SIN-CHN SCIENTIFIC PRESS PTE.LTD.

*Molecular & Cellular Biomechanics* 2024, 21(4), 1091.
https://doi.org/10.62617/mcb1091

Article

# Combined role of PEAR1 and GP genes in the mechanism of aspirin resistance and its clinical significance

**Yu Ning[1], Yan Gao[1,*], Wei Gao[2]**

[1] Beihua University Affiliated Hospital, Jilin 132001, China
[2] Jilin City People's Hospital, Jilin 132001, China
*** Corresponding author:** Yan Gao, yanjiuyouxiang163@163.com

**CITATION**

Ning Y, Gao Y, Gao W. Combined role of PEAR1 and GP genes in the mechanism of aspirin resistance and its clinical significance. Molecular & Cellular Biomechanics. 2024; 21(4): 1091.
https://doi.org/10.62617/mcb1091

**ARTICLE INFO**

Received: 12 December 2024
Accepted: 19 December 2024
Available online: 27 December 2024



**Abstract:** Exploring the mechanism of aspirin resistance helps to improve the prevention and treatment of ischemic stroke diseases and reduce the interference of resistance mechanism on the therapeutic effect of aspirin. In this paper, the Affiliated Hospital of Beihua university, together with two other hospitals, was chosen as the research unit, from which 260 AIS patients were selected for aspirin resistance study. The joint role of PEAR1 gene and GP gene in the mechanism of aspirin resistance was discussed by genotyping, TEG assay, univariate analysis, and binary logistic analysis methods. Among the GP1BA genes in AIS patients, the frequency of CC-type genes was as high as 92.03%, and the risk of aspirin resistance was significantly increased in CC-type relative to TT-type. The incidence of rs5924 allele T was significantly higher in the AR group than in the AS group (OR = 1.632, $p$ = 0.006), and there was a significant effect of diabetic conditions on aspirin resistance at the 1% level. Diabetes significantly inhibits plasma proteins in AIS patients and makes aspirin less inhibitory to platelets. Therefore, clarifying the influence of PEAR1 and GP genes in the mechanism of aspirin resistance can be used to obtain the precise loci from the gene therapy point of view to enhance the disease treatment effect of aspirin.

**Keywords:** PEAR1 gene; GP gene; aspirin; resistance mechanism; binary logic analysis

## 1. Introduction

Ischemic stroke is one of the major diseases that threaten human life and health, and is a serious public health problem all over the world. It not only has a high morbidity, disability, and mortality rate, but also has a high recurrence rate, which not only causes a certain degree of physiological and psychological damage to the patients, leading to a decline in their quality of life, but also brings heavy economic pressure to their families and society [1–3]. Globally, ischemic stroke is the second leading cause of death after heart disease, accounting for the prevalence of the total number of deaths worldwide, and the risk factors have been universally exposed, and at present, the incidence of ischemic stroke in China is increasing year by year, and there is a gradual upward trend in the rate of youth and morbidity, especially in rural areas. From China's point of view, with the arrival of the aging society and the development of urbanization, the trend of unhealthy lifestyle [4,5]. Although great progress has been made in the treatment of ischemic stroke, it is still the disease with the highest single-disease disability rate and a high recurrence rate, so it is particularly important to take the necessary preventive and curative measures to control the controllable risk factors and prevent the recurrence of ischemic stroke [6,7].

With the rapid development of human genome research and molecular biology technology, more and more studies have confirmed that ischemic stroke is an extremely complex disease caused by a combination of multiple genetic and environmental factors, and that genetic factors play an important role in the pathogenesis of stroke [8,9]. Genetic variations affect platelet function, leading to increased platelet activity and influencing platelet aggregation and thrombosis, which are closely related to the occurrence of ischemic stroke. As the current first-line drug for antiplatelet aggregation in clinical practice, aspirin has been widely used in clinical practice, and in terms of antiplatelet effect, most of the studies have confirmed that the application is more effective [10–12]. Many large group studies of cardiac, cerebral and peripheral vascular disease suggest that those taking aspirin therapy reduce the crisis of serious vascular accidents by approximately 25%, but aspirin is being challenged by novel antiplatelet agents, the latter in combination with aspirin can increase its maximum benefit, and it has been found that some patients are resistant to aspirin, and once detected, it can be replaced with other drugs [13–15]. The mechanism of aspirin resistance involves the molecular level, glycoprotein III is a membrane receptor in the integrin family, the latter causes platelets to bind to fibrinogen, and receptor nucleotide polymorphisms increase platelet aggregation in allele-dependent individuals, relevant clinical studies are inconclusive because different responses to aspirin occur through different pathways, and it is possible that other polymorphisms (e.g., glycoprotein Ia) may play a role in this [16–18]. Therefore, the combined role of platelet endothelial aggregation receptor-1 (PEAR1) and platelet glycoprotein (GP) in the mechanism of aspirin resistance and its clinical significance can be explored to find the theoretical basis for individualized and tailored rational and precise ischemic stroke prevention programs [19,20].

Aspirin is widely used in the prevention and treatment of ischemic stroke as a first-line drug for antiplatelet coagulation; however, not all patients benefit from the treatment, and aspirin resistance occurs in some patients. In this paper, 260 patients with AIS from the Affiliated Hospital of Beihua university were selected as study samples and divided into AS and AR groups. The detection of aspirin genotyping was compared by genotyping detection method and combined with TEG to detect platelet aggregation in AIS patients. The Hardy-Weinberg method was utilized for the balance test of the relevant data of AIS patients, and the gene frequency and allele expression of aspirin resistance genes were explored by comparing the polymorphisms of PEAR1 gene and GP gene, and the specific influencing factors of AR were analyzed by combining with binary logistic regression, so as to provide a new research basis for enhancing the disease treatment effect of aspirin.

