

Original Article

Correlation between aspirin resistance and glycoprotein IIb HPA-3 polymorphism and recurrent ischemic stroke

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Abstract: We discussed the correlation between aspirin resistance (AR) and glycoprotein (GP) IIb HPA-3 polymorphism and recurrent ischemic stroke. Patients with acute ischemic stroke and healthy age- and gender-matched volunteers by physical examination were included. Adenosine diphosphate (ADP) and rachidonic acid (ARA) were used as inducing agents. Platelet aggregation rate (PAgT) was determined by using flow cytometry, and $\text{PAgT}_{\text{ADP}} \geq 39.27\%$ coupled with $\text{PAgT}_{\text{ARA}} \geq 39.27\%$ was considered as AR. PCR-RFLP technique was adopted to detect GP IIb HPA-3 polymorphism. A total of 24 acute ischemic stroke (case group) and 98 healthy volunteers (control group) were included. In the case group, 162 cases were of the first onset (the first onset group), and 62 cases were recurrent (recurrent group). The incidence of AR was 15.18%, and the incidence of AR in the recurrent group was significantly higher than that of the first onset group (27.42% vs. 10.49%; $\chi^2 = 9.977$, $P = 0.002$). The frequency of bb genotype and frequency of b allele in the case group were considerably higher than those of the control group ($P < 0.001$); frequency of bb genotype ($P = 0.004$) and frequency of b allele ($P = 0.001$) in the recurrent group were significantly higher than those of the first onset group. As to cases with acute ischemic stroke, frequency of bb genotype ($P = 0.02$) and frequency of b allele ($P = 0.004$) in AR cases were significantly higher compared with non-AR cases. Multivariate logistic regression indicated that AR (odds ratio (OR) 2.933%, 95% confidence interval (CI) 1.326-6.486, $P = 0.008$) and bb genotype (OR 2.198, 95% CI 1.164-4.149, $P = 0.015$) were the independent risk factors of recurrent ischemic stroke. AR and GP IIb HPA-3 bb genotype may be correlated with recurrent ischemic stroke.

Keywords: Cerebral ischemia, aspirin, glycoprotein, GP IIb HPA-3 polymorphism

Introduction

The incidence of recurrent ischemic stroke is as high as 20%-40% [1]. Therefore it is highly important to screen the risk factors of recurrent ischemic stroke so as to formulate individualized antiplatelet therapy in secondary prevention. However, some patients still suffer from thromboembolic events after long-term aspirin treatment, from which the concept of aspirin resistance (AR) arises [2]. Biochemical AR refers to the phenomenon that platelet activity still maintains a high level in vitro test during aspirin treatment [3]. So far there is no established gold standard for detecting AR, and neither is there a consensus over the diagnosis of biochemical AR [4]. Study shows that AR is related to ischemic stroke [5]. Thrombosis plays an important role in the onset of ischemic stroke, and binding of glycoprotein (GP) IIb/IIIa to fibrinogen is the last pathway in thrombosis.

Therefore it is inferred that GP IIb HPA-3 polymorphism may be associated with ischemic stroke via its influence on thrombosis [6]. We used flow cytometry (FCM) to detect AR and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to detect GP IIb HPA-3 polymorphism. The correlation between AR and GP IIb HPA-3 polymorphism and recurrent ischemic stroke was analyzed based on the detection.

Subjects and method

Subjects

From May 2012 to April 2014 patients with acute ischemic stroke hospitalized at Department of Neurology, the Second Affiliated Hospital of Tianjin Medical University, were included. These patients met the following inclusion criteria: Diagnosed by 2010 Chinese

Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke [7]; having new loci confirmed by brain CT/MRI; Admitted to hospital within 7 days after onset. The exclusion criteria: Hemorrhagic cerebrovascular diseases; Allergic to aspirin; Temporary cerebral ischemia; Complicated by acute myocardial infarction, autoimmune diseases, rheumatic disease, tumors, infections, trauma and liver or renal insufficiency. The age- and gender-matched healthy volunteers without cerebrovascular diseases, diabetes or hypertension during the same period were included as controls. The protocol was approved by the Ethics Committee of the Second Affiliated Hospital of Tianjin Medical University, and informed consent was obtained from all subjects.

