Association between CYP2C19 genotype and the additional effect of cilostazol to clopidogrel resistance in neuroendovascular therapy

Hayato Tajima¹, Takashi Izumi², Shigeru Miyachi³, Noriaki Matsubara⁴, Masashi Ito², Tasuku Imai², Masahiro Nishihori², Kazunori Shintai², Sho Okamoto², Yoshio Araki², Yasuo Kumakura², Yoko Furukawa-Hibi⁵, Kiyofumi Yamada⁶, and Toshihiko Wakabayashi²

¹Department of Neurosurgery, Handa City Hospital, Handa, Japan
²Department of Neurosurgery, Nagoya University Graduate School of Medicine, Nagoya, Japan
³Department of Neurosurgery, Aichi Medical University, Nagakute, Japan
⁴Department of Neurosurgery, Osaka Medical College, Takatsuki, Japan
⁵Department of Neuropsychopharmacology and Hospital Pharmacy, Nagoya University Graduate School of Medicine, Nagoya, Japan

ABSTRACT

We investigated the association between CYP2C19 genotype and additional effect of cilostazol on clopidogrel resistance (CR) in neuroendovascular therapy. Between January 2012 and January 2016, 447 consecutive patients were administered with 75-mg cilostazol/day. The VerifyNow System was used for evaluating P2Y12 reaction units (PRU) > 230 and/or percentage inhibition of platelet function (% Inhibition) ≤ 20 as CR. Among 158 patients with CR, 31 were administered with additional 100- or 200-mg cilostazol/day and their platelet function was evaluated. According to CYP2C19 genotypes revealed using the Spartan RX and DNeasy Blood & Tissue Kit, patients were classified into three phenotypic groups: extensive metabolizer (EM, three patients), intermediate metabolizer (IM, 12 patients), and poor metabolizer (PM, 16 patients). Administration of additional cilostazol decreased PRU (EM group: 160.7 ± 85.2 after vs 278.3 ± 40.1 before, \(P = 0.15\); IM group: 205.6 ± 74.0 vs 254.3 ± 35.0, \(P = 0.02\); and PM group: 227.8 ± 52.2 vs 282.1 ± 30.4, \(P = 0.003\)), and increased % Inhibition (EM group: 40.0 ± 27.9 vs 9.3 ± 3.8, \(P = 0.25\); IM group: 31.4 ± 18.0 vs 11.8 ± 8.2, \(P = 0.001\); and PM group: 24.6 ± 15.0 vs 10.4 ± 9.3, \(P = 0.001\)). However, the rate of normalized-clopidogrel response, thromboembolic lesions, and bleeding complications were not significantly different among the three groups. Thus, the addition of cilostazol was effective on CR in terms of PRU, % Inhibition, rate of change of normalized-clopidogrel response, thromboembolic events, and bleeding complications irrespective of phenotype.

Keywords: CYP2C19 genotype, cilostazol, clopidogrel resistance, endovascular treatment, VerifyNow System

This is an Open Access article distributed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License. To view the details of this license, please visit (http://creativecommons.org/licenses/by-nc-nd/4.0/).
INTRODUCTION

The implication of dual antiplatelet therapy (DAPT) with aspirin and clopidogrel has been well recognized in neuroendovascular therapy to prevent thromboembolic events. However, clopidogrel resistance is often associated with an increased risk of thromboembolic complications.\textsuperscript{1-5) Moreover, a loss-of-function (LOF) polymorphism in cytochrome P450 2C19 (\textit{CYP2C19}) has been associated with clopidogrel resistance.\textsuperscript{6-8) Recently, the addition of cilostazol to DAPT has been reported to inhibit platelet activation and improve clinical outcomes following PCI.\textsuperscript{9) Furthermore, in neuroendovascular therapy, it has been reported that adjunctive cilostazol (triple antiplatelet therapy) in clopidogrel resistant patients reduces the rate of clopidogrel resistance and suppresses new ischemic lesions without hemorrhagic complications compared with DAPT in carotid artery stenting.\textsuperscript{10,11) However, the relationship between \textit{CYP2C19} genotypes and the additional effect of cilostazol to clopidogrel resistance has not been elucidated. Therefore, this study aimed to investigate the association between the additional effect of cilostazol to clopidogrel resistance and \textit{CYP2C19} genotypes.

