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Role of genetic factors on the effect of additional loading doses and two maintenance doses used to overcome clopidogrel hyporesponsiveness

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ABSTRACT

Background and objective: Additional loading doses and higher maintenance doses (MDs) have been used to overcome hyporesponsiveness of clopidogrel. We aimed to investigate whether genetic polymorphisms of two cytochromes (CYP2C19 and CYP2C9) and ABCB1 modify effect of such dose-adjustment strategy.

Materials and methods: We enrolled 118 patients undergoing elective or acute percutaneous coronary intervention (PCI) with drug eluting stent (DES). Platelet reactivity index (PRI) was measured using the vasodilator-stimulated phosphoprotein (VASP) index and a cut-off value of ≥60% was defined as hyporesponsiveness. Polymorphism of two cytochromes (CYP2C19, CYP2C9) and gene ABCB1 were determined. In patients hyporesponsive to the initial LD the dose-adjustment was performed using up to 3 additional 600 mg LDs in order to achieve PRI <60%, and both 150 mg and 75 mg MD were tested at the follow-up.

Results: Patients with at least one CYP2C19*2 allele had higher baseline PRI after the initial LD (78.2 ± 13.1 vs. 65.3 ± 19.5, $P = 0.005$). The PRI reduction with additional LD was significantly smaller in carriers of the CYP2C19*2 (25.2 ± 15.6 vs. 35.5 ± 16.8, $P = 0.025$) and similar trend was observed with subsequent additional LDs. Both MDs were less effective in presence of CYP2C19*2. Target PRI was, however, more frequently achieved with higher MD even in presence of CYP2C19*2 (in 70.6% vs. 23.5% of hyporesponders, $P = 0.008$). No such differences were observed for other polymorphisms.

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Conclusions: In patients hyporesponsive to a routine clopidogrel doses the potency of additional LD and higher MD of clopidogrel is compromised by presence of CYP2C19*2 allele. The dose-adjustment strategy is not affected by ABCB1 C3435T or CYP2C9 genotypes.
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1. Introduction

Dual antiplatelet therapy (DAPT) as a combination of aspirin and a $P2Y_{12}$-receptor antagonist reduces thrombotic complications in patients with acute coronary syndrome (ACS) or undergoing percutaneous coronary intervention (PCI), and it is a recommended treatment in current clinical guidelines [1]. For the last decade as a standard DAPT was the combination of aspirin and clopidogrel until newer generation more effective $P2Y_{12}$-receptor antagonists (prasugrel, ticagrelor) became available which provide more rapid, potent and reliable $P2Y_{12}$-receptor inhibition [1–3]. Although prasugrel and ticagrelor reduced the risk of cardiovascular death, myocardial infarction or stroke compared to clopidogrel in patients with ACS, the concerns of higher bleeding risk coupled with an increase in costs remain important shortcomings with the newer agents [4,5]. These considerations have encouraged the further investigation in a search for more personalized approach in each individual patient.

The pharmacodynamic response to clopidogrel varies among patients and standard doses of clopidogrel achieve suboptimal platelet inhibition. Hence, the "high on-treatment platelet reactivity" (HTPR) or hyporesponsiveness has been described in up to 50% of patients [2,6]. Numerous individual studies as well as several meta-analyses have demonstrated that HTPR is strongly associated with cardiovascular death, myocardial infarction and stent thrombosis (ST) in patients undergoing PCI [7].

Routine or platelet function testing-guided administration of higher or repeated clopidogrel loading doses (LDs) and higher maintenance doses (MDs) have failed to overcome hyporesponsiveness in a significant proportion of patients and yielded unsatisfactory long-term clinical results [8–15]. Genetic variants of CYP2C19 and ABCB1 genes have been associated with hyporesponsiveness and cardiovascular events among patients on treatment with clopidogrel [16]. Variations of these genes affect the rate of metabolism of clopidogrel that is pro-drug, and production of the active metabolite [16,17]. There are limited data if and how these polymorphisms affect the efficacy of tailored additional LDs and MDs used in hyporesponsive patients [18].

We aimed to investigate whether genetic polymorphisms of CYP2C19, ABCB1 and CYP2C9 modify effect of (i) additional 600 mg LDs of clopidogrel and (ii) higher MD (150 mg vs. 75 mg) in order to overcome hyporesponsiveness.

