Original Article

CYP2C19 Loss-of-Function Alleles and Clopidogrel Hyporesponsiveness in Patients with Reinfarction/Recurrent Myocardial Infarction after Coronary Stenting

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Background: CYP2C19 loss-of-function (LOF) alleles and clopidogrel hyporesponsiveness increased cardiovascular events in patients with newly diagnosed myocardial infarction (MI). However, data of CYP2C19 genetic polymorphism and clopidogrel hyporesponsiveness in patients with reinfarction/recurrent MI was lacked.

Objective: To investigate the prevalence and impact of CYP2C19 LOF alleles and clopidogrel hyporesponsiveness among patients with reinfarction/recurrent MI after coronary stenting.

Materials and Methods: All consecutive patients who were taking clopidogrel and presented with reinfarction/recurrent MI after coronary stenting at Queen Sirikit Heart Center of the Northeast and Srinagarind Hospital, Khon Kaen, Thailand, during December 2012 to December 2015 were enrolled. Genotype analysis of CYP2C19 alleles were investigated, which CYP2C19*2 and *3 were defined as LOF alleles, and clopidogrel responsiveness was assessed by VerifyNow©P2Y12 assay (Accumetrics, San Diego, CA, USA), which clopidogrel hyporesponsiveness was defined as $P2Y_{12}$ reaction unit (PRU) ≥240. Survival data of all patients were followed until December 2017.

Results: Sixty-seven patients were eligible, which mean age (SD) was 63 (10) years, 44 patients (65.7%) were male, and 27 patients (40.3%) presented with definite stent thrombosis (ST). Among overall patients, subgroup with definite ST, and subgroup without ST, the number of patients (%) with CYP2C19 LOF alleles were 41(61.2%), 16 (59.3%), and 25(62.5%), median PRU were 234, 260, and 215, number of patients (%) with clopidogrel hyporesponsiveness were 31(47.7%), 17(65.4%), and 14(35.9%), and five-year survival rate (95% confidence interval) were 67% (54-78%), 63% (42-78%), and 71% (53-83%), respectively. The presence of CYP2C19 LOF alleles was not associated with clopidogrel hyporesponsiveness either in overall patients or in any subgroup. Survival analysis showed no effect of either CYP2C19 LOF alleles or clopidogrel hyporesponsiveness on either short- or long-term mortality.

Conclusion: CYP2C19 LOF alleles and clopidogrel hyporesponsiveness is highly prevalent among Northeastern Thai patients with reinfarction/recurrent MI, however, the clinical impact of both disorders was not evidenced. Hence, routine platelet function testing and genetic testing in these particular patients may seems unnecessary.

Keywords: CYP2C19 loss-of-function allele, Clopidogrel hyporesponsiveness, Reinfarction, Recurrent myocardial infarction

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Clopidogrel is a $P2Y_{12}$ inhibitor which was widely used in conjunction with aspirin as dual antiplatelet therapy [DAPT] in patients with acute coronary syndrome [ACS] or patients who underwent percutaneous coronary intervention [PCI] with or without stenting to reduce the risk of recurrent myocardial infarction [MI] and stent thrombosis [ST][1,2]. To generate its active metabolite, two-step oxidation by hepatic cytochrome P450 [CYP450] is required[3], which CYP2C19 has been propose to be the

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major enzyme involved in such process[4,5]. Hence polymorphisms of CYP2C19 is inevitably affects the formation of clopidogrel active metabolite, which CYP2C19*2 and *3 are the two most frequent loss-of-function [LOF] alleles that lead to reduction of enzyme function, then reduce clopidogrel active metabolite, and consequently clopidogrel hyporesponsiveness[6-9].

Association between clopidogrel hyporesponsiveness or high on-treatment platelet reactivity [HPR] and ischemic events was strongly demonstrated in many case series and prospective observational studies among patients with coronary artery disease [CAD] who underwent PCI[9-14]. Nevertheless, many prospective randomized studies were failed to prove the beneficial concept of platelet function-guided therapy in such patients[15-18].

Carriers of CYP2C19 LOF alleles had increased risk of major adverse cardiovascular events (MACE) after PCI, especially stent thrombosis [ST][19,20], and point-of-care genetic testing for CYP2C19 profile could help in early optimization of $P2Y_{12}$ inhibition[21,22], however, no randomized trial has demonstrated clinical benefit of genetic testing in patients undergoing PCI.