MATERIALS AND METHODS

\textit{Study Design}

A total of 447 consecutive patients undergoing neuroendovascular therapy stent placement for carotid artery stenosis or vertebral artery stenosis and coiling for an intracranial aneurysm at Nagoya University Hospital for Neurosurgery between January 2012 and January 2016 were enrolled in the study. All patients received clopidogrel before the procedure and were tested for clopidogrel resistance using the VerifyNow System (Accriva Diagnostics, San Diego, California). Furthermore, the addition of cilostazol in patients with clopidogrel resistance was targeted. Patient background characteristics, diagnosis, procedure methods, intraprocedural complications, and diffusion-weighted imaging performed within 5 days were recorded and maintained in the database. \textit{CYP2C19} genotypes were evaluated using Spartan RX (Spartan Bioscience Inc. Ottawa, ON, Canada) and the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany), and phenotypes were classified as extensive metabolizer (EM), intermediate metabolizer (IM), poor metabolizer (PM) from the genotypic data.

\textit{Patient Background Characteristics}

We examined patients’ medical history for diabetes mellitus, hypertension, and dyslipidemia, which are risk factors of cerebrovascular disease. Diabetes mellitus was defined as hemoglobin A1c level of $\geq$ 6.5 \% or as patients undergoing diabetes treatment. Hypertension was defined as a systolic blood pressure of $\geq$ 140 mmHg or a diastolic blood pressure of $\geq$ 90 mmHg or patients on antihypertensive medication. Dyslipidemia was defined as a low-density lipoprotein cholesterol level of $\geq$ 140 mg/dL or patients on statin.

\textit{Evaluation of Platelet Function}

Platelet function was analyzed using the VerifyNow System. VerifyNow-P2Y12 assay results are expressed in P2Y12 Reaction Units (PRU) and \% inhibition of platelet function from baseline activation via thrombin receptor activating peptide (% Inhibition).\textsuperscript{12) Clopidogrel resistance was defined as PRU $>$ 230 or/and % Inhibition $\leq$ 20 according to previous studies.\textsuperscript{2,5,13)}}
Genotype related clopidogrel-cilostazol

Medication Regimen

Patients were administered with a combination of clopidogrel 75 mg/day and aspirin 100 mg/day or clopidogrel 75 mg/day from three weeks before neuroendovascular therapy and analyzed by VerifyNow System from two weeks before the procedures. Patients were subsequently identified as clopidogrel resistant and were prescribed with an additional cilostazol 100 mg/day or 200 mg/day at the discretion of surgeons. After approximately two weeks, the effect of the drug was measured again with VerifyNow System. In Japan, the recommended dose of cilostazol is 200 mg/day. However, in our study cilostazol, 100 mg/day was selected as the dose as the patient receiving cilostazol 200 mg/day was presented with a headache or tachycardia.

Genotype Data

Spartan RX and the DNeasy Blood & Tissue Kit were used for the genotypic analysis. Spartan RX is portable technology enables healthcare personnel with no previous training in genetic laboratory techniques to undertake genotyping.14,15) DNeasy Blood & Tissue Kit extracts genomic DNA from the samples by proteinase K digestion in combination, following the tissue protocol. We referred to some previous reports,7,16) and defined EM as CYP1C19*1/*1, IM as CYP2C19*1/*2 or CYP2C19*1/*3, and PM as CYP2C19*2/*2 or CYP2C19*2/*3 or CYP2C19*3/*3.

Statistical Analysis

All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).17) EZR is a modified version of R commander designed to add statistical functions frequently used in biostatistics. Continuous variables were presented as mean ± standard deviation. The relationship between the results of before and after the addition of cilostazol to clopidogrel was evaluated using a paired t test. However, as PRU and % Inhibition of EM group were not normally distributed, we used Wilcoxon signed rank test. To analyze significant differences among the three phenotype groups (EM group, IM group, and PM group), we compared categorical variables using Fisher’s exact test applying Bonferroni correction method and assessed continuous variables using one-way ANOVA. \( P < 0.05 \) was considered statistically significant.

RESULTS

The genotypic analysis of 447 patients undergoing neuroendovascular therapy with clopidogrel 75 mg/day was analyzed using VerifyNow platelet function assay.

