**Association of GPIa and COX-2 gene polymorphism with aspirin resistance**

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**Objective:** This study aimed to explore the association between GPIa, COX-2 gene polymorphisms and aspirin resistance in the ischemic stroke patients from the southern part of Jiangsu province.

**Methods:** In all, 97 patients with acute ischemic stroke were enrolled in the study. GPIa gene polymorphism at 807C>T (rs1126643) locus and COX-2 gene polymorphism at -765G>C (rs20417) locus were genotyped by PCR pyrosequencing technology. Patients were divided into the aspirin sensitivity (AS) group and aspirin resistance (AR) group according to the platelet aggregation rate. The relationship between the two gene polymorphisms and aspirin resistance was investigated and analyzed.

**Results:** The distribution of the genotype (CC, CT, TT, CT + TT, and CC) and the frequency of allele T of GPIa gene at 807C>T locus were significantly different in AS and AR groups in female patients (P <.05). Logistic regression analysis showed that the genotype of CT+TT at 807C>T locus was significantly correlated with AR after adjustment for relative factors (P = .047, OR = 4.856, 95% CI: 1.020–23.108). There were no significant differences in the genotype distribution and allele frequency of the COX-2 gene -765G>C site between two groups (P >.05).

**Conclusion:** GPIa gene polymorphism at 807C>T locus was associated with AR in Chinese Han females, and the expression of allele T increased the incidence of AR. The gene polymorphism of COX-2 gene at -765G>C locus was not significantly correlated with AR.

**KEYWORDS**

aspirin resistance, COX-2, gene polymorphism, GPIa, ischemic stroke

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**1 INTRODUCTION**

Stroke is one of the leading causes of human mortality and disability. Early neurologic exercise and recurrent ischemic stroke (IS) are common and are associated with poor prognosis in acute IS patients. Antiplatelet drugs, statins, and anti-hypertensive drugs are the third cornerstone of stroke prevention. As a routine therapeutic agent, aspirin is prescribed widely for the prophylaxis of cardio-thrombotic events. The effect of aspirin is achieved by suppressing thromboxane production and further by inhibiting platelet activation and aggregation. Substantial number of patients on aspirin therapy fails to reach this desired effect, and then they experience major adverse vascular events, a phenomenon known as aspirin resistance (AR). Since the discovery of this phenomenon, to unravel the mechanisms of aspirin insensitivity so far remains a daunting task. Evidence is mounting suggests that a strong genetic component underlies aspirin insensitivity.
Comparison of clinic characteristics between AS and AR patients

| Groups | AS group | AR group | P value |
|--------|----------|----------|---------|
| Gender (female %) | 20 (37.0%) | 17 (39.5%) | .801* |
| LDL (mmol/L) | 2.73 ± 0.81 | 2.79 ± 1.05 | .770 |
| CRP (mg/L) | 1.95 ± 1.5 | 2.31 (1.33) | .660** |
| PLT (10^9) | 192 (65) | 196 (85) | .885** |

*represents the use of chi-square test.
**represents the use of nonparametric tests.

A meta-analysis showed that the risk of recurrent ischemic cardiovascular events was 3.85 times higher in patients with biochemical AR and sixfold increased risk of vascular death events. The incidence of AR may be related to a variety of factors, such as patient compliance, drug dose, age, hypertension, diabetes, smoking, and genetic factors.

Aspirin acts by inhibiting platelet cyclooxygenase enzymes (COX), preventing generation of thromboxane A2 (TXA2) from arachidonic acid (AA). TXA2 binds to glycoprotein-coupled receptor (GP Ib/IIa) leading to phospholipase C activation and platelet aggregation. Faraday et al. have shown that gene polymorphism affects the response of platelets to aspirin, and the relationship between AR and gene polymorphism is important for understanding its mechanism. A number of studies have recorded the association of AR with single nucleotide polymorphisms (SNPs) in COX and the above-mentioned receptors genes. For example, polymorphisms in COX-1, COX-2, GPIIa, P2Y1, and P2Y12 were reported to contribute to AR.

