Acta Pharmacologica Sinica (2016) 37: 1442–1448
 1671-4083/16
www.nature.com/aps

Original Article

Associations of *MDR1*, *TBXA2R*, *PLA2G7*, and *PEAR1* genetic polymorphisms with the platelet activity in Chinese ischemic stroke patients receiving aspirin therapy

Ling-ling PENG[1, 3, #], Yuan-qi ZHAO[2, #], Zi-yi ZHOU[2], Jing JIN[1], Min ZHAO[2], Xin-meng CHEN[1], Ling-yan CHEN[1], Ye-feng CAI[2, *], Jia-li LI[1, *], Min HUANG[1, *]

[1]*Institute of Clinical Pharmacology, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, China;* [2]*Department of Neurology, Guangdong Provincial Hospital of Chinese Medicine, Guangzhou 510120, China;* [3]*Department of Pharmacy, General Hospital of Guangzhou Military Command of PLA, Guangzhou 510110, China*

Aim: Aspirin resistance has an incidence of 5%–65% in patients with ischemic stroke, who receive the standard dose of aspirin, but the platelet function is inadequately inhibited, thereby leading to thrombotic events. Numerous evidence shows that thromboxane A_2 receptor (TXA_2 receptor, encoded by *TBXA2R*), lipoprotein-associated phospholipase A_2 (Lp-PLA_2, encoded by *PLA2G7*) and platelet endothelial aggregation receptor-1 (*PEAR1*, encoded by *PEAR1*) are crucial in regulating platelet activation, and P-glycoprotein (P-gp, encoded by *MDR1*) influences the absorption of aspirin in the intestine. In this study we examined the correlation between *MDR1*, *TBXA2R*, *PLA2G7*, *PEAR1* genetic polymorphisms and platelet activity in Chinese ischemic stroke patients receiving aspirin therapy.
Methods: A total of 283 ischemic stroke patients receiving 100 mg aspirin for 7 d were genotyped for polymorphisms in *MDR1* C3435T, *TBXA2R* (rs1131882), *PLA2G7* (rs1051931, rs7756935), and *PEAR1* (rs12566888, rs12041331). The platelet aggregation response was measured using an automatic platelet aggregation analyzer and a commercially available TXB_2 ELISA kit.
Results: Thirty-three patients (11.66%) were insensitive to aspirin treatment. *MDR1* 3435TT genotype carriers, whose arachidonic acid (AA) or adenosine diphosphate (ADP)-induced platelet aggregation was lower than that of CC+CT genotype carriers, were less likely to suffer from aspirin resistance (odds ratio=0.421, 95% CI: 0.233–0.759). The *TBXA2R* rs1131882 CC genotype, which was found more frequently in the aspirin-insensitive group (81.8% *vs* 62.4%) than in the sensitive group, was identified as a risk factor for aspirin resistance (odds ratio=2.712, 95% CI: 1.080–6.810) with a higher level of AA-induced platelet aggregation. Due to the combined effects of *PLA2G7* rs1051931 and rs7756935, carriers of the AA-CC haplotype had a higher level of ADP-induced platelet aggregation, and were at considerably higher risk of aspirin resistance than noncarriers (odds ratio=8.233, 95% CI: 1.590–42.638).
Conclusion: A considerable portion (11.66%) of Chinese ischemic stroke patients are insensitive to aspirin treatment, which may be correlated with the *MDR1* C3435T, *TBXA2R* (rs1131882), and *PLA2G7* (rs1051931–rs7756935) polymorphisms.

Keywords: ischemic stroke; aspirin resistance; platelet activity; *MDR1*; *TBXA2R*; *PLA2G7*; *PEAR1*; genetic polymorphisms

Acta Pharmacologica Sinica (2016) 37: 1442–1448; doi: 10.1038/aps.2016.90; published online 19 Sep 2016

Introduction

Ischemic stroke is the rapid loss of brain functions due to a disturbance in the blood supply to the brain. It is a leading cause of disability and the second most common cause of death in adults around the world[1]. In China, with a population of 1.4 billion, the annual stroke death toll is approximately 1.6 million; thus, stroke has exceeded heart disease in becoming the major cause of death[2]. Aspirin, as a golden standard of antiplatelet therapy, is widely prescribed to treat ischemic stroke patients. However, recent studies have revealed that in certain cases, the platelet function was inadequately inhibited, thereby leading to thrombotic events despite therapy with the standard dose of aspirin. This phenomenon was called aspirin resistance and has an incidence of 5%–65% in patients with ischemic stroke[3]. Today, the underlying mechanism of aspirin resistance is still largely unknown, but it has been argued that genetic factors may be an important factor[4]. A number of studies have focused on the correlation between aspirin resis-