## 2. Information and methods

Aspirin, an antiplatelet agglutinating agent, reduces the incidence of ischemic stroke events, recurrence rates, and mortality, thereby improving the long-term prognosis of patients. Although low-dose aspirin has been shown to be an effective antiplatelet-agglutinating agent, however, not all patients benefit from it. Clinical observations have shown that 10% of patients with ischemic stroke still experience

thromboembolic events, i.e., the development of aspirin resistance or antiretroviral phenomenon (AR), after taking aspirin for a prolonged period of time. The development of AR has been associated with a variety of factors, such as poor patient adherence, and poor absorption of the drug. Some studies have continuously shown that the occurrence of AR is related to genetic polymorphisms. In this paper, the correlation between aspirin resistance and genetic polymorphisms was investigated in order to provide a theoretical basis for the use of aspirin in the individualized treatment of ischemic stroke.

## 2.1. PEAR1 gene and GP gene

(1) Gene encoding platelet endothelial aggregation receptor 1 (PEAR1)

PEAR1 is a molecule involved in the platelet activation pathway across the cell membrane, and its phosphorylation promotes platelet aggregation. Although its relationship with AR has been studied for a relatively short period of time, it has been studied with more positive results [21]. Relevant studies have shown that the rs12041331 gene polymorphism of PEAR1 is associated with the generation of PEAR1 and platelet aggregation capacity, and one genome-wide association study demonstrated that harboring the A allele was an independent determinant of platelet function and adverse clinical events in aspirin takers. Their findings indicated that AA or GA gene carriers had greater platelet activation compared to GG gene carriers [22].

(2) Genes encoding platelet membrane glycoprotein (GP) receptors

GP receptor is the key receptor for the final stage of platelet aggregation and is categorized into GP IIb/IIIa and GP Ia/IIa receptors. The most widely studied single nucleotide polymorphism in the GP IIIa receptor gene is T1565C (P1A1/A2). Existing relevant studies have analyzed 50 loci of 11 genes related to AR and concluded that T1565C mutation is significantly associated with AR in healthy individuals [23]. However, in patients with cardiovascular disease, the correlation is unclear and subsequent studies remain inconsistent. More studies on GP Ia receptor have been conducted on the C807 and T807 alleles. Carriers of the T807 allele have increased platelet GP Ia/IIa receptor density, whereas the opposite is true for C807. In addition, mutations at this locus and diabetes mellitus have been found to be strongly associated with AR in the post-stroke population. In recent years, a study that included 140 subjects with ischemic stroke risk factors in a comparative study with 80 healthy volunteers has confirmed that T807 is strongly associated with AR.

## 2.2. Mechanisms of aspirin resistance

There is no recognized definition of aspirin resistance. Clinical application of therapeutic doses of aspirin, there are still some patients with ischemic cerebrovascular disease events, increase the therapeutic dose, not only failed to achieve the therapeutic and preventive purposes, but also an increase in adverse effects, this phenomenon is known as aspirin resistance or aspirin resistance phenomenon.

The exact mechanism of inactivation of aspirin therapy is not well understood. Previous studies have focused primarily on linking laboratory-proven AR to

inappropriate aspirin dosages, with the idea that AR could be overcome by increasing aspirin dosage; however, there is no evidence that aspirin's antithrombotic effects are dose-related. For example, in patients undergoing carotid endarterectomy, the incidence of stroke, myocardial infarction, and death was lower in those with low-dose aspirin 80 mg or 325 mg. It is evident that clinical prognosis was not improved by changes in aspirin dose [24].

Platelet GP IIb/IIIa complex is a receptor for fibrinogen and platelet aggregation, and there are various genotypes in platelet GP IIIa subunit receptors, such as PIA1A1 pure type, PIA1A2 heterozygous type, and PIA2A2 pure type. Some studies have shown that PIA2 mutations are quite common and are associated with enhanced platelet reactivity and increased incidence of restenosis after stenting, with more than 30% of cardiovascular patients having this mutation type. In contrast, platelet activity in patients with PIA2 mutant phenotype is not only enhanced by agonists, but also less responsive to antiplatelet therapy such as aspirin [25]. In addition, the erythrocyte surface, which has the ability to coagulate and increase platelet activation, does not affect this cell-cell interaction for the purpose of preventing thrombosis because aspirin inhibits thromboxane formation, suggesting that there are other pathways available for thrombosis. In conclusion, the cause of AR may be related to a genetic abnormality, the mechanism of which needs to be further elucidated.

## 2.3. Clinical study information

Before sample extraction, this paper first estimated the sample size. In the validation study, the applicable research design type should be explained:

(1) Determine the $\alpha$ value

$\alpha$ value is a Type 1 error (false positive probability), usually a-0.05 or 0.01. Two-sided test is generally taken. To choose between unilateral or bilateral tests, follow the following principles. If the purpose is to infer whether there is a difference in the overall parameters (rate, mean) between the two groups, and the test group may be higher or lower than the control group, then a bilateral test is used. If there is firm evidence from expertise that the test group is not lower or higher than the control group, a unilateral test should be used.

(2) Determine the $\beta$ value or 1-$\beta$ value

The beta value is the false negative rate, usually $\beta$-0.10 or 0.20. Test efficacy or assurance = 1-$\beta$. If $\beta$-0.10, the test efficiency = 0.90. General inspection efficiency cannot be less than 0.80.

(3) Determine the effect indicators and their types

The main effect indicators should be selected for estimation, and the measurement data should be selected to be more objective.

This is a prospective, large-sample enrollment and follow-up study of the acute ischemic stroke (AIS) population in Region J. The Affiliated Hospital of Beihua university. Case recruitment was conducted from July 2022 to May 2024, and telephone/WeChat/face-to-face follow-up was conducted at the 1st, 4th, 8th, and 12th months after hospital discharge to observe and record the endpoint events (recurrence, adverse events, and death), and to explore the MR influencing factors of

AIS. The study was approved by the Medical Research Ethics Committee of the Affiliated Hospital of Beihua university and registered in the China Clinical Trial Registry.

The inclusion criteria were as follows:

(1) Aged 60–85 years;

(2) Diagnosed with ischemic stroke (atherosclerotic cerebral infarction, in accordance with the diagnostic criteria of the 2018 Chinese Guidelines for Diagnosis and Treatment of Acute Ischemic Stroke);

(3) During the acute period of onset (2 weeks);

(4) National Institutes of Health Stroke Scale (NIHSS) score ≤ 16;

(5) Voluntary participation and signed informed consent.