2. Materials and methods

In a prospective single-center study we included patients undergoing PCI with a drug eluting stent (DES) who received LD of clopidogrel according to the guidelines, namely, 300 mg or 600 mg for patients with scheduled or acute PCI, respectively [19–21]. The enrollment period was between September 2010 and December 2012. The following exclusion criteria were applied: expected noncompliance to therapy, congestive heart failure New York Heart Association functional class IV, bleeding or history of bleeding diathesis, platelet count $<100 \times 10^9$/L, oral anticoagulant therapy, chronic liver disease (cirrhosis, hepatitis) or serum bilirubin >2 mg/dL, hemorrhagic stroke or stroke of unspecified origin, malignancy or other concurrent severe illness with expected survival <1 year, contraindication to dual antiplatelet therapy as deemed by the treating physician. The protocol was approved by the local ethics committee and was according to the Declaration of Helsinki. Patients were included after two informed consents were obtained separately for each of two study components: treatment to clopidogrel and genetic investigation. Among initially included 118 patients only 94 patients fully adhered to the study design. One patient withdrew consent to the genetic analysis during the study therefore we report data on 93 patients. The remaining 24 patients were excluded during the study due to the following deviations from the protocol: incorrect use of clopidogrel doses ($n = 12$), treating physician changed clopidogrel to another antiplatelet drug ($n = 8$), patients refused a follow-up visit ($n = 4$). Minority of the patients ($n = 18$, 19.4%) underwent emergent or urgent PCI due to an acute coronary syndrome.

2.1. Blood samples

Blood samples for VASP phosphorylation analyses were drawn by atraumatic venipuncture of the antecubital vein. The first sample was taken after the PCI with DES on the second day after the routine LD. The subsequent samples were taken between 12 and 24 h after each additional LD, and at least 3 h after the last MD at the follow-up. Blood was collected into a vacutainer containing 3.8% trisodium citrate and filled to capacity. The vacutainer was inverted 3–5 times for gentle mixing and taken to the laboratory.

2.2. Platelet reactivity measurements

The VASP phosphorylation analysis was performed within 24 h of blood collection by an experienced investigator using Platelet VASP kits (PLT VASP/P2Y12, Biocytex, Marseille, France) according to the manufacturer's instructions [22]. A citrated blood sample was incubated with prostaglandin E_1 (PGE_1) and ADP 10 μmol/l for 10 min and fixed with paraformaldehyde, after which the platelets were permeabilized with a nonionic detergent. Analyses were performed on a Cytomics FC – 500 flow cytometer (Beckman Coulter, France), the platelet population was identified from its forward and side scatter distribution, and

5000 platelets were gated. VASP platelet reactivity index (VASP PRI) was calculated from the mean fluorescence intensity (MFI) of samples incubated with PGE_1 or PGE_1 and ADP according to the formula: VASP PRI = [(MFIc PGE1 − MFIc (PGE1 + ADP))/MFIc PGE1] × 100. According to the first VASP test patients were classified into responders or hyporesponders (VASP PRI <60% or ≥60%, respectively) to clopidogrel.

2.3. Treatment protocol

Hyporesponders received up to three additional LDs (each 600 mg), and the VASP PRI was repeated at least 12 h after each administration until a target VASP PRI (<60%) was reached. If these three additional LDs were unable to decrease the VASP PRI to <60%, patients were defined as resistant and were switched to ticagrelor. Hyporesponders received MD of 150 mg once daily for 30 days followed by MD of 75 mg till the end of 12 months in total. Responders received MD of 75 mg once daily for 12 months. Both groups were tested for VASP PRI on day 40 while on 75 mg. Hyporesponders had additional analysis of VASP PRI on day 10 while on MD of 150 mg.

2.4. Genetic polymorphisms

2.4.1. DNA isolation

DNA was acquired through the Latvian Genome Data Base (LGDB), a government funded biobank. DNA was extracted from white blood cells by standard phenol-chloroform protocol, DNA concentration measured by Nanodrop ND1000 spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA) and subsequently stored at −70 °C [23].

2.4.2. SNP genotyping

DNA samples were aliquoted from storage tubes into 96-well polymerase chain reaction (PCR) plates using a Tecan with Freedom Evo system (Tecan, Mannedorf, Switzerland) disposable filter tips. DNA concentration was normalized to 7 ng/mL. Genotyping was carried out using an Applied Biosystems TaqMan SNP Genotyping Assay with a modified protocol using 4.75 mL TaqMan Genotyping Mix (Life Technologies, Carlsbad, California, USA), 0.25 mL SNP genotyping assay ID C_25626674_20 (Life Technologies) and 5 mL Millipore H_2O (Millipore, Bedford, MA) on a Viia7 Real-Time PCR system (Life Technologies). All 7 SNPs had dbSNP identification numbers: rs4244285 (CYP2C19*2), rs4986893 (CYP2C19*3), rs56337013 (CYP2C19*5), rs12248560 (CYP2C19*17), rs1799853 (CYP2C9*2), rs1057910 (CYP2C9*3) and rs1045642 (ABCB1 C3435T), respectively. Probe and primer sequences are available on request. Variants were called using ViiA7 Software v1.2.1 (Life Technologies) [24].