According to the paucity of clinical benefit mentioned earlier, guidelines have recommended against either routine platelet function testing or genetic testing to adjust $P2Y_{12}$ inhibitor in general PCI, but have suggested to possibly consider both tests in specific situations, e.g. patients suffering from recurrent MI[1,2], despite lack of demonstrated evidence of CYP2C19 LOF allele and HPR among those particular patients.

The authors aimed to study the magnitude of CYP2C19 LOF alleles and clopidogrel hyporesponsiveness in patients with reinfarction/recurrent MI after PCI with stenting, which having this information could further improves clinical practice in tailoring DAPT in specific situation of MI.

Materials and Methods

Study background and patients

The authors conducted a single center study at Queen Sirikit Heart Center of the Northeast and Srinagarind Hospital, Khon Kaen University, Khon Kaen, Thailand. The present study is a sub-analysis from an entire population, which inclusion criteria are 1) male or female age ≥30 years, 2) history of ACS and underwent PCI with stenting, or coronary artery bypass graft [CABG], or medical therapy alone (only subgroup of patients who underwent PCI with stenting are included into this analysis), 3) diagnosis of reinfarction or recurrent MI, and 4) clopidogrel must be taken during presentation. All consecutive eligible patients were enrolled between December 2012 and December 2015. The patients who omitted clopidogrel for any dose within 1 week before presentation and who denied participation were excluded. All patients were provided written informed consent, and the study has been approved by the Khon Kaen University Ethics Committee in human research (HE551318).

Procedures

$P2Y_{12}$ inhibition activity and CYP2C19 genotype analysis were done in all enrolled patients. For assessment of $P2Y_{12}$ inhibition activity, the VerifyNow©P2Y12 assay (Accumetrics, San Diego, CA, USA) was used. Clopidogrel 75 mg od had to be taken for at least 7 days before testing and might be additional loaded with a dose of 300 or 600 mg during the coronary angiography [CAG]. A wash out period of 24 hours or 10 days joiyyvvjhuygbiu was required before VerifyNow©P2Y12 testing if eptifibatide or abciximab was used during PCI, respectively. The test was performed by research assistant and result was immediately informed to a researcher (BP), where clopidogrel was replaced by ticagrelor 90 mg bid in the patients who had evidence of clopidogrel hyporesponsiveness. For CYP2C19 genotype analysis, DNA sample were extracted from buffy coat by QIAamp® DNA Blood Mini kit according to the manufacturer's instruction. CYP2C19*1, CYP2C19*2 (681 G>A, rs4244285) and CYP2C19*3 (636 G>A, rs4986893) were examined by Real-time polymerase chain reaction (Real-time PCR) with specific TaqMan® probe and primer and performed by using the Light-Cycler 480 technology (Roche diagnostics, Meylan, France). All specific hydrolysis probes and primers were designed by Applied Biosystems, Foster city, U.S.A. PCR reaction contains TaqMan® Universal PCR Master Mix, TaqMan® SNP genotyping assay or TaqMan® Drug Metabolism Enzyme Genotyping assays [DME], gDNA and sterile water, and the PCR thermal cycler condition according to the manufacturer's instruction. All baseline clinical characteristics and laboratory results were reviewed and collected by researcher (BP), and survival of all patients was followed until December 2017.

Operational definition

MI, reinfarction, and recurrent MI were defined according to the universal definition of MI[23], which reinfarction was defined as MI that occurs within

28 days of an incident MI (individual's first MI), and recurrent MI as MI occurs after 28 days of an incident event. $P2Y_{12}$ inhibitory activity was determined by $P2Y_{12}$ reaction unit [PRU], which clopidogrel hyporesponsiveness was defined as the PRU ≥240[24]. Definite ST was defined according to the Academic Research Consortium [ARD] definition[25], and in-stent restenosis [ISR] was defined as the presence of >50% diameter stenosis in the stented segment[26].

In an analysis of CYP2C19 genotype, the *1/*1 alleles (normal function allele) was defined as extensive metabolizer (EM), *1/*2 and *1/*3 alleles (one LOF allele) were defined as intermediate metabolizer [IM], *2/*2, *2/*3, and *3/*3 alleles (two LOF alleles) were defined as poor metabolizer [PM], and LOF alleles was characterized as a combination of IM and PM.