# These authors contributed equally to this work.
*To whom correspondence should be addressed.
E-mail huangmin@mail.sysu.edu.cn (Min HUANG);
lijiali5@mail.sysu.edu.cn (Jia-li LI);
caiyefeng@126.com (Ye-feng CAI)
Received 2016-03-26 Accepted 2016-06-22

tance and genetic polymorphisms in cyclooxygenase (COX) but have yielded conflicting results[5, 6]. There may be other genetic factors accounting for the inter-individual differences in aspirin response.

The principal mechanism of the antithrombotic effect of aspirin is the irreversible inhibition of COX-1, which catalyzes the conversion of arachidonic acid (ARA) to prostaglandins G_2 and H_2, thus preventing the production of thromboxane A_2 (TXA_2). The binding of TXA_2 to its specific receptor (TXA_2 receptor, encoded by *TBXA2R*) could induce platelet activation and aggregation[7]. When the COX-dependent pathway is strongly inhibited by aspirin, maximal aggregation will then require the platelet activators in the COX-independent pathway. The platelet plasma membrane contains multiple activators that are responsible for activating the platelet physiological response. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2, encoded by *PLA2G7*) is synthesized in macrophages and transported into the circulation by HDL (high-density lipoprotein) and LDL (low-density lipoprotein) cholesterol particles[8]. Lp-PLA_2 is a strong platelet activator that has been reported to be a predictive factor for stroke and transient ischemic attack (TIA), and it might be associated with CHD (coronary heart disease) risk[9]. Platelet endothelial aggregation receptor-1 (PEAR1) is an epidermal growth factor repeat-containing transmembrane receptor that participates in platelet contact-induced activation[10]. *PEAR1* genetic polymorphisms have been associated with greater platelet aggregability in platelets functioning under native conditions. Therefore, genetic polymorphisms of *TBXA2R, PLA2G7,* and *PEAR1* may alter the aspirin response by affecting platelet function.

In its pharmacokinetic pathway, aspirin is quickly transformed into the inactive metabolite salicylic acid by carboxylesterase and is partly excreted by P-glycoprotein (P-gp, encoded by *MDR1*) in the gastric mucous membrane, plasma and red blood cells. Kugai *et al* reported that P-gp is involved in the pathogenesis of aspirin-induced intestinal epithelial injury[11]. It has been speculated that *MDR1* genetic polymorphisms may also contribute to inter-individual differences in aspirin response by influencing the absorption of aspirin.

Hence, the present study was embarked upon with the aim to study the association of *TBXA2R*, *PLA2G7*, *PEAR1,* and *MDR1* genetic polymorphisms with aspirin response and platelet activity in Chinese ischemic stroke patients. Six single-nucleotide polymorphisms (SNPs), which are commonly found in Asians (with a minor allele frequency higher than 0.1) and have been associated with the expression or function of a gene or protein, were chosen from candidate genes. To assess platelet reactivity during aspirin therapy, the ADP/AA-induced optical platelet aggregation method was used, and the synthesis of platelet TXB_2, a chemically stable and inactive product of TXA_2 hydrolysis, was assessed.

Materials and methods

Patients

In total, 283 ischemic stroke patients were enrolled from the Guangdong Provincial Hospital of Chinese Medicine from September 2012 to April 2014. Ischemic stroke was defined as a focal neurological deficit persisting for more than 24 h with evidence of cerebral infarction on neuroimaging. All patients who were ≥18 years old and who had taken 100 mg of aspirin (Bayer Healthcare Company Ltd, Beijing, China) for the previous 7 d were eligible for enrollment. Neurological severity was evaluated using the National Institutes of Health Stroke Scale (NIHSS). Exclusion criteria included current or past history neoplasm, bleeding disorders, abnormal renal function (creatinine >2.5 mg/dL), platelet count of <150 000/μL or >450 000/μL, and ingestion of clopidogrel, ticlopidine, dipyridamole, other nonsteroidal anti-inflammatory drugs, platelet glycoprotein IIb/IIIa (GPIIb/IIIa) inhibitors or fibrinolytics within the 30 d before the test. All participants submitted informed written consent before enrollment. The demographic data and relevant characteristics, such as age, gender, medical problems and lipid profile, of the patients were obtained from their medical records.

