = 772)	CYP2C19 Polymorphism		p Value
		*1*1 (n = 525)	*1*2 + *2*2 (n = 247)	
Definite/probable ST	24 (3.1%)	11 (2.1%)	13 (5.3%)	0.025
Definite	11 (1.4%)	5 (1.0%)	6 (2.5%)	0.100
Probable	13 (1.7%)	6 (1.1%)	7 (2.8%)	0.083
Cardiac mortality	18 (2.3%)	8 (1.5%)	10 (4.0%)	0.037
Composite of cardiac mortality and ST	29 (3.8%)	14 (1.8%)	15 (6.1%)	0.020

Table 3
Prevalence of CYP2C19 genotypes, ADP-RPR, and contemporary presence of ADP-RPR plus the CYP2C19*2 polymorphism according to the primary end point of stent thrombosis (ST) and the secondary end point of composite ST and cardiac mortality

Variables	No ST (n = 748)	ST (n = 24)	p Value	No ST + Cardiac Mortality (n = 743)	ST + Cardiac Mortality (n = 29)	p Value
CYP2C19 polymorphism						
2/2	24 (3.2%)	2 (8.3%)		24 (3.2%)	2 (6.9%)	
1/2	210 (28.1%)	11 (45.8%)	0.046	208 (28.0%)	13 (44.8%)	0.061
1/1	514 (68.7%)	11 (45.8%)		511 (68.8%)	14 (48.3%)	
ADP RPR						
Yes	102 (13.6%)	8 (33.3%)	0.001	100 (13.5%)	10 (34.5%)	0.001
No	646 (86.4%)	16 (66.7%)		643 (86.5%)	19 (65.5%)	
ADP RPR + CYP2C19*2						
Yes	34 (4.5%)	6 (25.0%)	<0.0001	33 (4.4%)	7 (24.1%)	<0.0001
No	714 (95.5%)	18 (75.0%)		710 (95.6%)	22 (75.9%)	

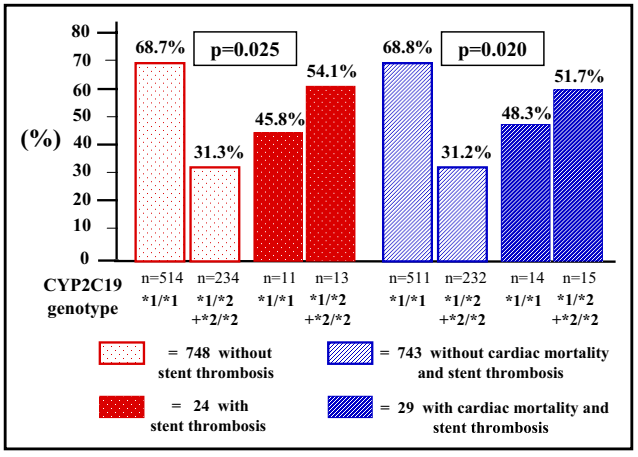

Figure 1. Prevalence of homozygote wild-type and rare allele carriers (heterozygotes and homozygotes) for the CYP2C19*2 polymorphism in patients with or without the occurrence of 6-month follow-up drug-eluting ST and the composite end point of ST and cardiac mortality.

We tested that the allele frequencies conformed to Hardy-Weinberg equilibrium proportions using chi-square test. Genotype and allele frequencies were compared between groups using chi-square analysis. Categorical variables were expressed as frequencies and percentages. Unless otherwise indicated, data were given as median and range. Comparisons of continuous variables between patients with and without ST or the composite of ST and cardiac mortality were performed using nonparametric Mann-Whitney U test.

Univariate and multivariate logistic regression analysis was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the development of ST or occurrence of the secondary end point. Multivariate logistic regression analysis was performed with ST or the composite of ST and cardiac mortality as the dependent variable and the CYP2C19*2 allele, ADP-RPR, traditional cardiovascular risk factors, and additional previously shown[9] clinical and procedural risk factors for ST (total chronic occlusion, multivessel disease, bifurcation lesion, acute myocardial infarction, previous myocardial infarction, total stent length, and left ventricular ejection fraction) as independent variables. A p value <0.05 was chosen as the cutoff for statistical significance.