Detection of PAgT

On the morning of the second day of admission, 5 ml of fasting blood sample was collected from elbow vein of each subject for routine blood test, routine coagulation test and detection of biochemical indicators. Then 1.8 ml of the collected blood sample was placed into a tube containing 3.8% sodium citrate anticoagulant. Flow cytometry (BD, USA) was performed within 30 min to detect PAgT. Three tubes containing 5 μ l of the blood sample and 3.8% sodium citrate anticoagulant were used and numbered. Into No. 1 tube 20 μ mol/L adenosine diphosphate (ADP) (Sigma, USA) was added; into No. 2 tube 2.5 g/L arachidonic acid (AA) (Sigma, USA) was added; No. 3 tube was the control tube without the addition of any reagent. Into all three tubes 10 μ l of chlorophyll protein-labeled GP IIIa monoclonal antibody (BD, USA) was added, mixed well and cultured at room temperature in the dark for 15 min. Next 1 ml of precooled 1% paraformaldehyde (PFA) (2°C-8°C) was added and mixed well. After fixation at 4°C for 30 min, the samples were analyzed by flow cytometry. PAgT was expressed as the reduction of platelet count.

Diagnostic criteria for AR

For healthy volunteers, 20 μ mol/L ADP and 2.5 g/L AA were used as inducing agents to detect PAgT, respectively. Thus the diagnostic criteria for normal population were set with respect to PAgT, i.e., $\text{PAg}_{\text{ADP}} 22.28 \pm 16.99\%$, and $\text{PAg}_{\text{AA}} 18.77 \pm 15.50\%$. For the case group, aspirin enteric-coated tablets were medicated (100 mg, Bayer AG, Germany) for ≥ 7 d. Then PAgT

was detected again, and $\text{PAg}_{\text{ADP}} \geq 39.27\%$ coupled with $\text{PAg}_{\text{AA}} \geq 34.27$ was considered as AR.

Detection of GP IIb HPA-3 polymorphism

From each case 200 μ l of blood sample was collected and subjected to DNA extraction according to the instruction of blood genomic DNA extraction kit (Beijing ComWin Biotech Co., Ltd.). Primers were designed as follows: upstream 5'-GTA AGA GCT GGG TGG AAG AAA GAC C-3', downstream 5'-CTC CTT AAC GTA CTG GGA AGC-3' [8]. The primers were synthesized by The Beijing Genomics Institute (BGI). PCR reaction system consisted of 2 \times Taq Master Mix 10 μ l, 5 pmol forward and reverse primers 1 μ l each and DNA template 3 μ l, distilled water 6 μ l, 20 μ l in total. Reaction conditions: predegeneration at 94°C for 5 min, degeneration at 94°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 30 s, 35 cycles; final extension at 72°C for 10 min. RLFP system: PCR product 10 μ l, BseGI restriction enzyme (Fermentas, Lithuania) 0.5 μ l, 10 \times digestion buffer 2 μ l, distilled water 7.5 μ l, 20 μ l in total. The samples were incubated at 55°C for 10-16 h and then analyzed by 2.5% agarose electrophoresis (Biowoest, USA) for 40 min. The target bands were visualized under the ultraviolet imaging system (Stata gene, USA) to determine the genotypes.

Statistical process

All statistical analyses were performed using SPSS 17.0 software. Measurement data were expressed as mean \pm standard deviation ($\bar{x} \pm s$). Independent samples t test was used for intergroup comparison. Count data were expressed as percentages (%), and intergroup comparison was carried out by χ^2 test. Multivariate logistic regression was adopted to control the influence of confounding variables. Relative risk was expressed by odds ratio (OR) and in terms of 95% CI (two-sided). $P < 0.05$ was considered significant.