Among the 447 patients tested using the VerifyNow System, a total of 158 were diagnosed with clopidogrel resistance. A total of 32 patients were administered with cilostazol. Among these, \( CYP2C19 \) genotypic analysis was performed on 31 patients. We could not analyze \( CYP2C19 \) genotypic analysis in the remaining single patient because the patient moved to other hospital. The genotypes were distinguished into the following the three groups: EM group (\( n = 3 \)), IM group (\( n = 12 \)), and PM group (\( n = 16 \)) (Fig. 1). There were no newly added drugs other than cilostazol during this study. No significant difference was found in the respective baseline demographic or medical history characteristics before the addition of cilostazol to clopidogrel resistance among the three groups (Table 1).

Results of PRU before and after the addition of cilostazol to clopidogrel resistance revealed that PRU after the addition of cilostazol was significantly lower than PRU before the addition of cilostazol in the IM and PM groups (IM group: 205.6 ± 74.0 vs 254.3 ± 35.0, \( P = 0.02 \) and
Table 1  Baseline characteristics before addition of cilostazol

| Phenotype | EM (n = 3) | IM (n = 12) | PM (n = 16) | p-value |
|-----------|------------|-------------|-------------|---------|
| General characteristics | | | | |
| Age ± SD | 69.3 ± 5.9 | 64.1 ± 10.9 | 63.9 ± 8.8 | 0.65 |
| Female (%) | 3 (100) | 7 (58.3) | 11 (68.8) | 0.55 |
| Risk factors | | | | |
| Diabetes mellitus (%) | 1 (33.3) | 3 (25.0) | 0 (0) | 0.07 |
| Hypertension (%) | 3 (100) | 8 (66.7) | 14 (87.5) | 0.32 |
| Dyslipidemia (%) | 1 (33.3) | 5 (41.7) | 7 (43.8) | 1 |
| Medications | | | | |
| ARB and/or CCB (%) | 3 (100) | 8 (66.7) | 14 (87.5) | 0.32 |
| Statin (%) | 1 (33.3) | 5 (41.7) | 7 (43.8) | 1 |
| Diagnosis | | | | |
| Aneurysm (%) | 3 (100) | 7 (58.3) | 12 (75.0) | 0.45 |
| ICS (%) | 0 (0) | 4 (33.3) | 4 (25.0) | 0.84 |
| VAS (%) | 0 (0) | 1 (8.3) | 0 (0) | 0.47 |
| VerifyNow assay | | | | |
| % Inhibition ± SD | 9.3 ± 3.8 | 11.8 ± 8.2 | 10.4 ± 9.3 | 0.88 |
| BASE ± SD | 307.7 ± 42.6 | 288.2 ± 29.9 | 312.5 ± 48.0 | 0.31 |
| PRU ± SD | 278.3 ± 40.1 | 254.3 ± 35.0 | 282.1 ± 30.4 | 0.1 |

Note: Values are expressed as numbers (%) or mean ± SD.

Abbreviations: EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; ARB, angiotensin II receptor blocker; CCB, calcium channel blocker; ICS, internal carotid artery stenosis; VAS, vertebral artery stenosis; % Inhibition, percentage inhibition of platelet function; BASE, baseline results; PRU, P2Y12 reaction units; and SD, standard deviation.

PM group: 227.8 ± 52.2 vs 282.1 ± 30.4, P = 0.003) (Fig. 2A). In the EM group, PRU after the addition of cilostazol to clopidogrel resistance did not differ significantly from PRU before the addition of cilostazol (160.7 ± 85.2 vs 278.3 ± 40.1; P = 0.15); however, the rate of change in PRU in the EM group was higher than that in the IM and PM groups.

As illustrated in Fig. 2B, % Inhibition after the addition of cilostazol to clopidogrel resistance was significantly higher than that before the addition of cilostazol to clopidogrel resistance in the IM and PM groups (IM group: 31.4 ± 18.0 vs 11.8 ± 8.2, P = 0.001 and PM group: 24.6 ± 15.0 vs 10.4 ± 9.3, P = 0.001). The % Inhibition after the addition of cilostazol to clopidogrel resistance was not significantly different from that before the addition of cilostazol to clopidogrel resistance in the EM group (40.0 ± 27.9 vs 9.3 ± 3.8, P = 0.25); however, the rate of change
Genotype related clopidogrel-cilostazol

447 patients underwent neuroendovascular therapy with clopidogrel at 75 mg/day

\[ \rightarrow 289 \text{ showed Clopidogrel normal} \]

158 had clopidogrel resistance

\[ \rightarrow 126 \text{ without cilostazol} \]

32 administered with additional cilostazol

\[ \rightarrow 1 \text{ lost} \]

31 assessed for CYP2C19 genotype

- 3 EM
- 12 IM
- 16 PM

**Fig. 1** Flow chart of study patients

**Fig. 2** Results of PRU before and after the addition of cilostazol to clopidogrel resistance (A) and % Inhibition (B). Results are expressed as mean (boxes) ± SD (error bars).

