

## Polygenic Pharmacogenetic Score Forecasts Outcomes in Stroke Patients Receiving Aspirin Therapy

Karim Mostafa<sup>1</sup>, Ahmed El Sayed<sup>1</sup>, Mohamed Farouk<sup>1\*</sup>, Youssef Nabil<sup>1</sup>

<sup>1</sup>Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt.

\*E-mail ✉ [mohamed.farouk.pg@outlook.com](mailto:mohamed.farouk.pg@outlook.com)

Received: 03 December 2021; Revised: 27 February 2022; Accepted: 01 March 2022

### ABSTRACT

Aspirin remains a key treatment for acute ischemic stroke (AIS), though patient responses can vary widely. This study sought to develop a pharmacogenetic polygenic response score (PgxRS) capable of predicting adverse clinical outcomes in AIS patients receiving aspirin therapy. A retrospective cohort of 828 AIS patients treated with aspirin was analyzed. Fifteen candidate single nucleotide polymorphisms (SNPs) within genes influencing aspirin's pharmacodynamics, transport, metabolism, and platelet activity were genotyped. Logistic regression was used to examine the relationship between each SNP and poor prognosis, defined as a modified Rankin Scale score exceeding 1 at 90 days. Multivariable predictive models integrating both genetic and clinical factors were then constructed. Patients carrying the ABCB1 rs1045642 GG genotype showed a lower likelihood of poor outcomes, while the P2Y1 rs1371097 T allele was linked to higher risk. A predictive model incorporating these SNPs alongside relevant clinical variables achieved moderate discriminative performance for forecasting adverse outcomes (AUC = 0.78, 95% CI: 0.74–0.81). The findings indicate that variation in the ABCB1 and P2Y1 genes contributes to differences in aspirin responsiveness among AIS patients. The PgxRS developed in this study, combining these genetic variants with clinical information, offers potential utility in guiding individualized antiplatelet therapy and stratifying patient risk. Validation in larger, ethnically diverse populations is necessary to confirm these results.

**Keywords:** Personalized medicine, Aspirin, Single nucleotide variant, Acute ischemic stroke, Prognosis, pharmacogenetics

**How to Cite This Article:** Mostafa K, El Sayed A, Farouk M, Nabil Y. Polygenic Pharmacogenetic Score Forecasts Outcomes in Stroke Patients Receiving Aspirin Therapy. J Pharmacogn Phytochem Biotechnol. 2022;2:91-102. <https://doi.org/10.51847/n7vgoQXWUI>

### Introduction

Stroke remains a major public health concern worldwide, ranking as the second leading cause of death and a primary contributor to long-term disability [1, 2]. Estimates from the World Stroke Organization indicate that over 12 million individuals experience a first-ever stroke annually, with stroke-related mortality exceeding 6 million per year [2]. Preventing recurrent ischemic events is a cornerstone of post-stroke care, and antiplatelet agents play a critical role in this context [3]. Among these agents, aspirin is widely recognized for its efficacy, reducing the risk of recurrent stroke by roughly 15% in secondary prevention scenarios [4], thus maintaining a central role in the management of cerebrovascular and cardiovascular conditions.

Nevertheless, patient responses to aspirin therapy are highly variable. A considerable proportion of patients continue to experience thrombotic events despite adherence to treatment, a phenomenon termed aspirin resistance or non-responsiveness [5]. Such variability complicates clinical management and is associated with poorer long-term outcomes. While demographic characteristics, comorbidities, and adherence explain part of this variability [6], they do not fully account for the differences observed across individuals. Increasing evidence suggests that genetic variability significantly influences aspirin efficacy [7]. Polymorphisms in genes related to aspirin's mechanism of action, pharmacokinetics, and platelet regulation appear to contribute to these interindividual differences.

Several specific genetic variants have been implicated in modulating aspirin response. For example, rs1330344 in the COX-1 gene, which encodes cyclooxygenase-1—a key enzyme targeted by aspirin for prostaglandin synthesis and platelet aggregation inhibition—has been associated with aspirin resistance. Carriers of the G allele at this locus exhibit high on-treatment platelet reactivity (HTPR) and an elevated risk of cardiovascular complications [8]. Interactions between COX-1 rs3842787 and COX-2 rs20417 have also been shown to increase the likelihood of aspirin resistance, reducing platelet inhibition independently of other risk factors [9].

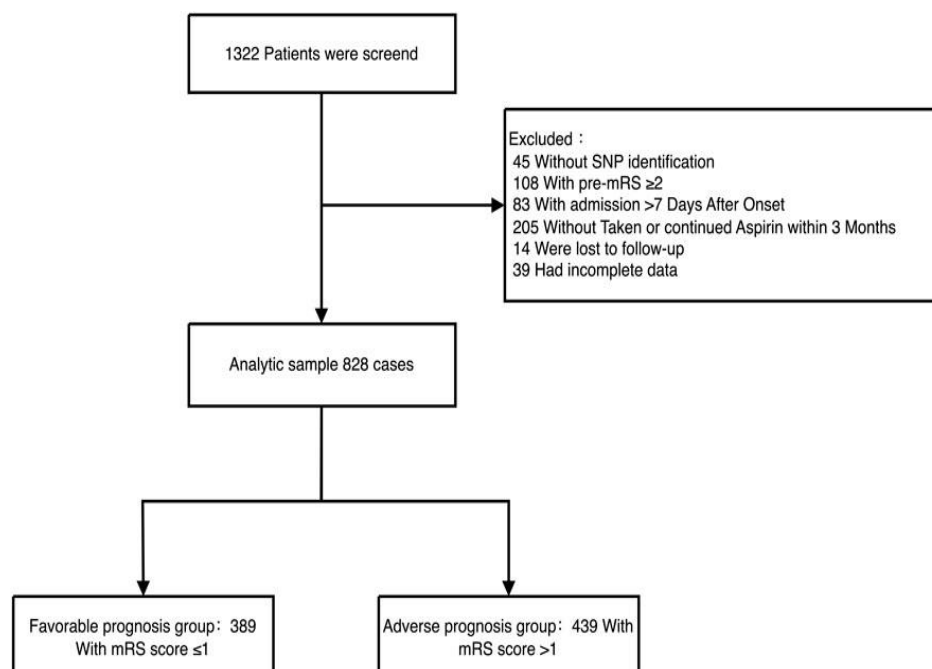
Other gene variants, such as COX-2 rs20417CC, P2Y1 rs1371097, and GPIIIa rs2317676, have similarly been linked to impaired aspirin response in stroke patients, likely through effects on platelet aggregation and activation [10]. Moreover, the ABCB1 gene variant rs1045642, which encodes the efflux transporter P-glycoprotein, has been associated with altered absorption and effectiveness of antiplatelet medications, including aspirin and clopidogrel, potentially contributing to therapeutic resistance [11].

