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Model based on single-nucleotide polymorphism to discriminate aspirin resistance patients

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ABSTRACT

Background Aspirin is widely used for preventing ischaemic events. About 20%–40% of patients have aspirin resistance (ASR), which prevents them from benefiting from aspirin medication. This study aimed to develop and validate a model based on single-nucleotide polymorphism (SNP) to distinguish ASR patients.

Methods We included patients with spontaneous intracerebral haemorrhage and continuing antiplatelet therapy from a multicentre, prospective cohort study as the derivation cohort. Thromboelastography (inhibition of arachidonic acid channel<50%) was used to identify ASR. Genotyping was performed to identify the ASR-related SNP. Based on the result of the logistic analysis, the aspirin resistance in the Chinese population score (ASR-CN score) was established, and its accuracy was evaluated using the area under the curve (AUC). Patients receiving dual antiplatelet therapy for unruptured intracranial aneurysm embolism were prospectively included in the validation cohort. After embolism, 30-day ischaemic events, including ischaemic stroke, new or more frequent transient ischaemic attack, stent thrombosis and cerebrovascular death, were recorded.

Results The derivation cohort included 212 patients (155 male patients and the median age as 59). 87 (41.0%) individuals were identified with ASR. The multivariate logistic analysis demonstrated six SNPs of *GP1BA, TBXA2R, PTGS2* and *NOS3* as risk factors related to ASR. The ASR-CN score integrating these SNPs performed well to discriminate ASR patients from non-ASR patients (AUC as 0.77). Based on the validation cohort of 372 patients receiving antiplatelet therapy after embolism (including 130 ASR patients), the ASR-CN score continued to distinguish ASR patients with good accuracy (AUC as 0.80). Patients with high a ASR-CN score were more likely to suffer from 30-day ischaemic events after embolism (OR, 1.28; 95% CI, 1.10 to 1.50; p=0.002).

Conclusion *GP1BA, TBXA2R, PTGS2* and *NOS3* were SNPs related to ASR. The ASR-CN score is an effective tool to discriminate ASR patients, which may guide antiplatelet therapy.

Clinical trial registration Surgical Treatments of Antiplatelet Intracerebral Hemorrhage cohort (unique identifier: ChiCTR1900024406, http://www.chictr.org.cn/edit.aspx?pid=40640&htm=4).

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ About 20%–40% of patients have aspirin resistance (ASR), which prevents them from benefiting from aspirin medication while increasing their risk of suffering from the side effect of aspirin. Genetic factors are the most prominent influences on ASR.

WHAT THIS STUDY ADDS

⇒ This study found single-nucleotide polymorphisms (SNPs) of four genes, including *GP1BA, TBXA2R, PTGS2* and *NOS3*, related to ASR in the Chinese population. The aspirin resistance in the Chinese population score (ASR-CN score) based on SNPs of the ASR could distinguish ASR patients and is related to ischaemic events in patients receiving antiplatelet therapy.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The ASR-CN score is an effective tool for guiding antiplatelet therapy in the Chinese population.

INTRODUCTION

Aspirin is widely used for preventing ischaemic cerebrovascular and cardiovascular diseases,[1 2] as well as ischaemic events after endovascular therapy.[3] However, a considerable number of individuals suffer from aspirin resistance (ASR), suggesting a potential failure of preventing ischaemic events in patients receiving aspirin. Previous studies reported that 20%–40% of patients who had an ischaemic stroke suffer from ASR.[4 5] Since antiplatelet medication has side effects, these patients cannot benefit from aspirin therapy. How to identify patients with the high risk of ASR is helpful to guide antiplatelet therapy.

Previous studies have revealed several factors of ASR, including aspirin dosage, medication adherence, systematic condition, comorbidities (eg, diabetes mellitus and dyslipidaemia), smokers and effects from combined medications (eg, proton pump inhibitors and carbonic anhydrase inhibitors).[6–8] Notably, genetic factor is the one of the influences on ASR. According to previous studies, numerous single-nucleotide polymorphisms (SNPs) were related to ASR potentially, including *MDR1, TBXA2R, PLA2G7* and

PEAR1.[9–11] These genes have been associated with the biological effect of platelet aggregation and activation of the cyclooxygenase pathway. However, there is no reliable diagnostic model to identify ASR patients.

This study aimed to investigate the factors associated with ASR based on two prospective cohorts. Subsequently, we developed and validated a diagnostic model for ASR.