Results

A total of 224 cases with acute ischemic stroke were recruited, including 139 males and 83 females aged 68.37 ± 11.19 years on average. The first onset group had 162 cases, including 102 males and 60 females aged 67.56 ± 11.29 on average; the recurrent group had 62 cases, including 31 males and 31 females aged

Recurrence of ischemic stroke

Table 1. Comparison of baseline information between each group

| A. Baseline comparison between case and control group | | | | |
|---|-----------------------------|--------------------------------|-------------------|---------|
| Item | Case group | Control group | t/ χ^2 value | P value |
| Number of females (n, %) | 85 (37.95) | 43 (43.88) | 1.001 | 0.317 |
| Smoking history (n, %) | 95 (42.41) | 41 (41.84) | 0.009 | 0.924 |
| Alcohol history (n, %) | 72 (32.14) | 34 (34.69) | 0.201 | 0.654 |
| Hypertension (n, %) | 172 (76.79) | 61 (62.24) | 7.207 | 0.007* |
| Type 2 diabetes (n, %) | 72 (32.14) | 10 (10.20) | 17.287 | 0.000* |
| Coronary heart disease (n, %) | 86 (38.39) | 38 (38.78) | 0.004 | 0.948 |
| Age (years) | 68.37±11.19 | 67.41±9.63 | 0.781 | 0.436 |
| Triacylglycerol (mmol/L) | 1.49±0.80 | 1.39±0.98 | 1.019 | 0.309 |
| Total cholesterol (mmol/L) | 4.87±1.04 | 4.74±1.35 | 0.885 | 0.377 |
| Low-density lipoprotein (mmol/L) | 3.28±1.01 | 2.75±1.08 | 4.258 | 0.000* |
| High-density lipoprotein (mmol/L) | 1.48±0.92 | 1.42±0.91 | 0.512 | 0.609 |
| White blood cell count ($10^9/L$) | 7.11±2.29 | 7.09±2.33 | 0.047 | 0.962 |
| Red blood cell count ($10^{12}/L$) | 4.46±0.53 | 4.45±0.46 | 0.162 | 0.872 |
| Platelet count ($10^9/L$) | 183.96±61.08 | 187.20±52.69 | 0.457 | 0.648 |
| Fibrinogen (g/L) | 3.29±0.88 | 3.21±0.94 | 0.591 | 0.555 |
| B. Baseline comparison between recurrent group and first onset group | | | | |
| Item | Recurrent group (n = 62) | First onset group (n = 162) | χ^2/t | p |
| Number of females (n, %) | 31 (50.0) | 60 (37.0) | 3.124 | 0.077 |
| Smoking history (n, %) | 24 (38.7) | 71 (43.8) | 0.481 | 0.488 |
| Alcohol history (n, %) | 16 (25.8) | 56 (34.6) | 1.578 | 0.209 |
| Coronary heart disease (n, %) | 25 (40.3) | 61 (37.7) | 0.135 | 0.713 |
| Hypertension (n, %) | 48 (77.4) | 124 (76.5) | 0.019 | 0.889 |
| Type 2 diabetes (n, %) | 28 (45.2) | 44 (27.2) | 6.661 | 0.01 |
| Age (years) | 70.48±10.70 | 67.56±11.29 | 1.761 | 0.08 |
| Baseline NIHSS score | 6.60±5.68 | 4.78±5.07 | 2.313 | 0.022 |
| White blood cell count ($10^9/L$) | 7.13±2.20 | 7.04±2.54 | 0.261 | 0.795 |
| Red blood cell count ($10^{12}/L$) | 4.46±0.55 | 4.45±0.48 | 0.109 | 0.914 |
| Platelet count ($10^9/L$) | 223.18±67.86 | 220.71±74.71 | 0.224 | 0.823 |
| Hcy ($\mu\text{mol}/L$) | 19.94±10.93 | 20.52±11.73 | 0.295 | 0.768 |
| CRP (mg/d) | 0.81±1.57 | 2.28±5.45 | 1.872 | 0.065 |
| D-dimer (mg/L) | 1.47±2.44 | 0.65±1.31 | 1.515 | 0.141 |
| Fibrinogen (g/L) | 3.39±1.03 | 3.25±0.82 | 0.972 | 0.334 |
| Total cholesterol (mmol/L) | 4.96±1.08 | 4.97±1.34 | 0.055 | 0.956 |
| Triacylglycerol (mmol/L) | 1.51±1.05 | 1.60±1.03 | 0.55 | 0.583 |
| High-density lipoprotein (mmol/L) | 0.93±0.25 | 0.95±0.27 | 0.404 | 0.686 |
| Low-density lipoprotein (mmol/L) | 3.29±0.88 | 3.28±1.07 | 0.059 | 0.953 |
| C. Baseline comparison between AR group and Non-AR group | | | | |
| Item | AR group | Non-AR group | t/ χ^2 value | P value |
| Number of females (n, %) | 14 (41.18) | 77 (40.53) | 0.206 | 0.65 |
| Smoking history (n, %) | 15 (44.18) | 83 (43.58) | 0.945 | 0.831 |
| Alcohol history (n, %) | 11 (32.35) | 67 (35.26) | 0.439 | 0.508 |
| Hypertension (n, %) | 26 (76.47) | 147 (77.37) | 0.019 | 0.889 |
| Type 2 diabetes (n, %) | 12 (35.29) | 58 (30.53) | 0.008 | 0.767 |
| Coronary heart disease (n, %) | 13 (38.24) | 89 (46.84) | 1.66 | 0.198 |
| Age (years) | 67.56±11.29 | 70.48±10.70 | 1.761 | 0.08 |
| Triacylglycerol (mmol/L) | 1.53±0.84 | 1.39±0.68 | 1.161 | 0.247 |