This study examines 15 candidate single nucleotide polymorphisms (SNPs) in 828 patients with acute ischemic stroke receiving aspirin therapy, focusing on genes involved in drug action, transport, metabolism, and platelet regulation. The primary aim is to develop a pharmacogenetic polygenic response score (PgxRS) capable of predicting adverse clinical outcomes. Identifying genetic determinants of aspirin response may support personalized antiplatelet therapy strategies, improving patient prognosis and reducing the global burden of stroke.

## Materials and Methods

### Study design and participants

We conducted a retrospective, single-center analysis of 1,322 patients admitted with acute ischemic stroke to the Neurology Department of Yangpu District Central Hospital, Shanghai, between September 2016 and October 2020. Inclusion criteria required patients to start aspirin therapy (100 mg daily) within 24 hours of admission, continue long-term aspirin monotherapy (100 mg daily) beyond 21 days, and have complete clinical and genetic datasets. After applying predefined exclusion criteria (**Figure 1**), 828 patients were retained for analysis. Stroke subtypes were categorized according to the TOAST (Trial of ORG 10172 in Acute Stroke Treatment) classification. The study was approved by the Ethics Committee of Yangpu District Central Hospital, and written informed consent was obtained from all participants or their legal representatives.



**Figure 1.** Patient Selection Workflow. This diagram depicts the stepwise process used to identify eligible ischemic stroke patients at the Neurology Department of Yangpu District Central Hospital from September 2016 to October 2020. It outlines the initial cohort, the criteria and steps applied to screen patient records, reasons for exclusions, and the final number of patients included in the study.

*Outcome measures*

The primary endpoint was the patient's functional status at 90 days after stroke, assessed using the modified Rankin Scale (mRS). Patients with scores of 0 or 1 were considered to have favorable outcomes, whereas scores above 1 were classified as unfavorable [12].

Secondary endpoints included a composite of vascular events, covering recurrent ischemic or hemorrhagic stroke, myocardial infarction, and vascular mortality. Additionally, early neurological deterioration—defined as an increase of at least 2 points on the NIHSS within the first 3 days of admission—was evaluated [13]. All evaluations were verified independently by a minimum of two neurologists to ensure accuracy.

*Genomic DNA isolation*

Participants provided blood samples (3 mL) drawn from the brachial vein into EDTA-containing tubes and stored at  $-20^{\circ}\text{C}$ . Genomic DNA was subsequently extracted using magnetic bead-based separation with the Lab-Aid 820 Nucleic Acid Extraction Midi Kit, following the manufacturer's instructions, and stored at  $-20^{\circ}\text{C}$  for subsequent genotyping.

*Selection of genetic variants*

A total of 15 single nucleotide polymorphisms (SNPs) were chosen based on previous literature (**Table 1**). These variants were grouped according to their biological roles:

Aspirin Pharmacodynamics: COX-1 (rs1330344) and COX-2 (rs20417), which regulate prostaglandin synthesis and platelet aggregation [8-10].

Drug Transport and Metabolism: ABCB1 (rs1045642, rs1128503, rs4148727), CYP2C9 (rs1057910, rs1799853), CYP3A5 (rs776746), and NR1I2 (rs13059232), involved in drug absorption, distribution, and metabolic processing [11, 14-18].

Platelet Function: P2Y12 (rs16863323, rs2046934, rs9859538), P2Y1 (rs1371097), and GPIIIa (rs2317676, rs5918), which influence platelet activation and aggregation [9, 10, 15, 19-21].

All selected SNPs were verified for adherence to Hardy-Weinberg Equilibrium prior to analysis.

**Table 1.** Major SNPs Reported for aspirin.

SNP	Gene	Participants	Ethnic group	Genetic associations and outcome	References
Mechanism of action					
rs1330344	COX-1	A total of 431 ischemic stroke patients receiving aspirin therapy were included in the study.	Chinese	The presence of the G allele at the COX-1 rs1330344 locus has been linked to reduced responsiveness to aspirin.	[8]
rs20417	COX-2	The study included 850 patients diagnosed with ischemic stroke.	Chinese	Aspirin resistance has been linked to the combined effect of COX-2 (rs20417CC), P2Y1 (rs1371097), and GPIIIa (rs2317676) variants.	[9, 10]
Genetics variants influencing pharma codynamics					
rs1045642	ABCB1	The study enrolled 60 patients diagnosed with coronary artery disease.	Germany	The ABCB1 C3435T variant reduces P-glycoprotein activity, potentially limiting the absorption of antiplatelet agents like aspirin and clopidogrel and contributing to drug resistance.	[11]
rs1128503	ABCB1	A total of 157 patients receiving dual antiplatelet therapy with aspirin and clopidogrel were included.	Chinese	The ABCB1 rs1128503 variant may be associated with a lower risk of recurrent ischemic stroke in patients with intracranial arterial stenosis.	[15]
rs4148727	ABCB1	The study included 3,010 patients who had experienced a minor stroke or transient ischemic attack (TIA).	Chinese	Dual antiplatelet therapy with clopidogrel plus aspirin is associated with a reduced risk of stroke recurrence in patients with the ABCB1 -154TT (rs4148727) and 3435 CC (rs1045642) genotypes, compared to	[16]

