Department of Neurology, Nanjing First Hospital, Nanjing Medical University, Nanjing, China

Paraoxonase-1 Q192R polymorphism is not associated with clopidogrel response in Chinese stroke patients

YANG JIE, ZHOU JUN-SHAN, TAN JIE, HE BANG-SHUN, ZOU JIAN-JUN

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Zou Jian-Jun, Department of Clinical Pharmacology, Nanjing First Hospital, Nanjing 210006, China
zoujianjun100@126.com

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It is well known that CYP2C19*2/*2 is associated with attenuated response to clopidogrel, but recent findings indicated that in white patients, paraoxonase-1 (PON1) 192Q/Q was a major determinant of clopidogrel efficacy. The objective of this research was to assess the impact of PON1 Q192R polymorphism on the maximum platelet aggregation (MPA) and the anti-platelet effect of clopidogrel in clopidogrel-treated Chinese stroke patients. The study recruited 183 eligible Chinese stroke patients treated with a loading dose of 300-mg clopidogrel and a 75-mg daily maintenance dose. CYP2C19*2 and PON1 Q192R were genotyped, a subcohort of 13 patients with CYP2C19 *2/*2 genotype was excluded. Finally 170 patients with CYP2C19*1/*1 (wild-type homozygotes, n = 87) or CYP2C19*1/*2 (mutant heterozygotes, n = 83) were enrolled in the study population. These patients were divided into three groups according to their PON1 Q192R genotype: wild-type homozygotes, PON1 192QQ, n = 17; mutant heterozygotes, PON1 192QR, n = 81; mutant homozygotes, PON1 192RR, n = 72. MPA was measured by light transmittance aggregometry (LTA) to assess platelet function after seven 75-mg maintenance doses of clopidogrel before discharge. In those patients who were carriers of 1 mutant allele (PON1 Q/R192), ADP-induced MPA were not significantly different compared with wild-type homozygous patients [30.5% (IQR, 17.5 to 49.1%) versus 25.0% (IQR, 10.0 to 52.5%), respectively; P = 0.910]. In addition, in the patients who were carriers of the 2 mutant allele (PON1 R/R192), MPA were also not significantly different from wild-type homozygous patients [29.2% (IQR, 15.0 to 43.4%) versus 25.0% (IQR, 10.0 to 52.5%), respectively; P = 0.717]. Results of a multivariable linear regression model demonstrated that PON1 192R allele carriage was not independently associated with ADP-induced MPA measurements (P = 0.408). PON1 Q192R polymorphism does not seem to exhibit any impact on MPA and clopidogrel response at all.

1. Introduction

Clopidogrel, an antagonist of the platelet P2Y12 adenosine diphosphate (ADP) receptor, is widely used for the prevention of recurrent ischaemic stroke (Aw et al. 2012; Fukuoka et al. 2011; Milionis et al. 2011). However, a higher proportion of stroke patients cannot avoid clopidogrel resistance after regularly taking clopidogrel as anti-platelet therapy (Fukuoka et al. 2011). Many clinical studies have almost consistently demonstrated that loss-of-function *CYP2C19*2* polymorphisms are associated with attenuated response to clopidogrel (Mega et al. 2009; Xie et al. 2011; Mega et al. 2010; Shuldiner et al. 2009). However, a more recent finding observed that paraoxonase-1 (PON1), rather than CYP2C19, was associated with reduced clopidogrel responsiveness in clopidogrel-treated patients (Bouman et al. 2011). Opposite to the above, several most recently published studies consistently support the earlier findings that *CYP2C19*2* rather than *PON1* Q192R polymorphism was associated with decreased platelet response to clopidogrel (Lewis et al. 2011; Fontana et al. 2011). However, all these studies were performed in white or black patients. The conclusion may not be fully generalized or extrapolated to the Chinese people due to the marked ethnic variability in the disposition of and response to some drugs (Xie et al. 2001; Xie 2010). Several studies have shown that ethnicity is one of the important covariates that could affect clopidogrel metabolism and response (Xie et al. 2011; Li et al. 2009). Moreover, there is a lower frequency of the reduced-function 192Q allele in the *PON1* Q192R polymorphism in Chinese subjects (36%) (Ko et al. 1998) than in white and black subjects (58%–73%) (Lewis et al. 2011; Fontana et al. 2011; Aynacioglu et al. 1999; Cataño et al. 2006). However, their associations with clopidogrel responsiveness have not yet been studied in Chinese stroke patients. To this end, the present study was performed in 170 eligible Chinese stroke patients receiving clopidogrel therapy and analyzed the impact of *PON1* polymorphism on the ADP-induced maximum platelet aggregation (MPA) and clopidogrel anti-platelet effect.