METHODS

Study population

Surgical Treatments of Antiplatelet Intracerebral Hemorrhage (SAP-ICH) study (unique identifier: ChiCTR1900024406, https://www.chictr.org.cn/showproj.html?proj=40640) references a prospective multicentre cohort study (September 2019 to December 2022, of patients with severe cerebral haemorrhage from seven regional medical centres). This study followed the Strengthening the Reporting of Observational Studies in Epidemiology protocol. The protocol of SAP-ICH study was published previously.[12]

The data generation is summarised in figure 1A. We included all intracerebral haemorrhage (ICH) patients in the SAP-ICH cohort receiving antiplatelet therapy according to the following inclusive criteria: (1) severe ICH and Glasgow Coma Score<13; (2) patient had no cerebrovascular diseases and intracranial tumours; (3) no haemorrhagic transformation of cerebral infarction or haemorrhage caused by venous thrombosis; (4) patients had no severe coagulation disorder (eg, haemophilia) or coagulation dysfunction (caused by malignant tumour, hypohepatia, renal dysfunction, thrombocytopaenia, coagulation diseases and so on); (5) patients did not receive other anticoagulation medications (vitamin K antagonist and new oral anticoagulants). In order to control the factors related to aspirin metabolism and absorption, we excluded patients based on the following criteria: (1) patients consumed drugs that had potential effects on the absorption and metabolism of aspirin, such as proton pump inhibitors and carbonic anhydrase inhibitors, within 1 month before haemorrhage; (2) patients discontinued aspirin>3 days; (3) patients did not take aspirin; (4) patients had liver diseases (hepatic failure) and kidney diseases (renal dysfunction, including chronic renal failure (phase II) and renal dysfunction after admission (glomerular filtration rate<90 mL/(min×1.73 m^2) and/or serum creatinine>133 µmol/L)); and (5) patients had no thromboelastography (TEG) data. Patients in the derivation cohort usually took 100 mg aspirin and/or 75 mg clopidogrel. Only seven patients took aspirin plus dipyridamole or ticagrelor. Thus, these patients were grouped into the dual antiplatelet.

For the validation cohort, we prospectively recruited patients who received stent-assisted coiling for unruptured intracranial aneurysms (UIAs) at our institution from January 2022 to August 2022. Patients were enrolled according to the following criteria: (1) patients received stent-assisted coiling for UIAs; (2) patients aged 18–75

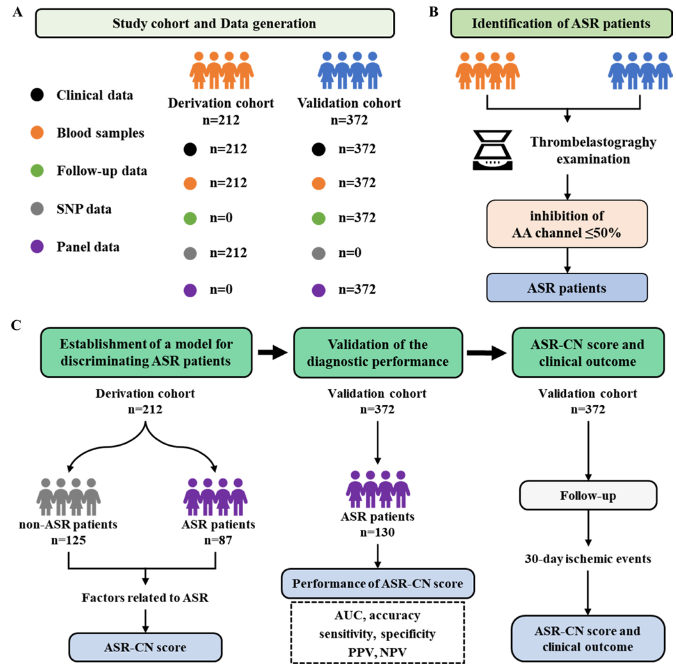

Figure 1 Data generation and study design. (A) The summary of data generation. (B) The flowchart of identifying ASR patients. (C) The flowchart of study design. We included 212 ICH patients in the Surgical Treatments of Antiplatelet Intracerebral Hemorrhage cohort who discontinued aspirin <5 days in the derivation cohort. In total, 87 patients of them were identified as ASR. In addition, we recruited 372 patients with UIA undergoing interventional procedures as a validation cohort. Among them, 130 patients were identified as ASR. Within the derivation cohort, we investigated the SNPs related to ASR, and constructed a scoring system (aspirin resistance in Chinese population score (ASR-CN score)) to identify ASR patients. Within the validation cohort, we validated the accuracy of the ASR-CN score in identifying ASR patients and whether the ASR-CN score is related to clinical outcomes (30-day ischaemic events after UIA embolisation). AA, arachidonic acid channel in the thromboelastography; ASR, aspirin resistance; ICH, intracerebral haemorrhage; NPV, negative predictive value; PPV, positive predictive value; SNP, single-nucleotide polymorphism; UIA, unruptured intracranial aneurysm.

years; (3) patients with a modified Rankin scale score≤2; (4) patients who agreed to receive follow-up and sign a written informed form. We then excluded patients who: (1) had compilations related to the surgical accidents and quality of the material, such as coil hesitation and incompletely opened stents; (2) received simultaneous treatment for other cerebrovascular diseases, such as arteriovenous malformations; (3) could not bear dual antiplatelet therapy (aspirin plus clopidogrel), because of an allergy to aspirin or clopidogrel; (4) aneurysms were recurrent or incompletely embolised; (5) received postoperative prophylactic use of glycoprotein IIb/IIIa antagonists (eg, tirofiban); (6) had malignant diseases, such as malignant tumours, renal failure or hepatic failure; (7) took medications that interfere with aspirin absorption and metabolism within 1 month before embolisation; (8)

were with poor medication compliance (Morisky Medication Adherence Scale<6).