In this study, the GPIa gene 807C>T site (rs1126643) and COX-2 gene -765G>C site (rs20417) were selected to observe the relationship between single nucleotide polymorphism and AR. It is expected to provide the basis for the individual selection of medication so that stroke patients benefit from the treatment of aspirin.

2 | MATERIALS AND METHODS

2.1 | Study population

This study was approved by the Human Ethics Committees of the PLA Rocket Force General Hospital, and informed consent was obtained from each patient. Approximately, 97 patients with acute ischemic stroke admitted to the PLA Rocket Force General Hospital from March 2015 to April 2016, including 60 males and 37 females, the average age of 68 ± 11 years old, are the southern Han population. After entering the hospital, patients were given daily oral administration of aspirin 100 mg. Exclusion criteria were as follows: (i) acute myocardial infarction, infection, blood system diseases, cancer, severe liver, and kidney dysfunction or autoimmune diseases; (ii) nearly a week to use other antiplatelet drugs or anticoagulant drugs, taking nonsteroidal antiinflammatory drug; (iii) recent trauma or surgery history; and (iv) platelet count <100 × 10^9/L or >450 × 10^9/L.

2.2 | Platelet aggregation test

Venous blood (5 mL) was drawn from each patient’s antecubital veins before and after 7 days of aspirin treatment. Platelet aggregation was measured by PL-11 platelet analyzer (Nanjing British Novartis Medical Technology Co., Ltd., Nanjing, China). The maximal platelet aggregation rate (MAR) was calculated using arachidonic acid (AA) as an inducer, and MAR ≥ 33.3% was defined as biochemical AR. Otherwise, patients were considered aspirin sensitive (AS).

2.3 | Genotyping (Gene single nucleotide polymorphisms detection)

Genomic DNA was isolated from whole blood using the QIAGEN Blood Mini Kit (QIAGEN, Germany) according to the manufacturer’s instructions. A forced-mutation polymerase chain reaction (PCR)-based restriction fragment length polymorphism analysis was developed to detect the GPIa and COX-2 gene polymorphisms. Sequencing of the PCR products was carried out using a pyrophosphoric acid sequencer (QIAGEN PyroMark Q24, Qi Jiagen Enterprise Management Co., Ltd., Shanghai, China), and the results were directly analyzed by the machine.

2.4 | Statistical analysis

SPSS20.0 statistical software was used to analyze the data. The normative test was used to analyze the normality. The normative continuous variables were analyzed by independent sample T-test. The data were expressed as mean ± standard deviation. Non-normative data were analyzed by nonparametric analysis. The results were measured by median and quartile Spacing said. The categorical variables were expressed as a count and a percentage, and the χ2 test was used for comparison between groups. The genotype and allele frequencies of the two groups were compared by χ2 test or Fisher exact test, and multivariate logistic regression was used to correct the related risk factors. Hardy–Weinberg genetic balance was evaluated using χ2 test. The difference was statistically significant with $P < .05$ (bilateral).

3 | RESULTS

3.1 | AS and AR patients between the general data comparison

Pertinent clinical characteristics of the study population are shown in Table 1. Among the 97 patients, AS group has 54 cases (55.7%)
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and AR group has 43 cases (44.3%). There were no significant differences in age, TC, LDL, Lp-PLA2, CRP, and PLT between the two groups (P > .05). There was no significant difference between the two groups in the clinical data of AS and AR patients.

3.2 | Association of GPIa gene 807C>T polymorphism with AR

GPIa gene 807C>T genotype distribution frequency accorded with Hardy–Weinberg genetic balance (P > .05), with population representation. AS group: CC 23 cases (42.6%), CT 26 cases (48.1%), TT 5 cases (9.3%), C 72 (66.7%), and T 36 (33.3%). AR group: CC 12 cases (27.9%), CT 23 cases (53.5%), TT 8 cases (18.6%), C 47 (54.7%), and T 39 (45.3%). There were no significant differences (P > .05) between AS and AR group in 807C>T locus genotype and allele (Table 2).