aspirin treatment alone					
rs1057910	CYP2C9	A total of 578 patients with coronary artery disease were included in the study.	Chinese	The rs1057910 polymorphism is associated with an increased risk of elevated platelet reactivity.	[18]
rs1799853	CYP2C9	The study included 26 patients with NSAID-related gastroduodenal bleeding confirmed by endoscopy and 56 control participants.	Italy	The CYP2C9*2 (R144C) and CYP2C9*3 (I359L) variants have been reported to decrease CYP2C9 enzyme activity.	[14]
rs776746	CYP3A5	A total of 578 patients diagnosed with coronary artery disease were included in the study.	Chinese	The rs776746 polymorphism is associated with a reduced risk of elevated platelet reactivity.	[18]
rs13059232	NR1I2	The study included 634 patients receiving treatment with either aspirin or clopidogrel.	Chinese	The NR1I2 rs13059232 polymorphism has been identified as an independent predictor of long-term clinical outcomes in patients treated with clopidogrel, whereas no significant association was observed in the aspirin-treated cohort.	[17]
Genetics variants of platelet function					
rs16863323	P2Y12	426 patients diagnosed with acute minor ischemic stroke	Chinese	A significant interaction exists between the rs16863323 and rs2317676 polymorphisms, both of which are independently linked to reduced antiplatelet drug responsiveness and a higher risk of major cardiovascular events in patients with minor stroke.	[21]
rs2046934	P2Y12	268 patients with symptomatic extracranial or intracranial arterial stenosis	Chinese	Individuals carrying the A allele of P2Y12 rs2046934 show a significantly higher risk of recurrent ischemic events.	[20]
rs9859538	P2Y12	157 patients receiving dual antiplatelet therapy (aspirin plus clopidogrel)	Chinese	The P2Y12 rs9859538 and rs10935842 polymorphisms are linked to a higher risk of recurrent ischemic events.	[15]
rs1371097	P2Y1	850 ischemic stroke patients treated with aspirin	Chinese	Aspirin resistance is associated with the combined presence of the rs20417CC, rs1371097TT, and rs2317676GG genotypes.	[9, 10]
rs2317676	GPIIIa	850 patients diagnosed with ischemic stroke	Chinese	The GPIIIa rs2317676GG variant in the platelet glycoprotein gene is linked to both aspirin resistance and an increased risk of recurrent vascular events.	[9, 21]
rs5918	GPIIIa	30 PIA1/A1 and 30 PIA1/A2 patients with coronary artery disease	America	Patients carrying the PIA2 risk allele demonstrate elevated platelet reactivity while undergoing aspirin therapy.	[19]

### SNP genotyping

In this study, all 15 targeted SNPs were analyzed using a custom multiplexed tagged-amplicon deep sequencing approach previously established by our group. For this purpose, 15 specific primer pairs were designed with Primer3 and synthesized to include universal adapter sequences at the 5' ends, as outlined by Zou *et al.* (2018). The workflow involved pre-amplification of the target regions, enzymatic cleanup with SAP-Exo1, a second PCR incorporating unique barcodes, followed by DNA library quantification and purification. Sequencing was performed on the NovaSeq 6,000 platform. Raw 150 bp paired-end reads were mapped to the human reference genome using Burrows-Wheeler Aligner (v0.7.17). Subsequent steps, including local realignment, recalibration of base quality scores, variant calling, and filtering, were carried out using the Genome Analysis Toolkit (v4.3), following established procedures.

*Statistical analysis*

Data analysis was performed in SPSS version 22. Continuous variables were checked for normal distribution using the Kolmogorov-Smirnov test. Variables with normal distribution are expressed as mean  $\pm$  standard deviation (SD) and compared with independent t-tests, while non-normal variables are reported as median (interquartile range, IQR) and analyzed using the Mann-Whitney U test. Categorical variables are presented as counts and percentages, and evaluated using Pearson's chi-square or Fisher's exact test depending on data characteristics.

Associations between SNP genotypes and the likelihood of unfavorable outcomes were assessed using multivariate logistic regression under additive, dominant, and recessive models. The recessive model considered only homozygous variants as relevant (BB vs. AA + AB), the dominant model combined heterozygous and homozygous variants (AB + BB vs. AA), and the additive model assumed a stepwise genotype effect (BB vs. AB vs. AA). Variables found to differ significantly between outcome groups in univariate analysis were included in multivariate models, and strongly correlated factors were removed to prevent multicollinearity.

Sex-specific subgroup analyses were performed using multivariate logistic regression to investigate SNP associations with outcomes separately in males and females. A Pharmacogenetic Polygenic Response Score (PgxRS) was calculated by weighting each included variable according to its  $\beta$  coefficient from the multivariate regression. The combined predictive ability of SNPs and clinical factors was evaluated with receiver operating characteristic (ROC) curve analysis, and the area under the curve (AUC) was used to measure discriminative performance. Statistical significance was defined as  $p < 0.05$ .

**Results and Discussion***Patient characteristics*

Among the 828 participants, 389 (47.0%) achieved a favorable 90-day outcome (mRS  $\leq 1$ ), whereas 439 (53.0%) had an unfavorable outcome (mRS  $> 1$ ). Patients with poorer outcomes were generally older, presented with higher NIHSS scores at admission, and more often had a history of diabetes or previous stroke. In contrast, they were less likely to be male and had a lower frequency of smoking history (**Table 2**).

**Table 2.** Characteristics of baseline clinical data according to prognosis group.

	Favorable prognosis group	Adverse prognosis group	P
Number of cases, n	389	439	
Age (years)	65.62 $\pm$ 10.95	73.41 $\pm$ 12.01	0.00
Age Groups			0.00
0–60	120 (30.85)	69 (15.72)	
61–70	155 (39.85)	122 (27.79)	
71–80	69 (17.74)	97 (22.10)	
>80	45 (11.57)	151 (34.40)	
Gender (Male, n (%))	274 (70.44)	265 (60.36)	0.00
Body Mass Index (BMI, kg/m <sup>2</sup> )	24.15 $\pm$ 3.41	24.42 $\pm$ 3.59	0.29
Medical history, n (%)			
Hypertension	294 (75.58)	331 (75.40)	0.98
Diabetes	112 (28.79)	157 (35.76)	0.04
Coronary heart disease	38 (9.77)	60 (13.67)	0.10
Stroke	63 (16.20)	111 (25.28)	0.00
Smoking	198 (50.90)	180 (41.00)	0.01
Admission NIHSS score, Median (IQR)	2 (1, 4)	4 (2, 8)	0.00
TOAST classification, n (%)			0.00
Large artery atherosclerosis	168 (43.19)	230 (52.39)	