Abbreviations: % Inhibition, percentage inhibition of platelet function; PRU, P2Y12 reaction units; SD, standard deviation
of % Inhibition between before and after the addition of cilostazol to clopidogrel resistance in EM group was higher than that in the IM and PM groups.

Normalized-clopidogrel response was observed in 1/3 patients (33.3%) in the EM group, in 7/12 patients (58.3%) in the IM group, and in 6/16 patients (37.5%) in the PM group (Table 2). The ratio of the patients with a normalized-clopidogrel response after the addition of cilostazol was not significantly different among the three groups.

There was no significant difference in thromboembolic lesions and bleeding complications in each group (Table 2). However, one patient in the PM group had transient thalamic aphasia as an ischemic event, which was completely recovered.

**DISCUSSION**

Two important clinical findings were discovered. First, irrespective of CYP2C19 genotype, the addition of cilostazol to clopidogrel significantly decreased the PRU and increased the % Inhibition. Second, there was no significant difference in the prevalence of thromboembolic events and bleeding complications among the CYP2C19 genotypes with the addition of cilostazol to clopidogrel resistance.

Previously, it was demonstrated that the addition of cilostazol to clopidogrel resistance decreased PRU and increased % Inhibition. However, the relationship between CYP2C19 genotypes which is one of the factors responsible for clopidogrel resistance and effect of the addition of cilostazol remains unclear. Particularly, PM usually demonstrates a significant reduction in platelet inhibition, patients in the PM group are likely to become clopidogrel resistant. In the present study, the PM group exhibited the highest frequency of clopidogrel resistance. However, after the addition of cilostazol, the frequency of normalized-clopidogrel response did not differ significantly among all the groups. Therefore, this study indicated that the addition of cilostazol to clopidogrel resistance was effective in particular patients with a PM.

Several reports have suggested that clopidogrel resistance was associated with the increased periprocedural thromboembolic events in neurovascular therapy. Conversely, reduced rate of thromboembolic events without increasing the rate of bleeding complications was also reported after the addition of cilostazol to clopidogrel resistance. In the present study, there was no significant difference in the thromboembolic events and the bleeding complications among all groups classified by CYP2C19 genotypes. Therefore, the results indicated that the addition of cilostazol to clopidogrel resistance irrespective of CYP2C19 genotypes prevented the thromboembolic events without bleeding complications.

Clopidogrel is metabolized to active thiol metabolite in the liver in two oxidation stages which involve several CYP enzymes; CYP2C19 particularly plays a significant role in this conversion. When the active thiol metabolite inhibits binding of adenosine diphosphate (ADP) to
the P2Y12 receptor, the synthesis of cyclic adenosine monophosphate (cAMP) is promoted. As a consequence, the activity of platelet aggregation is blocked.\(^{20,24}\) Thus, \textit{CYP2C19} LOF alleles, which exhibit a poor metabolic function, cause a reduction in the formation of active thiol metabolite and lead to a lack of platelet aggregation inhibition. However, a part of cilostazol is metabolized in the liver by P450 enzymes, and cilostazol enhances cAMP within the platelets by blocking phosphodiesterase-3A.\(^{19,25}\) These mechanisms explained that the addition of cilostazol to clopidogrel augment platelet aggregation inhibition. Because cilostazol alone cannot affect ADP and P2Y12 receptor, these mechanisms cannot explain the changes in PRU and \% Inhibition after the addition of cilostazol to clopidogrel in the present study. Kim \textit{et al.} suggested that the additional effect of cilostazol to clopidogrel was maximized in patients in the PM group of genotype \textit{CYP3A5*3/*3} owing to the lack of decrease in the concentration of the thiol metabolite by cilostazol in \textit{CYP3A5*3/*3} carriers.\(^{26}\) Future studies are required to clarify the mechanism that is not influenced by \textit{CYP2C19} genotypes.