Exclusion was based on fulfillment of any of the following points:

(1) Those with hemorrhagic stroke, mixed stroke, and tumor stroke;

(2) Those who have recently received whole blood or platelet transfusions, have coagulation disorders or other blood disorders;

(3) Patients with alteplase thrombolysis and some patients subject to anticoagulation or dual antiplatelet (e.g., PCI);

(4) Comorbidities with severe cardiac, pulmonary, and hepatic system diseases;

(5) Renal dysfunction (24-hour urine output less than 1200 ml and blood creatinine greater than 200 mmol/L);

(6) Cancer patients;

(7) Patients with active gastric ulcer or gastric bleeding;

(8) Patients who, in the judgment of the investigator, have poor compliance and are unable to complete follow-up;

(9) Currently participating in any clinical trials of other drugs or medical devices.

The occurrence of any of the following will result in immediate termination of the study:

(1) Cases of misdiagnosis or misinclusion that, in the judgment of the investigator, do not meet the inclusion criteria or meet the exclusion criteria;

(2) A patient who voluntarily requests to be withdrawn from the study;

(3) Those judged by the investigator to be unable to continue effective clinical observation for medical or other reasons;

(4) Patients who stopped antiplatelet medication in the middle of the study, or patients who switched to anticoagulants in the middle of the study.

A total of 260 patients with AIS were obtained, including 133 males and 127 females. By estimating the sample size, it was found that the $\alpha$ value of 260 samples was < 0.01, which passed the bilateral test. The $\beta$ value is 0.10 and the test efficiency is 0.9 > 0.8, indicating that the samples selected in this study are good and the conclusion value obtained is highly reliable.

## 2.4. Grouping criteria and related indicators

The electronic medical record system of our hospital was used to query the results of head magnetic resonance examination after admission. The machine model

was 3.0T-MR (Discovery750, GE Healthcare USA). The subgroups and pharmacologic interventions were as follows:

(1) Grouping. All enrolled patients underwent thromboelastography after admission to the hospital, and were divided into aspirin resistance (AR) and aspirin sensitivity (AS) groups based on the results of AA inhibition rate by TEG. According to China's Expert Consensus on Clinical Application of Aspirin (2012), if there are indications for clinical use of aspirin, AA inhibition rate <55% is defined as aspirin resistance (AR).

(2) Clinical intervention. For the aspirin-resistant group, different intervention modalities were given according to the degree of platelet drug resistance, and the regimen included increasing the dose of aspirin, combining aspirin with clopidogrel therapy, or switching to other antiplatelet drugs (e.g., clopidogrel hydrogensulfate tablets, cilostazol, disopyramide, etc.).

(3) Intervention adherence. For the subjects enrolled in the group, during the admission period, we strengthened the educational work in accordance with the Chinese secondary prevention guidelines for ischemic stroke, informing the necessity of taking medication, and at the same time, we retained complete information data and contact information, and carried out regular follow-up work on them at 1, 4, 8, and 12 months after discharge from the hospital.

The patients were followed up for a period of 12 months, starting from the time of admission to the hospital and ending with the occurrence of cerebrovascular events (including recurrent ischemic stroke, hemorrhagic stroke, etc.) and death, which included the improvement of the patients' symptoms, their daily life, taking medication, and whether they had a recurrence of adverse ischemic events, etc. The patients' symptoms and daily life, medication, and whether they had a recurrence of adverse ischemic events, etc. were also monitored. The primary observation index was ischemic stroke (including progressive exacerbation of primary cerebral infarction, new cerebral infarction at other sites, and TIA). The secondary observation indexes were hemorrhagic stroke (with clinical symptoms and confirmed by cranial CT) and all-cause death (stroke-related death, non-stroke-related death).

All patients were required to complete the collection of clinical data through bedside interviews or medical records, including gender, age, body mass index (BMI), smoking history, alcohol consumption, hypertension, diabetes mellitus, coronary artery disease, atrial fibrillation, cerebral infarction, cerebral hemorrhage, and the use of aspirin and statins and other related medical histories. All patients were required to complete the blood routine, coagulation routine and biochemistry related indexes after admission to the hospital.

## 2.5. Statistical methods

All data were exported as Excel files and then the files were saved in SPSS statistical software format for further analysis and the significance level *p*-value for all statistics was set at 0.05 (two-sided). The distribution of the measurements was tested for balance using one-sample Hardy-Weinberg. Data were expressed as mean ± standard deviation if they satisfied normal distribution. Count data such as risk factors such as gender, smoking, alcohol consumption, hypertension, diabetes

mellitus, coronary heart disease and hyperlipidemia were expressed as event proportions. A multifactor logistic regression model was used to include the influences with $P < 0.25$ in the model, analyze the independent influences of MR for AIS, and calculate the OR and 95% CI.

## 3. Results and analysis

Aspirin is one of the most widely used drugs to inhibit platelet aggregation and activation in the treatment and prevention of ischemic stroke, but some patients do not always achieve the desired effect of preventing ischemic stroke after receiving a standardized oral dose of aspirin.AR has led to the inability of patients to get the desired effect from aspirin in the prevention and treatment of cerebrovascular disease, which has limited its use. The inhibition of platelet aggregation by aspirin is an intricate process, and the mechanisms affecting its efficacy and resistance are complex and may involve multiple aspects of its inhibition of platelet aggregation. Based on the experimental design of AR in the previous section, this chapter focuses on the quantitative analysis of the data for its experimental results, so as to explore the correlation between AIS gene expression and AR.

### 3.1. General clinical data of patients in both groups

Based on the general clinical data of the patients admitted to the hospital, the acquired data were collected and organized and entered into SPSS software to obtain the results of the comparison of the general clinical data of the two groups of patients as shown in **Table 1**. Among them, total cholesterol (TC), low-density lipoprotein (LDL), lipoprotein-associated phospholipase A2 (Lp-PLA2), C-reactive protein (CRP), platelets (PLT), red blood cells (RBC), fasting glucose (FPG), uric acid (UA), and *, ** represent the use of chi-square test and non-parametric test, respectively.