2.5. Statistical analysis

Continuous variables were compared with Student *t* test (for two groups) or ANOVA (for more than 2 groups). Categorical

Table 1 – Baseline demographic, clinical, angiographic and biologic characteristics (*n* = 94).

	All patients (*n* = 93)	Responders (*n* = 26)	Hyporesponders (*n* = 67)	*P*
Age, years	63.0 (9.7)	63.6 (10.9)	63.6 (10.9)	0.719
Men, *n* (%)	49 (52.7)	13 (50.0)	36 (53.7)	0.819
BMI, kg/m^2	29.7 (4.6)	27.1 (2.8)	30.7 (4.8)	0.001
Previous myocardial infarction, *n* (%)	33 (35.5)	8 (30.8)	25 (37.3)	0.554
History of CABG, *n* (%)	2 (2.2)	1 (3.8)	1 (1.5)	0.647
Cardiovascular risk factors				
Current smoker, *n* (%)	11 (11.8)	2 (7.7)	9 (13.6)	0.579
Diabetes mellitus, *n* (%)	28 (30.1)	9 (34.6)	19 (28.4)	0.555
Hypertension, *n* (%)	83 (89.2)	24 (92.3)	59 (89.4)	0.797
Laboratory evaluation				
WBC, ×10^9/L	7.8 (2.2)	7.5 (1.5)	7.9 (2.4)	0.415
Hemoglobin, g/L	1.4 (0.2)	1.4 (1.1)	1.4 (2.2)	0.540
Platelets, ×10^3/L	225.9 (51.4)	239.1 (48.8)	220.6 (51.8)	0.115
Fibrinogen, g/L	3.4 (1.3)	3.2 (0.8)	3.4 (1.4)	0.516
Creatinine, μmol/L	83.2 (23.9)	80.2 (16.2)	84.4 (26.3)	0.451
GFR, mL/min	63.2 (23.9)	55.8 (15.3)	66.0 (26.0)	0.072
TC, mmol/L	4.5 (1.4)	4.3 (1.1)	4.6 (1.4)	0.315
HDL-C, mmol/L	1.2 (0.4)	1.3 (0.4)	1.2 (0.4)	0.654
LDL-C, mmol/L	2.6 (1.1)	2.5 (0.9)	2.7 (1.2)	0.437
TG, mmol/L	1.4 (0.9)	1.2 (0.7)	1.5 (1.0)	0.175
Angiography and intervention				
Number of treated vessels	1.1 (0.2)	1.1 (0.3)	1.0 (0.2)	0.542
Number of stents per patient	1.4 (0.6)	1.4 (0.6)	1.4 (0.6)	0.981
Number of DES per patient	1.2 (0.4)	1.2 (0.4)	1.2 (0.5)	0.946
GP IIb/IIIa inhibitor use, *n* (%)	44 (47.3)	12 (46.2)	32 (48.5)	0.840

Values are mean (standard deviation) unless otherwise stated.
BMI, body mass index; WBC, white blood cells; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; GFR, glomerular filtration rate; DES, drug-eluting stent; GP, glycoprotein.

variables were compared with Pearson χ^2 test or Fisher exact test as appropriate. Repeated measurements were compared with paired *t*-test and McNemar test for continuous and categorical data, respectively. Correspondence of genotype distribution to Hardy–Weinberg equilibrium was tested with chi-square goodness of fit test. When gene dose effect was analyzed, Spearman correlation was used. Two-sided *P* value <0.05 was regarded as statistically significant. All statistical analyses were performed using SPSS version 19.0 (SPSS Inc., Chicago, IL).

3. Results

3.1. Patients

Baseline characteristics of the study population are summarized in Table 1. There were no differences between responders and hyporesponders regarding demographic and clinical data, except for BMI (*P* = 0.001; Table 1).

3.2. Platelet parameters

The mean PRI after the initial dose of clopidogrel (PRI1) was 68.4 ± 18.9%. We observed large inter-individual variability in clopidogrel responsiveness, with PRI ranging from 8% to 94% (Fig. 1). The majority of patients (*n* = 67, 72.0%) were hyporesponsive (PRI1 ≥ 60%) to initial clopidogrel LD. Fig. 2A summarizes the effect of each additional clopidogrel LD on PRI in hyporesponders. The target PRI was attained with one, two and three additional LDs in 43 (66.2%), 13 (20.0%) and 9 (13.8%) patients, respectively.

