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Letter to the Editor

CYP2C19*2 genotype influence in acute coronary syndrome patients undergoing serial clopidogrel dose tailoring based on platelet function testing: Analysis from randomized controlled trial NCT02096419☆,☆☆

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Certain number of acute coronary syndrome (ACS) patients, regardless of the type of a $P2Y_{12}$ inhibitor being used, will develop a new ischemic event [1,2]. Despite the introduction of new antiplatelet agents, clopidogrel is still often administered in this setting. Patients with high on-treatment platelet reactivity (HTPR) on clopidogrel have an increased risk for new ischemic events [3]. Whether HTPR is a modifiable risk factor is not clear. In the time of progressive personalized medicine, effective, safe and widely available strategies are warranted to minimize the ischemic risk without increasing the bleeding risk. Previously, our group conducted a randomized controlled trial which evaluated how serial clopidogrel dose adjustment based on continuous platelet function testing (PFT) effects platelet reactivity (PR) levels and clinical outcomes in HTPR patients after percutaneous coronary intervention (PCI) in ACS. Briefly, patients in the interventional group took up to two additional 600 mg loading doses and 75–300 mg clopidogrel maintenance dose while patients in the control group were assigned to standard clopidogrel regimen. PFT was performed on Multiplate® function analyzer (Roche Diagnostics, Mannheim, Germany) in both groups on multiple occasions following PCI (days 1, 2, 3, 7, 30; months 2, 3, 6, 9, 12) with dose adjustment in the interventional group at each measurement to achieve optimal PR (19-46U). Patients in the interventional group maintained better PR during 12 month follow-up and had a better outcome [4].

Since clopidogrel requires bioactivation significantly catalyzed by CYP2C19 isoenzyme [5], we sought to evaluate the effect of CYP2C19*2 allele — the most common variant associated with the formation of a dysfunctional protein [6], on PR and ischemic outcome in both study groups.

This study was approved by the Ethics Committees of University Hospital Center Zagreb and University of Zagreb School of Medicine in concordance with Declaration of Helsinki. All patients gave informed consent before enrollment. Blood samples for CYP2C19 genotyping were collected either at the time of randomization or early during follow-up. Two patients in the control group died before blood sampling.

Genomic DNA was isolated from the whole blood containing EDTA with BioSprint 15 DNA Blood Kit (Qiagen, Germany) on the KingFisherML (Thermo Labsystems, USA). The CYP2C19*2 (rs4244285) genotype was determined in the allelic discrimination reaction performed with TaqMan ®Drug Metabolism Genotyping Assay (ID C_25986767_70) on 7500 Real Time PCR System, according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA).

CYP2C19 genotypes are shown as frequencies and prevalence. Kolmogorov–Smirnov test was used to test for normal distribution of continuous data. Appropriate parametric and non-parametric tests were used according to the results. Differences in PR status (HTPR vs. non-HTPR) between CYP2C19*2 carriers and non-carriers were tested with χ^2 test. The difference in clinical outcome was tested by χ^2 test. The differences in patients' data were tested by Student's t-test of independent samples or ANOVA for multiple independent

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samples. Logistic regression analysis was performed to characterize the relation between CYP2C19*2 genotype and ischemic outcomes. p values < 0.05 were regarded statistically significant. Statistical analysis was performed using SPSS software version 21 (IBM Corporation, USA).

Eighty-five (97.7%) patients were enrolled in the genetic substudy: 43 (100%) from the interventional group and 42 (95.4%) from the control group. Only 36.5% of patients had at least one CYP2C19*2 allele. There were no significant differences in CYP2C19 genotype between the study groups. In the interventional group CYP2C19*2 non-carriers were older and had a higher rate of arterial hypertension and dyslipidemia compared to carriers (Table 1). There was no difference in PR during follow-up between CYP2C19*2 carriers and non-carriers in the interventional group while CYP2C19*2 carriers had significantly higher PR levels than non-carriers in the control group and in the entire study population, as well (Fig. 1). The CYP2C19*2 allele was significantly associated with ischemic adverse events in the total study population (OR 3.310; CI 1.08–10.13; p = 0.036), but this correlation was not seen in the interventional group separately (p = 0.243).