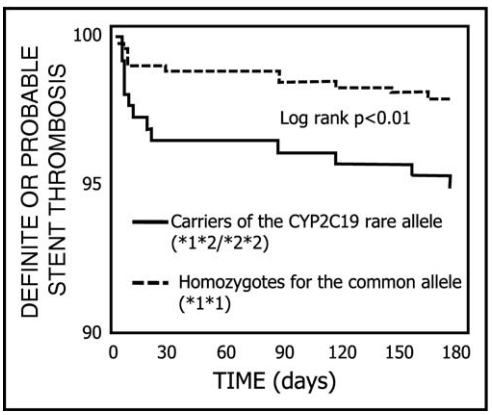

Figure 2. Kaplan-Meier analysis of the primary end point for the presence of the CYP2C19*2 polymorphism.

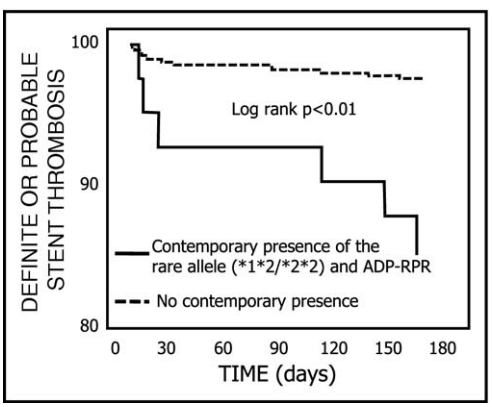

Figure 3. Kaplan-Meier analysis of the primary end point for the contemporary presence of the CYP2C19*2 polymorphism and ADP-RPR.

Results

Demographic and clinical characteristics of patients are listed in Table 1.

The genotype distribution of the CYP2C19*2 polymorphism in the overall patient population was 3.4% homozygotes *2/*2, 28.6% heterozygotes *1/*2, and 68.0% homozygotes *1/*1. The rare allele frequency in the overall patient population of the CYP2C19*2 polymorphism was

Table 4
The CYP2C19*2 allele and ADP-RPR odds ratios for the occurrence of stent thrombosis (ST) or the composite of ST and cardiac mortality in patients with acute coronary syndrome undergoing percutaneous coronary intervention

	Univariate Analysis	p Value	Multivariate Analysis*	p Value
ST				
CYP2C19*2 allele	2.59 (1.15–5.88)	0.022	3.43 (1.01–12.78)	0.047
ADP RPR	3.17 (1.32–7.59)	0.010	3.08 (1.23–7.72)	0.016
ST and cardiac mortality				
CYP2C19*2 allele	2.36 (1.12–4.97)	0.024	2.70 (1.00–8.42)	0.049
ADP RPR	3.38 (1.53–7.49)	0.003	2.90 (1.08–12.98)	0.019

* Multivariate logistic regression analysis was performed with ST or the composite of ST and cardiac mortality as the dependent variable and the CYP2C19*2 allele, ADP-RPR, traditional cardiovascular risk factors, and additional previously shown[9] clinical and procedural risk factors for ST (total chronic occlusion, multivessel disease, bifurcation lesion, acute myocardial infarction, previous myocardial infarction, total stent length, and left ventricle ejection fraction) as independent variables.

0.177. The CYP2C19*2 polymorphism was in Hardy-Weinberg equilibrium.

No statistically significant difference was observed between carriers of the CYP2C19*2 rare allele (heterozygotes plus homozygotes) and wild-type homozygotes for all the considered demographic (age and gender), clinical (smoking habit, hypertension, diabetes, dyslipidemia, previous myocardial infarction, previous percutaneous coronary intervention, previous coronary artery bypass grafting, stable angina, unstable angina, acute myocardial infarction, multivessel disease, left ventricular ejection fraction, chronic total occlusion, bifurcation lesion, and total stent length), and therapeutic (β blockers, calcium antagonists, statins, angiotensin-converting enzyme inhibitors, glycoprotein IIb/IIIa inhibitors, and proton pump inhibitors) variables (data not shown).