Optical platelet aggregation determination

Blood samples were drawn after the administration of the last dose of aspirin. Two tubes of whole blood, anticoagulated with 3.8% sodium citrate (4.5 mL each) were collected from each patient for platelet analysis. Turbidimetric platelet aggregation was performed in platelet-rich plasma with a platelet count adjusted to $250\times10^3/mm^3$. Platelets were stimulated with 0.5 mg/mL (1.6 mmol/L) arachidonic acid (AA) and 5 and 20 μmol/L adenosine diphosphate (ADP). Aggregation was performed with a LBY-NJ4A automatic platelet aggregation analyzer (Precil Inc, Beijing, China). The extent of aggregation was defined as the maximal amount of light transmission within 6 min of the addition of the agonist, with platelet-poor plasma used as a reference. Aspirin resistance was defined as a mean aggregation of ≥70% with 10 μmol/L ADP and a mean aggregation of ≥20% with 0.5 mg/mL AA. Aspirin semi-resistance was defined as meeting one but not both of the above criteria[12]. In general, the analysis in this study combined patients with aspirin resistance and those with aspirin semi-resistance into an aspirin-insensitive group.

Serum TXB_2 (s-TXB_2) concentration quantitation

TXB_2 concentrations were determined in serum samples using a commercially available ELISA kit (Thromboxane B_2 EIA Kit; Cayman Chemical, San Antonio, TX, USA) following the manufacturer's instructions, and all serum samples were assayed in duplicate. The mean inter- and intra-assay coefficient of variation (CV, %) of the serum TXB_2 (s-TXB_2) concentration quantitation were 4.952% (1.758%–8.102%) and 6.729% (3.391%–13.89%), respectively.

Individual SNP genotyping

Total genomic DNA was extracted from the peripheral leukocytes according to a previously described method[13]. The six SNPs selected were *TBXA2R* (rs1131882), *PLA2G7* (rs1051931, rs7756935), *PEAR1* (rs12566888, rs12041331), and *MDR1* C3435T. Genotyping was performed using an Agena Biosci-

ence MassARRAY® system (Agena Bioscience, San Diego, CA, USA). The PCR and extension primers and MassARRAY genetic analysis spectrogram for each SNP are provided in the Supplementary data.

Statistical analysis

Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) statistical software (version 21.0). The recorded clinical data, when normally distributed in the analyzed group of patients, are presented as the mean and standard deviation (SD), and non-normally distributed data are presented as the median and interquartile range. The chi-squared test was used to compare the observed allele and genotype frequencies with the Hardy-Weinberg equilibrium prediction. The linkage disequilibrium (LD) was measured by an online calculator (http://www.oege.org/software/cubex/). Comparisons between continuous variables were performed using unpaired Student's *t*-tests because these variables were normally distributed, as determined by the Shapiro-Wilk test. Comparisons of genetic polymorphisms in the aspirin-sensitive and aspirin-insensitive groups were performed using the chi-squared or Fisher's exact tests, as appropriate, and are described with the odds ratio with the 95% confidence interval (CI). To account for multiple testing, the Benjamini-Hochberg false discovery rate (FDR) correction was applied. All probability values are 2-sided, and a P value <0.05 was considered statistically significant. The statistical power of the sample size was calculated using PASS (Power Analysis and Sample Size) software (version 11.0.7; PASS, NCSS, LLC).

Results

Characteristics of the patients

The demographic and clinical characteristics of the participants are summarized in Table 1. The platelet aggregation results indicate that among the 283 stroke patients, 33 patients (11.66%) were aspirin-insensitive patients and 250 patients (88.34%) were aspirin-sensitive patients. Gender, hypertension, smoking, diabetes, coronary artery disease, TC (total cholesterol), TG (triglyceride), HDL, LDL and the platelet count were not related to the aspirin response (P>0.05).

Table 1. The general characteristics and laboratory parameters of aspirin sensitive and insensitive patients.