Two hundred and sixty patients were enrolled, of whom 128 (49.23%) were in the AS group, 70 (54.69%) were males and 58 (45.31%) were females. 132 (50.77%) were in the AR group, 65 (49.24%) were males and 67 (50.76%) were females. The proportion of females was not statistically significant between the two groups ($P > 0.05$).Among the 260 patients, comparing the clinical baseline data between the AS and AR groups, it was found that patients' age, TC, LDL, RBC, and Lp-PLA2 levels were higher in the AR group than in the AS group, and the difference was not statistically significant in the comparison between the two groups ($P > 0.05$).The levels of UA were similar in the two groups, and the difference was not statistically significant. PLT, FBG, NIHSS score, CRP levels were not statistically significant between the two groups, and the incidence of hypertension and diabetes was higher in the AS group than in the AR group, with no statistically significant difference. There was no statistically significant difference in TOAST typing and OCSP typing between the two groups ($P > 0.05$).

**Table 1.** General clinical data of the two groups.

| **Index** | **AS Group (*N* = 128)** | **AR Group (*N* = 132)** | ***P* value** |
|---|---|---|---|
| Age | 70.17 ± 8.62 | 70.98 ± 7.45 | 0.357 |
| Female (Count/%) | 58(45.31%) | 67(50.76%) | 0.816* |
| TC (mmol/L) | 4.12 ± 1.03 | 4.23 ± 1.15 | 0.635 |
| LDL (mmol/L) | 2.79 ± 0.83 | 2.78 ± 0.92 | 0.172 |
| Lp-PLA2 (ng/mL) | 275.48 ± 93.27 | 276.01 ± 95.18 | 0.898 |
| CRP (mg/L) | 1.97(6.49) | 2.42(1.37) | 0.665** |
| PLT (*$10^9$) | 196.38 | 195.74 | 0.217** |
| RBC (*$10^{12}$) | 4.53 ± 0.52 | 4.45 ± 0.83 | 0.531 |
| FPG (mmol/L) | 5.15(1.28) | 5.12(1.31) | 0.242** |
| UA (μmol/L) | 322.38 ± 133.54 | 323.51 ± 89.79 | 0.973 |
| NIHSS score | 3.84(6.27) | 2.25(5.07) | 0.527** |
| History of hypertension (Count/%) | 103(80.47%) | 84(63.64%) | 0.062* |
| History of diabetes (Count/%) | 28(21.88%) | 21(15.91%) | 0.236* |
| TOAST type (Count/%): | - | - | - |
| Atherosclerosis | 70(54.69%) | 82(62.12%) | 0.531* |
| Interstitial cerebral infarction | 35(27.34%) | 38(28.79%) | |
| Cerebral embolism | 21(16.41%) | 11(8.33%) | |
| Unknown cause | 2(1.56%) | 1(0.76%) | |
| OCSP type (Count/%): | - | - | - |
| Partial pre-circulating type | 68(53.13%) | 67(50.76%) | 0.098* |
| Complete pre-circulation | 42(32.81%) | 8(6.06%) | |
| Interstitial cerebral infarction | 15(11.72%) | 11(8.33%) | |
| After circulation | 3(2.34) | 46(34.85%) | |

Numerous studies have been conducted on the mechanism of occurrence of aspirin resistance, but there is still no definitive conclusion. Hypertension, diabetes mellitus, and hyperlipidemia are risk factors for cerebral infarction, and some studies have shown that these risk factors similarly affect the antiplatelet effect of aspirin. Since patients with hyperlipidemia and hypertension are often associated with endothelial injury and lipid metabolism disorders, endothelial injury is followed by increased expression of multiple inflammatory factors and adhesion factors, and platelet activity is elevated. Lipid peroxidation leads to increased levels of oxidatively modified low-density lipoprotein (Ox-LDL), which enhances platelet aggregation. Comparisons were made between the AS and AR groups, and the results suggested that the levels of Lp-PLA2 and CRP tended to be elevated in the AR group compared with the AS group, with no statistically significant difference. CRP is a protein produced by the body or tissues after injury or inflammatory stimulation, which is a marker of inflammatory response, and is considered to be a risk factor for cardiovascular and cerebrovascular events. There is still controversy about whether CRP is a risk factor for the development of AR, and some studies have shown that CRP levels are not related to aspirin resistance and concluded that

CRP is not suitable as a screening indicator for aspirin resistance, so further in-depth studies on the relationship between CRP and AR are needed. While platelets are activated, active Lp-PLA2 frees arachidonic acid from membrane phospholipids, which in turn generates thromboxane A2 to induce thrombus formation. Thus Lp-PLA2, unlike CRP, is a novel inflammatory marker with vascular specificity. Some studies have shown that AR is associated with age and gender, and that advanced age and women are risk factors for AR. The association of AR with age may be related to the reduced bioavailability of aspirin in the elderly with degeneration of hepatic and renal function and weakened gastrointestinal function, and the concomitant risk factors for aspirin resistance with advancing age. However, the exact relationship between age and AR is currently controversial. However, the exact relationship between age and AR is currently controversial, and the present study did not find a correlation between age and AR, probably because of the small sample size and the inability to cover the full range.

### 3.2. Aspirin genotyping detection

Taking 132 cases of AIS patients in the AR group as an example, based on the genotyping detection methods and steps given in the previous section, a Fascan multichannel fluorescence quantitative analyzer was used, digoxigenin staining solution was used as a fluorescence staining in situ hybridization reagent, and the type of specimen was venous blood containing ethylenediaminetetraacetic acid (EDTA) anticoagulation for aspirin medication-related gene testing. There are three genotypes of genes in the GP1BA gene. There are three subtypes in the GP1BA gene, i.e., CC genotype group, CT genotype group and TT genotype group, and three subtypes in the PEAR1 gene, i.e., AA genotype group, GA genotype group and GG genotype group. **Figure 1** shows the genotyping detection of aspirin GP1BA and PEAR1 genes.