Statistical analysis

As the initial aim of the present study is to determine a prevalence of clopidogrel hyporesponsiveness, the authors use a formula of estimating a population proportion with specified absolute precision ($n = [Z^2_{1-\alpha/2}P(1-P)]/d^2$), which confidence level ($1-\alpha$) = 95%, anticipated population proportion (P) = 0.44[27], and absolute precision required (d) = 0.1, then the total calculated study population was 95 patients. However, due to the low prevalence of interested events, the enrollment was terminated on December 2015 with overall patients of 74.

Continuous variables are presented as mean, median, range, and standard deviation [SD]. The Shapiro-Wilk test of normality was used to assess data distribution, which variables with normal distribution were compared by Student's t-test, and variables with abnormal distribution were compared by Wilcoxon test. Categorical variables are presented as number and percentage, which comparison was performed by Chi-square test with continuity correction if the expected cell count was less than 5 in less than 20% of cases, and otherwise was compared by Fisher's exact test if the expected cell count was less than 5 in more than 20% of cases. Survival was compared using Kaplan-Meier estimates and Log-Rank testing, which data were censored after December 2017. The *p*-value of <0.05 was considered statistically significant. The analysis was performed using STATA version 10.0.

Results

Sixty seven patients with reinfarction/recurrent MI were enrolled during the 3-year period of enrollment, all patients underwent urgent or emergent CAG, which definite ST was found in 27 patients (40.3%), whereas the rest was presented without ST. In overall patients, the mean age was 63.1 years, 44 patients (65.7%) were male, over half of patients had diabetes or hypertension (53.7% and 50.8%, respectively), about one-fourth of patients had heart failure during presentation (26.9%), and most patients (71.6%) presented with non-ST-segment elevation ACS [NSTEACS]. In comparison between subgroup with ST vs. without ST, statistically significant differences were found in mean left ventricular ejection fraction [LVEF] (40.9% vs. 50.8%, $p = 0.01$), median time to index event (7 days vs. 230 days, $p \leq 0.001$), diagnosis of NSTEACS (55.6% vs. 82.5%, $p = 0.04$), use of beta-blocker (63.0% vs. 85.0%, $p = 0.38$), use of angiotensin-converting enzyme inhibitor/angiotensin receptor blocker [ACEI/ARB] (25.9% vs. 62.5%, $p = 0.003$), and use of proton-pump inhibitor [PPI] (70.4% vs. 38.5%, $p = 0.011$) (Table 1).

The median PRU and proportion of patient with clopidogrel hyporesponsiveness (PRU ≥240) in overall patients, subgroup with ST, and subgroup without ST were 234, 260, and 215, and 47.7%, 65.4%, and 35.9%, respectively. The subgroup with ST had a significantly higher proportion of patient with clopidogrel hyporesponsiveness ($p = 0.02$) (Table 1).

The proportion of EM, IM, PM, and LOF alleles in overall patients, subgroup with ST, and subgroup without ST were 38.8%, 40.7%, and 37.5%, and 47.8%, 44.4%, and 50.0%, and 13.4%, 14.8%, and 12.5%, and 64.2%, 59.3%, and 62.5%, respectively, which no statistically significant difference was found between subgroup with ST vs subgroup without ST (Table 1). And according to the CYP2C19 phenotype, the PRU was not significantly differed among each group of phenotypes in overall patients, subgroup with ST, and subgroup without ST (Table 2).

All-cause death in overall patient, subgroup with ST, and subgroup without ST were 21 patients (31.3%), 10 patients (37.0%), and 11 patients (27.5%), respectively, which the difference in mortality rate among subgroup with ST vs. without ST was not significant ($p = 0.40$). The 1-, 3-, and 5-year survival rate (95% confidence interval; CI) in overall patient was 82% (71 to 89%), 71% (59 to 81%), and 67% (54 to 78%), respectively (Figure 1).