	Aspirin sensitive (n=250)	Aspirin insensitive (n=33)	P-value
Age, years	65.14±11.78	65.13±13.07	0.997
Sex			
Male, *n* (%)	158 (63.2)	21 (63.6)	0.961
Female, *n* (%)	92 (36.8)	12 (36.4)	
Smoker, *n* (%)	98 (0.39)	5 (0.28)	0.347
Hypertension, *n* (%)	160 (64.0)	19 (57.6)	0.541
Coronary artery disease, *n* (%)	24 (13.7)	0 (0)	0.226
Diabetes, *n* (%)	42 (16.8)	4 (12.1)	0.143
Total cholesterol, mmol/L	4.77±1.07	4.47±1.09	0.143
Triglyceride, mmol/L	1.71±1.20	1.31±0.84	0.249
HDL cholesterol, mmol/L	1.19±0.38	1.19±0.26	0.971
LDL cholesterol, mmol/L	2.98±0.97	3.01±0.90	0.934
Platelet count, $\times 10^3/mm^3$	223±52.40	232.31±69.04	0.552

Allelic and genotype frequencies

Table 2 lists the allele and genotype frequencies of the variants examined in the study population. All genotypes of the examined SNPs were in Hardy-Weinberg equilibrium (P>0.05). *PLA2G7* rs1051931 was in perfect linkage disequilibrium with rs7756935 (r^2=1). *PEAR1* rs12041331 was in tight linkage disequilibrium with rs12566888 (r^2=0.83).

Associations of genetic polymorphisms with platelet aggregation and TXB_2 synthesis

As shown in Table 3–5, *MDR1* 3435 TT genotype carriers had significantly less AA/ADP-induced platelet aggregation than did 3435 CT/CC genotype carriers (P=0.002 and P<0.001, respectively), and similarly, they tended to have a lower s-TXB_2 concentration although the observed difference did not remain when the FDR correction was applied (adjusted P>0.05). For *TBXA2R* rs1131882, the AA-induced platelet aggregation in individuals with the rs1131882 CC genotype was higher than that in carriers of the other genotypes (P=0.014), and the ADP-induced aggregation and s-TXB_2 concentration tended to be higher in the rs1131882 CC genotype group; however, the differences were not statistically significant after adjustment. With the combined effects of *PLA2G7* rs1051931 and rs7756935, carriers of the AA-CC haplotype had considerably more ADP-induced platelet aggregation than did noncarriers (P=0.022), however, no association was observed between the *PLA2G7* genetic polymorphisms and AA-induced platelet aggregation/s-TXB_2 concentration. Neither the s-TXB_2 concentration nor the AA/ADP-induced platelet aggregation correlated with the *PEAR1* genetic polymorphisms (rs12041331 and rs12566888).

Associations of genetic polymorphisms with aspirin resistance

The genotypic distribution of polymorphisms of the candidate genes in aspirin-sensitive and aspirin-insensitive patients are listed in Table 6. Significant differences were observed in the following SNPs: *MDR1* C3435T, *TBXA2R* (rs1131882), and *PLA2G7* (rs1051931-rs7756935) (P=0.021, P=0.028 and P= 0.023, respectively). For *MDR1* C3435T, the proportion of TT genotype carriers was significantly lower in the aspirin-insensitive group than that in the sensitive group (6.1% *vs* 23.6%), and the risk of aspirin resistance was significantly lower in patients with the *MDR1* 3435 TT genotype than in the CT+CC genotype carriers (odds ratio=0.421, 95% CI: 0.233–0.759). The proportion of *TBXA2R* rs1131882 CC genotype carriers was significantly higher in the aspirin-insensitive group than that in the sensitive group (81.8% *vs* 62.4%), and the carriers of the CC genotype had a significantly higher risk of aspirin resistance compared with the risk of the T allele carriers (odds ratio=2.712, 95% CI: 1.080–6.810). Moreover, the proportion of *PLA2G7* rs1051931 AA-rs7756935 CC carriers was signifi-

Table 2. Alleles and genotypes frequencies for 6 SNPs in the investigated patients.

Gene name (SNP rs#)	Allele frequency	Genotypes frequency	HWE *P* value
MDR1 (rs1045642)	C (0.59); T (0.41)	CC (0.40); CT (0.38); TT (0.22)	0.83
TBXA2R (rs1131882)	C (0.63); T (0.37)	CC (0.35); CT (0.51); TT (0.14)	0.54
PLA2G7 (rs1051931)	G (0.87); A (0.13)	AA (0.02); AG (0.21); GG (0.77)	0.16
PLA2G7 (rs7756935)	A (0.87); C (0.13)	CC (0.02); AC (0.21); AA (0.77)	0.16
PEAR1 (rs12041331)	G (0.54); A (0.46)	GG (0.29); GA (0.47); AA (0.24)	0.87
PEAR1 (rs12566888)	G (0.51); T (0. 49)	GG (0.26); GT (0.48); TT (0.26)	0.76

Table 3. Associations of *MDR1*, *TBXA2R*, *PLA2G7*, and *PEAR1* polymorphisms with the serum TXB_2 concentration in ischemic stroke patients.