Cardioembolic	24 (6.17)	44 (10.02)	
Small artery occlusion	173 (44.47)	147 (33.49)	
Other etiology	4 (1.03)	8 (1.82)	
Unknown etiology	20 (5.14)	10 (2.28)	
Medication during hospitalization, n (%)			0.13
Aspirin	111 (28.53)	154 (35.08)	
Aspirin + Clopidogrel	267 (68.64)	274 (62.41)	
Aspirin + Other	11 (2.83)	11 (2.51)	
Statins medication	355 (91.26)	406 (92.48)	0.61
Laboratory indicators, Mean $\pm$ SD			
White Blood Cell count (WBC, $\times 10^9/L$ )	7.48 $\pm$ 2.47	7.83 $\pm$ 2.77	0.06
Red Blood Cell count (RBC, $\times 10^{12}/L$ )	4.53 $\pm$ 0.57	4.36 $\pm$ 0.64	0.00
Platelet count (PLT, $\times 10^9/L$ )	220.31 $\pm$ 65.01	218.07 $\pm$ 69.84	0.64
C-Reactive Protein (CRP, mg/L)	9.82 $\pm$ 18.61	16.59 $\pm$ 30.13	0.00
Neutrophil Count (NEUT#, $\times 10^9/L$ )	5.03 $\pm$ 2.35	5.60 $\pm$ 2.59	0.00
Lymphocyte Count (LYMPH#, $\times 10^9/L$ )	1.75 $\pm$ 0.70	1.56 $\pm$ 0.94	0.00
Creatinine (Cr, mg/dL)	74.25 $\pm$ 44.94	75.96 $\pm$ 60.99	0.67
Uric Acid (UA, mg/dL)	327.98 $\pm$ 101.11	319.29 $\pm$ 106.55	0.24
Fasting Plasma Glucose (FPG, mg/dL)	7.15 $\pm$ 3.16	7.64 $\pm$ 3.38	0.03
Glycated Hemoglobin (HbA1c, %)	6.83 $\pm$ 1.80	7.04 $\pm$ 1.89	0.10
Triglycerides (TG, mg/dL)	1.59 $\pm$ 0.97	1.56 $\pm$ 1.29	0.75
Total Cholesterol (CHOL, mg/dL)	4.80 $\pm$ 1.21	4.84 $\pm$ 1.30	0.68
High-Density Lipoprotein cholesterol (HDL-c, mg/dL)	1.12 $\pm$ 0.26	1.11 $\pm$ 0.29	0.81
Low-Density Lipoprotein cholesterol (LDL-c, mg/dL)	3.12 $\pm$ 0.89	3.14 $\pm$ 0.91	0.76
Homocysteine (Hcy, $\mu\text{mol}/L$ )	18.25 $\pm$ 10.84	19.66 $\pm$ 13.84	0.11
AA Inhibition Rate(%)	66.31 $\pm$ 23.67	68.53 $\pm$ 24.52	0.31
Endpoint events, n (%)			
Early neurological deterioration	10 (2.57)	70 (15.95)	0.00
cardiovascular and cerebrovascular composite events	20 (5.14)	41 (9.34)	0.03
Hospital readmission for treatment	64 (16.45)	139 (31.66)	0.00

Note: Bold values indicate statistical significance ( $P < 0.05$ ).

Laboratory results indicated that individuals who experienced an unfavorable prognosis had notably elevated fasting glucose, C-reactive protein (CRP), and neutrophil counts compared with those who recovered more favorably. Conversely, red blood cell and lymphocyte levels were reduced in this group (**Table 2**).

Regarding secondary outcomes, patients with poor prognosis faced significantly higher occurrences of early neurological decline, cardiovascular or cerebrovascular events, and subsequent hospital readmissions for treatment, with all differences reaching statistical significance ( $P < 0.05$ ).

#### *Genotype patterns and clinical associations*

After excluding two SNPs due to very low mutation rates, analysis of the remaining 15 variants revealed that the distribution of rs1045642 (ABCB1) and rs1371097 (P2Y1) differed significantly between patients with favorable versus unfavorable outcomes (**Table 3**).



**Table 3.** Characteristics of genotype data according to prognosis group.

SNPs	Ref/ Alt	Favorable prognosis group (n = 389)			Adverse prognosis group (n = 439)			Allele(P)	Genotype(P)		
		AA (%)	AB (%)	BB(%)	AA (%)	AB (%)	BB(%)		Dominant	Additive	Recessive
rs1045642	A>G	98 (27.61)	126 (35.49)	131 (36.90)	105 (25.80)	185 (45.45)	117 (28.75)	0.24	0.63	0.01	0.02
rs1057910	A>C	361 (92.80)	28 (7.20)	0 (0.00)	414 (94.31)	25 (5.69)	0 (0.00)	0.47	0.46	0.46	1
rs1128503	A>G	170 (43.70)	179 (46.02)	40 (10.28)	199 (45.33)	192 (43.74)	48 (10.93)	0.87	0.69	0.8	0.85
rs13059232	T>C	64 (16.45)	181 (46.53)	144 (37.02)	74 (16.86)	211 (48.06)	154 (35.08)	0.66	0.95	0.84	0.61
rs1330344	C>T	44 (11.31)	201 (51.67)	144 (37.02)	69 (15.72)	206 (46.92)	164 (37.36)	0.42	0.08	0.14	0.98
rs1371097	C>T	209 (53.73)	154 (39.59)	26 (6.68)	206 (46.92)	188 (42.82)	45 (10.25)	0.02	0.06	0.06	0.09
rs16863323	C>T	96 (24.81)	231 (59.69)	60 (15.50)	103 (23.46)	256 (58.31)	80 (18.22)	0.44	0.71	0.57	0.34
rs1799853	C>T	388 (99.74)	1 (0.26)	0 (0.00)	439 (100.00)	0 (0.00)	0 (0.00)	0.95	0.95	0.95	1
rs20417	C>G	346 (88.95)	40 (10.28)	3 (0.77)	384 (87.47)	52 (11.85)	3 (0.68)	0.63	0.58	0.77	0.79
rs2046934	G>A	7 (1.80)	117 (30.08)	265 (68.12)	10 (2.28)	137 (31.21)	292 (66.51)	0.62	0.81	0.82	0.68
rs2317676	A>G	245 (62.98)	130 (33.42)	14 (3.60)	284 (64.69)	134 (30.52)	21 (4.78)	0.94	0.66	0.52	0.5
rs4148727	A>G	307 (78.92)	80 (20.57)	2 (0.51)	353 (80.41)	82 (18.68)	4 (0.91)	0.78	0.66	0.64	0.79
rs5918	T>C	385 (98.97)	4 (1.03)	0 (0.00)	436 (99.77)	1 (0.23)	0 (0.00)	0.3	0.3	0.3	1
rs776746	C>T	207 (53.21)	151 (38.82)	31 (7.97)	220 (50.11)	187 (42.60)	32 (7.29)	0.62	0.41	0.54	0.81
rs9859538	G>A	299 (76.86)	78 (20.05)	12 (3.08)	328 (74.89)	96 (21.92)	14 (3.20)	0.59	0.56	0.8	0.91