Study design and identification of ASR

Patients with arachidonic acid channel in the TEG (TEG-AA)<50% (which was considered to be a failure of antiplatelet therapy)[13] were identified as the ASR, otherwise as the non-ASR (figure 1B). All TEG examinations were performed within 2 hours after blood sample collection. TEG was used to monitor the function of platelet with the assistance of Thrombelastograph Coagulation Analyzer 5000 (Haemoscope, Niles, Illinois, USA). The platelet inhibition rate was calculated according to the maximum amplitude. TEG-AA represented the inhibition of platelets by aspirin.

DNA extraction and genotyping

To identify genes associated with ASR, we first built a gene pool by searching the PharmGKB database (https://www.pharmgkb.org/) and discovered 75 genes with evidence levels as 3 or 2B. We included all genes related to the pharmacological efficacy of aspirin and excluded all genes associated with aspirin hypersensitivity reactions. Finally, we established a gene bank of 22 SNPs containing 19 genes to identify the genetic characteristics of ASR patients. We collected blood samples for genotyping before surgery. All samples were centrifuged at 3000 rpm for 10 min within 3 hours of collection and then stored in liquid nitrogen. Samples with obvious signs of haemolysis were considered unacceptable. According to the manufacturer's protocol, DNA was extracted from the whole blood sample using the DNA extraction kit (MyGenostics, Beijing, China). DNA library was then established using the DNA Library Prep Kits (MyGenostics) and Ampure beads method. To enrich targeted genes, 500 n/g DNA samples from each library were hybridised with the detector (P039-Exome, MyGenostics) and microbeads (MyGenostics). The purified DNA library was subsequently amplified using the Master Mix and Hotstar Enzyme (MyGenostics). Genotyping assays were performed using the Flowcell chips and NextSeq 500 (Illumina, California, USA). All raw data were in FASTq format and preprocessed using Cutadapt V.1.16. Data were then mapped based on the human genome (hg19) using BWA V.0.7.10, Samtools V.1.2 and Bamtool V.2.4.0. Base quality score was recalibrated using the GenomeAnalysisTK V.4.0.8.1, and SNPs were identified. Finally, SNPs were annotated using the ANNOVAR.

As for CYP2C19 metabolisers, because there were no ultrarapid metabolisers (*17/*17) in this study, the metabolisers were classified as extensive (EM, *1/*1, *1/*17), intermediate metaboliser (IM, *1/*2, *1/*3, *2/*17 and *3/*17) and poor metaboliser (PM, *2/*2, *2/*3, *3/*3), referring to previous study.[14 15]

Data collection

We collected baseline information, including age, gender, comorbidities (history of dyslipidaemia, diabetes mellitus, coronary artery (CA) disease and ischaemic stroke), antiplatelet therapy regimes prehaemorrhage (aspirin monotherapy and dual ant antiplatelet therapy (aspirin plus clopidogrel), dosage, frequency and duration), tobacco and alcohol consumption, laboratory findings (platelet count, activated partial thromboplastin time (APTT), prothrombin time (PT) and fibrinogen (Fbg)). For patients with diabetes mellitus, the glycosylated haemoglobin (HbA1c), triglyceride and high-density lipoprotein (HDL) before surgery were collected. Alcohol consumption was categorised as regular (one or more drinks per week) and non-regular.[16] Current smoking status (patients never quit smoking or have smoking cessation less than 1 year) was also recorded.[17]

For the derivation cohort, the radiologist and neurosurgeon recorded the location of the haematoma (supratentorial lobar, supratentorial deep and cerebellar) based on the CT at admission and independently calculated the haematoma volume using the ABC/2 method, blinded to clinical information. These discrepancies were resolved by consultation with a senior neurosurgeon.

For the validation cohort, aneurysm locations (anterior cerebral artery, anterior communicating artery, internal carotid artery, middle cerebral artery and posterior circulation) and aneurysm size were determined by a radiologist and a neurointerventional surgeon, who were blinded to clinical information. These discrepancies were resolved by consultation with a neurointerventional surgeon.

Follow-up and outcomes in the validation cohort

For patients receiving conventional stent-assisted coil embolisation, dual antiplatelet therapy (aspirin 100 mg plus clopidogrel 75 mg) was maintained for 6 weeks, and aspirin (100 mg) monotherapy continued for 6 months. All patients in the validation cohort were followed regularly by telephone interviews or outpatient visits until 30 days after embolisation. Ischaemic events within 30 days were recorded. The primary outcome was ischaemic events within 30 days,[18] which was a combination of ischaemic stroke, new or more frequent transient ischaemic attacks, stent thrombosis, urgent revascularisation and cerebrovascular death.

Statistical analysis

Category variables were compared using the χ^2 test or Fisher's exact test, and independent samples t-test and Mann-Whitney U test were performed for continuous variables.