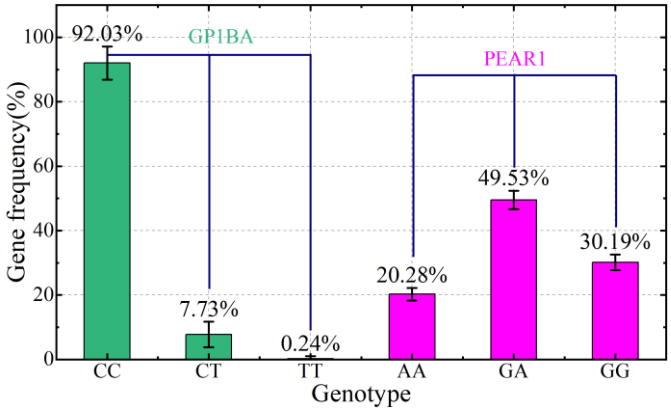


**Figure 1.** Aspirin GP and PEAR1 gene fractal detection.

The results of aspirin PEAR1 and GP1BA genotyping tests in 132 ischemic stroke patients were analyzed retrospectively. As can be seen from the figure, the frequency of CC-type genes in GP1BA genes was as high as 92.03%, and according to related studies, CC-type significantly increased the risk of aspirin resistance relative to TT-type. The ratio of AA-type genes, GA-type genes and GG-type genes in PEAR1 genes was 2:5:3. Molecular testing of drug-related genes is a prerequisite

for the implementation of individualized drug therapy. Currently, genes related to aspirin efficacy or adverse effects include GP1BA, CYP2C19, HLA-DPB1, PEAR1, etc. In the guidelines for individualized pharmacy services for antiplatelet drugs based on pharmacogenomics, the recommended loci for aspirin-related gene testing are GP1BA and PEAR1. The level of evidence for GP1BA is 2B, and related studies have reported that GP1BA gene polymorphisms are associated with aspirin sensitivity in patients, with a significantly increased risk of aspirin resistance in CC type compared to TT type. Existing studies have found that pure mutations in the PEAR1 gene increase the risk of ischemic stroke recurrence, and carriers of the PEAR1 allele have an increased risk of ischemic events in Chinese patients with acute coronary syndromes (ACS); therefore, polymorphisms in the PEAR1 gene will lead to significant individualized differences in the efficacy of aspirin antiplatelet therapy. Different races, different regions, and the use of different platelet function assays result in a wide range of fluctuations in the reported incidence of aspirin resistance.

### 3.3. Hardy-Weinberg equilibrium tests

After the detection of GP1BA and PEAR1 gene loci polymorphisms were found in 132 patients, in order to verify the reliability of the resultant genotyping test results, this paper used Hard-Weinberg's law to perform a balanced test. **Table 2** shows the distribution of genotypes and the results of Hard-Weinberg balanced test, and then the 132 patients were divided into the first recurrence group and recurrence group, and the two genotypes were compared with the allele frequency, and the results of the comparison of the genotypes and allele frequency of GP1BA and PEAR1 were obtained as shown in **Table 3**.

Testing of 132 patients for the polymorphisms at each locus of the three genes revealed 39 cases of the GG genotype, 65 cases of the GA genotype, and 26 cases of the AA genotype of the PEAR1 gene (rs12041338), with a frequency of 55.43% for allele G and 44.57% for allele A. The results were summarized in the following table. GP1BA gene (rs5924) TT genotype in 1 case, CT genotype in 10 cases, CC genotype in 121 cases, allele C frequency was 98.62% and allele C frequency was 1.38%. The PEAR1 and GP1BA gene polymorphisms were tested to be consistent with the Hard-Weinberg law, indicating that the samples in this study were representative of the population.

PEAR1 is a type I cell surface receptor whose gene consists of 23 exons and 22 introns and is highly expressed in platelets and endothelial cells as well as in other cell types, which can participate in and influence platelet activation and aggregation, and play an important role in thrombus formation. In this study, genetic testing of 132 AIS patients revealed that carriers of the A allele at rs12041338 in AIS patients treated with aspirin significantly increased the risk of myocardial infarction compared with GG purists, and that carriers of the A allele at the rs12041338 locus were more likely to have a cardiovascular thrombotic event or die compared with GG purists. Although different from the subjects of some of the studies, the present study with its both found that the pure AA genotype increased the risk of recurrent thrombotic events. Taken together, it is hypothesized that the A allele may increase

PEAR1 protein expression or alter the protein structure, enhancing the role of PEAR1 in platelet activation and leading to an increased risk of thrombosis. Platelets have a variety of transmembrane glycoprotein receptors on their surface, some of which are highly polymorphic and encoded by two or more allelic isoforms, and substitutions between alleles resulting in differences in amino acid sequences can affect the tertiary structure of the GP receptor, leading to subtle changes in its function. In this study, only 10 and 12 cases of heterozygous TT genotypes were found in the first and recurrent groups, respectively, by genetic testing of 132 patients with AIS, and no individuals with pure mutant phenotypes were detected. There were no significant differences in allele and genotype frequencies among patients with AIS. Similar to the results of other studies, PLA1/A2 polymorphisms are rare in the Chinese population, so they may not be a risk factor for ischemic stroke recurrence. The reason for the difference between the results of foreign studies and the present study may be due to the difference in the ethnicity of the study population.