Notes:

- A: Denotes the reference (wild-type or common) allele.
- B: Denotes the alternative (variant or minor) allele.
- AA: Homozygous genotype carrying two reference alleles.
- AB: Heterozygous genotype carrying one reference allele and one alternative allele.
- BB: Homozygous genotype carrying two alternative alleles.
- Allele (P): P-value assessing differences in allele frequencies between groups.
- Genotype (P): P-value evaluating differences in genotype distribution between groups.

Values shown in bold indicate statistically significant differences ( $P < 0.05$ ).

**Table 4** shows that, after accounting for potential confounding factors, individuals with the rs1045642 GG genotype had a notably lower likelihood of experiencing an unfavorable prognosis under the recessive model of logistic regression (Model 1: adjusted OR = 0.66, 95% CI: 0.48–0.92,  $P = 0.01$ ; Model 2: adjusted OR = 0.67, 95% CI: 0.49–0.93,  $P = 0.01$ ). In contrast, the presence of the rs1371097 T allele was associated with an elevated risk of poor outcomes when analyzed using the additive model, indicating a dose-dependent effect (Model 1: adjusted OR = 1.27, 95% CI: 1.00–1.61,  $P = 0.05$ ; Model 2: adjusted OR = 1.30, 95% CI: 1.03–1.64,  $P = 0.03$ ).

**Table 4.** Correlation between SNPs and unfavorable prognosis in ischemic stroke after adjusting for confounding factors.

	Genotype	Favorable prognosis group (n = 389)		Adverse prognosis group (n = 439)		Model1			Model2		
		n	%	n	%	OR	95% CI	P	OR	95% CI	P
rs1045642	A-G										
Dominant	AA	98	27.61%	105	25.80%	1.02	[0.72, 1.45]	0.91	1.01	[0.71, 1.44]	0.96
	GG + AG	257	72.39%	302	74.20%						
Additive	AA	98	27.61%	105	25.80%	0.88	[0.74, 1.04]	0.12	0.88	[0.74, 1.04]	0.13
	AG	126	35.49%	185	45.45%						
	GG	131	36.90%	117	28.75%						
Recessive	AA + AG	224	63.10%	290	71.25%	0.66	[0.48, 0.91]	0.01	0.67	[0.48, 0.92]	0.01
	GG	131	36.90%	117	28.75%						
rs1371097	C-T										
Dominant	CC	209	53.73%	206	46.92%	1.29	[0.95, 1.76]	0.10	1.33	[0.97, 1.81]	0.07
	TT + CT	180	46.27%	233	53.08%						
Additive	CC	209	53.73%	206	46.92%	1.27	[1.0, 1.62]	0.05	1.30	[1.02, 1.66]	0.03
	CT	154	39.59%	188	42.82%						
	TT	26	6.68%	45	10.25%						
Recessive	CC + CT	363	93.32%	394	89.75%	1.57	[0.9, 2.73]	0.11	1.63	[0.94, 2.84]	0.08
	TT	26	6.68%	45	10.25%						

• Model 1: Adjusted for age, sex, prior history of diabetes, previous stroke, TOAST classification, and NIHSS score at admission.

• Model 2: Includes all variables from Model 1 except for diabetes history, and additionally adjusts for fasting blood glucose, red blood cell count, C-reactive protein (CRP), neutrophil count, and lymphocyte count.

Note: Values shown in bold represent statistically significant differences ( $P < 0.05$ ).

### Subgroup analysis

To explore potential sex-specific effects, analyses were conducted separately for male ( $n = 539$ ) and female ( $n = 289$ ) participants. Among male patients, 274 (approximately 51%) achieved favorable functional outcomes. After controlling for relevant confounders, the rs1045642 GG genotype in the recessive model was linked to a lower likelihood of adverse prognosis (Model 1: adjusted OR = 0.66, 95% CI: 0.45–0.97,  $P = 0.04$ ; Model 2: adjusted OR = 0.67, 95% CI: 0.45–0.98,  $P = 0.04$ ), whereas rs1371097 did not demonstrate any significant association with outcomes ( $P > 0.05$ ).

In the female subgroup, 115 patients (about 40%) had favorable outcomes. Similar to males, the rs1045642 GG genotype was inversely associated with poor prognosis in the recessive model (Model 1: adjusted OR = 0.66, 95% CI: 0.48–0.90,  $P = 0.01$ ; Model 2: adjusted OR = 0.66, 95% CI: 0.48–0.91,  $P = 0.01$ ). Additionally, when analyzed under the dominant model, carriers of the rs1371097 TT or TC genotypes were at higher risk for adverse outcomes (Model 1: adjusted OR = 1.28, 95% CI: 1.00–1.62,  $P = 0.05$ ; Model 2: adjusted OR = 1.30, 95% CI: 1.03–1.66,  $P = 0.03$ ).

### Construction of a predictive model for adverse stroke outcomes

Building on these findings, two multivariate logistic regression models (Model 1 and Model 2) (**Table 5**) were developed that combined the rs1045642 and rs1371097 genotypes with key clinical factors to estimate the risk of poor prognosis following stroke.

**Table 5.** Results of multivariable logistic regression analysis for rs1045642 and rs1371097 SNP genotypes in predicting adverse prognosis in stroke.