To identify factors associated with ASR, we compared the differences between ASR patients and non-ASR patients. Significant parameters in univariate analysis were then assessed by logistic regression models to identify independent risk factors associated with ASR. The results were presented in the form of OR, and 95% CI were also calculated. Based on the β value of multivariate logistic analysis, we evaluated the importance of each parameter and established a diagnostic model (aspirin resistance in Chinese population score (ASR-CN) score). The accuracy of the model to discriminate ASR patients

Table 1 The demographic and clinical features of patients in the derivation cohort

Characteristics	ASR n=87	Non-ASR n=125	P value
Age, years, m (IQR)	56 (48–66)	61 (51–71)	0.902
Male gender, n (%)	64 (73.6%)	91 (72.8%)	0.117
Comorbidities, n (%)			
Dyslipidaemia	7 (8.0%)	13 (10.4%)	0.565
Diabetes mellitus	25 (28.7%)	36 (28.8%)	0.992
CA diseases	67 (77.0%)	107 (85.6%)	0.110
Ischaemic stroke or TIA	22 (25.3%)	43 (34.4%)	0.158
Ever-or-now smokers, n (%)	29 (33.3%)	25 (20.0%)	0.029*
Regular drinkers, n (%)	3 (3.4%)	4 (3.2%)	0.921
Laboratory findings			
Platelet count, $\times 10^9$, m (IQR)	216 (192–238)	216 (200–226)	0.790
APTT, m (IQR)	24.8 (24.5–31.7)	24.8 (24.4–30.4)	0.323
PT, m (IQR)	1.01 (1.00–1.07)	1.00 (0.96–1.04)	0.059
Fibrinogen, g/L, m (IQR)	2.8 (2.3–3.1)	2.8 (2.4–3.6)	0.100
Antiplatelet therapy, n (%)			0.218
Aspirin	71 (81.6%)	93 (74.4%)	
Dual antiplatelet	16 (18.4%)	32 (25.6%)	
Haematoma location, n (%)			0.838
Supratentorial lobar	23 (26.4%)	43 (34.4%)	
Supratentorial deep	57 (65.5%)	62 (49.6%)	
Cerebella	7 (8.0%)	20 (16.0%)	
Haematoma volume, mL, m (IQR)	51.8 (33.3–75.3)	49.7 (36.2–82.2)	0.477

*The difference was significant.
APTT, activated partial thromboplastin time; ASR, aspirin resistance; CA, coronary artery; PT, prothrombin time; TIA, transient ischaemic attack.

from non-ASR patients was evaluated using the receiver operator characteristic curve and area under the curve (AUC). Models with AUC>0.7 were considered to have good discriminatory accuracy. Using the highest Youden Index (sensitivity+specificity−1), we divided all patients into a high-risk group and a low-risk group. Accuracy, specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV) were calculated.

To investigate whether the ASR-CN score is related to ischaemic events, we compared the differences between patients suffering and not suffering from ischaemic events. Univariate and multivariate logistic regression analyses were performed to investigate the variables associated with 30-day ischaemia incidents.

RESULTS

Baseline characteristics of patients in the derivation cohort

The study design is presented in figure 1C. The derivation cohort included 212 patients with ICH from 308 patients on continuous antiplatelet therapy in the SAP-ICH cohort (online supplemental figure 1). Baseline information is summarised in table 1. Of all included patients, 155 patients were men and the median age was 59 years. In total, 54 (25.5%) patients were ever-or-now smokers.

A total of 87 (41.0%) patients were identified as ASR by TEG (AA<50%). More ASR patients were ever-or-now smokers, compared with non-ASR patients (33.3% vs 20.0%, p=0.029). There were no significant differences in age, gender, comorbidities, alcohol consumption, platelet count, APTT, PT, Fbg, antiplatelet therapy regimen, haematoma location and haematoma volume (all p>0.05) between ASR and non-ASR patients.

SNPs and factors associated with ASR

To investigate the SNP characteristics of ASR patients and non-ASR patients, we examined 22 SNP potentially related to ASR (figure 2A). The characteristics of genetic type are summarised in online supplemental table 1. There were significant differences in *GP1BA~rs6065, TBXA2R* (*~rs1131882, ~rs4523*), *PTGS2* (*~rs12042763, ~rs20417*) and *NOS3~rs1799983* between ASR patients and non-ASR patients (all p<0.05). Here, we found that the SNP mutations of *GP1BA, TBXA2R, PTGS2* and *NOS3* resulted in a higher risk of ASR (figure 2B). We further classified SNP mutations in these four genes as no mutation, single mutation and multiple mutations. The univariate logistic analyses revealed that parameters, including ever-or-now smokers, genetic type of *GP1BA, TBXA2R, PTGS2* and