The relationship between biochemical AR and GPIa gene polymorphism was studied after chi-square analysis by sex. In male patients, there were no significant differences in the distribution of the three genotypes in AS and AR groups (P = .930). In female patients, the distribution of CC, CT, and TT genotypes in the two groups was statistically significant different (P < .05) (Table 3).

There were significant differences between the AS and AR groups in the 807C>T locus dominant model and the recessive model in female patients (P < .05). We further corrected the risk factors of AR (age, total cholesterol, low-density lipoprotein, hypertension, and history of diabetes mellitus) using logistic regression analysis model. The results showed that the genotype of 807C>T CT + TT of GPIa gene was still related to the occurrence of AR (P = .047, OR = 4.856, 95% CI: 1.020–23.108), suggesting that genotype of 807C>T CT + TT of GPIa might be an independent risk factor for AR. The multivariate logistic regression of the invisible model was associated with a risk factor of P = .05. (Table 4)

3.3 | Association of COX-2 gene -765G>C polymorphism with AR

After removing incomplete cases, there were remaining 91 cases. AR group: GG 17 cases (42.5%), GC 12 cases (30.0%), CC11 cases (27.5%), G 46 cases (57.5%), and C 34 cases (42.5%). AS group: GG23 cases (45.1%), GC group 23 cases (45.1%), CC5 cases (9.8%), G 69 cases (67.6%), and C 33 cases (32.4%). There were no significant differences in genotype and allele distribution between AS and AR groups (P > .05). (Table 5)

4 | DISCUSSION

Most of the ischemic strokes are caused by unstable atherosclerotic plaque rupture, leading to thrombosis, and thus the blood vessels of the brain tissue ischemic necrosis. Platelet adhesion, activation is the initiation of thrombosis. The platelet surface GPIa/IIa complex, as the collagen initial receptor, plays an important role in the platelet and damaged vascular wall adhesion and aggregation process. In recent years, it has been found that the two-linked single nucleotide polymorphisms of the C807T (rs126643) and G873A alleles are associated with the density of GPIa/IIa receptors on the platelet membrane surface in the platelet membrane GPIa gene coding sequence. Therefore, it is speculated that the GPIa gene polymorphism of platelet membrane may be a genetic predisposing factor of thrombotic disease. In this study, the results of χ2 stratification test showed that the differences among the three genotypes were statistically significant in female patients. Due to the small number of TT genotypes, in this paper, χ2 test was performed on the distribution of genotype (CT + TT/CC) and recessive model (CC + CT/TT) between the two groups. The results showed that the distribution of the two genotypes was statistically different. The logistic regression model was

| Gender | n | genotype | AS | AR | χ² | P value |
|--------|---|----------|----|----|----|---------|
| Male   | 60| CC       | 9 (26.5%) | 8 (30.8%) | 0.337 | .93 |
|        |   | CT       | 21 (61.8%) | 16 (61.5%) |         |     |
|        |   | TT       | 4 (11.8%) | 2 (7.7%) |         |     |
| Female | 37| CC       | 12 (60.0%) | 4 (23.5%) | 7.377 | .024 |
|        |   | CT       | 7 (35.0%) | 7 (41.2%) |         |     |
|        |   | TT       | 1 (5.0%) | 6 (35.5%) |         |     |