Table 2 lists clinical outcomes at 6 months. The ST rate at 6 months was 3.1%. There were no differences in ST rates among patients receiving sirolimus- or paclitaxel-eluting stents or both type of stents (3.4%, 2.3%, and 5.5%, respectively). Most ST (64%) was subacute and occurred at a mean of 54 $\pm$ 62 days (range, 4 to 180) from stent implantation, whereas no patient had acute ST. The incidence of the composite end point of cardiac death and ST was 3.8%. The incidence of the primary and composite end points significantly differed between wild-type homozygous patients (*1/*1) and carriers of the CYP2C19*2 rare allele (*1/*2 plus *2/*2 genotypes; Table 2). Concerning analysis with definite and probable ST separately, similar results were observed even if the full statistical significance was not reached (Table 2).

Genotype distribution of the CYP2C19*2 polymorphism was significantly different between patients with or without ST and showed a trend to statistical significance between patients with or without the secondary end point (ST and cardiac mortality; Table 3). The prevalence of carriers of the rare allele was significantly higher in patients with (54.1%) than without ST (31.3%) and in patients with ST and cardiac mortality (51.7%) than in patients without ST and cardiac mortality (31.2%; p = 0.025 and p = 0.020, respectively; Figure 1).

Patients with ST had significantly higher (p = 0.048) platelet aggregation using ADP (56%, range 14% to 100%) than patients without the occurrence of ST (46%, range 1% to 100%).

Carriers of the rare allele of the CYP2C19 polymorphism had significantly higher (p = 0.001) ADP-induced platelet aggregation than noncarriers of the rare allele (51%, range 2% to 100% vs 45%, range 1% to 100%, respectively).

In patients with ST, ADP-RPR was more prevalent (33.3%) with respect to patients without ST (13.6%; p = 0.001; Table 3). Moreover, in patients with ST, a statistically significant (p <0.0001) higher prevalence of subjects with the contemporary presence of ADP-RPR plus the CYP2C19*2 polymorphism (25%) with respect to patients without ST (4.5%) was observed (Table 3).

Similar results were observed concerning the secondary end point of composite cardiac mortality and ST (Table 3).

Event-free survival curves of ST (primary end point) according to the presence of the rare allele of the CYP2C19*2 polymorphism and the contemporary presence of ADP-RPR plus CYP2C19*2 are shown in Figures 2 and 3, respectively.

Univariate and multivariate logistic regression analyses for the occurrence of ST and the composite end point of ST and cardiac mortality were listed in Table 4. At multivariate logistic regression analysis adjusted for the presence of ADP-RPR, traditional cardiovascular risk factors, and additional previously shown[9] clinical and procedural risk factors for ST, the CYP2C19*2 gene polymorphism resulted an independent risk factor for ST and the composite end point of cardiac mortality and ST (Table 4). Moreover, in this analysis, ADP-RPR was associated with a higher risk of ST and the composite end point of cardiac mortality and ST independently of the CYP2C19*2 polymorphism (Table 4).

At multivariate logistic regression analysis adjusted for traditional cardiovascular risk factors and clinical and procedural risk factors for ST, the contemporary presence of ADP-RPR plus the CYP2C19*2 polymorphism was a strong independent risk factor for ST (OR 5.79, 95% CI 1.04 to 39.01, p = 0.033). Similar results at multivariate regression analysis were obtained concerning the composite end point of cardiac mortality and ST (OR 11.45, 95% CI 1.84 to 71.27, p = 0.009).

Discussion

In this study, we investigated the role of the CYP2C19*2 loss-of-function polymorphism in determining the risk of occurrence of drug-eluting ST and the composite end point of cardiac mortality and ST in patients treated with combined

clopidogrel and aspirin therapy enrolled in the RECLOSE trial. The present study provided the novel finding that the 2C19*2 allele of the CYP2C19 gene was an independent risk factor for drug-eluting ST. Moreover, RPR to ADP in the acute periprocedural phase was associated with a higher risk of ST independently of the CYP2C19*2 polymorphism, thus underlying the predictive role of both acute platelet hyperreactivity and the CYP2C19*2 genetic determinant.