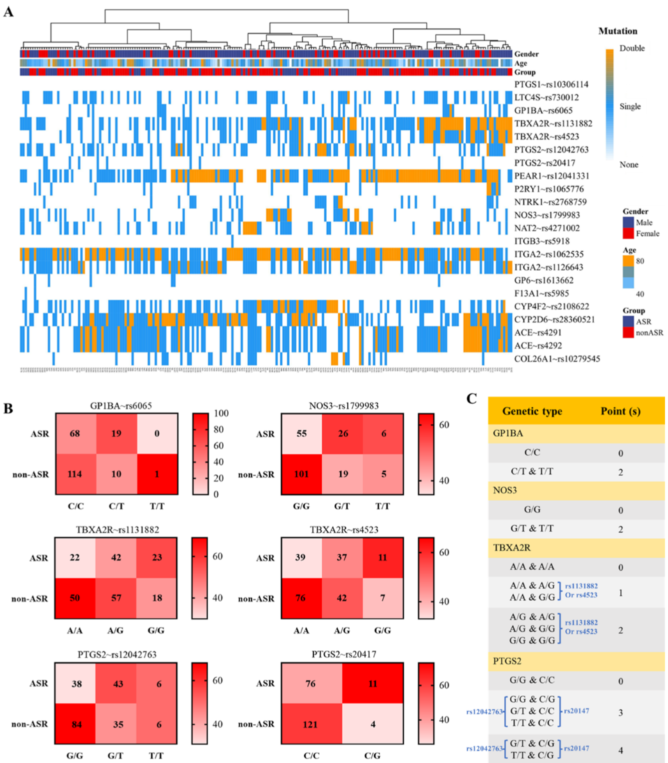

Figure 2 The genetic mutation associated with ASR and the aspirin resistance in the Chinese population score (ASR-CN) scores (A) The heatmap presents the mutations of single-nucleotide polymorphism related to aspirin's metabolism and efficacy, based on the derivation cohort. (B) The confusion matrixes show the number and percentage of ASR in each genetic type for *GP1BA, NOS3, TBXA2R* and *PTGS2*, within the derivation cohort. (C) The ASR-CN score was used to identify patients at high risk of ASR. ASR, aspirin resistance.

NOS3, were the risk factors of ASR (online supplemental table 2). The subsequent multivariate logistic analysis demonstrated that the genetic type of *GP1BA, TBXA2R, PTGS2* and *NOS3* was independent risk factor for ASR (table 2).

SNPs-based scoring model for ASR patients

Based on the coefficient and weight of each genetic type of *GP1BA, TBXA2R, PTGS2* and *NOS3* (online supplemental table 3), we established the ASR-CN score (figure 2C). The ASR-CN score was higher in ASR patients compared with non-ASR patients (online supplemental figure 2A–C). After adjustment by factors that may be associated with ASR (age, sex, dyslipidaemia, diabetes mellitus and coagulation factors), the ASR-CN score can still discriminate ASR patients from non-ASR patients (online supplemental figure 2D). The highest degree of discrimination between ASR patients and non-ASR patients was achieved by the ASR-CN score (AUC as 0.77, online supplemental figure 2E). Based on the highest Youden Index at ASR-CN score as 3 (≥3 as the high-risk group), the accuracy, sensitivity, specificity, PPV and NPV of ASR-CN score for ASR were 0.73, 0.90, 0.62, 0.62 and 0.90, respectively (online supplemental figure 2C). A high ASR-CN score was also associated with ASR after controlling for potential ASR-related factors (online supplemental figure 3).

Validation of the performance of ASR-CN score to identify the ASR patients

The validation cohort included 372 patients, and 130 (34.9%) patients were diagnosed with ASR by TEG. In online supplemental table 4, we concluded the baseline information of the validation cohort. Significance was found in the genetic type of *GP1BA, TBXA2R* and *PTGS2*, between ASR and non-ASR patients (all $p<0.05$). Moreover, ASR patients had higher ASR-CN scores compared with non-ASR patients (figure 3A, $p<0.001$). The ASR-CN score had the highest accuracy in distinguishing ASR patients from non-ASR patients (AUC as 0.80), compared with the genotypes of *GP1BA, TBXA2R, PTGS2* and

Table 2 Multivariate logistic analysis of factors related to aspirin resistance based on the derivation cohort

	Crude		Adjusted*	
Parameters	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
Ever-or-now smokers	1.73 (0.86 to 3.46)	0.124		
GP1BA (C/C vs C/T and T/T)	2.54 (1.06 to 6.09)	0.037	2.77 (1.13 to 6.78)	0.025
TBXA2R				
A/A and A/A	Reference		Reference	
A/A and A/G, A/A and G/G	1.41 (0.59 to 3.38)	0.442	1.39 (0.57 to 3.35)	0.468
A/G and A/G, A/G and G/G, G/G and G/G	2.44 (1.18 to 5.06)	0.016	2.31 (1.10 to 4.84)	0.026
PTGS2				
G/G and C/C	Reference		Reference	
G/G and C/G, G/T and C/C, T/T and C/C	3.12 (1.67 to 5.84)	<0.001	3.2 (1.69 to 6.10)	<0.001
G/T and C/G, T/T and C/G	13.07 (1.32 to 128.97)	0.028	13.50 (1.33 to 136.83)	0.028
NOS3 (G/G vs G/T and T/T)	2.16 (1.06 to 4.39)	0.033	2.33 (1.13 to 4.82)	0.023

*The result was adjusted by age, gender, dyslipidaemia, diabetes mellitus, regular drinkers and platelet count.