**Table 2.** The results of the balance test for the Hard-Weinberg.

| **Group** | **Num** | **GP1BA** | | | **PEAR1** | | |
|---|---|---|---|---|---|---|---|
| | | **CC** | **CT** | **TT** | **AA** | **GA** | **GG** |
| Observed | 132 | 121 | 10 | 1 | 26 | 65 | 39 |
| Actual | 132 | 125 | 7 | 0 | 28 | 67 | 37 |
| $x^2$ | - | 0.002 | | | 2.127 | | |
| *P* | - | 1.078 | | | 0.389 | | |

**Table 3.** The results of the GP1BA and the PEAR1 gene and the allele frequency.

| **Group** | **Num** | **PEAR1 (Count/%)** | | | **Allele (Count/%)** | |
|---|---|---|---|---|---|---|
| | | **AA** | **GA** | **GG** | **G** | **A** |
| Starting | 68 | 7(10.29%) | 43(63.24%) | 18(26.47%) | 73(55.30%) | 59(44.70%) |
| Relapse | 64 | 15(23.44%) | 40(62.50%) | 9(14.06%) | 51(38.64%) | 81(61.36%) |
| $x^2$ | - | 8.514 | | | 6.325 | |
| *P* | - | 0.015 | | | 0.011 | |
| Group | Num | GP1BA (Count/%) | | | Allele (Count/%) | |
| | | CC | CT | TT | C | T |
| Starting | 72 | 32(44.44%) | 30(41.67%) | 10(13.89%) | 48(36.36%) | 84(63.64%) |
| Relapse | 60 | 10(16.67%) | 38(63.33%) | 12(20.00%) | 56(42.42%) | 76(57.58%) |
| $x^2$ | - | 5.895 | | | 5.428 | |
| *P* | - | 0.054 | | | 0.035 | |

## 3.4. Comparison of polymorphisms in PEAR1 and GP genes

In order to further analyze the gene locus expression of PEAR1 gene and GP gene in AIS patients, their single nucleotide polymorphisms (SNPs) were analyzed from both AS and AR groups. The allele and genotype frequencies of the rs12041338 and rs5924 loci of the PEAR1 gene and the GP gene were detected and analyzed in group comparisons to assess the correlation with AR in AIS patients.

**Table 4** shows the allele frequency and genotype distribution characteristics of each locus between the two groups.

As can be seen from the table, the incidence of rs5924 allele T was significantly higher in the AR group than in the AS group (OR = 1.632, $p = 0.006$), where the genotype of rs5924 (CT + TT) was significantly higher in the AR group than in the AS group (OR = 2.138, $p = 0.005$). And the frequency and genotype distribution of alleles at the rs12041338 locus were not significantly different between the two groups (OR = −0.204 vs. −0.316, *p*-value greater than 0.05, respectively).

In the study herein, the genotypes and allele frequencies of the 2 SNPs of the aspirin gene were examined separately, and the results showed that in the AR group, the T distribution of the rs5924 allele of the PEAR1 gene and GP gene was significantly higher than that of the AS group, and the frequency of the gene distribution of (CT + TT) was also significantly higher than that of the AS group. This indicates a significantly higher risk of AR in patients with CT/TT genotype. In contrast, there was no significant difference in the frequency and genotype distribution of alleles at the rs12041338 locus between the two groups. Our experimental results are consistent with the existing findings of related experts, who found that PEAR1 gene and GP gene polymorphisms can affect the activity or function of P-gp, which is closely related to the different individual responses to different drugs among individuals. However, there are not many studies on the gene expression of PEAR1 gene and GP gene in Chinese Han Chinese ischemic stroke patients. Overseas scholars and others found that the T allele at the rs5924 locus was associated with increased expression of P-gp in the human duodenum, as well as reduced bioavailability of P-gp-transported substrates. A recent meta-analysis also showed that the TT genotype at the rs5924 locus plays an important role in refractory epilepsy. Another study found a strong association between the TT genotype at the C1236T locus and the development of steroid resistance in childhood idiopathic nephrotic syndrome. A literature on Indian population reported that stroke patients with TT genotype at rs5924 locus had a significantly higher risk of AR than those with CC genotype and TT genotype was more associated with AR in patients with intracranial large atherosclerotic stroke. The results of our experiment are similar to those of the Indian population, i.e., the T allele of rs5924 may play a role in the development of AR.

**Table 4.** The frequency and genotype distribution characteristics of the point allele.

| - | AR | AS | $x^2$ | *P* | OR | 95%CI |
|---|---|---|---|---|---|---|
| Allele | | | | | | |
| rs12041338 | - | - | 3.452 | 0.237 | −0.204 | (−0.415, 1.306) |
| A | 34(31.78%) | 114(38.64%) | - | - | - | - |
| G | 73(68.22%) | 181(61.36%) | - | - | - | - |
| rs5924 | - | - | 7.524 | 0.006 | 1.632 | (1.126, 2.548) |
| C | 88(55.00%) | 182(43.33%) | - | - | - | - |
| T | 72(45.00%) | 238(56.67%) | - | - | - | - |

**Table 4.** (*Continued*).

| - | AR | AS | $x^2$ | *P* | OR | 95%CI |
|---|---|---|---|---|---|---|
| Genotype | | | | | | |
| rs12041338 | - | - | 7.084 | 0.225 | −0.316 | (−0.182, 0.937) |
| AA | 38(40.86%) | 61(27.36%) | - | - | - | - |
| AG | 42(45.16%) | 73(32.74%) | - | - | - | - |
| GG | 13(13.98%) | 89(39.90%) | - | - | - | - |
| rs5924 | - | - | 8.354 | 0.012 | - | - |
| CC | 22(25.88%) | 82(34.17%) | - | - | - | - |
| CT | 35(41.18%) | 107(44.58%) | 7.124 | 0.005 | 2.138 | (1.102, 3.984) |
| TT | 28(32.94%) | 51(21.25%) | - | - | - | - |

In addition, the correlation between PEAR1 gene and GP gene of aspirin gene on platelet aggregation rate was analyzed in this paper, and the specific results were shown in **Table 5**. The median (quartile) of platelet aggregation rate in patients with CC genotype was 18.42 (11.26–45.58) %, and the platelet aggregation rate in patients with CC genotype was significantly higher than that in patients with CT/TT genotype. The risk of aspirin resistance was higher in patients with CC genotype in GP1BA polymorphism, and this difference was statistically significant ($P < 0.01$). While the platelet aggregation rate of AA/AG/GG genotypes of PEAR1 gene ranged from 0.85% to 3.19% and the difference was statistically significant ($P < 0.01$), comparatively speaking, GP1BA gene had a higher effect on aspirin resistance than PEAR1 gene. It was shown that during the initial adhesion of platelets, platelet receptor glycoprotein 1B effectively binds to vWF and subendothelial collagen to form glycoprotein Ib/IX/V complexes, which together participate in platelet aggregation. At the same time, PEAR1 gene and GP1B gene can interact on vWF, which makes the reduction of platelet flow velocity at the site of mediated vascular injury and prolongs the contact time with reactive components of cellular matrix, which contributes to platelet activation. This process is key to inducing thrombosis leading to ischemic stroke.

