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PON1 Q192R is associated with high platelet reactivity with clopidogrel in patients undergoing elective neurointervention: A prospective single-center cohort study

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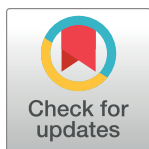
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Abstract

Background and purpose

The impact of the paraoxonase-1 (PON1) polymorphism, Q192R, on platelet inhibition in response to clopidogrel remains controversial. We aimed to investigate the association between carrier status of PON1 Q192R and high platelet reactivity (HPR) with clopidogrel in patients undergoing elective neurointervention.

Methods

Post-clopidogrel platelet reactivity was measured using a VerifyNow® P2Y12 assay in P2Y12 reaction units (PRU) for consecutive patients before the treatment. Genotype testing was performed for PON1 Q192R and CYP2C19*2 and *3 (no function alleles), and *17. PRU was corrected on the basis of hematocrit. We investigated associations between factors including carrying ≥1 PON1 192R allele and HPR defined as original and corrected PRU ≥208.

Results

Of 475 patients (232 men, median age, 68 years), HPR by original and corrected PRU was observed in 259 and 199 patients (54.5% and 41.9%), respectively. Carriers of ≥1 *PON1* 192R allele more frequently had HPR by original and corrected PRU compared with non-carriers (91.5% vs 85.2%, P = 0.031 and 92.5% vs 85.9%, P = 0.026, respectively). In multivariate analyses, carrying ≥1 *PON1* 192R allele was associated with HPR by original (odds ratio [OR] 1.96, 95% confidence interval [CI] 1.03–3.76) and corrected PRU (OR 2.34, 95% CI 1.21–4.74) after adjustment for age, sex, treatment with antihypertensive medications, hematocrit, platelet count, total cholesterol, and carrying ≥1 *CYP2C19* no function allele.

Conclusions

Carrying ≥1 *PON1* 192R allele is associated with HPR by original and corrected PRU with clopidogrel in patients undergoing elective neurointervention, although alternative results related to other genetic polymorphisms cannot be excluded.

Introduction

Dual antiplatelet therapy (DAPT), most commonly with aspirin and clopidogrel, is regularly used for antiplatelet premedication to prevent thromboembolic complications following endovascular treatment. However, there is marked inter-patient variability of platelet inhibition in response to clopidogrel, and high platelet reactivity (HPR) with clopidogrel is associated with an increased risk of thromboembolic complications following endovascular treatment [1–3].

Clopidogrel is a thienopyridine that blocks the P2Y12 receptor. The antiplatelet activity of clopidogrel depends on its conversion to an active metabolite mainly by the cytochrome P450 family 2 subfamily C member 19 (CYP2C19). *CYP2C19* is a highly polymorphic gene. *CYP2C19*2* and **3* were reported to be the most common single-nucleotide polymorphisms (SNPs) that decreased enzyme activity in a multi-ethnic population. Carrying ≥1 *CYP2C19* no function allele was associated with HPR with clopidogrel and thromboembolic complications following endovascular treatment [4–6]. Conversely, *CYP2C19*17* is an increased function SNP that contributes to enhanced platelet inhibition in response to clopidogrel [7].

Paraoxonase-1 (PON1) is another enzyme that participates in the second step of clopidogrel metabolism, and the *PON1* Q192R SNP is a major determinant of PON1 activity [8]. Compared with carriers of ≥1 *PON1* 192R allele, non-carriers have lower PON1 activity and lower platelet inhibition in response to clopidogrel, which leads to a higher risk of stent thrombosis following coronary artery stenting [8]. However, unlike *CYP2C19* SNPs, the impact of *PON1* Q192R on platelet inhibition in response to clopidogrel remains controversial; the association between *PON1* Q192R and HPR with clopidogrel has not been replicated in other studies [9–12]. Conversely, recent studies have shown that carriers of ≥1 *PON1* 192R allele have reduced platelet inhibition in response to clopidogrel compared with non-carriers [13–16].

A recent study demonstrated that HPR also led to thromboembolic complications in patients undergoing pipeline embolization device placement for intracranial aneurism (IA) [17]. However, to date, little is known about the association between *PON1* Q192R and HPR with clopidogrel in patients undergoing neurointervention [18]. Therefore, this study investigated the impact of *PON1* Q192R on HPR with clopidogrel in patients undergoing elective neurointervention.

Materials and methods

Ethics statement

The ethics committee at Kyushu University Hospital (389–01) and Kokura Memorial Hospital approved data collection from patients undergoing elective neurointervention at Kokura Memorial Hospital and genotype testing at Kyushu University. Written informed consent was obtained from all participants.

Study patients

Five hundred and sixty-seven patients who underwent scheduled endovascular treatment for cerebrovascular disease from May 2010 to September 2015 were enrolled in this observational study. All patients were on DAPT before neurointervention. Alongside low-dose acetylsalicylic acid therapy, 75 mg clopidogrel was administered for ≥7 days before treatment. For patients administered clopidogrel <7 days before treatment, clopidogrel was administered at a loading dose of 300 mg followed by 75 mg daily. The following clinical information was systematically extracted from medical records: age, sex, body mass index, vascular risk factors (hypertension, diabetes mellitus, dyslipidemia, and current smoking status), ischemic heart disease, and concomitant treatment with antihypertensive medications (calcium channel blockers, beta blockers, and/or angiotensin-converting enzyme inhibitors [ACEI]/angiotensin receptor blockers [ARB]) and proton pump inhibitors. Laboratory data, including hematocrit, platelet count, and serum cholesterol levels were obtained from routine laboratory testing principally performed a few days before treatment.