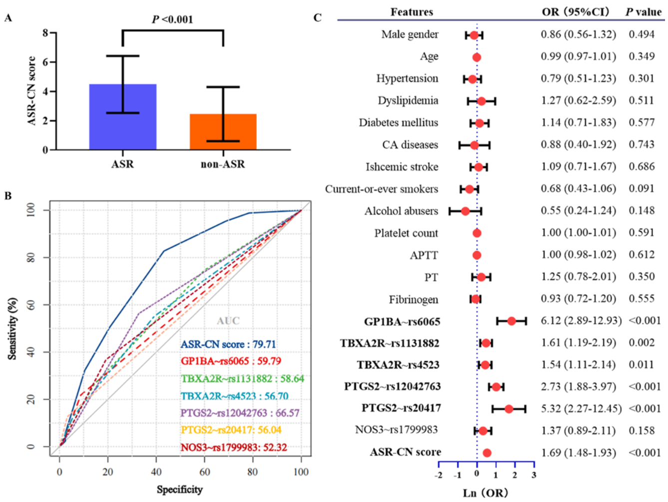

Figure 3 Validation of the performance of the ASR-CN scores to identify ASR patients. (A) Within the validation cohort, ASR patients had higher ASR-CN scores compared with non-ASR patients. (B) The performance of ASR-CN score and other parameters to discriminate ASR patients from non-ASR patients. (C) The forest plot displayed the result of the univariate logistic analysis for ASR within the validation cohort. The mutations in *GP1BA, NOS3, TBXA2R* and *PTGS2* were related to ASR. High ASR-CN was a risk factor for ASR. AUC, area under the curve; APTT, activated partial thromboplastin time; ASR, aspirin resistance; ASR-CN; aspirin resistance in the Chinese population; CA, coronary artery; PT, prothrombin time.

NOS3 (figure 3B). Subsequent univariate logistic analysis (figure 3C) and multivariate logistic analysis (online supplemental table 5) displayed that a high ASR-CN score was a risk factor for ASR. Based on the validation cohort, the accuracy, sensitivity, specificity, PPV and NPV of ASR-CN score (≥3 as the high-risk group) for ASR were 0.76, 0.87, 0.71, 0.61 and 0.91, respectively (online supplemental figure 4).

We further investigated whether ASR-CN score could discriminate ASR and non-ASR patients within the patients with diabetes mellitus. There was no difference in HbA1c, triglyceride, HDL level and triglyceride/HDL ratio between ASR and non-ASR patients (all p>0.05, online supplemental figure 5A–D). ASR-CN score could discriminate ASR patients, even being adjusted by HbA1c and triglyceride/HDL ratio (online supplemental figure 5E).

ASR-CN score is related to ischaemic events after aneurysm embolisation

Based on the validation cohort, we further investigated whether the ASR-CN score is related to 30-day ischaemic events after aneurysm embolisation. The differences in clinical, radiological and laboratory characteristics between patients with and without ischaemic events are summarised in table 3. In total, 45 (12.1%) patients suffered from ischaemic events 30 days after aneurysm embolisation (online supplemental table 6). More patients with 30-day ischaemic events were identified as ASRs (figure 4A, p=0.036), and CYP2C19 IM or PM metabolisers (figure 4B, p<0.001). 30-day ischaemia occurrences were more frequent in those with higher ASR-CN scores (figure 4C). Univariate logistic regression analysis indicated that the history of ischaemic stroke (p=0.044), ASR-CN score (p=0.010), CYP2C19 IM metabolisers (p=0.001) and PM metabolisers (p<0.001) were risk factors for patients with 30-day ischaemic events (figure 4D and online supplemental table 7). The multivariate logistic analysis further demonstrated that the history of ischaemic stroke (OR, 2.08; 95% CI, 1.04 to 4.16; p=0.038), high ASR-CN score (OR, 1.28; 95% CI, 1.10 to 1.50; p=0.002), CYP2C19 IM metabolisers (OR, 6.61; 95% CI, 2.24 to 19.48; p=0.001) and CYP2C19 PM metabolisers (OR, 17.83; 95% CI, 5.04 to 63.10; p<0.001) were risk factors independently associated with 30-day ischaemic events (table 4).

We performed subgroup analysis on different CYP2C19 metabolisers (online supplemental figure 6). For IM and PM metabolisers, the high-risk patients had a higher percentage of ischaemic events compared with low-risk patients (all p<0.05).

DISCUSSION

Because aspirin treatment not only lowers the risk of ischaemic events but also has side effects (eg, hypersensitivity and asthma), it is not beneficial for ASR patients. In this study, we found SNPs of four genes, including *GP1BA, TBXA2R, PTGS2* and *NOS3*, were related to ASR. With the integration of these SNPs, the ASR-CN score could distinguish ASR patients from non-ASR patients with good accuracy. Based on the validation cohort, we discovered that patients with high ASR-CN scores were at higher risk of ischaemic events, even if receiving double antiplatelet treatment, after UIA embolisation. This study provided the first tool to discriminate ASR patients in the Chinese population, which may guide antiplatelet therapy.

There is controversy regarding how to recognise people who are resistant to aspirin. Failure to inhibit platelet aggregation in the laboratory is probably the optimal definition for ASR. However, several laboratories assay methods had been proposed in previous studies.[19 20] TEG evaluates changes in viscoelasticity during whole blood coagulation, which can simulate the actual coagulation and is used to detect platelet dysfunction in stroke patients and brain trauma patients.[19 20] Moreover, we included individuals who continued their aspirin medication based on two prospective cohort studies. According to our study, we could investigate the risk factors associated with ASR.