Two patients (2.2%) were identified as resistant to clopidogrel as the three additional LDs were unable to achieve PRI below 60%. Both participants responded, however, to ticagrelor 180 mg (Fig. 3), which reduced PRI statistically significantly compared to PRI4 (12.0 ± 1.4 vs. 73.0 ± 4.2, *P* = 0.042).

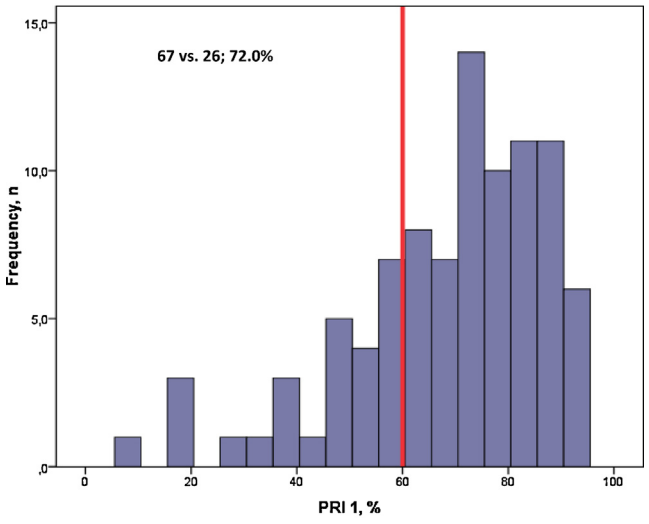

Fig. 1 – Baseline vasodilator-stimulated phosphoprotein platelet reactivity index (PRI) after the initial loading dose of clopidogrel (*n* = 93).

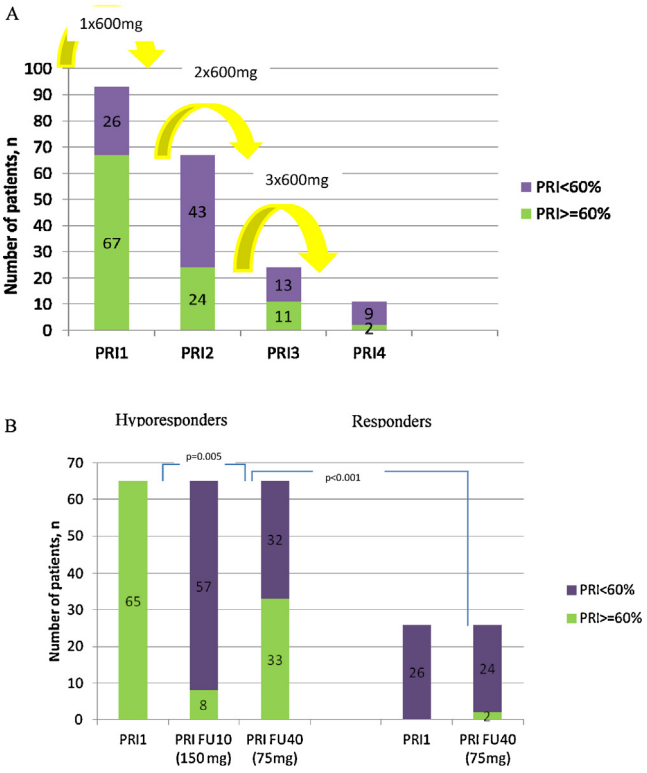

Fig. 2 – Effect of each additional loading dose (600 mg) in hyporesponders on platelet reactivity index (PRI) (A). Effect of 150-mg and 75-mg maintenance doses at 10 and 40 days, respectively, in hyporesponders (*n* = 65, two resistant patients switched to ticagrelor excluded) and effect of 75-mg maintenance dose in responders (*n* = 26) (B).

Effect of the two MDs (150 mg and 75 mg) on PRI was investigated during follow-up on day 10 and 40, respectively, in hyporesponders, and on day 40 (75 mg MD) in responders (Fig. 2B). Only 8 (12.3%) hyporesponders had PRI ≥60% while on 150 mg MD on day 10 compared to 32 (49.2%) patients while on 75 mg MD on day 40 (*P* = 0.005). On day 40, fewer patients in responders group had PRI ≥60% (*n* = 2, 7.7%) compared to hyporesponders (*n* = 33, 50.8%) while on 75 mg MD (*P* < 0.001).