Recurrence of ischemic stroke

| | | | | |
|---|--------------|--------------|-------|-------|
| Total cholesterol (mmol/L) | 4.85±1.04 | 4.92±1.07 | 0.428 | 0.669 |
| Low-density lipoprotein (mmol/L) | 3.27±1.06 | 3.30±0.88 | 0.175 | 0.861 |
| High-density lipoprotein (mmol/L) | 1.47±0.94 | 1.50±0.89 | 0.252 | 0.801 |
| White blood cell count (10 ⁹ /L) | 7.13±2.20 | 7.04±2.55 | 0.261 | 0.795 |
| Red blood cell count (10 ¹² /L) | 4.46±0.55 | 4.45±0.48 | 0.109 | 0.914 |
| Platelet count (10 ⁹ /L) | 182.21±57.91 | 188.51±68.97 | 0.639 | 0.525 |
| Fibrinogen (g/L) | 3.25±0.82 | 3.39±1.03 | 1.072 | 0.285 |

Note: *P < 0.05, indicating statistically significant difference.

Table 2. Comparison of PAgT and AR incidence between case group and control group ($\bar{x} \pm s$, %)

| Group | Case | Before treatment | | After treatment | | AR [case (%)] |
|-------------------|------|--------------------------|--------------------------|--------------------------|--------------------------|------------------------|
| | | ADP | AA | ADP | AA | |
| First onset group | 162 | 26.11±11.46 | 22.72±12.11 | 22.85±8.43 | 18.14±9.06 | 24(14.81) |
| Recurrent group | 62 | 30.75±11.87 ^a | 28.43±13.53 ^a | 27.23±15.29 ^b | 24.78±16.48 ^a | 21(33.87) ^b |
| Case group | 224 | 27.72±11.64 ^c | 24.73±12.52 ^c | 24.34±10.64 | 20.35±11.49 | 45 (20.09) |
| Control group | 98 | 22.28±8.67 | 18.77±7.91 | - | - | - |

Compared with first onset group, ^aP < 0.01, ^bP < 0.05; ^ccompared with the control group, P < 0.01; PAgT: platelet aggregation rate; ADP: adenosine diphosphate; AA: arachidonic acid; AR: aspirin resistance.