This study identified six genotypes of four genes as independent risk factors strongly related to ASR. GP Ib, encoded by *GP1BA*, is a platelet surface membrane glycoprotein that plays an essential role in platelet aggregation.[21] The SNP (rs6065 C>G/C>T) leads to amino acid changes that may affect the structure of GP Ib proteins, a risk factor for ischaemic stroke.[22] Nitric

Table 3 The difference of patients in the validation cohort, grouped by 30-day ischaemic events

Characteristics	Ischaemic events n=45	No ischaemic events n=327	P value
Age, years, m (IQR)	57 (47–62)	55 (49–62)	0.443
Male gender, n (%)	19 (42.2%)	158 (48.3%)	0.868
Comorbidities, n (%)			
Hypertension	18 (40.0%)	124 (37.9%)	0.788
Dyslipidaemia	6 (13.3%)	29 (8.9%)	0.337
Diabetes mellitus	14 (31.1%)	91 (27.8%)	0.647
CA diseases	6 (13.3%)	25 (7.6%)	0.196
Ischaemic stroke or TIA	30 (66.7%)	165 (50.5%)	0.042*
Ever-or-now smokers, n (%)	18 (40.0%)	115 (35.2%)	0.527
Regular drinkers, n (%)	2 (4.4%)	32 (9.8%)	0.244
Laboratory findings			
Platelet count, $\times10^9$, m (IQR)	216 (210–244)	216 (206–229)	0.215
APTT, m (IQR)	24.8 (23.2–30.4)	24.8 (24.5–30.4)	0.506
PT, m (IQR)	1 (1–1)	1 (1–1)	0.058
Fibrinogen, g/L, m (IQR)	2.80 (2.42–3.58)	2.80 (2.34–3.33)	0.498
Aneurysm locations, n (%)			0.198
AcomA/ACA	5 (11.1%)	27 (8.3%)	
ICA	30 (66.7%)	201 (61.5%)	
MCA	10 (22.2%)	87 (26.6%)	
PC	0 (0.0%)	12 (3.7%)	
Aneurysm size, mL, m (IQR)	6.4 (6.1–8.8)	6.3 (6.0–8.7)	0.499
ASR (TEG-AA<50%), n (%)	22 (48.9%)	108 (33.0%)	0.037*
ASR-CN score, m (IQR)	4 (2–5)	3 (2–4)	0.036*
CYP2C19 metaboliser, n (%)			<0.001*
EM	4 (8.9%)	134 (41.0%)	
IM	30 (66.7%)	162 (49.5%)	
PM	11 (24.4%)	31 (9.5%)	

*The difference was significant.
ACA, anterior cerebral artery; AcomA, anterior communicating artery; APTT, activated partial thromboplastin time; ASR, aspirin resistance; ASR-CN, aspirin resistance in the Chinese population; CA, coronary artery; EM, extensive metaboliser; ICA, internal carotid artery; IM, intermediate metaboliser; MCA, middle cerebral artery; PC, posterior circulation; PM, poor metaboliser; PT, Prothrombin time; TEG-AA, arachidonic acid channel in the thromboelastography; TIA, transient ischaemic attack.

oxide signalling is a key regulator of vascular tone and platelet aggregation. The *NOS3* SNP (rs1799983 T>A/T>G) will enhance the process of platelet aggregation by suppressing endothelial nitric oxide synthase activity.[23] *TBXA2R* encodes a member of the G protein-coupled receptor family that interacts with thromboxane A2 to induce platelet aggregation and regulate haemostasis. Mutations in *TBXA2R* might affect the transcription and/or translation efficiency of both isoforms of the *TBXA2R* gene.[10 24] *PTGS2* encodes the inducible isozyme that may affect the biological effect of cyclooxygenase 2-derived prostaglandins. Mutations in *PTGS2* have been implicated in promoting platelet dysfunction.[25] Therefore, it is evident that NOS3, GP1BA, TBXA2R and PTGS2 are involved in regulating platelet aggregation. Mutations in these genes aberrantly enhance platelet function and increase the risk of ischaemic events. Previous studies also reported that *MDR1, PLA2G7* and *PEAR1* were related to ASR.[9–11] However, the SNPs of these genes failed to be significant between ASR patients and non-ASR patients in this current study. This phenomenon may be due to the interaction among these genes, which did not be studied in previous works.

Contrary to clopidogrel, ASR may be influenced by multiple genes. Integrating the SNPs of *GP1BA, TBXA2R, PTGS2* and *NOS3*, we established the ASR-CN score to identify the ASR patients. Without waiting for a period of taking aspirin and subsequently testing the platelet function, ASR-CN could effectively discriminate ASR patients with high sensitivity. For patients with ASR-CN score≥3, given they may not benefit from aspirin therapy (no antiplatelet effect, but it may lead to asthma and