**Table 5.** Gene polymorphism and platelet aggregation.

| Genotype | | Num | Platelet aggregation (%) | *Z* | *P* |
|---|---|---|---|---|---|
| | CC | 121 | 18.42(11.26~45.58) | | |
| GP1BA | CT | 10 | 10.57(8.41~82.87) | −6.982 | 0.000 |
| | TT | 1 | 1.24(0.35~6.93) | | |
| | AA | 26 | 3.19(1.58~6.95) | | |
| PEAR1 | AG | 67 | 1.72(1.14~3.28) | −7.541 | 0.009 |
| | GG | 39 | 0.85(0.26~1.83) | | |

## 3.5. Logistic regression analysis of AR influencing factors

The AR group was compared with the AS group with whether it was AR as the dependent variable, 1 = yes, 0 = no. A total of 21 analyzed indicators included in this study were screened for statistical significance after one-way screening including

low-density lipoprotein (LDL), platelet (PLT), fasting glucose (FPG), history of hypertension (HOH), and history of diabetes mellitus (HOD), and the assignment of the value of "1" for yes and '0' for no, were analyzed by unconditional binary logistic regression, and the results are shown in **Table 6**. From the coefficients of the fitted model, it can be seen that the regression coefficient of history of diabetes mellitus is 1.295 and its significance *p*-value is 0.008 < 0.01, which possesses significance at 1 level. This indicates that diabetes mellitus has a significant effect on the development of aspirin resistance, suggesting that diabetes mellitus may be an independent risk factor for the development of aspirin resistance.

By univariate and binary logistic regression analysis in the AR and AS groups, the 95% confidence intervals of PLT, LDL, and FPG did not contain 0, and all of them were positively associated with the vocalization of AR, which suggests that PLT, LDL, and FPG may be independent risk factors for the development of AR. In the univariate analysis of AR in this study, elevated LDL may be a risk factor for the development of AR in acute ischemic stroke, whereas there was no significant relationship between TC, TG, and HDL-C and the development of AR. In addition, diabetes mellitus is highly associated with acute coronary syndrome, atherosclerosis, recurrence of ischemic events and stent thrombosis. Also, many clinical studies have shown that diabetes mellitus is a risk factor for platelet hyperreactivity (HPR) in patients with ischemic stroke, and aspirin resistance is mainly associated with accelerated platelet renewal and platelet hyperreactivity, so diabetes mellitus is closely related to the development of AR.

**Table 6.** The logical regression analysis of AR influencing factors.

| Variable | Beta | S.E. | Wald | *P* | OR | 95%CI | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower | Upper |
| PLT | 0.931 | 0.193 | 3.176 | 0.179 | 2.553 | 1.248 | 3.749 |
| LDL | 0.165 | 0.327 | 0.208 | 0.624 | 1.182 | 0.527 | 2.356 |
| FPG | 0.027 | 0.055 | 0.087 | 0.761 | 1.027 | 0.895 | 1.128 |
| HOH | -0.163 | 0.462 | 0.115 | 0.735 | 0.845 | 0.351 | 2.114 |
| HOD | 1.279 | 0.485 | 6.249 | 0.008 | 3.939 | 1.382 | 9.585 |

## 4. Conclusion

Some studies have found that aspirin can partially improve the clinical effect of cerebral hemorheology and cerebral hemodynamics in the treatment of cerebral infarction, but the specific effect of aspirin varies from person to person, and some patients may also suffer from cardiovascular disease events, namely AR [26]. The article analyzed the joint role of PEAR1 gene and GP gene in the mechanism of aspirin resistance using 260 AIS patients from the Affiliated Hospital of Beihua university as study samples. The frequency of CC-type gene in GP1BA gene was as high as 92.03%, and the risk of CC-type was significantly increased in relation to TT-type of aspirin resistance.The ratio of AA-type gene, GA-type gene and GG type genes were 2:5:3. The incidence of rs5924 allele T was significantly higher in the AR group than in the AS group (OR = 1.632, $p = 0.006$), and the genotype of rs5924 (CT+TT) was significantly higher in the AR group than in the AS group (OR =

2.138, $p = 0.005$). History of diabetes possessed a high independent risk effect on the mechanism of aspirin resistance, with a Beta coefficient of 1.279 and a significant effect at the 1% level. Diabetes causes inhibition of aspirin acetylation, and the associated plasma proteins are competitive antagonists of the PEAR1 and GP1BA genes, which reduces platelet reactivity and leads to diminished platelet inhibition by aspirin. Thus, the PEAR1 and GP1BA genes may be related genes that influence aspirin resistance.

**Author contributions:** Conceptualization, YN and YG; methodology, YN; software, YN; validation, YN, YG and WG; formal analysis, YN; investigation, YN; resources, YN; data curation, YN and WG; writing—original draft preparation, YN and YG; writing—review and editing, YN and WG; visualization, YN; supervision, YG; project administration, YG; funding acquisition, YG. All authors have read and agreed to the published version of the manuscript.

**Funding:** Project of Jilin City Science and Technology Bureau, Project specification number: 20210409067. Project: Analysis of the Correlation between PEAR1, GP Gene Interactions, and Aspirin Resistance.

**Ethical approval:** The study was conducted in accordance with the Declaration of Helsinki, and approved by Medical Ethics Committee of Beihua University Affiliated Hospital (20240059). Informed consent was obtained from all subjects involved in the study.