TABLE 2 Distribution of GPIa gene 807C>T polymorphism in AS and AR

| Groups | n | Genotype n (%) | Allele n (%) |
|--------|---|----------------|--------------|
|        |   | CC | CT | TT | C | T |
| AS     | 54| 23 (42.6%) | 26 (48.1%) | 5 (9.3%) | 72 (66.7%) | 36 (33.3%) |
| AR     | 43| 12 (27.9%) | 23 (53.5%) | 8 (18.6%) | 47 (54.7%) | 39 (45.3%) |
| χ²     |    | 3.126 | 2.915 |
| P value | .210 | .088 |

TABLE 3 Distribution of GPIa gene polymorphism by gender in both groups
used to calibrate the age, hyperlipidemia, low-density lipoprotein, hypertension, and diabetes mellitus. Genotype (CT + TT) was still associated with AR, suggesting that it might be an independent risk factor for biochemical AR. The frequency of T allele in AR group was higher than that in AS group, the difference was statistically significant, suggesting that carrying 807T allele in women increased the incidence of biochemical AR. Studies have found that the expression of the 807T allele increased the level of cholesterol from atherosclerosis. In this study, genotype CT + TT/CC grouping, compared between the two groups of total cholesterol, low-density lipoprotein, blood glucose, uric acid, and other related factors, no significant differences were found, and the mechanism need for further study. In this study, female patients were aged between 40 and 86 years old, and only two patients <50 years old, the rest were all menopausal women, these maybe one reason why women are more prone to AR. Indication that women carry 807T allele were more prone to AR. Studies have shown that carrying the 807T allele increased the risk of stroke in young women, suggesting that genetic factors affect cerebrovascular disease. These suggest that some of the reasons why our findings regarding the association of platelet GPIa/IIa gene polymorphisms with AR are partly due to the demographic characteristics of the study population, and that genetic gene polymorphisms affecting young women or menopausal women may be more significant, so stratification of the population is particularly important.

Studies have shown that the expression of COX-2 in atherosclerotic plaques was 10–20 times of the normal tissues. COX-2 can be induced by inflammation, and it can induce the production of prostaglandin H2 through the non-COX-1 pathway. This process cannot be completely inhibited by the conventional dose of aspirin, which in turn produces thromboxane A2, stimulates platelet aggregation. So COX-2 compensatory generation may be an important reason for the occurrence of AR. Studies have shown that the 765 G>C (rs20417) of the promoter region of the COX-2 gene is associated with AR, and the mutant allele may upregulated COX-2 expression by altering the activity of the promoter. The relationship between COX-2 gene-765G>C polymorphism and AR is controversial, and the results are different at home and abroad. The study of Sharma et al included 450 patients with ischemic stroke and treated with aspirin. The patients were followed up, and then divided into two groups with good prognosis and poor prognosis, and the distribution of two genotypes was compared. They found that the CC and GC genotypes were associated with adverse vascular events and that patients carrying the C allele were more likely to develop AR than those without carriers. Cipollone F et al have shown that the incidence of cerebral infarction and myocardial infarction in patients with CC and GC genotypes is relatively low and this may be associated with the reduced risk of ischemic cardiovascular and cerebrovascular disease. In this study, no single nucleotide polymorphisms at -765G>C were found to be associated with AR.

There are some shortcomings and limitations in this study. First, this study is a single-center retrospective study, some important information is difficult to collect completely; thus, the analysis of variables is not comprehensive, such as smoking, drinking, obesity, and other risk factors were not included, and the amount of samples collected is small. Furthermore, aspirin antiplatelet is involved in a variety of enzymes and receptors in the process, each enzyme and receptor gene has multiple loci polymorphism, and gene–gene and gene–environment differences may affect the results. Therefore, it is necessary to carry out a large sample size, multiple gene loci, multi-factor, multi-center study, to further clarify the pathogenesis of aspirin resistance in patients with cerebral infarction, and to provide the basis for clinical individualization for stroke primary and secondary prevention.

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REFERENCES

1. GBD 2013 Mortality and Causes of Death Collaborators. Global, regional, and national age-specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015; 385: 117-171.