Platelet function measurements

Blood samples (1.8 ml) were taken from the median cubital vein in vacuum collection tubes containing 0.2 ml of 3.2% sodium citrate using a 21-G blood collection needle before the procedure on the day of neurointervention. Platelet reactivity was evaluated using a VerifyNow® P2Y12 assay (Accumetrics, San Diego, CA, USA) according to the manufacturer's instructions. Platelet reactivity was expressed as P2Y12 reaction units (PRU). Because hematocrit alters VerifyNow P2Y12 assay results, we calculated the corrected PRU by subtracting 7.5 PRU for every % of hematocrit below and adding 7.5 PRU for every % of hematocrit above, 42% as previously proposed [19]. We defined HPR as an original PRU of ≥208 [20], as well as a corrected PRU of ≥208.

DNA extraction and genotyping

Genomic DNA was extracted from 200 μl of whole blood collected in ethylenediaminetetraacetate-coated tubes using a NucleoSpin® Blood QuickPure kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. To evaluate the quantity and quality of extracted DNA, the optical density was measured using a NanoDrop™ 1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). The DNA concentration was adjusted to 10 ng/μl before assays were performed.

Genotyping was performed using the TaqMan™ Drug Metabolism Genotyping Assay (Applied Biosystems, Foster City, CA, USA) for the following SNPs: *PON1* Q192R (rs662; c.575A>G rs662 or p.Gln192Arg), *CYP2C19*2* (rs4244285; c.681G>A or p.Pro227Pro), *CYP2C19*3* (rs4986893; c.636G>A or p.Trp212Ter), and *CYP2C19*17* (rs12248560; c.-806C>T). The total reaction volume (25 μl) consisted of 2 × TaqMan Universal Polymerase Chain Reaction Master Mix II (Applied Biosystems), 20 × primer and probe mix (TaqMan Drug Metabolism Genotyping Assay Mix), and 20 ng of template DNA according to the

manufacturer's instructions. Polymerase chain reaction was performed using the 7500 Real-Time PCR System (Applied Biosystems). Allele discrimination was manually and automatically performed using the 7500 System Sequence Detection software (Applied Biosystems).

Statistical analysis

All statistical analyses were performed with JMP statistical software, version 9.0 (SAS Institute, Cary, NC, USA). Data are expressed as medians and interquartile ranges for continuous variables and counts and percentages for categorical variables. All SNPs were tested for deviation from Hardy–Weinberg equilibrium. Clinical characteristics and carrier status of at least one variant allele of Q192R of *PON1* and no function (*2* and *3*) and increased function (*17*) alleles of *CYP2C19* were compared between patients with and without HPR by original and corrected PRU using the Chi-squared test, Fisher's exact test, or the Wilcoxon rank-sum test as appropriate. A multivariate logistic regression analysis was performed to investigate factors associated with HPR by original and corrected PRU using forced entry and stepwise selection procedures. Age, sex, and carrier status of ≥ 1 *CYP2C19* no function allele and ≥ 1 *PON1* 192R allele were forced in; other variables were chosen by stepwise selection with a significance level of $\alpha = 0.10$ for entry and $\alpha = 0.10$ for removal. A P-value < 0.05 was considered statistically significant.

Results

Of 567 patients who underwent scheduled endovascular treatment and were registered during the study period, 92 were excluded because of no clopidogrel use (n = 6), initiation of clopidogrel <7 days without a loading dose (n = 14), lack of PRU data (n = 37), or lack of genomic data (n = 35). Finally, 475 patients (232 male; median age, 68 years) were included in the following analysis. Two hundred and seventy-six patients received simple/balloon or stent-assisted coil embolization for ruptured (n = 13) or unruptured (n = 263) IA, 197 received stent placement for symptomatic (n = 117) or asymptomatic (n = 80) carotid artery stenosis, 1 received embolization for carotid-cavernous fistula, and 1 received parent artery occlusion.

Results from genetic testing are shown in Table 1. Four hundred and twenty-one patients (88.6%) carried ≥ 1 *PON1* 192R allele. Two hundred and fifty-two patients (53.1%) carried ≥ 1 *CYP2C19*2* allele and 105 patients (22.1%) carried ≥ 1 *CYP2C19*3* allele. Thus, 321 patients

Table 1. Genotype distribution among the study population.

Alleles		Total (n = 475)	MAF (%)	Hardy-Weinberg equilibrium (*P* value)	MAF from the 1000 Genomes Project (East Asian, %)
PON1 Q192R	A/A	54 (11.4)	64.9	0.421	66.6
(rs662, c.575A>G)	A/G	225 (47.4)			
	G/G	196 (41.3)			
*CYP2C19*2*	G/G	223 (46.9)	30.7	0.333	31.2
(rs4244285, c.681G>A)	G/A	212 (44.6)			
	A/A	40 (8.4)			
*CYP2C19*3*	G/G	370 (77.9)	12.1	0.194	5.6
(rs4986893, c.636G>A)	G/A	95 (20.0)			
	A/A	10 (2.1)			
*CYP2C19*17*	C/C	472 (99.4)	0.3	1.000	1.5
(rs12248560, c.-806C>T)	C/T	3 (0.6)			
	T/T	0 (0)			

Data are presented as n (%). MAF, minor allele frequency.