SNP	TXB_2 concentration (pg/mL)	*P*-value
MDR1 (rs1045642)		
TT	18.44±22.54	0.015[a]
TC+CC	138.50±217.86	
TBXA2R (rs1131882)		
CC	234.59±449.53	0.037[a]
CT+TT	73.77±138.77	
PLA2G7 (rs1051931–rs7756935)		
AA-CC carriers	68.91±67.05	0.750
AA-CC noncarriers	122.71±290.30	
PEAR1 (rs12041331–rs1256888)		
AA-GG carriers	54.60±88.31	0.223
AG-GG noncarriers	87.29±216.19	

[a] After Benjamini-Hochberg false discovery rate correction, *P*>0.05.

Table 4. Associations of *MDR1*, *TBXA2R*, *PLA2G7*, and *PEAR1* polymorphisms with the maximal ADP-induced aggregation in ischemic stroke patients.

SNP	ADP-induced aggregation	*P*-value
MDR1 (rs1045642)		
TT	33.60±16.61	<0.001
TC+CC	44.73±15.96	
TBXA2R (rs1131882)		
CC	45.77±12.88	0.048[a]
CT+TT	40.34±14.33	
PLA2G7(rs1051931-rs7756935)		
AA-CC carriers	57.68±13.83	0.022
AA-CC noncarriers	43.33±15.10	
PEAR1 (rs12041331-rs1256888)		
AA-GG carriers	49.04±17.05	0.377
AG-GG noncarriers	42.79±14.80	

[a] After Benjamini-Hochberg false discovery rate correction, *P*>0.05.

Table 5. Associations of *MDR1*, *TBXA2R*, *PLA2G7*, and *PEAR1* polymorphisms with the maximal AA-induced aggregation in ischemic stroke patients.

SNP	AA-induced aggregation	*P*-value
MDR1 (rs1045642)		
TT	6.64±0.62	0.002
TC+CC	16.41±20.19	
TBXA2R (rs1131882)		
CC	17.46±15.85	0.014
CT+TT	8.97±6.93	
PLA2G7 (rs1051931–rs7756935)		
AA-CC carriers	20.54±11.94	0.182
AA-CC noncarriers	12.02±15.40	
PEAR1 (rs12041331–rs1256888)		
AA-GG carriers	13.35±16.57	0.391
AG-GG noncarriers	11.18±15.32	

cantly higher in the aspirin-insensitive group than in the sensitive group (9.1% *vs* 1.2%), and the risk of aspirin resistance in rs1051931 AA-rs7756935 CC genotypes carriers was considerably higher than the risk in the carriers of the other genotypes (odds ratio=8.233, 95% CI: 1.590–42.638).

However, neither of the two SNPs (rs12566888, rs12041331) of *PEAR1* was correlated with the aspirin response (*P*>0.05).

Discussion

The role of aspirin in preventing ischemic stroke has been well documented[14, 15]. However, some patients are not responsive to aspirin and can thus still suffer from ischemic stroke and cardiovascular events. The definition of aspirin resistance is debated; hence, the reported incidence rate varies broadly from 5% to 65% depending on the assay used for identification and the population studied[16, 17]. In this study, the prevalence of aspirin insensitivity was 11.66%, which was evaluated by the classical gold standard, optical platelet aggregation. In addition, the level of platelet TXB_2 synthesis was assessed, which is an alternative method of evaluating platelet reactivity and aspirin response[18].

The thromboxane A_2 produced by aggregating platelets is

Table 6. Associations of MDR1, TBXA2R, PLA2G7 and PEAR1 polymorphisms with aspirin resistance in ischemic stroke patients.

SNP *n* (%)	Aspirin insensitive (*n*=33)	Aspirin sensitive (*n*=250)	*P*-value	OR	95% CI
MDR1 (rs1045642)					
TT	2 (6.1)	59 (23.6)	0.021	0.421	0.233–0.759
TC+CC	31 (93.9)	191 (76.4)			
TBXA2R (rs1131882)					
CC	27 (81.8)	156 (62.4)	0.028	2.712	1.080–6.810
CT+TT	6 (18.2)	94 (37.6)			
PLA2G7 (rs1051931–rs7756935)					
AA-CC carriers	3 (9.1)	3 (1.2)	0.023	8.233	1.590–42.638
AA-CC noncarriers	30 (90.9)	247 (98.8)			
PEAR1 (rs12041331–rs1256888)					
AA-GG carriers	6 (18.2)	63 (25.2)	0.378	0.660	0.260–1.671
AG-GG noncarriers	27 (81.8)	187 (74.8)			