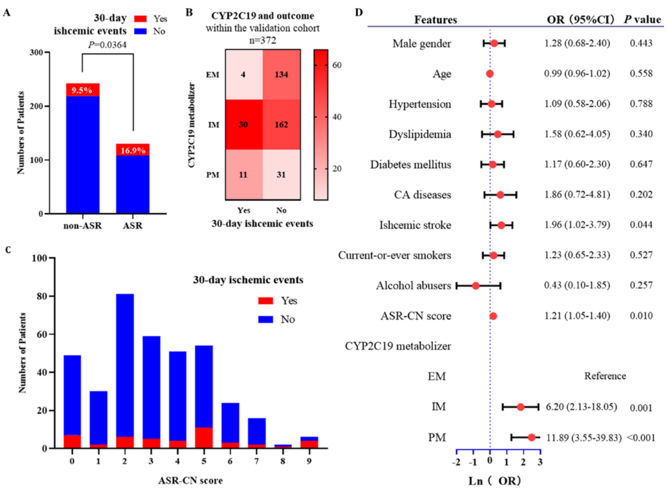

Figure 4 A high ASR-CN score is associated with a high risk of ischaemic events in patients receiving intracranial aneurysm embolisation. (A) More ASR patients (with a arachidonic acid channel in the thromboelastography<50%) experienced ischaemic events within 30 days after aneurysm embolisation compared with the non-ASR patients. (B) The confusion matrix presents the percentage of patients suffering from 30-day ischaemic events in each CYP2C19 metaboliser. Patients with PM metaboliser had the highest percentage of 30-day ischaemic events. (C) The distribution of patients with 30-day ischaemic events in each ASR-CN score. (D) The forest plot shows the results of the univariate logistic analysis for 30-day ischaemic events. ASR, aspirin resistance; ASR-CN, aspirin resistance in the Chinese population; CA, coronary artery; EM, extensive metaboliser; IM, intermediate metaboliser; PM, poor metaboliser.

hypersensitivity), aspirin could not be considered as the appropriate choice for antiplatelet therapy. Instead, other antiplatelet medications, for example, ticagrelor, cilostazol, clopidogrel and other, could be considered. For patients with ASR-CN score<3, they could take aspirin for antiplatelet therapy regularly.

CYP2C19 metaboliser can affect the pharmacological effects of clopidogrel and are related to ischaemic events. Numerous previous investigations revealed PM and IM metabolisers as ischaemic stroke risk factors.[26 27] In the current study, we found that for PM and IM metabolisers, patients with a high ASR-CN score had a high risk of suffering from 30-day ischaemic events, suggesting that the efficacy of aspirin is related to outcome. However, for EM metabolisers, there was no difference in ischaemic events between ASR and non-ASR patients, which suggested that clopidogrel plays a protective role in 'clopidogrel responsive but aspirin resistant' patients during the dual antiplatelet therapy. Thus, combined with ASR-CN score and CYP2C19 metabolisers, clinicians could understand the effects of antiplatelet therapy and make appropriate clinical decisions.

Although exciting, there are several limitations in this study. First, we enrolled patients with severe ICH in the derivation cohort. Considering that inflammatory conditions and some treatments after admission (dehydration and methylprednisolone) may affect platelet function, some patients may exhibit pseudo-ASR. Thus, patient selection bias may limit our conclusion. Second, to validate the clinical utility of the ASR-CN score, we performed a cohort study including only patients who received stent-assisted coiling. All patients in the validation cohort received dual antiplatelet therapy. Although some patients were ASR, clopidogrel treatment may prevent them from ischaemic events, which may underestimate the clinical impact of ASR for ischaemic events. Bias in patient selection and treatment protocol may prevent us from drawing certain conclusions. Third, a gene pool of ASR-associated SNPs was established based on a database search. There may be other potential ASR genes, which were not considered in this study. Fourth, all included patients were Chinese, and this study took only ICH and intracranial aneurysm into consideration. It is unclear whether our findings apply to other populations. Therefore, the generality of ASR-CN remains unclear. Despite the above limitations, this study demonstrated SNPs associated with ASR and provided a useful tool (ASR-CN score) to identify ASR patients. The ASR-CN score can help physicians develop antiplatelet therapy strategies to prevent ischaemic events.

CONCLUSION

Based on two prospective cohort studies, we identified SNPs of *GP1BA, TBXA2R, PTGS2* and *NOS3* as risk factors

Table 4 Multivariate logistic analysis of factors related to 30-day ischaemic events based on the validation cohort

	Crude		Adjusted*	
Parameters	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
Ischaemic stroke	2.08 (1.04 to 4.16)	0.038	2.08 (1.03 to 4.22)	0.041
ASR-CN score	1.28 (1.10 to 1.50)	0.002	1.28 (1.09 to 1.50)	0.002
CYP2C19 metaboliser				
EM	Reference		Reference	
IM	6.61 (2.24 to 19.48)	0.001	6.52 (2.20 to 19.29)	0.001
PM	17.83 (5.04 to 63.10)	<0.001	19.50 (5.44 to 69.89)	<0.001

*The result was adjusted by age, gender and aneurysm size.
ASR-CN, aspirin resistance in the Chinese population; EM, extensive metaboliser; IM, intermediate metaboliser; PM, poor metaboliser.

related to ASR. The ASR-CN score is an effective tool for directing antiplatelet therapy. For patients with ASR-CN score≥3, more than 60% of them were likely to be ASR.

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Competing interests None declared.

Patient consent for publication Consent obtained from parent(s)/guardian(s).

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