(67.6%) carried ≥ 1 *CYP2C19* no function allele. Three patients (0.6%) had the *CYP2C19***17* allele. All tested SNPs were in Hardy–Weinberg equilibrium. In this study, the minor allele frequency of *CYP2C19***3* was higher compared with the East Asian population assessed in the 1000 Genomes Project (12.1% vs 5.6%) [21].

HPR by original and corrected PRU of ≥ 208 was observed in 259 and 199 patients (54.5% and 41.9%), respectively. After the correction of PRU on the basis of hematocrit, 70 patients with HPR were re-classified as non-HPR and 10 patients without HPR were re-classified as HPR. The re-classification rate was 16.8% and the correction of PRU reduced the prevalence of HPR by 23.2%. There were 17 patients without the *PON1* 192R allele or *CYP2C19* no function allele, 137 carrying ≥ 1 *PON1* 192R allele but not the *CYP2C19* no function allele, 37 carrying ≥ 1 *CYP2C19* no function allele but not the *PON1* 192R allele, and 284 carrying ≥ 1 *PON1* 192R allele and the *CYP2C19* no function allele. Among them, the frequencies of HPR by original PRU were 17.7%, 38.7%, 51.4%, and 64.8%, respectively, and those by corrected PRU were 11.8%, 23.4%, 35.1%, and 53.5%, respectively.

Patient characteristics are presented in Table 2. Patients with HPR by original PRU were more frequently female (59.1% vs 41.7%, respectively; P < 0.001) with lower platelet counts (median, $19.9 \times 10^4/\mu l$ vs $21.0 \times 10^4/\mu l$, respectively; P < 0.001), and hematocrit levels (median, 38.7% vs 40.5%, respectively; P < 0.001) compared with those without HPR. Compared with non-carriers, carriers of ≥ 1 *PON1* 192R allele (91.5% vs 85.2%, respectively; P = 0.031) and ≥ 1 *CYP2C19* no function allele (78.4% vs 54.6%, respectively; P < 0.001) more frequently had HPR by original PRU. A similar trend was observed when comparing patients with and

Table 2. Univariate analysis of high platelet reactivity in patients undergoing elective neurointervention.

Variables	Total (n = 475)	HPR by original PRU			HPR by corrected PRU		
		HPR (n = 259)	Non-HPR (n = 216)	*P* value	HPR (n = 199)	Non-HPR (n = 276)	*P* value
Sex, male	232 (48.8)	106 (40.9)	126 (58.3)	<0.001	86 (43.2)	146 (52.9)	0.037
Age (years)	68 (58–75)	68 (59–75)	67 (57–75)	0.336	68 (59–74)	68 (58–76)	0.862
Body mass index	22.9 (21.0–25.0)	22.8 (20.8–24.9)	23.0 (21.1–25.2)	0.483	23.2 (21.0–25.2)	22.5 (21.0–24.6)	0.128
Hypertension	284 (59.8)	159 (61.4)	125 (57.9)	0.436	122 (61.3)	162 (58.7)	0.567
Dyslipidemia	269 (56.6)	144 (55.6)	125 (57.9)	0.619	113 (56.8)	156 (56.5)	0.955
Diabetes mellitus	113 (23.8)	58 (22.4)	55 (25.5)	0.434	45 (22.6)	68 (24.6)	0.609
Ischemic heart disease	91 (19.2)	49 (18.9)	42 (19.4)	0.885	33 (16.6)	58 (21.0)	0.226
Current smoking	56 (11.8)	24 (9.3)	32 (14.8)	0.062	20 (10.1)	36 (13.0)	0.318
Calcium channel blocker	230 (48.4)	134 (51.7)	96 (44.4)	0.113	101 (50.8)	129 (46.7)	0.388
β-blocker	57 (12.0)	31 (12.0)	26 (12.0)	0.982	21 (10.6)	36 (13.0)	0.410
ACEI/ARB	177 (37.3)	102 (39.4)	75 (34.7)	0.296	78 (39.2)	99 (35.9)	0.459
Proton pump inhibitor	351 (73.9)	194 (74.9)	157 (72.7)	0.584	148 (74.4)	203 (73.6)	0.841
Clopidogrel <7 days	74 (15.6)	45 (17.4)	29 (13.4)	0.237	34 (17.1)	40 (14.5)	0.442
Platelet count ($\times 10^4/\mu l$)	20.5 (17.3–23.8)	19.9 (16.5–23.1)	21.0 (18.4–24.9)	<0.001	19.9 (16.9–22.7)	21.0 (18.0–25.0)	<0.001
Hematocrit (%)	39.4 (36.5–42.2)	38.7 (35.7–40.9)	40.5 (38.0–43.5)	<0.001	39.7 (37.0–42.6)	39.2 (36.2–42.0)	0.118
Total cholesterol (mg/dl)	185 (158–211)	188 (160–212)	182 (157–210)	0.389	190 (161–215)	180 (155–209)	0.024
HDL-C (mg/dl)	54 (47–65)	55 (47–68)	53 (46–63)	0.064	56 (48–67)	54 (46–63)	0.074
LDL-C (mg/dl)	110 (87–127)	110 (86–124)	110 (87–128)	0.688	110 (88–131)	110 (86–125)	0.298
≥ 1 *PON1* 192R allele	421 (88.6)	237 (91.5)	184 (85.2)	0.031	184 (92.5)	237 (85.9)	0.026
≥ 1 *CYP2C19* no function allele	321 (67.6)	203 (78.4)	118 (54.6)	<0.001	165 (82.9)	156 (56.5)	<0.001
≥ 1 *CYP2C19* increased function allele	3 (0.6)	3 (1.2)	0 (0)	0.255	3 (1.5)	0 (0)	0.073