	<b>Beta</b>	<b>OR</b>	<b>P</b>	<b>0.025</b>	<b>0.975</b>
const	-4.49	0.01	0.00	-5.59	-3.38
rs1045642	-0.41	0.66	0.01	-0.73	-0.09
rs1371097	0.24	1.27	0.05	0.00	0.48
sex	-0.03	0.97	0.88	-0.37	0.32
age	0.05	1.06	0.00	0.04	0.07
history of diabetes	0.45	1.57	0.01	0.12	0.78
history of stroke	0.41	1.51	0.04	0.03	0.79
TOAST classification	-0.03	0.97	0.67	-0.17	0.11
NIHSS score at admission	0.18	1.19	0.00	0.13	0.22

The table summarizes the regression coefficients, odds ratios (ORs), and corresponding p-values for each SNP and genotype included in the prediction models (Model 1 and Model 2). These metrics reflect both the magnitude and statistical significance of the relationship between the genetic variants and the risk of adverse stroke outcomes, after accounting for the covariates incorporated in each model.

Adverse prognosis prediction model 1:

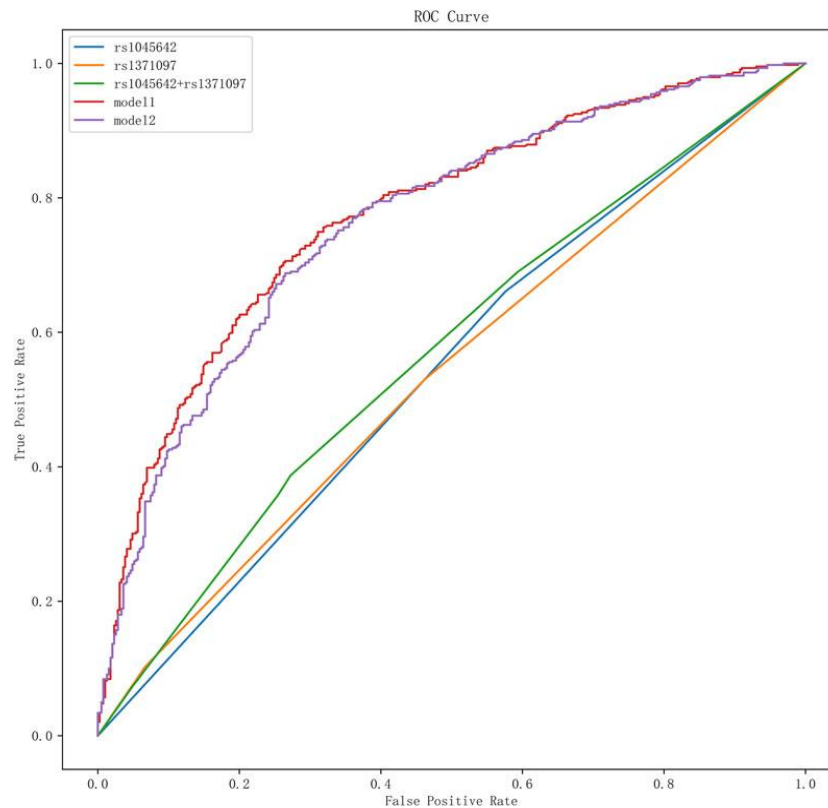
$$\hat{y} = \frac{1}{1 + e^{4.50 - 0.41x_1 + 0.23x_2 - 0.03x_3 + 0.05x_4 + 0.45x_5 + 0.18x_6 - 0.03x_7 + 0.41x_8}} \quad (1)$$

In the prediction models, the variables were defined as follows: x1 corresponds to the rs1045642 genotype (0 = AA or AG, 1 = GG), x2 to the rs1371097 genotype (0 = CC, 1 = CT, 2 = TT), x3 to sex (0 = female, 1 = male), x4 to age in years, x5 to a history of diabetes (0 = no, 1 = yes), x6 to the NIHSS score at admission, x7 to TOAST classification (1 = large artery, 2 = cardioembolic, 3 = small artery occlusion, 4 = other causes, 5 = undetermined), and x8 to prior stroke history (0 = no, 1 = yes).

Receiver operating characteristic (ROC) curve analysis showed that Model 1, using an optimal cutoff of 0.53, achieved 70% sensitivity and 75% specificity for predicting unfavorable outcomes. The area under the curve (AUC) for Model 1 was 0.78 (95% CI: 0.74–0.81,  $P < 0.05$ ), reflecting moderate discriminative ability. Model 2, which incorporated additional clinical parameters, did not improve predictive performance over Model 1 (AUC = 0.78, 95% CI: 0.75–0.81,  $P < 0.05$ ). Detailed AUC values and other model performance metrics are presented in **Table 6** and illustrated in **Figure 2**.

**Table 6.** Diagnostic values of the two prediction models.

	<b>Model1</b>	<b>Model2</b>
AUC	0.78	0.78
AUC Confidence Interval	[0.74,0.81]	[0.75,0.81]
Optimal Cutoff Value	0.53	0.51
Sensitivity	0.70	0.72
Specificity	0.75	0.74



**Figure 2.** Receiver operating characteristic (ROC) curves illustrating the predictive performance of individual SNPs and combined models for identifying patients at risk of poor stroke outcomes. Each curve represents the ability of a specific genetic marker or model to discriminate between favorable and unfavorable prognosis.

In this study, we examined the role of genetic variation in influencing aspirin response among patients with acute ischemic stroke, focusing on 15 candidate SNPs. Our analysis revealed that the GG genotype of rs1045642 within the ABCB1 gene was associated with a decreased likelihood of poor outcomes, whereas the presence of the T allele in rs1371097 of the P2Y1 gene corresponded to an increased risk. These findings underscore the importance of genetic differences in modulating aspirin effectiveness and suggest avenues for personalized antiplatelet therapy.

The ABCB1 gene encodes the drug transporter P-glycoprotein, which is critical for regulating the absorption and systemic distribution of medications, including aspirin [22]. Previous reports have linked the rs1045642 variant to altered transporter function and aspirin resistance [16, 23, 24]. Consistent with these studies, our data suggest that individuals carrying the GG genotype may experience enhanced aspirin uptake, potentially resulting in greater therapeutic benefit. Moreover, evidence indicates that ABCB1 overexpression in the small intestine can protect against aspirin-induced epithelial damage [25], which may reduce gastrointestinal complications and improve long-term adherence to therapy. These observations highlight the need for further research into how ABCB1 variants influence both drug response and adverse gastrointestinal effects in chronic aspirin users.