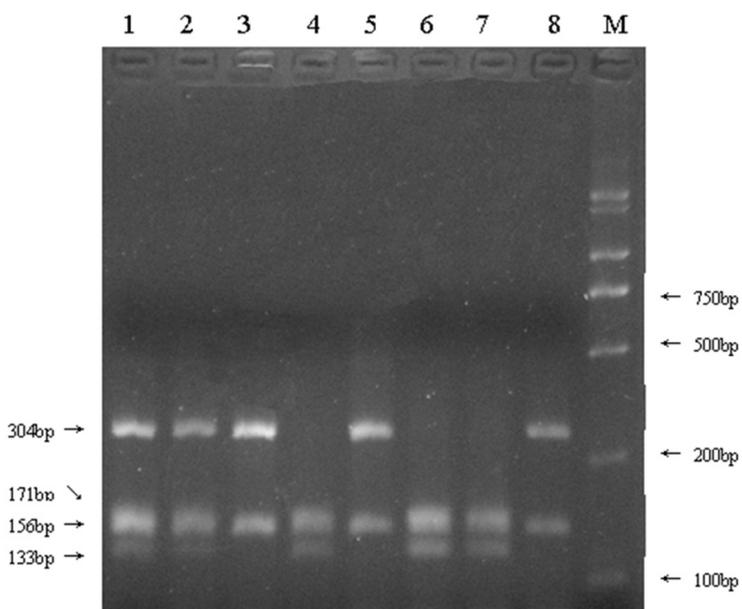


Figure 1. Genotyping results of GP IIb HPA-3 gene (M: 100 bp DNA Marker; 1, 2 is ab; 4, 6, 7 is aa; 3, 5, 8 is bb).

67.56±11.29 on average. For the control group consisting of 98 cases, there were 55 males and 43 females, aged 67.41±9.63 on average.

Comparison of demographic and clinical data between recurrent group and the first onset group

National Institutes of Health Stroke Scale (NIHSS) scores of recurrent group and the first

onset group upon admission were 6.60±5.68 and 4.78±5.07, respectively ($t = 2.313$, $P = 0.022$). Moreover, the percentage of cases with type 2 diabetes in the former was significantly higher than that of the latter (45.2% vs. 27.2%; $\chi^2 = 6.661$, $P = 0.010$). The two groups did not differ significant in other aspects ($P > 0.05$) (Table 1).

Comparison of PAgT and AR incidence between case group and control group

Before aspirin treatment, PAg_{TADP} ($t = 4.350$, $P < 0.001$) and PAg_{TAA} ($t = 4.737$, $P < 0.001$) in the case group were both significantly higher compared with the control group; PAg_{TADP} ($t = 2.682$, $P = 0.008$) and PAg_{TAA} ($t = 3.059$, $P = 0.002$) in the recurrent group were considerably higher than those of the recurrent group. After aspirin treatment, PAg_{TADP} ($t = 2.134$, $P = 0.036$) and PAg_{TAA} ($t = 3.005$, $P = 0.004$) in the recurrent group were much higher compared with the first onset group (Table 2). AR incidence was 15.18% in the case group, and AR incidence of the recurrent group was significantly higher than that of the first onset group

Recurrence of ischemic stroke

Table 3. Comparison of frequencies of GP IIb HPA-3 genotypes and alleles between the case group and the control group (n, %)

| Group | Case | Genotype | | | χ^2 | P | Allele | | χ^2 | P |
|-------------------|------|------------|------------|-------------|----------|-------|-------------|-------------|----------|-------|
| | | aa | ab | bb | | | a | b | | |
| Case group | 224 | 39 (17.41) | 84 (37.50) | 101 (45.90) | 40.618 | 0.000 | 215 (36.16) | 233 (63.84) | 18.429 | 0.000 |
| Control group | 98 | 47 (47.96) | 36 (36.73) | 15 (15.31) | - | - | 130 (66.33) | 66 (33.67) | - | - |
| Subgroup | | | | | | | | | | |
| Recurrent group | 62 | 7 (11.29) | 16 (25.81) | 39 (62.90) | 11.004 | 0.004 | 30 (24.19) | 94 (75.82) | 10.637 | 0.001 |
| First onset group | 162 | 32 (19.75) | 68 (41.98) | 62 (38.27) | - | - | 132 (40.74) | 192 (59.26) | - | - |
| AR group | 34 | 2 (5.88) | 10 (29.41) | 22 (64.71) | 7.180 | 0.028 | 14 (20.59) | 54 (79.41) | 8.422 | 0.004 |
| No-AR group | 190 | 37 (19.47) | 74 (38.95) | 79 (41.58) | | | 148 (38.95) | 232 (61.05) | | |