The present study has several limitations. First, it was performed using a small sample size, specifically in the EM group, and it also lacked a comprehensive prospective design. Second, the decrease in the risk of thromboembolic events owing to the addition of cilostazol to clopidogrel is unclear because the results were not directly compared with clopidogrel resistance. Therefore, a prospective, multi-center study is suggested in the future to further confirm the efficacy of addition of cilostazol to clopidogrel resistance.

\textbf{CONCLUSIONS}

In conclusion, this study provided novel and important information regarding the additional effect of cilostazol to clopidogrel resistance. The results also demonstrated that lower PRU and higher \% Inhibition and the rate of change of normalized-clopidogrel response, thromboembolic events, and bleeding complications were not associated with \textit{CYP2C19} genotypes.

\textbf{CONFLICT OF INTEREST}

The authors declare that they have no competing interests.

\textbf{ACKNOWLEDGEMENTS}

I am grateful to all participants of the study.

\textbf{REFERENCES}

1) Flechtenmacher N, Kammerer F, Dittmer R, Budde U, Michels P, Rother J, \textit{et al.} Clopidogrel resistance in neurovascular stenting: correlations between light transmission aggregometry, verifyNow, and the multiplate. \textit{AJNR Am J Neuroradiol}, 2015; 36: 1953–1958.
2) Fifi JT, Brockington C, Narang J, Leesch W, Ewing SL, Bennet H, \textit{et al.} Clopidogrel resistance is associated with thromboembolic complications in patients undergoing neurovascular stenting. \textit{AJNR Am J Neuroradiol}, 2013; 34: 716–720.
3) Rho GJ, Shin WR, Kong TS, Kim MS, Lee CJ, Lee BH. Significance of clopidogrel resistance related to the stent-assisted angioplasty in patients with atherosclerotic cerebrovascular disease. \textit{J Korean Neurosurg Soc}, 2011; 50: 40–44.
4) Kim B, Kim K, Jeon P, Kim S, Kim H, Byun H, \textit{et al.} Thromboembolic complications in patients with
clopidogrel resistance after coil embolization for unruptured intracranial aneurysms. AJNR Am J Neuroradiol, 2014; 35: 1786–1792.

5) Asai T, Miyachi S, Izumi T, Matsubara N, Haraguchi K, Yamanouchi T, et al. Relationship between low response to clopidogrel and periprocedural ischemic events with coil embolization for intracranial aneurysms. J Neurointerv Surg, 2016; 8: 752–755.

6) Mega JL, Simon T, Collet JP, Anderson JL, Antman EM, Bliden K, et al. Reduced-function CYP2C19 genotype and risk of adverse clinical outcomes among patients treated with clopidogrel predominantly for PCI: a meta-analysis. JAMA, 2010; 304: 1821–1830.

7) Nakata T, Miyahara M, Nakatani K, Wada H, Tanigawa T, Komada F, et al. Relationship between CYP2C19 loss-of-function polymorphism and platelet reactivities with clopidogrel treatment in Japanese patients undergoing coronary stent implantation. Circ J, 2013; 77: 1436–1444.

8) Wang Y, Cai H, Zhou G, Zhang Z, Liu X. Effect of CYP2C19*2 and *3 on clinical outcome in ischemic stroke patients treated with clopidogrel. J Neurol Sci, 2016; 369: 216–219.

9) Geng DF, Liu M, Jin DM, Wu W, Deng J, Wang JF. Cilostazol-based triple antiplatelet therapy compared to dual antiplatelet therapy in patients with coronary stent implantation: a meta-analysis of 5,821 patients. Cardiology, 2012; 122: 148–157.

10) Nakagawa I, Wada T, Park HS, Nishimura F, Yamada S, Nakagawa H, et al. Platelet inhibition by adjunctive cilostazol suppresses the frequency of cerebral ischemic lesions after carotid artery stenting in patients with carotid artery stenosis. J Vasc Surg, 2014; 59: 761–767.

11) Hwang G, Huh W, Lee JS, Villavicencio JB, Villamor RB, Jr., Ahn SY, et al. Standard vs modified antiplatelet preparation for preventing thromboembolic events in patients with high on-treatment platelet reactivity undergoing coil embolization for an unruptured intracranial aneurysm: a randomized clinical trial. JAMA Neurol, 2015; 72: 764–772.