2. Investigations and results

2.1. PON1 Q192R genotyping

In the cohort of 183 eligible stroke patients, a sub-cohort of 13 patients with CYP2C19 *2/*2 genotype was excluded. Finally 170 patients with CYP2C19*1/*1 (wild-type homozygotes, n = 87) or CYP2C19*1/*2 (mutant heterozygotes, n = 83) were enrolled in the study population. The results of *PON1* Q192R (rs662) genotyping are shown in Fig. 1. These stroke patients were divided into 3 groups according to their *PON1*

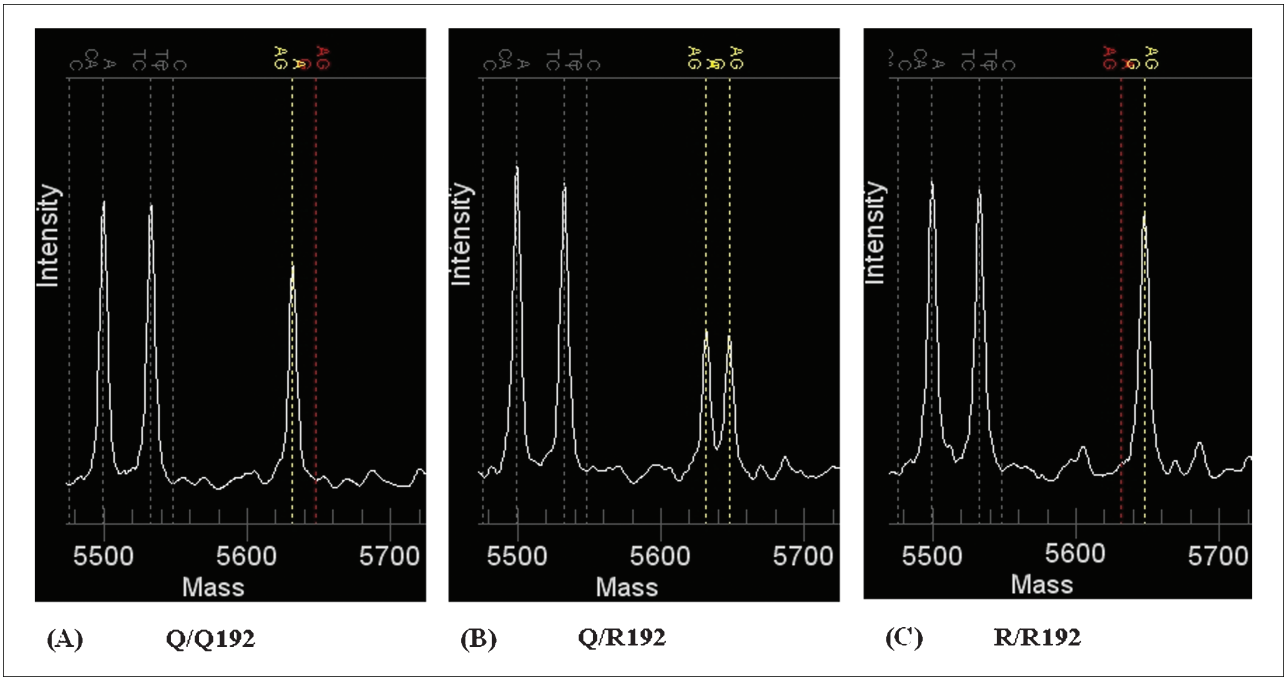

Fig. 1: Typical mass spectrograms for genotyping of PON1 Q/Q192 (A), Q/R192 (B) and R/R192 (C)