a potent platelet activator and vasoconstrictor, and its action is mediated by the thromboxane A_2 receptor (TBXA2R). The association between *TBXA2R* polymorphism and platelet activity was analyzed previously in healthy volunteers; the analysis indicated that the *TBXA2R* rs1131882C allele might be correlated with higher platelet activity[19]. Consistent with this finding, the present study found that the rs1131882 CC genotype was associated with higher platelet aggregation in response to ADP/AA and was correlated with an increased level of platelet TXB_2 synthesis. In addition, the rs1131882 CC genotype was a risk factor for aspirin resistance in Chinese ischemic stroke patients. The results are the first to show the association of the *TBXA2R* rs1131882 with platelet reactivity during aspirin therapy in subjects with ischemic stroke. *TBXA2R* rs1131882 is located in the common coding portion of isoforms alpha and beta, and it represents a synonymous substitution. As such, *TBXA2R* rs1131882 may affect the transcription and/or translation efficiency of both isoforms of the *TBXA2R* gene by being in linkage disequilibrium with one or several SNPs in the promoter region of the gene in the intronic silencer region or in the enhancer region. Further study is required to assess our speculations and reveal the underlying mechanisms.

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is related to lipoprotein metabolism and inflammatory pathways, and it is a potent mediator of hypersensitivity and inflammatory reactions. Lipoprotein-associated phospholipase A_2 is a platelet activator that has been reported to be a predictive factor for stroke and TIA[9, 20]. Lp-PLA_2 is encoded by the *PLA2G7* gene, which is located on chromosome 6q21-p12. One non-synonymous polymorphism, A379F (rs1051931) in exon 11 of *PLA2G7*, has been associated with Lp-PLA_2 enzymatic activity, and another non-synonymous SNP (rs7756935), which was in perfect linkage disequilibrium with rs1051931, was shown to be associated with Lp-PLA_2 activity. In the present study, we found that the rs1051931A-rs7756935C haplotype was associated with higher platelet aggregation in response to ADP and was correlated with an increased risk of aspirin resistance in Chinese ischemic stroke patients. The current results were supported by the findings of Kruse *et al,* which indicated a decrease in the affinity of 379Valine (rs1051931AA genotype) in Lp-PLA_2 recombinant protein for its substrate (platelet-activating factor, PAF), resulting in two-fold lower Lp-PLA_2 activity *in vitro*[21]. In addition, Grallert *et al* demonstrated that rs7756935 was associated with Lp-PLA_2 activity or mass[22], and a subsequent study reported that the rs7756935C allele was associated with the risk of coronary heart disease (CHD)[23]. To date, this study is the first to report an association between aspirin resistance and *PLA2G7* polymorphisms in Chinese ischemic stroke patients.

Platelet endothelial aggregation receptor-1 (PEAR1) is a recently identified platelet transmembrane protein that is activated by platelet contact. During platelet aggregation by various agonists, the membrane expression of PEAR1 and its tyrosine phosphorylation (phosphorylated at Tyr-925 and Ser-953/1029) increase. The polymorphisms of *PEAR1*, which contribute to altered platelet function, are deemed an important factor in aspirin resistance[24]. A recent GWAS study identified that a variant in intron 1 of the *PEAR1* gene (rs12566888) was associated with ADP and epinephrine-induced aggregation, and the rs12566888T allele was associated with a decrease in aggregation response[25]. Another common variant (rs12041331) in intron 1 was in tight linkage disequilibrium with rs12566888, and it has been reported that the G allele was associated with greater platelet aggregation in the presence and absence of aspirin treatment in African Americans. In addition, the researchers found that the PEAR1 protein expression was greatest for the GG homozygote, intermediate for the GA heterozygote and least for the AA homozygote[26]. However, in our study, no association was observed between plate-

let activity during aspirin therapy and rs12566888/rs12041331. It was speculated that the allele frequencies of *PEAR1* SNPs might have significant inter-ethnic differences, and a possible limitation in this study is the limited sample size. Therefore, further studies are required to clarify the precise mechanism involved in our findings.