P2Y1 encodes a platelet surface receptor central to platelet activation and aggregation [26]. Prior studies have suggested that rs1371097 can alter platelet responsiveness to aspirin [10, 27]. A meta-analysis indicated that the TT + CT genotypes of this SNP were more prevalent among stroke patients exhibiting aspirin resistance [10]. In line with this, our study found that carriers of these genotypes had a higher risk of poor outcomes, likely due to increased platelet aggregation and reduced aspirin efficacy.

Overall, our findings support the significant contribution of rs1045642 and rs1371097 variants to stroke prognosis. Importantly, analyses stratified by sex revealed differing effects of these SNPs. The protective effect of rs1045642 GG was observed in both males and females. In contrast, rs1371097 showed a sex-specific influence: the TT + TC genotypes were linked to adverse outcomes in female patients but not in males. This pattern remained after adjusting for confounding factors, suggesting that rs1371097 may affect stroke outcomes through mechanisms that differ between men and women.

Previous research has shown that although stroke occurs less frequently in females than in males, women tend to experience worse outcomes after stroke [28]. Variations in hormone levels, gene expression, and sensitivity to inflammatory responses between sexes may modulate the effect of rs1371097 on stroke prognosis [29]. For example, estrogen has been reported to provide neuroprotective effects in females [30]. However, whether the rs1371097 variant interacts with estrogen signaling to influence stroke outcomes remains unclear. Investigating this potential pathway could shed light on sex-based differences in stroke prognosis and should be addressed in future studies.

In this work, we developed a molecular prediction model incorporating rs1045642, rs1371097, and key clinical variables—including age, sex, diabetes history, prior cerebral infarction, TOAST classification, admission NIHSS score, fasting blood glucose, red blood cell count, CRP, neutrophil count, and lymphocyte count. This integrative model demonstrated superior predictive performance for adverse stroke outcomes compared with any single factor. Given the multifactorial nature of stroke prognosis, individual gene variants have modest effects on clinical outcomes. No single factor can fully predict risk or prevent adverse events, highlighting the value of combining genetic and clinical information. We therefore recommend constructing predictive scoring systems that integrate multiple factors to better evaluate the long-term efficacy of aspirin therapy in acute stroke patients. Such comprehensive models can more accurately stratify patient risk and inform clinical decision-making.

Several limitations should be noted. First, as a single-center, retrospective study conducted exclusively in the Han Chinese population, the generalizability of our findings to other ethnic groups is uncertain and requires further validation. Second, although all participants were on aspirin, the concomitant use of other antiplatelet agents was not strictly controlled, and potential interactions between genetic variants and other medications were not explored. Third, despite collecting detailed follow-up data—including 90-day mRS, NIHSS scores, cardiovascular and cerebrovascular events, and mortality—platelet reactivity measurements were unavailable for many patients, limiting mechanistic interpretation. Fourth, rs1045642 did not conform to Hardy-Weinberg equilibrium (HWE) in this cohort; while this may indicate a role in disease susceptibility, deviations from HWE require cautious interpretation. Larger, multi-ethnic studies are needed to clarify its impact on stroke outcomes. Finally, after Bonferroni correction for multiple comparisons, no genetic variant reached statistical significance, likely due to limited sample size, small effect sizes, or genetic heterogeneity. Future investigations with larger cohorts integrating genetic and clinical data are essential to validate these findings.

Despite these limitations, this study proposes a practical polygenic pharmacogenetic risk score and identifies potential associations between specific SNPs and adverse stroke outcomes in aspirin-treated patients. Further research into these genetic variants could help guide personalized treatment strategies, optimize drug efficacy, and reduce the risk of poor outcomes.

## Conclusion

Our findings indicate that the rs1045642 GG genotype is linked to a lower likelihood of unfavorable outcomes, whereas the rs1371097 T allele is associated with increased risk in acute ischemic stroke patients receiving aspirin. Incorporating these SNPs along with relevant clinical factors into a predictive scoring system may provide a valuable tool for assessing long-term prognosis. Although further validation is needed, this study underscores the potential of pharmacogenetic approaches to support personalized antiplatelet therapy and improve outcomes in stroke patients.

**Acknowledgments:** We are grateful to all the people who participated in our study.

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Financial Support:** The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This study is supported by the National Natural Science Foundation of China (32170582), the Special Clinical Research Project of Yangpu District (YPM202305). The Science and Technology Commission of Shanghai Municipality (18411970100). Shanghai Municipal Planning Commission of science and Research Fund (20204Y0123).