Q192R genotype: wild-type homozygotes, *PON1* 192QQ, n = 17; mutant heterozygotes, *PON1* 192QR, n = 81; mutant homozygotes, *PON1* 192RR, n = 72 (Table 1). The baseline characteristics of the stroke patients were well balanced between the three groups of genotypes of *PON1* Q192R. Moreover, the genotype distributions *PON1* were not deviated from the Hardy-Weinberg equilibrium (P = 0.40), the frequency of the Q allele of *PON1* Q192R was 33.8%.

2.2. Genotypes and MPA

The median value of ADP-induced MPA in the study population was 27.6% (IQR, 14.9 to 47.6%). ADP-induced MPA was not significantly different between the 3 genotype groups (P = 0.772) (Table 1). As demonstrated in Fig. 2, in the 81 patients who were carriers of 1 mutant allele (PON1 Q/R192), ADP-induced MPA were not significantly different compared with wild-type homozygous (n = 17) patients [30.5% (IQR, 17.5 to 49.1%) versus 25.0% (IQR, 10.0 to 52.5%), respectively; P = 0.910]. In addition, In the 72 patients who were carriers of the 2 mutant allele (PON1 R/R192), MPA were not significantly different compared with wild-type homozygous (n = 17) patients [29.2% (IQR, 15.0 to 43.4%) versus 25.0% (IQR, 10.0 to 52.5%), respectively; P = 0.717]. Results of a multivariable linear regression model demonstrated that PON1 192R allele carriage was not independently associated with ADP-induced MPA measurements (partial $R^2 = 0.052$, P = 0.408). (Table 2), suggesting that the *PON1* 192R allele may not lead to increased MPA.

3. Discussion

This study is the first to explore the effects of the genetic polymorphisms of *PON1* Q192R on ADP-induced MPA in clopidogrel-treated Chinese patients with stroke. In terms of the

Table 1: Demographic, baseline clinical and procedural characteristics of the patients undergoing PCI according to *PON1* Q192R genotypes

	PON1 Q/Q192 (n = 17)	PON1 Q/R192 (n = 81)	PON1 R/R192 (n = 72)	p value
Age, yrs	61.4 ± 8.7	63.3 ± 8.5	62.4 ± 8.4	0.622
Male (%)	14(82.4)	56(69.1)	50(69.4)	0.502
BMI, kg/m^2	25.7 ± 2.9	24.6 ± 2.6	24.5 ± 3.0	0.321
Hypertension	15(88.2)	59(72.8)	50(69.4)	0.245
Hyperlipidemia	10(58.8)	47(58.0)	42(58.3)	0.998
Diabetes mellitus	6(35.3)	18(22.2)	19(26.4)	0.523
Current smoking	8(47.1)	18(22.2)	29(40.3)	0.022
Statins	17(100)	78(96.3)	65(90.3)	0.107
ACE inhibitor	9(52.9)	27(33.3)	31(43.1)	0.229
Calcium-channel blocker	9(52.9)	34(42.0)	29(40.3)	0.637
Omeprazole	15(88.2)	61(75.3)	50(69.4)	0.231
Platelet count, × 10^9/L	195.3 ± 92.5	194.9 ± 51.5	209.4 ± 53.6	0.302
HDL-cholesterol, mmol/L	0.96 ± 0.24	0.99 ± 0.27	1.04 ± 0.38	0.528
LDL-cholesterol, mmol/L	2.8 ± 1.0	2.5 ± 0.7	2.7 ± 0.7	0.213
MPA (%)	34.4 ± 29.6	33.7 ± 20.9	32.2 ± 22.3	0.772

Values are n (%) or mean ± SD. PON1 = paraoxonase-1; BMI = body mass index; Statins, including atorvastatin, lovastatin, or simvastatin; ACE = angiotensin-converting enzyme; HDL = high-density lipoprotein; LDL = low-density lipoprotein, MPA = maximum platelet aggregation.