The mean VASP PRI for elective cases who received 300 mg LD of clopidogrel was not significantly lower compared to acute

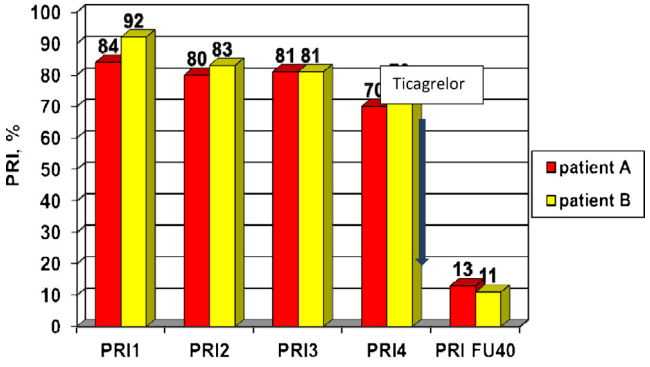

Fig. 3 – Platelet reactivity index (PRI) in clopidogrel-resistant patients and the effect of ticagrelor.

Table 2 – Genotype distributions and allele frequencies of all investigated genetic variations.

Polymorphism	Genotype	Patients, *n* (%)	P[a]	Allele	Allele frequency
CYP2C19					
CYP2C19*2	GG (wt/wt)	71 (76.3)	0.686	G	0.876 (163)
(*G681A/rs4244285*)[b]	AG (wt/*2)	21 (22.6)		A	0.124 (23)
	AA (*2/*2)	1 (1.1)			
CYP2C19*3	GG (wt/wt)	92 (98.9)	0.958	G	0.995 (185)
(*G636A/rs4986893*)	AG (wt/*3)	1 (1.1)		A	0.005 (1)
CYP2C19*5	CC (wt/wt)	90 (100.0)	NA	C	1.000 (90)
(*C1297T/rs56337013*)	CT (wt/*5)				
CYP2C19*17	CC (wt/wt)	36 (38.7)	0.523	C	0.634 (118)
(*C806T/rs12248560*)	CT (wt/*17)	46 (49.5)		T	0.366 (68)
	TT (*17/*17)	11 (11.8)			
CYP2C9					
CYP2C9*2	CC (wt/wt)	86 (92.5)	0.706	C	0.962 (179)
(*C430T/rs1799853*)	CT (wt/*2)	7 (7.5)		T	0.038 (7)
CYP2C9*3	AA (wt/wt)	79 (84.9)	0.433	A	0.925 (172)
(*A1075C/rs1057910*)	CA (wt/*3)	14 (15.1)		C	0.075 (14)
ABCB1					
ABCB1	CC	17 (18.2)	0.410	C	0.452 (84)
(*C3435T/rs1045642*)	CT	50 (53.8)		T	0.548 (102)
	TT	26 (28.0)			

NA, not applicable; wt, wild-type; SNP, single-nucleotide polymorphism.
[a] Hardy–Weinberg equilibrium.
[b] According to National Center for Biotechnology Information.

patients who received 600 mg LD of clopidogrel (67.2 ± 19.7 vs. 74.0 ± 8.0, *P* = 0.285).

3.3. *Genotyping results*

No deviations from the Hardy–Weinberg equilibrium were detected. Genotype distribution and allele frequencies of the genetic variations studied are presented in Table 2. For the CYP2C19 genotype, one patient was homozygote for the *2 mutant allele of CYP2C19 (1.1%), 21 (22.6%) were heterozygotes and 71 (76.3%) were homozygotes for the wild-type allele.

3.4. *Relationship of genotypes with PRI*

Table 3 summarizes platelet reactivity after the first routine dose (PRI1) by genotypes. Carriers of the CYP2C19*2 allele (wt/*2 and *2/*2) had significantly higher PRI1 compared with patients homozygous for CYP2C19 wild-type genotype (78.2 ± 13.1 vs. 65.3 ± 19.5, *P* = 0.005).

Platelet activity remained higher in carriers of the CYP2C19*2 allele (wt/*2 and *2/*2) after the first additional LD (PRI2) compared with homozygotes of CYP2C19 wild-type genotype (57.0 ± 19.1 vs. 40.8 ± 21.5, *P* = 0.006) (Fig. 4). The absolute decrease of mean PRI following the first additional LD (PRI1–PRI2) was significantly smaller in carriers vs. noncarriers of *2 (25.2 ± 15.6 vs. 35.5 ± 16.8, *P* = 0.025) (Table 4).

The platelet reactivity with two different MDs of clopidogrel in the whole group and responders/non-responders stratified by the genotypes is summarized in Table 5. Both MDs were less effective in the presence of CYP2C19*2. Among hyporesponders, carriers of CYP2C19*2 (wt/*2 and *2/*2) had significantly higher PRI on day 10 while on the 150 mg MD (53.3 ± 12.1 vs. 40.3 ± 13.5, *P* = 0.001) and on day 40 while on the 75 mg MD (65.5 ± 10.4 vs. 56.3 ± 14.5, *P* = 0.020) compared with homozygous for CYP2C19 wild type genotype. In the whole study group carriers of CYP2C19 *2 had higher PRI with 75 mg MD (63.1 ± 11.3 vs. 50.3 ± 17.1, *P* = 0.002).