Data are presented as n (%) or median (interquartile range). ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; HDL-C, high-density lipoprotein-cholesterol; HPR, high platelet reactivity; LDL-C, low-density lipoprotein-cholesterol; PRU, P2Y12 reaction unit.

without HPR by corrected PRU, except when comparing higher total cholesterol levels (median, 190 mg/dl vs 179 mg/dl, P = 0.024) between patients with HPR by corrected PRU or those without HPR.

In a multivariate analysis, carrying ≥1 *PON1* 192R allele (odds ratio [OR] 1.96, 95% confidence interval [CI] 1.03–3.76), male sex (OR 0.50, 95% CI 0.32–0.78), treatment with a calcium channel blocker (OR 1.69, 95% CI 1.11–2.59), hematocrit (OR 0.87, 95% CI 0.83–0.92, per 1% increase), platelet count (OR 0.92, 95% CI 0.88–0.96, per 1×10^4/μl increase), and carrying ≥1 *CYP2C19* no function allele (OR 4.17, 95% CI 2.67–6.60) were associated with HPR by original PRU. Similarly, carrying ≥1 *PON1* 192R allele was associated with HPR by corrected PRU (OR 2.34, 95% CI 1.21–4.74) after adjustment for age, sex, treatment with ACEI/ARB, hematocrit, platelet count, total cholesterol, and carrying ≥1 *CYP2C19* no function allele (Table 3).

Discussion

This study clarified the associations between the carrier status of *PON1* Q192R and HPR with clopidogrel in patients undergoing elective neurointervention. Carrying ≥1 *PON1* 192R allele as well as ≥1 *CYP2C19* no function allele was associated with HPR, which occurred in approximately half of the patients with clopidogrel in the present study. The prevalence of HPR was markedly higher in patients carrying both ≥1 *PON1* 192R allele and the *CYP2C19* no function allele compared with noncarriers.

Previous studies reported the prevalence of HPR with clopidogrel on the basis of a PRU of ≥208 ranged from 18.3% to 57.1% [22,23]. Together with multiple clinical, hematological, and biochemical parameters, the *CYP2C19* no function allele was associated with the inter-patient variability of platelet inhibition in response to clopidogrel [4,24,25]. In the present study, more than two-thirds of patients were carriers of ≥1 *CYP2C19* no function allele, which is in

Table 3. Multivariate analysis of high platelet reactivity in patients undergoing elective neurointervention.

Variables	OR (95% CI)	*P* value
HPR by original PRU		
Sex, male	0.50 (0.32–0.78)	0.002
Age (per 1-year increase)	1.00 (0.98–1.02)	0.854
Treatment with calcium channel blocker	1.69 (1.11–2.59)	0.015
Hematocrit (per 1% increase)	0.87 (0.83–0.92)	<0.001
Platelet count (per 1×10^4/μl increase)	0.92 (0.88–0.96)	<0.001
Total cholesterol (per 10 mg/dl increase)	1.05 (0.99–1.11)	0.082
≥1 *PON1* 192R allele	1.96 (1.03–3.76)	0.039
≥1 *CYP2C19* no function allele	4.17 (2.67–6.60)	<0.001
HPR by corrected PRU		
Sex, male	0.54 (0.34–0.83)	0.005
Age (per 1-year increase)	1.00 (0.98–1.02)	0.993
Treatment with ACEI/ARB	1.47 (0.97–2.26)	0.073
Hematocrit (per 1% increase)	1.05 (1.00–1.11)	0.032
Platelet count (per 1×10^4/μl increase)	0.92 (0.88–0.95)	<0.001
Total cholesterol (per 10 mg/dl increase)	1.06 (1.00–1.12)	0.033
≥1 *PON1* 192R allele	2.34 (1.21–4.74)	0.011
≥1 *CYP2C19* no function allele	4.53 (2.87–7.30)	<0.001

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CI, confidence interval; HPR, high platelet reactivity; OR, odds ratio; PRU, P2Y12 reaction unit.

accordance with the observation that the frequency of carriers of ≥1 *CYP2C19* no function allele is markedly higher in East Asian populations compared with Western populations [6,26].