Design of our investigation was based on the assumption that determining PR at more occasions would lead to a better insight of platelet response during follow-up. In this way we were able to assess the effect of CYP2C19*2 genotype on temporal PR variations in the study population. Our results indicate that serial adjustment of $P2Y_{12}$ inhibition based on PFT during twelve months might lead to better PR control and outcome in ACS patients regardless of the underlying genotype. This implies that determining PR phenotype might be more important and clinically useful than genotyping for HTPR. This is in correlation with previous claims that CYP2C19*2 genotype alone has a relatively modest effect on clopidogrel's pharmacodynamics [7]. We believe that CYP2C19 genotyping cannot be a good tool to identify patients with HTPR as it has a low negative predictability for HTPR. In the present analysis, 54 patients (63.5%) were CYP2C19*2 non-carriers. This was also confirmed in a recent meta-analysis which showed that CYP2C19*2 genotype had a negative predictive value for HTPR phenotype of only 52.3% [8]. As CYP2C19*2 allele is strongly associated with increased ischemic risk, especially stent thrombosis [9], CYP2P19 genotyping might be helpful to stratify patients' risk, but its routine clinical use, especially in guiding antiplatelet therapy is controversial, as there is no convincing evidence to support it. The routine use of PFT is currently not recommended after PCI and/or ACS either [10,11] as large, randomized trials brought negative results [12,13]. These trials, however, recruited mostly non-ACS patients and did not consider temporal PR changes which might have altered the pharmacodynamic effect of their intervention.

Screening for HTPR to guide antiplatelet therapy in ACS patients seems reasonable as it has the ability to account for many factors that effect PR including genotype, compliance, ACS, underdosing, comorbidities and concomitant therapy. In our interventional group, even though CYP2C19*2 non-carriers were older and had a higher rate of arterial hypertension and dyslipidemia, their PR levels during follow-up did not differ significantly compared to CYP2C19*2 carriers. This implies that serial PFT to guide antiplatelet therapy might also reduce the effect of other factors that contribute to HTPR.

As the benefit of clopidogrel is consistent during 12 months post-PCI [7] and PR is a dynamic variable with large inter- and intraindividual fluctuations [4,12,14] we believe that further studies should be more focused on overcoming HTPR phenotype and maintaining optimal PR continuously rather than determining HTPR genotype to select optimal therapy. PFT might be important with the use of newer $P2Y_{12}$ inhibitors also to predict the bleeding risk as low PR is more often present compared to clopidogrel.

Whether routine genotyping and PFT could help reduce future events in ACS patients is still debatable. A relatively small sample cannot guarantee significant power of our results. Only large-scale, randomized trials which take temporal PR variations into consideration will be able to fully investigate these strategies.

Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

Table 1
Patients' data.

	Interventional group (N = 43)			Control group (N = 42)		
	CYP2C19*2 carriers (N = 16)	CYP2C19*2 non-carriers (N = 27)	p	CYP2C19*2 carriers (N = 15)	CYP2C19*2 non-carriers (N = 27)	p
Age, mean (SD)	58.13 (12.99)	66.48 (11.42)	**0.033**	62.73 (10.26)	63.07 (13.30)	0.932
Men, n (%)	11 (68)	11 (40.7)	0.076	9 (60.0)	17 (62.9)	0.850
ACS type, n UA:NSTEMI:STEMI	1:4:11	7:6:14	0.270	3:3:9	3:7:17	0.708
AH, n (%)	6 (37.4)	22 (81.4)	**0.003**	8 (53.3)	17 (62.9)	0.542
DM, n (%)	3 (27.2)	11 (40.7)	0.186	5 (33.3)	6 (22.2)	0.433
HLP, n (%)	6 (37.5)	19 (70.3)	**0.035**	8 (53.3)	11 (40.7)	0.432
Smokers, n (%)	4 (25.0)	5 (18.5)	0.706	5 (33.3)	6 (22.2)	0.433
Family history for CAD, n (%)	3 (27.2)	4 (14.8)	1.000	0 (0.0)	6 (22.2)	0.073
Previous MI, n (%)	1 (6.2)	5 (18.5)	0.386	2 (13.3)	1 (3.7)	0.287
Previous PCI, n (%)	1 (6.2)	5 (18.5)	0.386	2 (13.3)	0 (0.0)	0.122
BMI, kg/m^2 mean (SD)	29.18 (4.51)	28.78 (4.50)	0.776	28.21 (3.61)	26.90 (4.45)	0.362
Index event data						
CAD						
Single vessel, n	11	13		5	5	
Two vessel, n	4	9	0.169	3	11	0.822
Three vessel, n	1	5		7	11	
Culprit lesion, n						
LAD: LCx: RCA	6:5:5	10:4:13	0.371	5:3:7	10:3:14	0.825
Mean total stent length, mm (SD)	24.81 (14.74)	27.19 (14.39)	0.607	33.07 (15.77)	27.15 (16.08)	0.742

ACS — acute coronary syndrome; AH — arterial hypertension; BMI — body mass index; CAD — coronary artery disease; DM — diabetes mellitus, HLP — hyperlipoproteinemia; LAD — left anterior descending; LCx — left circumflex artery; NSTEMI — non ST-elevation myocardial infarction; MI — myocardial infarction; PCI — percutaneous coronary intervention; RCA — right coronary artery; SD — standard deviation; STEMI — ST-elevation myocardial infarction; UA — unstable angina.
Bold-faced values represent statistically significant differences (p < 0.05).