Target PRI <60% was achieved in 70.6% vs. 93.8% of patients with 150 mg MD (*P* = 0.024), and 23.5% vs. 58.3% with 75 mg MD (*P* = 0.014) in carriers and non-carriers, respectively. The success

Table 3 – Repartition of genetic polymorphisms of CYP2C19, CYP2C9 and ABCB1 according to response to the initial dose of clopidogrel (*n* = 93).

Variable	PRI1, % (SD)	P	Hyporesponders (*n* = 67)	P
*CYP2C19*2*				
wt/wt	65.3 (19.5)	0.016	48/71 (67.6)	0.220
wt/*2	77.7 (13.2)		18/21 (85.7)	
*2/*2	89.0 (–)		1/1 (100)	
*CYP2C19*3*				
wt/wt	68.2 (19.0)	0.472	66/92 (71.7)	1.000
wt/*3	82.0 (–)		1/1 (100)	
*CYP2C19*17*				
wt/wt	73.0 (15.0)	0.140	29/36 (80.6)	0.307
wt/*17	64.7 (20.7)		30/46 (65.2)	
*17/*17	68.7 (20.8)		8/11 (72.7)	
*CYP2C9*2*				
wt/wt	68.6 (18.9)	0.687	63/86 (73.3)	0.361
wt/*2	65.6 (20.6)		4/7 (57.1)	
*CYP2C9*3*				
wt/wt	69.1 (17.7)	0.385	58/79 (73.4)	0.488
wt/*3	64.3 (25.2)		9/14 (64.3)	
ABCB1				
CC	67.0 (22.5)	0.379	11/17 (64.7)	0.683
CT	66.5 (18.7)		36/50 (72.0)	
TT	72.8 (16.9)		20/26 (76.9)	

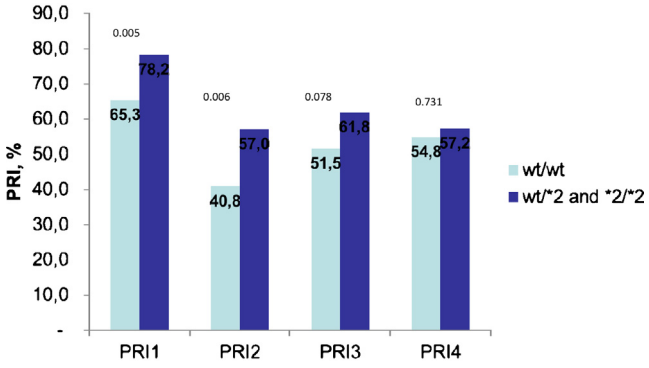

Fig. 4 – Platelet reactivity after clopidogrel each loading dose depending on genetic polymorphism of CYP2C19*2 (*n* = 93).

rate was statistically significantly improved with higher MD both in carriers and non-carriers of CYP2C19*2 ($P = 0.008$ and $P < 0.001$, respectively).

Patients with CYP2C19*17 allele (wt/*17 and *17/*17) had lower PRI1 compared with homozygous for CYP2C19 wild-type genotype (65.4 ± 20.6 vs. 73.0 ± 15.0, $P = 0.060$) (Table 3). Carriers of both gain-of-function allele of the CYP2C19*17 (*17/*17) had similar PRI1 compared with patients with one gain-of-function allele or homozygotes of the wild-type allele (wt/*17 and wt/wt) (68.3 ± 18.8 vs. 68.7 ± 20.8, $P = 0.947$). No other polymorphism in recessive or dominant model had significant association with baseline or any other PRI.

The two clopidogrel-resistant patients had the following genotypes: (i) patient A, CYP2C9*3 wt/wt, CYP2C9*2 wt/wt, CYP2C19*2 wt/*2, CYP2C19*3 wt/wt, CYP2C19*5 wt/wt, CYP2C19*17 wt/*17, ABCB1 C/T, and (ii) patient B, CYP2C9* 3wt/wt, CYP2C9*2 wt/wt, CYP2C19*2 wt/*2, CYP2C19*3 wt/wt, CYP2C19*5 wt/wt, CYP2C19*17 wt/wt, ABCB1 T/T.

Table 4 – Influence of the first additional loading dose of clopidogrel on the platelet reactivity according to the CYP2C19, CYP2C9 and ABCB1 genotypes.