2. Yi X, Lin J, Wang C, Zhang B, Chi W. A comparative study of dual versus monoantiplatelet therapy in patients with acute large-artery atherosclerosis Stroke. J Stroke Cerebrovasc Dis. 2014;23:1975-1981.

3. Buchanan MR, Verma S. Biological basis and clinical implications of acetylsalicylic acid resistance. Can J Cardiol. 2006;22:149-151.

4. Grinstein J, Cannon CP. Aspirin resistance: current status and role of tailored therapy. Clin Cardiol. 2012;35:673-681.

5. Kim SH, Sanak M, Park HS. Genetics of hypersensitivity to aspirin and nonsteroidal anti-inflammatory drugs. Immunol Allergy Clin North Am. 2013;33:177-194.

6. Palikhe NS, Kim SH, Park HS. What do we know about the genetics of aspirin intolerance? J Clin Pharm Ther. 2008;33:465-472.

7. Krasopoulos G, Brister SJ, Beattie WS, Buchanan MR. Aspirin resistance and risk of tubular morbidity: systematic review and meta-analysis. BMJ. 2008;336:195-198.

8. Hankey GJ, Eikelboom JW. Aspirin resistance. Lancet. 2006;367: 606-617.

9. Nakahata N. Thromboxane A2: physiology/pathophysiology, cellular signal Transduction and pharmacology. Pharmacol Ther. 2008;118:18-35.

10. Faraday N, Yanek LR, Mathias R, et al. Heritability of platelet responsiveness to aspirin in activation pathways directly and indirectly related to cyclooxygenase-1. Circulation. 2007;115:2490-2496.

11. Goodman T, Sharma P, Ferro A. The genetics of aspirin resistance. Int J Clin Pract. 2007;61:826-834.

12. Sharma V, Kaul S, Al-Hazzani A, Alhawatwi AA, Jyothy A, Munshi A. Association of COX-2 rs20417 with aspirin resistance. J Thromb Thrombolyis. 2013;35:95-99.

13. Halushka MK, Walker LP, Halushka PV. Genetic variation in cyclooxygenase 1: effects on response to aspirin. Clin Pharmacol Ther. 2003;73:122-130.

14. Goodman T, Ferro A, Sharma P. Pharmacogenetics of aspirin resistance: a thoroughly prepared review. Br J Clin Pharmacol. 2008;66:222-232.

15. Li Q, Chen BL, Ozdemir V, et al. Genetic polymorphisms of COX1, GPIIa and P2Y1 in a Chinese population and association with attenuated response to aspirin. Pharmacogenomics. 2007;8:577-586.

16. Grinshtein VI, Kosinova AA, Grinshtein IY. Aspirin resistance candidate genes and their association with the risk of fatal NGOs events. Ter Arkh. 2013;85:95-100.

17. Colaiizzo D, Fofi L, Tistica G, et al. The COX-2 G/C -765 polymorphism may modulate the occurrence of cerebrovascular ischemia. Blood Coagul Fibrinolysis. 2006;17:93-96.

18. Lu JX, Lu ZQ, Zhang SL, Zhi J, Chen ZP, Wang WX. Polymorphism in integrin ITGA2 is associated with ischemic stroke and altered serum cholesterol in Chinese individuals. Balkan Med J. 2014;31:55-59.

19. Reiner AP, Kumar PN, Schwartz SM, et al. Genetic variants of platelet glycoprotein receptors and risk of stroke in young women. Stroke. 2000;31:1628-1633.

20. Cambria-Kiely JA, Gandhi PJ. Aspirin resistance and genetic polymorphisms. J Thromb Thrombolyis. 2002;14:51-58.

21. Cipollone F, Toniato E, Martonetti S, et al. Identification of New Elements of Plaque Stability (INES) Study Group. A polymorphism in the cyclooxygenase 2 gene as an inherited protective factor against myocardial infarction and stroke. JAMA. 2004;291:2221-2228.

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