In a recent analysis, *MDR1* C3435T was reported to be associated with clinical outcome of aspirin treatment evaluated by the modified Rankin Scale score in ischemic stroke patients. The authors speculated that *MDR1* C3435T might be involved in the mechanism responsible for aspirin resistance[27]. Our study evaluated a different ischemic stroke population from China using both the optical platelet aggregation method and assessment of platelet TXB_2 synthesis. We found that the homozygous mutant (TT genotype) was associated with lower platelet aggregation in response to ADP/AA and was correlated with a decreased level of platelet TXB_2 synthesis. Our data suggest that the 3435 TT genotype of *MDR1* is a protective factor against aspirin resistance in Chinese ischemic stroke patients. The current results were supported by the functional findings of Wang *et al*, who showed that the 3435TT genotype resulted in lower expression and function of P-gp[28], thus decreasing the efflux and increasing the bioavailability of aspirin.

In summary, we have shown that *MDR1* C3435T, *TBXA2R* (rs1131882) and *PLA2G7* (rs1051931–rs7756935) may be associated with platelet activity during aspirin therapy. The *MDR1* 3435 TT genotype is a protective factor, while the *TBXA2R* rs1131882 CC, *PLA2G7* rs1051931 AA-rs7756935 CC genotypes are risk factors for aspirin resistance. To date, our study is the first to report the associations of *MDR1*, *TBXA2R,* and *PLA2G7* polymorphisms with platelet reactivity in Chinese ischemic stroke patients receiving aspirin therapy. These results may be helpful for aspirin treatment in ischemic stroke. However, due to the relatively small sample size, a further study with a larger sample size is needed to provide sufficient power for drawing firm conclusion about our findings.

Acknowledgements

We appreciate the financial support provided by a National Science and Technology Major Project in Science and Technology grant from the Science and Technology Ministry of China (Grant No 2012ZX09506001-004), the Key Laboratory Foundation of Guangdong Province (Grant No 2011A060901014), the Major Scientific and Technological Project of Guangdong Province (Grants 2011A080300001 and 2012A080202013), a China Postdoctoral Science Foundation-funded project (No 2013M530858) and a project supported by the Guangdong Provincial Department of Science and Technology joint special fund (No 2012A032500016).

Author contribution

Ling-ling PENG, Jia-li LI, Jing JIN, Min HUANG, and Ye-feng CAI designed the research; Ling-ling PENG, Yuan-qi ZHAO, Zi-yi ZHOU, Min ZHAO, Xin-meng CHEN and Lin-yan CHEN performed the research; Ling-ling PENG and Jia-li LI analyzed the data; Ling-ling PENG and Jia-li LI wrote the paper.

Supplementary information

Supplementary information is available at the website of Acta Pharmacologica Sinica.

References

1 Feigin VL. Stroke epidemiology in the developing world. Lancet 2005; 365: 2160–1.
2 Liu L, Wang D, Wong KS, Wang Y. Stroke and stroke care in China: huge burden, significant workload, and a national priority. Stroke 2011; 42: 3651–4.
3 Ozben S, Ozben B, Tanrikulu AM, Ozer F, Ozben T. Aspirin resistance in patients with acute ischemic stroke. J Neurol 2011; 258: 1979–86.
4 Faraday N, Becker DM, Becker LC. Pharmacogenomics of platelet responsiveness to aspirin. Pharmacogenomics 2007; 8: 1413–25.
5 Arisawa T, Tahara T, Shibata T, Nagasaka M, Nakamura M, Kamiya Y, *et al*. Genetic polymorphisms of cyclooxygenase-1 (COX-1) are associated with functional dyspepsia in Japanese women. J Womens Health (Larchmt) 2008; 17: 1039–43.
6 Cipollone F. COX-2 and prostaglandins in atherosclerosis. Lupus 2005; 14: 756–9.
7 Zhao J, Zheng L, Fei Q, Fu Y, Weng Y, Wu H, *et al*. Association of thromboxane A_2 receptor gene polymorphisms with cerebral infarction in a Chinese population. Neurol Sci 2013; 34: 1791–6.
8 Karabina SA, Ninio E. Plasma PAF-acetylhydrolase: an unfulfilled promise? Biochim Biophys Acta 2006; 1761: 1351–8.
9 Delgado P, Chacon P, Penalba A, Pelegri D, Garcia-Berrocoso T, Giralt D, *et al*. Lipoprotein-associated phospholipase A_2 activity is associated with large-artery atherosclerotic etiology and recurrent stroke in TIA patients. Cerebrovasc Dis 2012; 33: 150–8.
10 Nanda N, Bao M, Lin H, Clauser K, Komuves L, Quertermous T, *et al*. Platelet endothelial aggregation receptor 1 (PEAR1), a novel epidermal growth factor repeat-containing transmembrane receptor, participates in platelet contact-induced activation. J Biol Chem 2005; 280: 24680–9.
11 Kugai M, Uchiyama K, Tsuji T, Yoriki H, Fukui A, Qin Y, *et al*. MDR1 is related to intestinal epithelial injury induced by acetylsalicylic acid. Cell Physiol Biochem 2013; 32: 942–50.
12 Gum PA, Kottke-Marchant K, Poggio ED, Gurm H, Welsh PA, Brooks L, *et al*. Profile and prevalence of aspirin resistance in patients with cardiovascular disease. Am J Cardiol 2001; 88: 230–5.
13 Loparev VN, Cartas MA, Monken CE, Velpandi A, Srinivasan A. An efficient and simple method of DNA extraction from whole blood and cell lines to identify infectious agents. J Virol Methods 1991; 34: 105–12.
14 Collaborative overview of randomised trials of antiplatelet therapy — I: Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. Antiplatelet Trialists' Collaboration. BMJ 1994; 308: 81–106.
15 Pujol Lereis VA, Ameriso S, Povedano GP, Ameriso SF. Ischemic stroke in patients receiving aspirin. J Stroke Cerebrovasc Dis 2012; 21: 868–72.
16 Algra A, van Gijn J. Aspirin at any dose above 30 mg offers only modest protection after cerebral ischaemia. J Neurol Neurosurg Psychiatry 1996; 60: 197–9.
17 Zheng AS, Churilov L, Colley RE, Goh C, Davis SM, Yan B. Association of aspirin resistance with increased stroke severity and infarct size.