Recently, the CYP2C19*2 allelic variant, encoding a deficient drug-metabolizing enzyme CYP2C19, was associated with impaired platelet inhibition after acute and long-term oral clopidogrel administration in healthy subjects.[17–20] The association between the CYP2C19*2 polymorphism and platelet reactivity has been shown in patients with acute coronary syndromes in our recent report of 1,419 patients,[22] an additional study of 603 patients with non–ST elevation acute coronary syndromes,[23] and this study.

Our data showed that the CYP2C19*2 polymorphism was an independent risk factor for ST and also the composite end point of cardiac mortality and ST when the presence of the CYP2C19*2 polymorphism was evaluated in multivariate logistic regression analysis adjusted for ADP-RPR, traditional cardiovascular risk factors, and additional possible clinical and procedural risk factors for ST (total long-term occlusion, multivessel disease, bifurcation lesion, acute myocardial infarction, previous myocardial infarction, total stent length, and left ventricle ejection fraction). Interestingly, the present findings indicated that the contemporary presence of ADP-RPR and the CYP2C19*2 polymorphism was the strongest predictor of ST complication or the composite of ST and cardiac mortality with respect to the CYP2C19*2 polymorphism and ADP-RPR per se.

Our data provided the 2 insights that (1) mining of the CYP2C19*2 polymorphism as a risk factor for ST was only partially linked to its role in determining the ADP-RPR phenotype observed in the acute periprocedural phase, and (2) other genetic and acquired determinants of RPR in addition to the CYP2C19*2 polymorphism might have a role in determining the 6-month clinical outcome in these high-risk vascular patients. Recently, a CYP2C9 polymorphism in a different isoform of CYP was associated with diminished pharmacodynamic response and poor responder status to clopidogrel therapy in healthy subjects.[18] Moreover, other polymorphisms, such as glycoprotein Ia C807T, glycoprotein IIIa Leu33Pro, cyclooxygenase-1, and cyclooxygenase-2, might contribute to the increased thrombotic risk in these patients.[11–13] Moreover, omeprazole was shown to significantly decrease the effect of clopidogrel on platelet activation.[26] Dual-antiplatelet therapy was frequently associated with proton pump inhibitors to prevent gastrointestinal bleeding, and the isoenzyme CYP2C19 was also involved in the metabolism of proton pump inhibitors.[27] However, in our study, there was no difference between patients with or without ST in the prevalence of proton pump inhibitor treatment.

By considering the contemporary presence of ADP-RPR plus the CYP2C19*2 polymorphism, we might identify about 95% of subjects who did not develop ST with specificity of 0.95 and sensitivity of 0.25. Consequently, a strong effort should be made to identify new genetic and nongenetic risk factors for ST and, most importantly, evaluate the impact of the contemporary presence of different functional polymorphisms of CYP2C19 and other genes involved in antiplatelet therapy responsiveness, as well as other RPR determinants to develop a combined score for better prediction of ST and cardiac mortality.

Recently, Trenk et al[28] confirmed an association between the CYP2C19*2 polymorphism and platelet function phenotype, as well as an association of high platelet reactivity on clopidogrel therapy with poor clinical outcome, which we had already reported in 2 previous studies,[9,13] but they did not show a significant association between the CYP2C19*2 polymorphism and clinical outcomes. It was conceivable that the higher number of thrombotic events related to the higher risk profile of our patients and the different choice of clinical outcomes allowed us to detect a significant association between the CYP2C19*2 polymorphism and ischemic events, namely ST and cardiac death.

The different timing of venous blood sampling in glycoprotein IIb/IIIa inhibitor–treated patients might be a limitation of the study. Nevertheless, if present, the residual effect of glycoprotein IIb/IIIa inhibitors on platelet aggregation was modest and would result in mild underestimation of the number of patients with RPR. In addition, data from published reports showed that platelet aggregation induced using ADP fully recovered 5 days after discontinuation of abciximab therapy.[29]

By increasing the clopidogrel maintenance dose (>75 mg) or using other drugs (i.e., prasugrel) that are not influenced by the polymorphism, it could be possible to increase the entity of platelet inhibition or override, at least in part, the nonresponsiveness to the standard maintenance dose. Our findings emphasized the need for additional studies aimed to clarify whether a more intense therapy designed for correction of inadequate suppression of platelet reactivity could improve outcome in this clinical setting without increasing the major bleeding complications.

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