**Ethics Statement:** None.

## References

1. Ma Q, Li R, Wang L, Yin P, Wang Y, Yan C, et al. Temporal trend and attributable risk factors of stroke burden in China, 1990-2019: an analysis for the Global Burden of Disease Study 2019. *Lancet Public Health*. 2021;6(12):e897-e906.
2. Feigin VL, Brainin M, Norrving B, Martins S, Sacco RL, Hacke W, et al. World stroke organization (WSO): global stroke fact sheet 2022. *Int J Stroke*. 2022;17(1):18-29.
3. Bhatia K, Jain V, Aggarwal D, Vaduganathan M, Arora S, Hussain Z, et al. Dual antiplatelet therapy versus aspirin in patients with stroke or transient ischemic attack: meta-analysis of randomized controlled trials. *Stroke*. 2021;52(6):e217-e23.
4. Hankey GJ. Secondary stroke prevention. *Lancet Neurol*. 2014;13(2):178-94.
5. Kim JT, Heo SH, Lee JS, Choi MJ, Choi KH, Nam TS, et al. Aspirin resistance in the acute stages of acute ischemic stroke is associated with the development of new ischemic lesions. *PLoS One*. 2015;10(4):e0120743.
6. Yi X, Zhou Q, Lin J, Chi L. Aspirin resistance in Chinese stroke patients increased the rate of recurrent stroke and other vascular events. *Int J Stroke*. 2013;8(7):535-9.
7. Silva G, Lopes BM, Moser V, Ferreira LE. Impact of pharmacogenetics on aspirin resistance: a systematic review. *Arq Neuropsiquiatr*. 2023;81(1):62-73.
8. Li XL, Cao J, Fan L, Wang Q, Ye L, Cui CP, et al. Genetic polymorphisms of HO-1 and COX-1 are associated with aspirin resistance defined by light transmittance aggregation in Chinese Han patients. *Clin Appl Thromb Hemost*. 2013;19(5):513-21.
9. Yi X, Cheng W, Lin J, Zhou Q, Wang C. Interaction between COX-1 and COX-2 variants associated with aspirin resistance in Chinese stroke patients. *J Stroke Cerebrovasc Dis*. 2016;25(9):2136-44.
10. Yi X, Wang C, Zhou Q, Lin J. Interaction among COX-2, P2Y1 and GPIIIa gene variants is associated with aspirin resistance and early neurological deterioration in Chinese stroke patients. *BMC Neurol*. 2017;17(1):4.
11. Taubert D, von Beckerath N, Grimberg G, Lazar A, Jung N, Goesser T, et al. Impact of P-glycoprotein on clopidogrel absorption. *Clin Pharmacol Ther*. 2006;80(5):486-501.
12. Chen S, Chen Z, Xu Q, Jiang X, Lin C, Ji J. Dual effects of serum urate on stroke risk and prognosis: insights from Mendelian randomization. *Front Neurol*. 2024;15:1359292.
13. Gong C, Huang J, Qiu Z, Guo M, Chen L, Sang H, et al. Association of conscious sedation with dexmedetomidine and outcome in stroke patients undergoing thrombectomy in the DEVT and RESCUE-BT trials. *Neurology*. 2024;103(11):e209953.
14. Pilotto A, Seripa D, Franceschi M, Scarcelli C, Colaizzo D, Grandone E, et al. Genetic susceptibility to nonsteroidal anti-inflammatory drug-related gastroduodenal bleeding: role of cytochrome P450 2C9 polymorphisms. *Gastroenterology*. 2007;133(2):465-71.
15. Li X, Jiang L, Sun S, Li W, Li X, Miao Z, et al. The influence of ABCB1 and P2Y12 genetic variants on clinical outcomes in Chinese intracranial artery stenosis patients. *Clin Exp Pharmacol Physiol*. 2018;45:978-82.
16. Pan Y, Chen W, Wang Y, Li H, Johnston SC, Simon T, et al. Association between ABCB1 polymorphisms and outcomes of clopidogrel treatment in patients with minor stroke or transient ischemic attack: secondary analysis of a randomized clinical trial. *JAMA Neurol*. 2019;76(5):552-60.
17. Chen YB, Zhou ZY, Li GM, Xiao CX, Yu WB, Zhong SL, et al. Influences of an NR1I2 polymorphism on heterogeneous antiplatelet reactivity responses to clopidogrel and clinical outcomes in acute ischemic stroke patients. *Acta Pharmacol Sin*. 2019;40(6):762-8.
18. Zou X, Deng XL, Wang YM, Li JH, Liu L, Huang X, et al. Genetic polymorphisms of high platelet reactivity in Chinese patients with coronary heart disease under clopidogrel therapy. *Int J Clin Pharm*. 2020;42(1):158-66.
19. Cooke GE, Liu-Stratton Y, Ferketich AK, Moeschberger ML, Frid DJ, Magorien RD, et al. Effect of platelet antigen polymorphism on platelet inhibition by aspirin, clopidogrel, or their combination. *J Am Coll Cardiol*. 2006;47(3):541-6.

20. Li XQ, Ma N, Li XG, Wang B, Sun SS, Gao F, et al. Association of PON1, P2Y12 and COX1 with recurrent ischemic events in patients with extracranial or intracranial stenting. *PLoS One*. 2016;11(2):e0148891.
21. Yi X, Zhou Q, Wang C, Liu P, Fu C. Platelet receptor Gene (P2Y12, P2Y1) and platelet glycoprotein Gene (GPIIIa) polymorphisms are associated with antiplatelet drug responsiveness and clinical outcomes after acute minor ischemic stroke. *Eur J Clin Pharmacol*. 2017;73(4):437-43.
22. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci U S A*. 1987;84(21):7735-8.
23. Peng LL, Zhao YQ, Zhou ZY, Jin J, Zhao M, Chen XM, et al. Associations of MDR1, TBXA2R, PLA2G7, and PEAR1 genetic polymorphisms with the platelet activity in Chinese ischemic stroke patients receiving aspirin therapy. *Acta Pharmacol Sin*. 2016;37(11):1442-8.
24. Sharma V, Kaul S, Al-Hazzani A, Prabha TS, Rao PPKM, Dadheech S, et al. Association of C3435T multi drug resistance gene-1 polymorphism with aspirin resistance in ischemic stroke and its subtypes. *J Neurol Sci*. 2012;315(1-2):72-6.
25. Kugai M, Uchiyama K, Tsuji T, Yoriki H, Fukui A, Qin Y, et al. MDR1 is related to intestinal epithelial injury induced by acetylsalicylic acid. *Cell Physiol Biochem*. 2013;32(4):942-50.
26. Storey RF. Biology and pharmacology of the platelet P2Y12 receptor. *Curr Pharm Des*. 2006;12(10):1255-9.
27. Timur AA, Murugesan G, Zhang L, Aung PP, Barnard J, Wang QK, et al. P2RY1 and P2RY12 polymorphisms and on-aspirin platelet reactivity in patients with coronary artery disease. *Int J Lab Hematol*. 2012;34(5):473-83.
28. Naveed H, Almasri M, Kazani B, Nauman A, Akhtar N, Singh R, et al. Women and stroke: disparities in clinical presentation, severity, and short- and long-term outcomes. *Front Neurol*. 2023;14:1147858.
29. Bushnell C, McCullough LD, Awad IA, Chireau MV, Fedder WN, Furie KL, et al. Guidelines for the prevention of stroke in women: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. 2014;45(5):1545-88.
30. Villa A, Gelosa P, Castiglioni L, Cimino M, Rizzi N, Pepe G, et al. Sex-specific features of microglia from adult mice. *Cell Rep*. 2018;23(12):3501-11.