Genotype	*n*	PRI1–PRI2, % (SD)	*P*
CYP2C19*2			
wt/wt	48	35.5 (16.8)	0.025
wt/*2 and *2/*2	19	25.2 (15.6)	
CYP2C19*17			
wt/wt	29	31.7 (16.2)	0.705
wt/*17 and *17/*17	38	33.3 (17.8)	
CYP2C19*3			
wt/wt	66	32.8 (17.1)	0.392
wt/*3	1	18.0 (–)	
CYP2C9*2			
wt/wt	63	32.3 (17.1)	0.659
wt/*2	4	36.3 (17.4)	
CYP2C9*3			
wt/wt	58	32.9 (17.5)	0.691
wt/*3	9	30.4 (14.3)	
ABCB1			
CC	17	32.9 (17.1)	0.719
CT/TT	76	30.8 (17.3)	

4. Discussion

Our study confirms the previous reports that the patients carrying the loss-of-function CYP2C19*2 allele have significantly higher PRI following an initial LD of clopidogrel than wild-type homozygotes [2]. In addition, we have demonstrated that PRI reduction (PRI1–PRI2) after the first additional 600 mg LD of clopidogrel is significantly smaller in CYP2C19*2 allele carriers. Similar trend was observed after the second and subsequent loading doses, which was non-significant presumably due to smaller number of patients requiring more than one additional LD.

Previous studies reporting on the impact of ABCB1 C3435T genotypes on clopidogrel treatment efficacy have provided inconsistent results. For instance, in the PLATO trial the ABCB1 3435CC high-expression genotype was associated with increased risk of cardiovascular death, myocardial infarction, and stroke in the clopidogrel arm of the study [17], whereas in the TRITON-TIMI 38 trial, the highest event rates were observed in carriers of the opposite (3435TT) ABCB1 genotype [25]. Importantly, in a meta-analysis by Su et al. T allele was associated with increased early and long-term risk of major cardiovascular events with 300 mg LD, but not with 600 mg LD [26]. Our data go in line with this analysis as we observed similar, although nonsignificant, trend of higher PRI values with TT genotype after the initial LD.

Of note, in none of the studies included in the meta-analysis dose adjustment guided by platelet function testing was performed. Such study, however, has been recently published by Bonello et al. [18], in which they found that CYP2C19*2, but neither PON1 nor ABCB1 genotype, was associated with HTPR after the initial LD, while only ABCB1 was responsible for the failure of the additional LD strategy. Since there were only two patients in whom dose-adjustment failed in our study, the statistical comparison with the other patients was not feasible. Both clopidogrel-resistant patients, however, were carriers of T allele (heterozygote and homozygote), as well as heterozygotes for CYP2C19*2, which supports the notion that both alleles may be involved in resistance, but not the only responsible factors. Importantly, both patients had pronounced response to ticagrelor, underscoring the advantage of the newer drug.

There are several differences between our study and the study reported by Bonello et al. [18]. In our study the sample was smaller, most of patients underwent scheduled PCI and thus received 300 mg initial LD in line with the current guidelines [1], as well as the target PRI was <60%. In contrast, Bonello et al. included only patients with acute coronary syndrome receiving 600 mg of initial LD, and set the target PRI <50%. Importantly, first blood sample for VASP analysis was taken after the PCI in our study as opposed to before the intervention in the study by Bonello et al. One may argue that mechanical injury itself may augment platelet reactivity and confound the findings. It remains unclear, however, to what extent, if any, the different findings regarding effects of ABCB1 C3435T can be attributed to the above-mentioned factors. Altogether our data suggest, that CYP2C19*2 has much stronger impact on PRI reduction with additional LD than ABCB1 T allele.

Table 5 – Influence of the two maintenance doses of clopidogrel on the platelet reactivity according to the genetic polymorphisms.