Similar to a previous study [19], the current study showed a significant negative association between hematocrit and PRU. Correcting for hematocrit resulted in an 8% re-classification and 15.4% reduction in the prevalence of HPR using a PRU threshold of ≥208, which may more accurately identify patients that would benefit from alternative antiplatelet therapy. Compared with the previous study, the current study showed a larger re-classification rate of 16.8% and a reduction in the prevalence of HPR of 23.2%. These differences between studies might be related to the relatively lower hematocrit levels in the current study that might explain the trend for re-classification as non-HPR by corrected PRU. In addition to the significant impact of carrying ≥1 *CYP2C19* no function allele, this study revealed that carrying ≥1 *PON1* 192R allele was a contributing factor for HPR based on the original and corrected PRU with clopidogrel in patients undergoing elective neurointervention. About 85% of clopidogrel is metabolized by CES1 and the remaining 15% is metabolized into a biologically active thiol metabolite via two steps. First, monooxygenation of the thiophene ring produces 2-oxo-clopidogrel. Second, oxidative cleavage of the thiolactone ring of 2-oxo-clopidogrel yields sulfenic acid, which is subsequently reduced to a thiol, an active metabolite bearing an exocyclic double bond (Fig 1). CYP2C19 contributes to both steps in the clopidogrel bioactivation pathway—the formation of 45% of 2-oxo-clopidogrel and 21% of the active metabolite [27]—whereas the PON1-mediated hydrolysis of 2-oxo-clopidogrel generates an endo metabolite, in which the double bond migrates from an exocyclic to an endocyclic position in the piperidine ring [28]. Unlike the active metabolite, the endo metabolite is unstable and ineffective for platelet inhibition [29]. These findings suggest that carriers of ≥1 *PON1* 192R allele have higher PON1 activity that accelerates the increase in the endo metabolite of clopidogrel compared with non-carriers. Moreover, lower CYP2C19 activity might further reinforce this cascade via the delayed metabolism of 2-oxo-clopidogrel by CYP2C19, which allows PON1 to metabolize 2-oxo-clopidogrel into the endo metabolite. Therefore, compared with Western populations, the impact of *PON1* Q192R on HPR with clopidogrel might be more apparent in East Asian populations, in which carriers of *CYP2C19* no function alleles are common. This study showed a markedly higher prevalence of HPR by original and corrected PRU in patients carrying ≥1 *PON1* 192R allele and the *CYP2C19* no function allele compared with noncarriers. Although no significant association between *PON1* polymorphisms and response to clopidogrel was reported by Saiz-Rodríguez et al. [18], this might be due to ethnic differences or combined assessment of the *PON1* polymorphisms, *PON1* Q192R, L55M (rs854560; c.163T>A or p. Leu55Met), and C-108T (rs705379; c.-108C>T). Our results are consistent with previous studies [13–16] but were opposite to those of Bouman et al. [8]. This suggests a difference in the role of PON1 between Western and East Asian populations. PON1 contributes to the antioxidative function of high-density lipoprotein-cholesterol, which potentially suppresses platelet aggregation [30]. Therefore, in addition to *CYP2C19* and *PON1* polymorphisms, differences in lipoprotein levels and their association with vascular disease risk might explain these results [31]. Our findings may have clinical utility in that *PON1* and *CYP2C19* genotyping may provide prognostic information on the efficacy of clopidogrel in patients undergoing neurointervention. Further study is needed to investigate the relative risk of thromboembolic complications between the carrier status of *PON1* 192R allele and *CYP2C19* no function allele, the benefit of alternative antiplatelet therapy with ticagrelor or prasugrel in patients carrying ≥1 *PON1* 192R allele and the *CYP2C19* no function allele, and whether the de-escalation of DAPT to clopidogrel monotherapy can avoid more bleeding events in non-carriers.
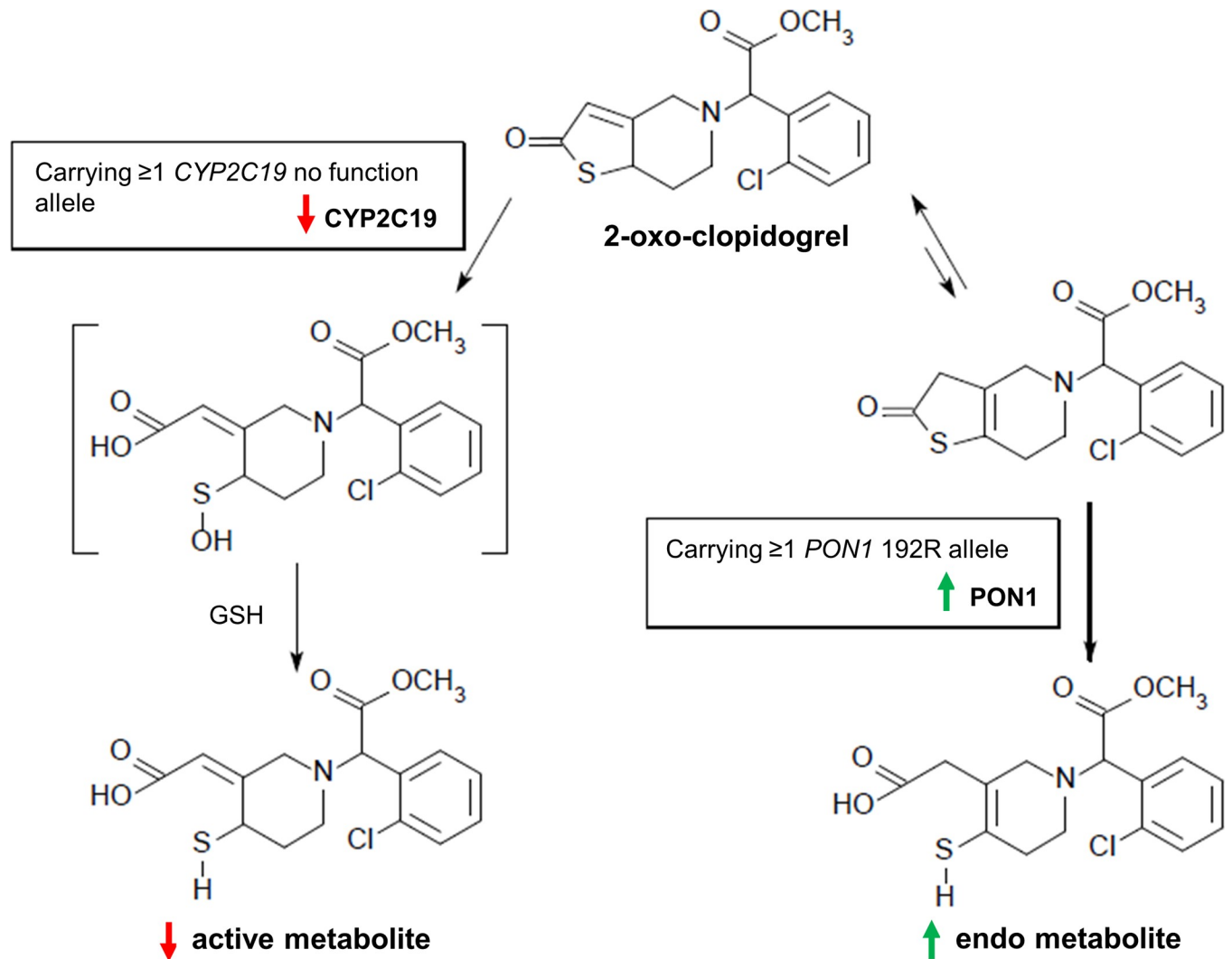
Fig 1. Pathway involved in the formation of the active and endo metabolites of clopidogrel in patients with polymorphisms in cytochrome P450 family 2 subfamily C member 19 (*CYP2C19*) and paraoxonase-1 (*PON1*). In carriers of ≥1 *PON1* 192R allele, high PON1 activity may accelerate an increase in the production of the endo metabolite of clopidogrel. Low CYP2C19 activity in carriers of ≥1 *CYP2C19* no function allele may reinforce this cascade via the delayed metabolism of 2-oxo-clopidogrel into an active metabolite, which allows PON1 to metabolize 2-oxo-clopidogrel into the endo metabolite of clopidogrel. GSH, glutathione.