Table 4. Comparison of frequencies of GP IIb HPA-3 genotypes and alleles between AR group and non-AR group (n, %)

| Group | Case | Genotype | | | Allele | |
|--------------|------|------------|------------|------------|-------------|-------------|
| | | aa | ab | bb | a | b |
| AR group | 34 | 2 (5.88) | 10 (29.41) | 22 (64.71) | 14 (20.59) | 54 (79.41) |
| Non-AR group | 190 | 37 (19.47) | 74 (38.95) | 79 (41.58) | 148 (38.95) | 232 (61.05) |
| χ^2 | - | | 7.180 | | | 8.422 |
| P | - | | 0.028* | | | 0.004* |

Note: * $P < 0.05$, indicating statistically significant difference.

Table 5. Logistic regression results

| Variables | OR | 95% CI | P value |
|----------------------|-------|-------------|---------|
| Diabetes | 2.072 | 1.081~3.968 | 0.028* |
| Baseline NIHSS score | 1.056 | 0.998~1.117 | 0.060 |
| AR | 2.933 | 1.326~6.486 | 0.008* |
| bb genotype | 2.198 | 1.164~4.149 | 0.015* |

Note: * $P < 0.05$, indicating statistically significant difference.

(27.42% vs. 10.49%, $\chi^2 = 9.977$, $P = 0.002$) (Table 2).

Comparison of frequencies of GP IIb HPA-3 genotypes and alleles between the case group and control group

After restriction enzyme digestion, 3 genotypes were produced, namely, aa (171 bp, 156 bp, 133 bp), bb (304 bp, 156 bp) and ab (304 bp, 171 bp, 156 bp, 133 bp), as shown in Figure 1. The case group showed significant differences in genotype frequency ($\chi^2 = 40.618$, $P < 0.001$) and allele frequency ($\chi^2 = 18.429$, $P < 0.001$) compared with the control group. The frequencies of bb genotype and b allele were significantly higher than those of the control group ($P < 0.001$). Genotype frequency ($\chi^2 = 11.004$, $P = 0.004$) and allele frequency ($\chi^2 = 10.637$, $P =$

0.001) in the recurrent group were remarkably different from those in the first onset group; the frequencies of bb genotype and b allele ($P < 0.001$) in the recurrent group were both significantly higher than those in the first onset group (Table 3).

Comparison of frequencies of GP IIb HPA-3 genotypes and alleles between AR group and non-AR group

Genotype frequency ($\chi^2 = 7.180$, $P = 0.028$) and allele frequency ($\chi^2 = 8.422$, $P = 0.004$) in AR group had a considerable difference compared with non-AS group. The frequencies of bb genotype and b allele were both significantly higher in AR group than in non-AR group (Table 4).

Multivariate logistic regression for risk factors of recurrent ischemic stroke

Taking whether the ischemic stroke recurred or not as the dependent variable, variables with $P < 0.1$ in single factor analysis were subjected to multivariate logistic regression. Results indicated that type 2 diabetes ($OR = 2.072$, 95% CI: 1.081-3.968, $P = 0.0028$), AR ($OR = 2.933$, 95% CI: 1.326-6.486, $P = 0.008$) and bb genotype ($OR = 2.198$, 95% CI: 1.164-4.149, $P =$

0.015) were all independent risk factors of recurrent ischemic stroke (Table 5).

Discussion

FCM is commonly used for the analysis of cell surface markers, intracellular antigens and receptors and immune cells. The use of FCM for the detection of PAgT is comparatively rare. By labeling antibodies with fluorescence, FCM can specifically detect nearly all platelets. Single platelets or platelet aggregates can be differentiated based on certain parameters. PAgT is usually expressed as the reduction of single platelets in blood samples by smart computing. In this way, platelet aggregation is quantitatively and accurately evaluated [9]. Research shows that the incidence of AC among patients with ischemic stroke is 5%-40% [10]. Our results (15.18%) fell within this scope, demonstrating the effectiveness of FCM in the detection of biochemical AR.