According to the CYP2C19 phenotype, having LOF alleles had no effect on either short- or long-term survival, as the survival rate of subgroup

Table 1. Baseline characteristics

Character	All patients (n = 67)	With stent thrombosis (n = 27)	Without stent thrombosis (n = 40)	*p*-value
Age (year)	63.1±10.3 (36 to 83)	62.7±11.8 (36 to 81)	63.3±10.0 (45 to 83)	0.80
Male	44 (65.7)	19 (70.4)	25 (62.5)	0.50
Cardiovascular risk				
Diabetes	36 (53.7)	14 (51.9)	22 (55.0)	0.80
Hypertension	34 (50.8)	13 (48.2)	21 (52.5)	0.72
Stroke	4 (6.0)	1 (3.7)	3 (7.5)	0.52
Current smoker	1 (1.5)	0	1 (2.5)	0.40
Heart failure	18 (26.9)	9 (33.3)	9 (22.5)	0.32
BMI (kg/m^2)	24.3±4.2 (15.0 to 42.3)	23.7±2.9 (16.0 to 29.1)	24.7±4.9 (15.0 to 42.3)	0.34
Heart rate (bpm)	80.9±18.4 (44 to 140)	86.6±22.2 (60 to 140)	77.1±14.4 (44 to 104)	0.03
SBP (mmHg)	128.5±23.5 (91 to189)	125.3±24.8 (91 to 189)	130.6±22.6 (95 to 179)	0.37
DBP (mmHg)	74.5±14.7 (49 to 128)	75.1±16.4 (49 to 115)	74.1±13.7 (49 to 128)	0.77
LVEF (%)	46.8±16.2 (11 to 79)	40.9±14.8 (11 to 67)	50.8±16.1 (20 to 79)	0.01
FBS (mg/dl)	169.6±82.8 (75 to 441)	187.8±91.0 (75 to 434)	155.3±74.2 (85 to 441)	0.14
HbA1C	7.1±1.8 (4.2 to 11.6)	7.0±2.0 (4.2 to 11.6)	7.1±1.7 (4.2 to 10.7)	0.85
LDL-C (mg/dl)	98.2±34.2 (28 to 194)	103.0±36.7 (46-194)	95.1±32.6 (28 to 184)	0.38
eGFR ($ml/min/1.73\ m^3$)	70.5±34.5 (1.9 to 185.2)	65.1±38.7 (5.7 to 145.6)	74.2±31.4 (1.9 to 185.2)	0.30
PRU*	234 (8 to 368)	260 (8 to 316)	215 (78 to 368)	0.32
Clopidogrel hyporesponsiveness (PRU ≥240)	31 (47.7)	17 (65.4)	14 (35.9)	0.02
Time to index event (days)	58 (1 to 3,220)	7 (1 to 330)	230 (1 to 3,220)	<0.001
Index event diagnosis				
NSTEACS	48 (71.6)	15 (55.6)	33 (82.5)	0.04
STEMI	19 (28.4)	12 (44.4)	7 (17.5)	0.23
Previous medications				
Aspirin	67 (100.0)	27 (100.0)	40 (100.0)	>0.99
Beta-blocker	51 (76.1)	17 (63.0)	34 (85.0)	0.038
ACEI/ARB	32 (47.8)	7 (25.9)	25 (62.5)	0.003
Statins	60 (89.6)	24 (88.9)	36 (90.0)	0.88
CCB	8 (11.9)	1 (3.7)	7 (17.5)	0.08
Nitrates	39 (58.2)	13 (48.2)	26 (65.0)	0.17
Diuretics	13 (19.4)	4 (14.8)	9 (22.5)	0.43
PPI use	34 (51.5)	19 (70.4)	15 (38.5)	0.011
Omeprazole	31 (91.2)	16 (84.2)	15 (100.0)	0.10
Lanzoprazole	1 (2.9)	1 (5.3)		
Esomeprazole	2 (5.9)	2 (10.5)		
CYP2C19 phenotype				
Extensive metabolizer	26 (38.8)	11 (40.7)	15 (37.5)	0.86
Intermediate metabolizer	32 (47.8)	12 (44.4)	20 (50.0)	0.76
Poor metabolizer	9 (13.4)	4 (14.8)	5 (12.5)	0.91
Intermediate + poor metabolizer	41 (61.2)	16 (59.3)	25 (62.5)	0.83

Data are presented as mean±standard deviation (range) or n (%), except median (range) for PRU and time to index event.
ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blocker; BMI, body mass index; CCB, calcium channel blocker; CYP, cytochrome P; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FBS, fasting blood sugar; HbA1C, hemoglobin A1C; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; NSTEACS, non-ST-segment elevation acute coronary syndrome; PPI, proton-pump inhibitor; PRU, $P2Y_{12}$ reactivity unit; SBP, systolic blood pressure; STEMI, ST-segment elevation myocardial infarction.
* The PRU data was collected in 65 patients (26 in subgroup with ST, and 39 in subgroup without ST)
# Extensive metabolizer = *1/*1, Intermediate metabolizer = *1/*2, *1/*3, poor metabolizer = *2/*2, *2/*3, *3/*3