12) Malinin A, Pokov M, Spergling D, Defrancro A, Schwartz K, Schwartz D, et al. Monitoring platelet inhibition after clopidogrel with the VerifyNow-P2Y12(R) rapid analyzer: the VERIfy Thrombosis risk ASsessment (VERITAS) study. Thromb Res, 2007; 119: 277–284.

13) Price MJ, Berger PB, Teirstein PS, Tanguay JF, Angiolillo DJ, Spriigs 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.

14) Roberts JD, Wells GA, Le May MR, Labinaz M, Glover C, Froeschl M, et al. Point-of-care genetic testing for personalisation of antiplatelet treatment (RAPID GENE): a prospective, randomised, proof-of-concept trial. Lancet, 2012; 379: 1705–1711.

15) Wirth F, Zahra G, Xuereb RG, Barbara C, Fenech A, Azzopardi LM. Comparison of a rapid point-of-care and two laboratory-based CYP2C19*2 genotyping assays for personalisation of antiplatelet therapy. Int J Clin Pharm, 2016; 38: 414–420.

16) Pare G, Mehta SR, Yusuf S, Anand SS, Connolly SJ, Hirsh J, et al. Effects of CYP2C19 genotype on outcomes of clopidogrel treatment. N Engl J Med, 2010; 363: 1704–1714.

17) Kanda Y. Investigation of the freely available easy-to-use software ‘EZR’ for medical statistics. Bone Marrow Transplant, 2013; 48: 452–458.

18) Jeong YH, Lee SW, Choi BR, Kim IS, Seo MK, Kwak CH, et al. Randomized comparison of adjunctive cilostazol versus high maintenance dose clopidogrel in patients with high post-treatment platelet reactivity: results of the ACCEL-RESISTANCE (Adjuvant Cilostazol Versus High Maintenance Dose Clopidogrel in Patients With Clopidogrel Resistance) randomized study. J Am Coll Cardiol, 2009; 53: 1101–1109.

19) Maruyama H, Takeda H, Dembo T, Nagoya H, Kato Y, Fukuoka T, et al. Cilostazol resistance and the effect of combination cilostazol in patients with ischemic stroke or carotid artery stenting using the VerifyNow P2Y12 assay. Intern Med, 2011; 50: 695–698.

20) Maruyama H, Fukuoka T, Deguchi I, Ohe Y, Nagoya H, Kato Y, et al. Dual antiplatelet therapy clopidogrel with low-dose cilostazol intensifies platelet inhibition in patients with ischemic stroke. Intern Med, 2013; 52: 1043–1047.

21) Mao L, Jian C, Changzhi L, Dan H, Suihua H, Wenyi T, et al. Cytochrome CYP2C19 polymorphism and risk of adverse clinical events in clopidogrel-treated patients: a meta-analysis based on 23,035 subjects. Arch Cardiowasc Dis, 2013; 106: 517–527.

22) Ge H, Lv X, Ren H, Jin H, Jiang Y, He H, et al. Influence of CYP2C19 genetic polymorphisms on clinical outcomes of intracranial aneurysms treated with stent-assisted coiling. J Neurointerv Surg, 2017; 9: 958–962.

23) Scott SA, Sangkuhl K, Gardner EE, Stein CM, Hulot JS, Johnson JA, et al. Clinical pharmacogenetics implementation consortium guidelines for cytochrome P450-2C19 (CYP2C19) genotype and clopidogrel therapy. Clin Pharmacol Ther, 2011; 90: 328–332.
24) Gallego-Fabrega C, Krupinski J, Fernandez-Cadenas I. Drug resistance and secondary treatment of ischaemic stroke: The genetic component of the response to acetylsalicylic acid and clopidogrel. *Neurologia*, 2015; 30: 566–573.

25) Hiratsuka M, Hinai Y, Sasaki T, Konno Y, Imagawa K, Ishikawa M, et al. Characterization of human cytochrome p450 enzymes involved in the metabolism of cilostazol. *Drug Metab Dispos*, 2007; 35: 1730–1732.

26) Kim HS, Lim Y, Oh M, Ghim JL, Kim EY, Kim DH, et al. The pharmacokinetic and pharmacodynamic interaction of clopidogrel and cilostazol in relation to CYP2C19 and CYP3A5 genotypes. *Br J Clin Pharmacol*, 2016; 81: 301–312.