JAMA Neurol 2013; 70: 208–13.

18 Cattaneo M. Laboratory detection of 'aspirin resistance': what test should we use (if any)? Eur Heart J 2007; 28: 1673–5.

19 Fontana P, Gandrille S, Remones V, Dupont A, Reny JL, Aiach M, *et al.* Identification of functional polymorphisms of the thromboxane A_2 receptor gene in healthy volunteers. Thromb Haemost 2006; 96: 356–60.

20 Lp-PLA_2 Studies Collaboration, Thompson A, Gao P, Orfei L, Watson S, Di Angelantonio E, *et al.* Lipoprotein-associated phospholipase A_2 and risk of coronary disease, stroke, and mortality: collaborative analysis of 32 prospective studies. Lancet 2010; 375: 1536–44.

21 Kruse S, Mao XQ, Heinzmann A, Blattmann S, Roberts MH, Braun S, *et al.* The Ile198Thr and Ala379Val variants of plasmatic PAF-acetylhydrolase impair catalytical activities and are associated with atopy and asthma. Am J Hum Genet 2000; 66: 1522–30.

22 Grallert H, Dupuis J, Bis JC, Dehghan A, Barbalic M, Baumert J, *et al.* Eight genetic loci associated with variation in lipoprotein-associated phospholipase A_2 mass and activity and coronary heart disease: meta-analysis of genome-wide association studies from five community-based studies. Eur Heart J 2012; 33: 238–51.

23 Xu LM, Zhou JQ, Huang S, Huang Y, Le YP, Jiang DJ, *et al.* An association study between genetic polymorphisms related to lipoprotein-associated phospholipase A_2 and coronary heart disease. Exp Ther Med 2013; 5: 742–50.

24 Lewis JP, Ryan K, O'Connell JR, Horenstein RB, Damcott CM, Gibson Q, *et al.* Genetic variation in PEAR1 is associated with platelet aggregation and cardiovascular outcomes. Circ Cardiovasc Genet 2013; 6: 184–92.

25 Johnson AD, Yanek LR, Chen MH, Faraday N, Larson MG, Tofler G, *et al.* Genome-wide meta-analyses identifies seven loci associated with platelet aggregation in response to agonists. Nat Genet 2010; 42: 608–13.

26 Faraday N, Yanek LR, Yang XP, Mathias R, Herrera-Galeano JE, Suktitipat B, *et al.* Identification of a specific intronic PEAR1 gene variant associated with greater platelet aggregability and protein expression. Blood 2011; 118: 3367–75.

27 Sharma V, Kaul S, Al-Hazzani A, Prabha TS, Rao PP, Dadheech S, *et al.* Association of C3435T multi drug resistance gene-1 polymorphism with aspirin resistance in ischemic stroke and its subtypes. J Neurol Sci 2012; 315: 72–6.

28 Wang D, Johnson AD, Papp AC, Kroetz DL, Sadee W. Multidrug resistance polypeptide 1 (MDR1, ABCB1) variant 3435C>T affects mRNA stability. Pharmacogenet Genomics 2005; 15: 693–704.