We found that PAgT in the recurrent group was significantly higher than that in the first onset group, indicating that the platelets maintained a high activity level. This result agreed with that by Htun et al. [11]. Acute ischemic stroke is usually associated with vascular endothelial injury and exposure of collagen, which promotes platelet adhesion and aggregation and hence thrombosis. The recurrent cases might have more severe vascular endothelial injury than the first onset causes with more platelet being activated, leading to increased platelet aggregation.

At 7 d of aspirin treatment, PAgT and incidence of AR in recurrent group still showed significant increase compared with the first onset group. Gengo et al. [12] also indicated the incidence of AR in recurrent ischemic stroke was as high as 36%, which was comparable to our result. AR may lead to activation of serine 529 of acetylated cyclooxygenase 1, and a large amount of AA will be transformed into prostaglandin. As a result, thromboxane is produced in large quantity, and the platelets are highly activated, leading to thrombosis and recurrent ischemic stroke. Multivariate logistic regression showed that AR was the independent risk factor of recurrent ischemic stroke, consistent with the findings of Rafferty et al. [5].

GP IIb is localized at chromosome 17 in human, containing 30 exons and 29 introns and having a full length of about 17.2 kb. GP IIb usually exists on the platelet membrane in the form of Ca²⁺-dependent dimer. GP IIb HPA 3a/3b is the result of T-to-G mutation, with the substitution of soleucine (HPA3a) by serine (HPA3b). Reiner et al. [13] found that bb genotype played a role in ischemic stroke in young women. Duan et al. [6] also indicated that GP IIb HPA-3 Ile/Ser843 polymorphism was associated with ischemic stroke in males below 60 years. However, another research suggested that GP IIb HPA-3 polymorphism was irrelevant to ischemic stroke [14]. We discovered that the frequencies of GP IIb HPA-3 bb genotype and b allele in the case group were both significantly higher than those of the control group. Thus bb genotype was a genetic susceptibility factor of ischemic stroke.

It was also found that the frequencies of GP IIb HPA-3 bb genotype and b allele in AR group had considerable increase compared with non-AR group, suggesting that GP IIb HPA-3 bb genotype might be a risk factor of AR in ischemic stroke. Currently only a few studies are devoted to the correlation between GP IIb HPA-3 polymorphism and recurrent ischemic stroke. According to our results, the frequencies of GP IIb HPA-3 bb genotype and b allele in the recurrent group were significantly higher than those of the first onset group. Multivariate logistic regression was performed, and bb genotype was found to be an independent risk factor of recurrent ischemic stroke. GP IIb HPA-3 bb genotype may cause a change of configuration of GP IIb/IIIa, thus exposing more fibrinogen-binding sites. This in turn promotes the binding of platelet to fibrinogen, platelet adhesion and aggregation and hence thrombosis [6].

No consensus has been reached as to the correlation between recurrent ischemic stroke and other common risk factors. Some researchers consider hypertension, diabetes, coronary heart disease and hyperlipidemia as the risk factors of recurrent ischemic stroke. But others believe that only a few of them do contribute to the risk of recurrent ischemic stroke [15]. Through multivariate logistic regression, we found that type 2 diabetes was the independent risk factor. The discrepancies between the results may be due to different effect of man-

agement of underlying diseases in ischemic stroke patients from different regions.

The present research has the following limitations. Firstly, all cases came from the same hospital, which makes selection bias inevitably. Secondly, there were differences in patients' posture during blood sampling, blood sampling time, sample delivery and detection time. These may affect the reliability of PaGT detection. Thirdly, the sample size was limited. In spite of this, we confirmed the correlations between AR and bb genotype and recurrent ischemic stroke. Therefore individualized antiplatelet therapy can be formulated based on AR and bb genotype detection to reduce the recurrence risk.

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Disclosure of conflict of interest

None.

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Recurrence of ischemic stroke

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