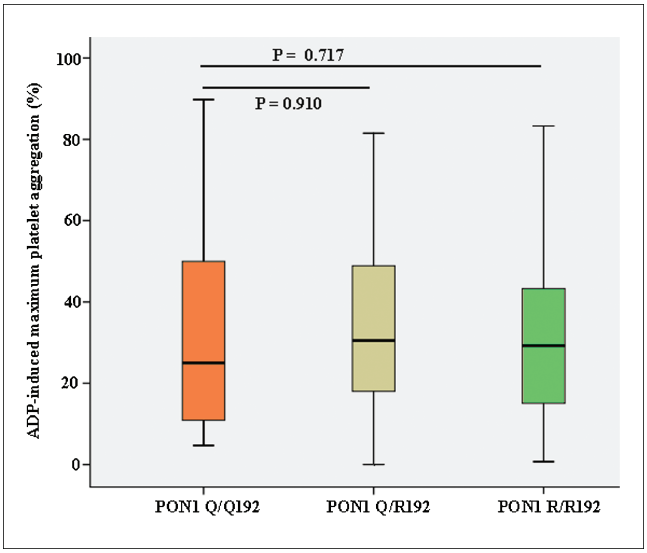

Fig. 2: PON1 Q192R genotypes and MPA. ADP-induced MPA (%) in relation to PON1 Q192R genotypes (Q/Q192, Q/R192, R/R192). Platelet aggregation values were compared across all genotype groups with the minor Kruskal-Wallis test (P = 0.772) and between groups with Mann-Whitney U test. PON1 = paraoxonase-1; MPA = maximum platelet aggregation

Table 2: Results of a multivariable linear regression model

	β Coefficient		
Variable	Value	SE	P
PON1 192R allele carriage	–2.31	1.78	0.408
Age	0.15	0.23	0.523
male	–8.13	4.09	0.069
Body mass index	–0.77	0.67	0.243
Hyperlipidemia	1.63	0.38	0.287
HDL-cholesterol	5.12	4.22	0.405
LDL-cholesterol	0.92	2.53	0.730
Diabetes mellitus	–6.77	4.29	0.110
Hypertension	8.22	4.23	0.064
Use of statin	3.62	2.28	0.645
Use of omeprazole	3.27	2.45	0.296

Results of the multivariable linear regression model are shown for ADP-induced maximum platelet aggregation as the dependent variable

fact that ethnicity of the patients has been identified as an important covariate that could affect the metabolism of and response to clopidogrel (Xie et al. 2011; Li et al. 2009), further investigations of anti-platelet effect of clopidogrel in Chinese patients with different genotype of *PON1* Q192R would be needed to benefit them most. In addition, this study has also confirmed that *PON1* Q192R polymorphism does not have a marked impact on both platelet response to clopidogrel in Chinese stroke patients as reported in the white patients (Lewis et al. 2011; Fontana et al. 2011). PON1 may not be an important metabolizing enzyme responsible for clopidogrel bio-activation and its pharmacological action, because *PON1* locus was not identified to link to clopidogrel anti-platelet effect in a genome-wide association study (Shuldiner et al. 2009). Therefore, it is not surprising that results of us and others (Lewis et al. 2011; Fontana et al. 2011) did not support any impact of a functionally altered *PON1* polymorphism (Q192R) on clopidogrel metabolism and response. These data suggest that routine genotyping of the *PON1* Q192R may not help identify patients who are at higher risk of developing ischemic events when clopidogrel is used as anti-platelet therapy to prevent ischemic events after stroke.