In addition to hematocrit, this study revealed clinical and biochemical parameters including sex, lower platelet count, and higher total cholesterol level that were also associated with HPR by original and corrected PRU with clopidogrel. A recent report demonstrated that lower platelet count was associated with larger mean platelet volume, which reflected greater pro-thrombotic activity [32]. Another report demonstrated an association between total cholesterol level and clopidogrel resistance [33]. However, these associations remain controversial and the mechanism is unclear. Further study with larger samples might provide reliable conclusions.

This study had several limitations. First, this study did not include genetic polymorphisms other than *CYP2C19**2, *3, and *17, and *PON1* Q192R on post-clopidogrel platelet reactivity. Notably, *PON1* L55M, which lowers the activity of PON1 [34], and has an allele frequency of 3.4% in East Asians [21], was not included in the analysis. Moreover, in addition to the partial role of CYP2C19, many other enzymes are involved in the metabolism of clopidogrel, and the

role of PON1 could be relatively minor. Therefore, this study cannot exclude alternative results that might be related to other genetic polymorphisms. Second, the study was performed at a single center with a relatively small number of patients with limited clinical and laboratory information, which may have introduced some selection bias. Third, previous studies showed a PRU of ≥208 predicted thromboembolic events following pipeline embolization device placement for IA [17]; however, we could not systematically evaluate associations between periprocedural thromboembolic complications and HPR due to the heterogeneity of endovascular procedures and additional treatment for HPR (e.g., some patients with HPR received additional antithrombotic agents before and after treatment). Fourth, the lack of a multiple-comparison correction might have led to false-positive results. Finally, participants in this study were mostly Japanese, which might limit the generalizability of the findings.

Conclusions

Alongside clinical, hematological, and biochemical parameters and the presence of ≥1 *CYP2C19* no function allele, carrying ≥1 *PON1* 192R allele is associated with HPR by original and corrected PRU with clopidogrel in patients undergoing elective neurointervention, although alternative results that might be related to other genetic polymorphisms cannot be excluded.

Supporting information

S1 File.
(XLSX)

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References

1. Müller I, Besta F, Schulz C, Massberg S, Schönig A, Gawaz M. Prevalence of clopidogrel non-responders among patients with stable angina pectoris scheduled for elective coronary stent placement. Thromb Haemost. 2003; 89:783–787. https://doi.org/10.1055/s-0037-1613462 PMID: 12719773

2. Matetzky S, Shenkman B, Guetta V, Shechter M, Beinart R, Goldenberg I, et al. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. Circulation. 2004; 109:3171–3175. https://doi.org/10.1161/01.CIR.0000130846.46168.03 PMID: 15184279

3. Ryu DS, Hong CK, Sim YS, Kim CH, Jung JY, Joo JY. Anti-platelet drug resistance in the prediction of thromboembolic complications after neurointervention. J Korean Nerosurg Soc. 2010; 48:319–324. https://doi.org/10.3340/jkns.2010.48.4.319 PMID: 21113358