**Conflict of interest:** The authors declare no conflict of interest.

## References

1. Powers, W. J. (2020). Acute ischemic stroke. New England Journal of Medicine, 383(3), 252-260.
2. Walter, K. (2022). What is acute ischemic stroke?. Jama, 327(9), 885-885.
3. Sommer, C. J. (2017). Ischemic stroke: experimental models and reality. Acta neuropathologica, 133(2), 245-261.
4. Khoshnam, S. E., Winlow, W., Farzaneh, M., Farbood, Y., & Moghaddam, H. F. (2017). Pathogenic mechanisms following ischemic stroke. Neurological Sciences, 38, 1167-1186.
5. Mendelson, S. J., & Prabhakaran, S. (2021). Diagnosis and management of transient ischemic attack and acute ischemic stroke: a review. Jama, 325(11), 1088-1098.
6. Catanese, L., Tarsia, J., & Fisher, M. (2017). Acute ischemic stroke therapy overview. Circulation research, 120(3), 541-558.
7. Barthels, D., & Das, H. (2020). Current advances in ischemic stroke research and therapies. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1866(4), 165260.
8. Hermann, D. M., & Kleinschnitz, C. (2019). Modeling vascular risk factors for the development of ischemic stroke therapies. Stroke, 50(5), 1310-1317.
9. Heo, J., Youk, T. M., & Seo, K. D. (2021). Anemia is a risk factor for the development of ischemic stroke and post-stroke mortality. Journal of Clinical Medicine, 10(12), 2556.
10. Vasudeva, K., & Munshi, A. (2019). Genetics of platelet traits in ischaemic stroke: focus on mean platelet volume and platelet count. International Journal of Neuroscience, 129(5), 511-522.
11. Yi, X., Lin, J., Luo, H., Wang, C., & Liu, Y. (2017). Genetic variants of PTGS2, TXA2R and TXAS1 are associated with carotid plaque vulnerability, platelet activation and TXA2 levels in ischemic stroke patients. PLoS One, 12(7), e0180704.
12. Alqahtani, Z., & Jamali, F. (2018). Clinical outcomes of aspirin interaction with other non-steroidal anti-inflammatory drugs: a systematic review. Journal of Pharmacy & Pharmaceutical Sciences, 21(1s), 48s-73s.
13. Xu, X. R., Yousef, G. M., & Ni, H. (2018). Cancer and platelet crosstalk: opportunities and challenges for aspirin and other antiplatelet agents. Blood, The Journal of the American Society of Hematology, 131(16), 1777-1789.

14. Hannachi, N., Baudoin, J. P., Prasanth, A., Habib, G., & Camoin-Jau, L. (2020). The distinct effects of aspirin on platelet aggregation induced by infectious bacteria. Platelets, 31(8), 1028-1038.
15. Wu, Q. L., Dong, J., Zeng, H. W., Lv, C., Liu, A. J., & Zhang, W. D. (2020). Monitoring antiplatelet aggregation in vivo and in vitro by microtiter plate method. Journal of cardiovascular pharmacology, 75(4), 314-320.
16. Alyavi, A. L., Khodjanova, S. I., Uzokov, J. K., & Kadirova, S. (2021). Aspirin resistance in patients with chronic coronary syndrome. Indian Journal of Forensic Medicine & Toxicology, 15(3), 1843-1846.
17. Zimmermann, P., & Curtis, N. (2018). The effect of aspirin on antibiotic susceptibility. Expert Opinion on Therapeutic Targets, 22(11), 967-972.
18. Christiansen, M., Grove, E. L., & Hvas, A. M. (2021, October). Contemporary clinical use of aspirin: mechanisms of action, current concepts, unresolved questions, and future perspectives. In Seminars in Thrombosis and Hemostasis (Vol. 47, No. 07, pp. 800-814). Thieme Medical Publishers, Inc..
19. Baturina, O., Andreev, D., Fedina, L., Mirzaev, K., Ivashchenko, D., Ryzhikova, K., ... & Sychev, D. (2022). Influence of clinically significant genes on antiplatelet effect of clopidogrel and clinical outcomes in patients with acute coronary syndrome and atrial fibrillation. Pharmacology, 107(3-4), 216-226.
20. O'connor, C. T., Kiernan, T. J., & Yan, B. P. (2017). The genetic basis of antiplatelet and anticoagulant therapy: A pharmacogenetic review of newer antiplatelets (clopidogrel, prasugrel and ticagrelor) and anticoagulants (dabigatran, rivaroxaban, apixaban and edoxaban). Expert opinion on drug metabolism & toxicology, 13(7), 725-739.
21. Wen Zhou,Bin Zhang,Keyu Fan,Xiaojian Yin,Jinfeng Liu & Shaohua Gou. (2021). An Original Aspirin-Containing Carbonic Anhydrase 9 Inhibitor Overcomes Hypoxia-Induced Drug Resistance to Enhance the Efficacy of Myocardial Protection. Cardiovascular Drugs and Therapy(4),1-14.
22. Ma Ji,Fan Zhenhai,Tang Qiulin,Xia Hongwei,Zhang Tao & Bi Feng. (2020). Aspirin attenuates YAP and β-catenin expression by promoting β-TrCP to overcome docetaxel and vinorelbine resistance in triple-negative breast cancer. Cell death & disease(7),530.
23. Yi Xingyang,Wang Chun,Zhou Qiang & Lin Jing. (2017). Interaction among COX-2, P2Y1 and GPIIIa gene variants is associated with aspirin resistance and early neurological deterioration in Chinese stroke patients. BMC neurology(1),4.
24. Lucas Sobral de Rossi,Raquel Pires Nakama,Lucas Felipe Dos Santos,Leonardo Berto Pereira,Aparecida Donizette Malvezi,Maria Isabel Lovo Martins... & Phileno Pinge Filho. (2024). Metabolic syndrome promotes resistance to aspirin in mitigating bone loss in murine periodontal disease. Life Sciences123224-123224.
25. Jun Liu,Linkun Pan,Sheng Wang,Yueran Li,Yilai Wu,Jiajie Luan & Kui Yang. (2024). Predicting laboratory aspirin resistance in Chinese stroke patients using machine learning models by GP1BA polymorphism. Pharmacogenomics11-12.
26. Parsa kondelaji Mohammad & Mansouritorghabeh Hassan. (2023). Aspirin and clopidogrel resistance; a neglected gap in stroke and cardiovascular practice in Iran: a systematic review and meta-analysis. Thrombosis Journal(1),79-79.