The present study has its limitations that need to be discussed. First, the number of stroke patients was relatively small. Second, the active metabolite of clopidogrel in plasma was not measured due to its chemical instability and difficulty getting it as a chemical standard. Third, only one platelet function method was used in this study, although MPA measured by LTA is widely used to assess the functional status of platelets.

4. Experimental

4.1. Subjects

The present study was conducted in Han Chinese patients with stroke. A total of 183 consecutive stroke patients (aged 18–75 years) were eligible for the inclusion criteria in a single-center, prospective observational cohort in the department of neurology of Nanjing First hospital in China; the inclusion period lasted from July 2010 until July 2011. The exclusion criteria were active bleeding and bleeding diathesis, platelet count $< 100 \times 10^9$/L, severe renal or hepatic disorder, hematologic disorder, active malignancy, body mass index (BMI) < 18.5 or > 40 kg/m^2, use of hormone replacement therapy or contraceptives, and premature clopidogrel or aspirin cessation or nonadherence. The study protocol was approved by the ethics committee of Nanjing First Hospital, Nanjing Medical University. All patients signed their written informed consent prior to participation.

4.2. Study protocol

All patients received a loading dose of 300 mg of clopidogrel (Plavix®, Hangzhou Sanofi-Aventis Minsheng Pharmaceuticals Co. Ltd., Hangzhou, China), All patients received dual anti-platelet therapy (aspirin and clopidogrel). Aspirin (100 mg/day, lifelong), and clopidogrel (75 mg/day, for 12 months) were administered. The predischarge samples were drawn in the morning on day 7 but before intake of the maintenance dose of clopidogrel using tubes containing 3.8% sodium-citrate (NanGeer Biomedical Co., Ltd, SiChuan, China). Blood samples for aggregation testing were processed within 2 h after blood collecting. Information on the patients was obtained from the practicing physicians or derived from hospital readmission records.

4.3. PON1 Q192R genotyping

Genomic DNA was extracted using commercially available QIAamp DNA™ Blood Mini Kit (Qiagen, Venlo, the Netherlands). Primers were obtained from Sangon Biotech (Shanghai, China). Genotyping was performed in Shanghai Benegene Biotechnology Co., Ltd. (Shanghai, China) using the chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF) (Jackson et al. 2000) from MassARRAY Compact System (Sequenom, San Diego, CA, USA). To verify correct sample handling, genotyping was repeated in 20% of the randomly selected patients for all variants tested. Repeated genotyping revealed the identical results, and the call rate for *PON1* Q192R was 100%, respectively.

4.4. MPA assay

MPA was measured by LTA in native platelet-rich plasma (PRP) after stimulation with 20 μmol/L ADP (Sigma-Aldrich, Munich, Germany) using a 4-channel LBY-NJ aggregometer (PuLiSheng, Beijing, China) (Bouman et al. 2010). The PRP was prepared by centrifugation of citrated venous blood at 150 g for 15 min, and platelet-poor plasma (PPP) by centrifugation at 1,500 g for 20 min. PRP was adjusted to 200–250 $\times 10^9$ platelets/L by dilution with autologous PPP. Aggregation results were expressed as percentage of maximal light transmission using PPP from the same patient as reference (100% transmission). The coefficient of variation of our optical aggregometry assay was less than 10%. MPA was measured by the same laboratory staff unaware of patient's outcomes and genotyping results.

4.5. Statistical analysis

Data are expressed as mean ± SD or number (percentage). Categorical values or possible deviations of the genotype distribution from the Hardy-Weinberg equilibrium were analyzed with Chi-square or Fisher's exact test, as appropriate. Continuous variables with a Gaussian distribution were compared by means of the unpaired 2-tailed t test or ANOVA for > 2 groups, whereas continuous variables with a non-Gaussian distribution were compared by Kruskal-Wallis test or Mann-Whitney U test. Correlations between various genotypes and MPA were performed using multivariate linear regression analysis. All statistical analyses were performed with SPSS 16.0 (SPSS Inc, Chicago, III, USA). Statistical significance was accepted at $p < 0.05$.

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