4. Shuldiner AR, O'Connell JR, Bliden KP, Gandhi A, Ryan K, Horenstein RB, et al. Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. JAMA. 2009; 302:849–857. https://doi.org/10.1001/jama.2009.1232 PMID: 19706858

5. Simon T, Verstuyft C, Mary-Krause M, Quteineh L, Drouet E, Méneveau N, et al. Genetic determinants of response to clopidogrel and cardiovascular events. N Engl J Med. 2009; 360:363–375. https://doi.org/10.1056/NEJMoa0808227 PMID: 19106083

6. Jang JS, Cho KI, Jin HY, Seo JS, Yang TH, Kim DK, et al. Meta-analysis of cytochrome P450 2C19 polymorphism and risk of adverse clinical outcomes among coronary artery disease patients of different ethnic groups treated with clopidogrel. Am J Cardiol. 2012; 110:502–508. https://doi.org/10.1016/j.amjcard.2012.04.020 PMID: 22591668

7. Sibbing D, Koch W, Gebhard D, Schuster T, Braun S, Stegherr J, et al. Cytochrome 2C19*17 allelic variant, platelet aggregation, bleeding events, and stent thrombosis in clopidogrel-treated patients with coronary stent placement. Circulation. 2010; 121:512–518. https://doi.org/10.1161/CIRCULATIONAHA.109.885194 PMID: 20083681

8. Bouman HJ, Schömig E, van Werkum JW, Velder J, Hackeng CM, Hirschhäuser C, et al. Paraoxonase-1 is a major determinant of clopidogrel efficacy. Nat Med. 2011; 17:110–116. https://doi.org/10.1038/nm.2281 PMID: 21170047

9. Hulot JS, Collet JP, Cayla G, Silvain J, Allanic F, Bellemain-Appaix A. CYP2C19 but not PON1 genetic variants influence clopidogrel pharmacokinetics, pharmacodynamics, and clinical efficacy in post-myocardial infarction patients. Circ Cardiovasc Interv. 2011; 4:422–428. https://doi.org/10.1161/CIRCINTERVENTIONS.111.963025 PMID: 21972404

10. Sibbing D, Koch W, Massberg S, Byrne RA, Mehilli J, Schulz S, et al. No association of paraoxonase-1 Q192R genotypes with platelet response to clopidogrel and risk of stent thrombosis after coronary stenting. Eur Heart J. 2011; 32:1605–1613. https://doi.org/10.1093/eurheartj/ehr155 PMID: 21527445

11. Trenk D, Hochholzer W, Fromm MF, Zolk O, Valina CM, Stratz C, et al. Paraoxonase-1 Q192R polymorphism and antiplatelet effects of clopidogrel in patients undergoing elective coronary stent placement. Circ Cardiovasc Genet. 2011; 4:429–436. https://doi.org/10.1161/CIRCGENETICS.111.960112 PMID: 21685174

12. Mega JL, Close SL, Wiviott SD, Man M, Duvvuru S, Walker JR, et al. PON1 Q192R genetic variant and response to clopidogrel and prasugrel: pharmacokinetics, pharmacodynamics, and a meta-analysis of clinical outcomes. J Thromb Thrombolysis. 2016; 41:374–383. https://doi.org/10.1007/s11239-015-1264-9 PMID: 26573179

13. Li X, Zhang L, Chen X, Qu F, Li J, Ma C, et al. PON1 Q192R genotype influences clopidogrel responsiveness by relative platelet inhibition instead of on-treatment platelet reactivity. Thromb Res. 2013; 132:444–449. https://doi.org/10.1016/j.thromres.2013.08.004 PMID: 23993903

14. Li X, Wang Z, Wang Q, Xu Q, Lv Q. Clopidogrel-associated genetic variants on inhibition of platelet activity and clinical outcome for acute coronary syndrome patients. Basic Clin Pharmacol Toxicol. 2019; 124:84–93. https://doi.org/10.1111/bcpt.13110 PMID: 30098132

15. Peng W, Shi X, Xu X, Lin Y. Both CYP2C19 and PON1 Q192R genotypes influence platelet response to clopidogrel by thrombelastography in patients with acute coronary syndrome. Cardiovasc Ther. 2019; 2019:3470145. https://doi.org/10.1155/2019/3470145 PMID: 31772608

16. Zhang Z, Chen M, Zhang L, Zhao Q. The impact of cytochrome 450 and Paraoxonase polymorphisms on clopidogrel resistance and major adverse cardiac events in coronary heart disease patients after percutaneous coronary intervention. BMC Pharmacol Toxicol. 2020; 21:1. https://doi.org/10.1186/s40360-019-0378-7 PMID: 31900240

17. Adeeb N, Griessenauer CJ, Foreman PM, Moore JM, Shallwani H, Motiei-Langroudi R, et al. Use of Platelet Function Testing Before Pipeline Embolization Device Placement: A Multicenter Cohort Study. Stroke. 2017; 48:1322–1330. https://doi.org/10.1161/STROKEAHA.116.015308 PMID: 28411263

18. Saiz-Rodríguez M, Belmonte C, Caniego JL, Koller D, Zubiaur P, Bárcena E, et al. Influence of CYP450 enzymes, CES1, PON1, ABCB1, and P2RY12 polymorphisms on clopidogrel response in patients subjected to a percutaneous neurointervention. Clin Ther. 2019; 41:1199–1212.e2. https://doi.org/10.1016/j.clinthera.2019.04.037 PMID: 31128980