Table 2. CYP2C19 genotype and PRU*

	Overall population (n = 65)					With stent thrombosis (n = 26)					Without stent thrombosis (n = 39)				
	EM (n = 23)	IM + PM (n = 43)	IM (n = 34)	PM (n = 9)	*p*-value	EM (n = 10)	IM + PM (n = 16)	IM (n = 12)	PM (n = 4)	*p*-value	EM (n = 15)	IM + PM (n =25)	IM (n = 20)	PM (n = 5)	*p*-value
PRU	213 (90 to 368)	236 (8 to 357)	237 (8 to 357)	234 (62 to 353)	0.86[#] 0.98[+] 0.67[++]	262 (8 to 295)	260 (62 to 316)	266 (133 to 316)	256 (62 to 289)	0.44[#] 0.97[+] >0.99[++]	196 (140 to 368)	223 (78 to 357)	224 (126 to 357)	211 (78 to 353)	>0.99[#] >0.99[+] >0.99[++]

Data are presented as median (range).
CYP, cytochrome P; EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; PRU, $P2Y_{12}$ reactivity unit
* The PRU data was collected in 65 patients (26 in subgroup with ST, and 39 in subgroup without ST)
[#] *p*-value for EM vs. IM + PM, [+] *p*-value for EM vs. IM, [++] *p*-value for EM vs. PM

with EM and LOF alleles was literally equal ($p = 0.88$, 95% CI; 0.44 to 2.58), (Figure 2) and in analysis of subgroup with LOF alleles, having PM was not affected survival when comparing with IM ($p = 0.87$, 95% CI 0.50 to 1.78) (Figure 3). And according to the presence of clopidogrel hyporesponsiveness, PRU ≥240 during presentation with reinfarction/recurrent MI was not associated with either short- or long-term survival, as

Figure. 1 Kaplan-Meier Estimates of the probability of survival in overall patients. The 1-, 3-, and 5-year survival rate (95% confidence interval) in overall patients was 82% (71 to 89%), 71% (59 to 81%), and 67% (54 to 78%), respectively.

Figure. 2 Kaplan-Meier Estimates of the probability of survival, according to CYP2C19 phenotype. The 5-year survival rate (95% confidence interval) in subgroup with extensive metabolizer and with combined intermediate and poor metabolizer was 68% (46 to 83%), and 67% (50 to 79%), respectively, $p = 0.88$.

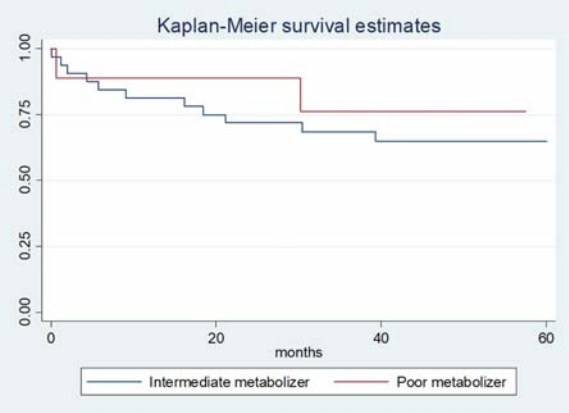

Figure 3. Kaplan-Meier Estimates of the probability of survival, according to subgroup of CYP2C19 loss-of-function allele. The 3-year survival rate (95% confidence interval) in subgroup with intermediate metabolizer and poor metabolizer was 68% (49 to 82%), and 76% (33 to 94%), respectively, $p = 0.86$.