Variable	*n*	Responders (*n* = 26)	*n*	Hyporesponders (*n* = 65)[a]		All patients (*n* = 91)[a]
		75 mg		150 mg	75 mg	75 mg
*CYP2C19*2*						
wt/wt	23	37.7 (15.4)	48	40.3 (13.5)	56.3 ± 14.5	50.3 (17.1)
wt/*2	3	49.7 (5.1)	16	51.9 (10.9)	64.7 ± 10.2	62.3 (11.0)
*2/*2	–		1	76.0 (–)	78.0 (–)	78.0 (–)
		P = 0.198		*P* = 0.001	*P* = 0.043	*P* = 0.006
*CYP2C19*3*						
wt/wt	21	38.8 (15.0)	56	43.1 (14.6)	59.5 (13.7)	52.8 (16.6)
wt/*3	5	40.0 (16.9)	9	47.8 (11.8)	53.7 (15.8)	81.0 (–)
		P = 0.877		*P* = 0.362	*P* = 0.247	*P* = 0.095
*CYP2C19*17*						
wt/wt	7	32.1 (8.0)	28	45.5 (15.1)	58.7 (11.8)	3.4 (15.4)
wt/*17	16	41.6 (17.7)	29	42.1 (14.6)	59.0 (16.2)	52.8 (18.6)
*17/*17	3	41.7 (7.5)	8	43.3 (9.7)	57.9 (14.3)	53.5 (14.6)
		P = 0.379		*P* = 0.665	*P* = 0.982	*P* = 0.984
*CYP2C9*2*						
wt/wt	23	39.8 (15.8)	61	43.8 (13.8)	59.0 (14.3)	53.7 (17.0)
wt/*2	3	33.3 (2.5)	4	42.0 (23.0)	55.0 (9.6)	45.7 (13.5)
		P = 0.495		*P* = 0.805	*P* = 0.588	*P* = 0.228
*CYP2C9*3*						
wt/wt	21	38.8 (15.0)	56	43.1 (14.6)	59.5 (13.7)	53.9 (16.8)
wt/*3	5	40.0 (16.9)	9	47.8 (11.8)	53.7 (15.8)	48.8 (17.0)
		P = 0.877		*P* = 0.362	*P* = 0.247	*P* = 0.299
ABCB1						
C/C	6	49.5 (18.3)	11	45.6 (12.0)	62.1 (13.6)	57.7 (16.1)
C/T	6	34.2 (12.0)	35	42.1 (15.1)	58.1 (14.0)	51.3 (17.2)
T/T	14	39.8 (14.9)	19	46.2 (14.2)	57.9 (14.8)	53.6 (16.5)
		P = 0.109		*P* = 0.595	*P* = 0.688	*P* = 0.404

[a] Two clopidogrel-resistant patients switched to ticagrelor were excluded.

In our view there is a discrepancy between the high prevalence of patients not reaching target PRI <50% reported in literature (from 16% to 50%) and much lower frequency of stent thrombosis rates observed in the randomized studies during the first year (<1%) [9,27,28]. We therefore attempted to test the safety of PRI <60% as a less conservative target for the dose-adjustment strategy. The one-year clinical follow-up data will be reported separately.

Despite the raised cut-off value, the prevalence of hyporesponders with PRI ≥60% after the initial LD was higher than expected (72.0%). One may speculate that DES is more frequently chosen in diabetics and complicated lesions, hence higher atherosclerosis burden, which in turn may be associated with higher platelet reactivity and lead to a selection bias. Use of 300 mg LD may be another factor contributing to higher PRI, although in our sample elective cases receiving 300 mg LD had lower PRI than acute patients receiving 600 mg LD.

We observed that CYP2C19*2 carriers had higher PRI with both 75 mg MD and 150 mg MD of clopidogrel. In these patients 150 mg MD was significantly more effective than 75 mg MD to maintain target PRI <60%. We did not find significant interaction of ABCB1 genotype and efficacy of either MD. These findings confirm previous reports [10,29,30] that 150 mg MD may be preferred to 75 mg in carriers of CYP2C19*2, although no benefit of such strategy has been shown to improve clinical outcomes. In our view this finding additionally supports importance of CYP2C19*2 over ABCB1 since effect of LD is short-lasting.

No other polymorphisms (CYP2C19*3, CYP2C19*5, CYP2C 19*17, CYP2C9*2, CYP2C9*3 and ABCB1 C3435T) were found to have clearly significant interaction with LDs or MDs. It should be noted, that minor alleles of these polymorphisms are rare, and therefore are unlikely to have as high clinical significance as CYP2C19*2 if the type 2 statistical error was present. Besides the smaller sample size, another limitation of the study is the heterogeneity of study group as about one fifth of the patients had ACS and received 600 mg initial LD.

The advantage of the present study is the use of the platelet VASP test (PLT-VASP), which is highly specific to $P2Y_{12}$ inhibition. This method has other significant logistical advantages including: (i) aspirin and other medications such as GPIIb/IIIa antagonists do not interfere with the results, (ii) analysis can be performed within 48 h from the collection of the blood, (iii) the samples can be stored at room temperature, (iv) only a single full citrate tube is required.

The results of the present study are of potential interest to help define a therapeutic strategy to improve platelet reactivity inhibition in hyporesponders. Integrated approach of testing both presence of CYP2C19*2 and degree of platelet reactivity may be superior to platelet function testing alone in order to achieve the most optimal P2Y12 inhibition in each individual patient.

5. Conclusions

In patients hyporesponsive to a routine clopidogrel doses the potency of additional LD and higher MD of clopidogrel is compromised by presence of CYP2C19*2 allele. The dose-adjustment strategy is not affected by ABCB1 C3435T or CYP2C9 genotypes.

Conflict of interest statement

The authors state no conflict of interest.

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