19. Kakouros N, Kickler TS, Laws KM, Rade JJ. Hematocrit alters VerifyNow P2Y12 assay results independently of intrinsic platelet reactivity and clopidogrel responsiveness. J Thromb Haemost. 2013; 11:1814–1822. https://doi.org/10.1111/jth.12376 PMID: 24118870

20. Price MJ, Berger PB, Teirstein PS, Tanguay JF, Angiolillo DJ, Spriggs D, et al. Standard- vs high-dose clopidogrel based on platelet function testing after percutaneous coronary intervention: the GRAVITAS randomized trial. JAMA. 2011; 305:1097–1105. https://doi.org/10.1001/jama.2011.290 PMID: 21406646

21. Genomes Project Consortium. A global reference for human genetic variation. Nature. 2015; 526 (7571):68–74. https://doi.org/10.1038/nature15393 PMID: 26432245

22. Yaseen IF, Farhan HA, Abbas HM. Clopidogrel non-responsiveness in patients undergoing percutaneous coronary intervention using the VerifyNow test: frequency and predictors. Eur J Hosp Pharm. 2019; 26:113–116. https://doi.org/10.1136/ejhpharm-2017-001359 PMID: 31157110

23. Waksman R, Maya J, Angiolillo DJ, Carlson GF, Teng R, Caplan RJ, et al. Ticagrelor versus clopidogrel in black patients with stable coronary artery disease: prospective, randomized, open-label, multiple-dose, crossover pilot study. Circ Cardiovasc Interv. 2015; 8:e002232. https://doi.org/10.1161/CIRCINTERVENTIONS.114.002232 PMID: 26152562

24. Cui H, Lin S, Chen X, Gao W, Li X, Zhou H, et al. Correlation between SNPs in candidate genes and VerifyNow-detected platelet responsiveness to aspirin and clopidogrel treatment. Cardiovasc Drugs Ther. 2015; 29:137–146. https://doi.org/10.1007/s10557-015-6585-6 PMID: 25860557

25. Voisin S, Bongard V, Tidjane MA, Lhermusier T, Carrié D, Sié P. Are P2Y12 reaction unit (PRU) and % inhibition index equivalent for the expression of P2Y12 inhibition by the VerifyNow assay? Role of haematocrit and haemoglobin levels. Thromb Haemost. 2011; 106:227–229. https://doi.org/10.1160/TH11-01-0046 PMID: 21544318

26. Desta Z, Zhao X, Shin JG, Flockhart DA. Clinical significance of the cytochrome P450 2C19 genetic polymorphism. Clin Pharmacokinet. 2002; 41:913–958. https://doi.org/10.2165/00003088-200241120-00002 PMID: 12222994

27. Kazui M, Nishiya Y, Ishizuka T, Hagihara K, Farid NA, Okazaki O, et al. Identification of the human cytochrome P450 enzymes involved in the two oxidative steps in the bioactivation of clopidogrel to its pharmacologically active metabolite. Drug Metab Dispos. 2010; 38:92–99. https://doi.org/10.1124/dmd.109.029132 PMID: 19812348

28. Dansette PM, Rosi J, Bertho G, Mansuy D. Cytochromes P450 catalyze both steps of the major pathway of clopidogrel bioactivation, whereas paraoxonase catalyzes the formation of a minor thiol metabolite isomer. Chem Res Toxicol. 2012; 25:348–356. https://doi.org/10.1021/tx2004085 PMID: 22103858

29. Gong IY, Crown N, Suen CM, Schwarz UI, Dresser GK, Knauer MJ, et al. Clarifying the importance of CYP2C19 and PON1 in the mechanism of clopidogrel bioactivation and in vivo antiplatelet response. Eur Heart J. 2012; 33:2856–2464a. https://doi.org/10.1093/eurheartj/ehs042 PMID: 22374717

30. Mackness MI, Arrol S, Abbott C, et al. Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. Atherosclerosis. 1993; 104:129–135. https://doi.org/10.1016/0021-9150(93)90183-u PMID: 8141836

31. Paré G, Çaku A, McQueen M, et al. Lipoprotein(a) Levels and the Risk of Myocardial Infarction Among 7 Ethnic Groups. Circulation. 2019; 139:1472–1482. https://doi.org/10.1161/CIRCULATIONAHA.118.034311 PMID: 30667276

32. Miller MM, Henninger N, Słowik A. Mean platelet volume and its genetic variants relate to stroke severity and 1-year mortality. Neurology. 2020; 95:e1153–e1162. https://doi.org/10.1212/WNL.0000000000010105 PMID: 32576634

33. Su J, Zheng N, Li Z, Huangfu N, Mei L, Xu X, et al. Association of GCK gene DNA methylation with the risk of clopidogrel resistance in acute coronary syndrome patients. J Clin Lab Anal. 2020; 34:e23040. https://doi.org/10.1002/jcla.23040 PMID: 31605429

34. Brophy VH, Jampsa RL, Clendenning JB, et al. Effects of 5' regulatory-region polymorphisms on paraoxonase-gene (PON1) expression. Am J Hum Genet. 2001; 68:1428–1436. https://doi.org/10.1086/320600 PMID: 11335891