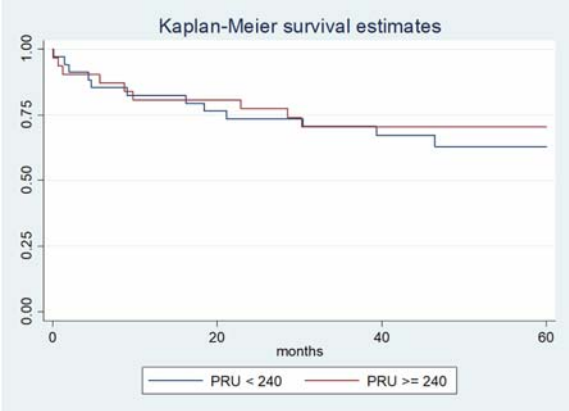

Figure 4. Kaplan-Meier Estimates of the probability of survival, according to P2Y12 reactivity unit (PRU). The 5-year survival rate (95% confidence interval) in patients with PRU <240 and ≥240 was 63% (44 to 77%), and 70% (51 to 83%), respectively, $p = 0.66$.

there was no significant difference in survival in subgroup with PRU ≥240 or PRU <240 ($p = 0.66$, 95% CI; 0.35 to 1.96) (Figure 4).

Discussion

In patients who previously underwent coronary stenting and presented with reinfarction/recurrent MI, our study demonstrated the high prevalence of CYP2C19 LOF alleles and clopidogrel hyporesponsiveness (61.2% and 47.7%, respectively), and a significantly higher prevalence of clopidogrel hyporesponsiveness in subgroup with ST (63.0%) compared with subgroup without ST (35.0%) ($p = 0.02$). However, the prevalence of CYP2C19 LOF alleles (IM, PM, and combined IM-PM) were similar between subgroup with and without ST, and the presence of either CYP2C19 LOF alleles or clopidogrel hyporesponsiveness in these particular patients had no effect on either short- or long-term survival.

Platelet reactivity was naturally increased during the time of ACS and was gradually reduced after commencement of appropriate treatment[16,28,29]. Previous PRU cutoff values for determining HPR were derived from various CAD patients and varied in levels (>208 to ≥240)[13,24]. The new cutoff valve of PRU >262 in patients with ACS was recently raised, which Nakamura M et al showed that PRU of 262 in Japanese ACS patients was the optimal cutoff valve for preventing MACE up to 3 days after PCI[28]. However, beneficial concept of platelet-function guided therapy specifically in patient with ACS has been denied[18], and has never been proof in patient with reinfarction/recurrent MI. In the present study, the patient subgroup with ST had higher median PRU and significantly higher prevalence of clopidogrel hyporesponsiveness (using traditional criteria of PRU ≥240) compared with patient subgroup without ST (260 vs. 215, $p = 0.32$, and 65.4% vs. 35.9%, $p = 0.02$, respectively). This finding may enlighten the alternative advantage of determining PRU in patient with reinfarction/recurrent MI, which presenting of high PRU or having clopidogrel hyporesponsiveness may indicate ST and urge the emergent CAG rather than conservative therapy.

Previous studies had demonstrated the significant independent effect of clopidogrel hyporesponsiveness or HPR on stent thrombosis, non-fatal MI, and cardiovascular death in short-term (<1 year) among patients who underwent PCI with drug-eluting stent (DES), including patients with ACS[12,30,31]. In the present study, however, we could not demonstrate such effect of clopidogrel hyporesponsiveness on mortality either in short- or long-term among patients with reinfarction/recurrent MI. This discordance might be explained by differences in patients' background, number of study population, and varying strategy of medical and invasive treatment between each center and era.

CYP2C19 LOF alleles attributed to clopidogrel hyporesponsiveness and resulted into MACE after PCI[19,20]. Genetic polymorphism of CYP2C19 is known

to be varied across ethnicity[32], Among the heathy native population of Northeastern Thailand, prevalence of CYP2C19 LOF alleles (*2 and *3), IM, and PM were 52.3%, 46.7%, and 5.6%, respectively[33]. In the present study, we found prevalence of CYP2C19 LOF alleles, IM, and PM in overall patients of 61.2%, 47.8%, and 13.4%, respectively. Comparing with healthy population, the prevalence of CYP2C19 LOF alleles is higher among patients with reinfarction/recurrent MI, and seemingly driven by higher prevalence in subgroup PM. However, since the present study has no direct healthy control subject, then it is impossible to conclude that CYP2C19 LOF alleles has an effect on development of reinfarction/recurrent MI, and the differences is possibly nothing but the play of chance. Nevertheless, the higher prevalence of CYP2C19 LOF alleles, especially PM, among these particular patients should not be simply overlooked, as this small signal may give way to the proof of causative relationship between CYP2C19 LOF alleles and incident of reinfarction/recurrent MI.

The presence of CYP2C19 LOF alleles was similar between subgroup with ST and subgroup without ST (59.3% vs 62.5%, respectively, $p = 0.83$), and without difference in any specific group of IM or PM. This finding could imply that CYP2C19 LOF alleles, although attributed to clopidogrel hyporesponsiveness, but could not directly impact the occurrence of ST like clopidogrel hyporesponsiveness did itself. This is because clopidogrel hyporesponsiveness resulted from multiple etiologies, whereas genetic polymorphisms is one among others[24], which there are also others gene that attributed to hepatic metabolism of clopidogrel, i.e. CYP3A4, and it was found that only 6-12% of the on-clopidogrel variability in platelet reactivity could be explained by differences in genotype[34,35].

The meaningless effect of genetic polymorphisms on clopidogrel responsiveness may also be revealed in our study, according to the depiction of PRU and CYP2C19 phenotype in varying group of patients (Table 2), there was no difference of PRU in any group of CYP2C19 phenotype among overall patients and both subgroups. And in survival analysis, the effect of CYP2C19 LOF alleles on mortality could not be demonstrated. Hence, even CYP2C19 LOF alleles did have effect on clopidogrel responsiveness, but it may not be the crucial factor, especially in such situation of ACS or reinfarction/recurrent MI, and eventually was unable to demonstrate the clear effect on clinical outcome.

The higher proportion of PPI use (mostly omeprazole) in subgroup patient with ST compared with subgroup without ST (70.4% vs. 38.5%, $p = 0.011$) may had contribution to the higher PRU and higher proportion of patient with clopidogrel hyporesponsiveness in subgroup with ST. An interaction between PPI and clopidogrel, as PPI could reduce platelet inhibitory effect of clopidogrel, was previously well described[36,37]. However, cardiovascular impact of such interaction was not apparent from a large randomized study[38]. The reason of discrepancy in PPI use in our study was unable to explain.

Many of limitations were contained in the present study, 1) this is a single-center study and the authors could not avoid the bias in recruitment and practice, 2) the sample size was smaller than expected, 3) there is the ethnicity difference in CYP2C19 polymorphisms and results from our particular population may not be generalized to the others, 4) the authors could not control the form of clopidogrel use (branded vs. generic) and method of clopidogrel loading (300 mg vs. 600 mg), which the effect of these discrepancies to PRU could not be dismissed, and 5) there was no data of other non-fatal events, i.e., non-fatal MI, stroke, and bleedings, which having complete data of important outcomes might give more understating to the clinical impact of CYP2C19 polymorphisms and clopidogrel hyporesponsiveness.

Conclusion

CYP2C19 LOF allele and clopidogrel hyporesponsiveness were prevalent in Northeastern Thai patients with reinfarction/recurrent MI after coronary stenting. The impact to either short- or long-term mortality of such problems, however, was negative. Routine use of platelet function testing and genetic testing in an attempt to tailor $P2Y_{12}$ inhibitor in these particular patients is seems unnecessary and automatic switch to potent $P2Y_{12}$ inhibitor is reasonable. Nevertheless, presence of high PRU or clopidogrel hyporesponsiveness may indicate ST and urge the invasive procedure, which probably making platelet function testing has its role in diagnostic pathway.

What is already known on this topic?

CYP2C19 genetic polymorphism and clopidogrel hyporesponsiveness portend adverse cardiovascular events in patients with CAD who underwent percutaneous coronary intervention with stenting. However, clinical trials of platelet function test-guided therapy were failed to prove additive

benefits, while information of genetic testing-guided therapy is lacked, and guidelines are consistently recommended against routine use of platelet function testing and genetic testing to tailor $P2Y_{12}$ inhibitor therapy in general conditions.

What this study adds?

The authors have added the information of CYP2C19 genetic polymorphism and clopidogrel hyporesponsiveness in exceptional condition of ACS (reinfarction/recurrent MI) among Northeastern Thai patients. The prevalence of CYP2C19 LOF alleles and clopidogrel hyporesponsiveness in such condition and population are high. Considering the weak clinical benefit of both tests and effectiveness with acceptable safety profile of new oral $P2Y_{12}$ inhibitors, automatic switching from clopidogrel to potent $P2Y_{12}$ inhibitors should be recommended rather than use of either platelet function testing or genetic testing to guide therapy in this particular condition.

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Potential conflicts of interest

The authors declare no conflict of interest.

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