Relationship between recurrent ischemic events in cerebrovascular disease and cytochrome *P450 2C19* gene polymorphism on the basis of thrombelastography

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Background: Recurrent ischemic events in cerebrovascular disease present a difficult problem in clinical practice. The predictive value of cytochrome *P450 2C19* (*CYP2C19*) gene polymorphism and high platelet reactivity for recurrent ischemic events in cerebrovascular disease is not clear.

Methods: A total of 295 patients with acute ischemic cerebrovascular disease admitted to the cerebrovascular disease center of Northern Theater General Hospital between January 1, 2020 and February 2, 2021 were enrolled in this study. Thrombelastography (TEG) was used to detect platelet reactivity and *CYP2C19* gene polymorphism.

Results: Among the 118 noncarriers, 97 had normal platelet reactivity and 21 had high platelet reactivity. Of the 177 carriers, 120 showed normal platelet reactivity and 57 showed high platelet reactivity. The area under the curve (AUC) of *CYP2C19* gene polymorphism in predicting recurrent ischemic events was 0.66. The regression coefficients of hypertension, stroke history, carriers, and high platelet reactivity with recurrent ischemic events were 0.341, 0.402, 0.358, and 0.281, respectively, with significant positive correlation ($P<0.05$).

Conclusions: Hypertension, stroke history, carriers, and high platelet reactivity are all independent risk factors for recurrent ischemic events. *CYP2C19* gene polymorphism and high platelet reactivity can be used as effective predictors of recurrent ischemic events in clinical cerebrovascular disease.

Keywords: Cerebrovascular disease; recurrent ischemic event; thrombelastography; cytochrome *P450* (*CYP*) *2C19* gene polymorphism; high platelet reactivity

Introduction

Ischemic stroke refers to cerebral blood supply disorders, ischemia, and hypoxia leading to ischemic necrosis or softening of local brain tissue (1,2). Statistics show that China's annual number of new strokes is 1.5–2 million, the annual incidence is 116–219/100,000, and annual mortality is 58–42/100,000, making stroke China's leading cause of death. Ischemic stroke usually occurs in people over 50–60 years of age, often with atherosclerosis, hypertension, rheumatic heart disease, coronary heart disease, or diabetes, and smoking, drinking, and other unhealthy lifestyle habits (3-5). Few ischemic stroke patients are completely cured, and there is a high recurrence rate. The annual recurrence rate of ischemic stroke in China is as high as 17.7%, while the recurrence rate of stroke in 10 years can reach 39.2% (6). Therefore, reducing recurrent ischemic events of acute ischemic cerebrovascular disease has significant economic and social benefits. Studies have shown that eroded or destroyed

atherosclerotic plaques, the basis of thrombosis, are exposed to circulating platelets (7,8). Through the endogenous coagulation pathway, platelets are rapidly activated, resulting in the secretion of its granular content including adenosine diphosphate (ADP), which stimulates platelet aggregation, eventually leading to thrombosis (9). Therefore, the prevention of platelet aggregation is important for preventing thrombosis in acute ischemic cerebrovascular disease.

When transformed into active thiol metabolite (SR 26334), clopidogrel inhibits ADP-induced platelet activation through the isoenzyme of hepatocyte cytochrome P450 (CYP). The activity of P450 isoenzyme is mainly affected by the cytochrome *P450 2C19* (*CYP2C19*) gene. Gu *et al.* [2014] extracted peripheral blood from 299 ischemic stroke patients and 295 healthy controls. Genotyping using polymerase chain reaction-restriction fragment length polymorphism showed that the *CYP2C19* 681AA genotype may be an independent risk factor for ischemic stroke and recurrent stroke (10). Mega *et al.* showed that compared with noncarriers, the active metabolite of clopidogrel decreased 32.4% in healthy subjects carrying *CYP2C19* functional deficiency genes. After taking clopidogrel, the platelet inhibition rate of carriers decreased by 9% compared with noncarriers (11). Pan *et al.* [2017] retrospectively explored the relationship between genetic polymorphism and the efficacy of clopidogrel in patients with ischemic stroke or transient ischemic attack, and proposed that carriers of *CYP2C19* allele loss (*2, *3 and *8) had a higher risk of stroke and complex vascular events than non-carriers (12). Yang *et al.* [2020] tested the effects of ticaggrel and clopidogrel on platelet reactivity in patients with mild stroke or transient ischemic attack (TIA), and divided them into carriers and non-carriers according to the status of *CYP2C19* allele loss. The results showed that ticagor/aspirin clopidogrel had a low platelet reactivity ratio in carriers, but not in noncarriers (13). Wu *et al.* [2018] enrolled 1,476 patients with mild stroke or transient ischemic attack who were treated with clopidogrel and aspirin, and investigated whether decreased renal function would change the relationship between *CYP2C19* gene variation and clinical outcomes. Classification based on the quartile of renal function estimated by glomerular filtration rate (eGFR) found that the *CYP2C19* allele carriers in the lowest quartile of renal function had a higher incidence of new stroke than non-carriers (14). Clinical detection methods for platelet function mainly include light transmittance aggregometry (LTA), platelet function analysis, VerifyNOW assay, and thrombelastography (TEG). TEG is an analyzer which can dynamically monitor the entire coagulation process (15).

Unlike a blood coagulation analyzer, TEG can fully reflect interaction among platelets, coagulation factors, fibrinogen, the fibrinolytic system, and other cellular components from coagulation to fibrinolysis by detecting a small amount of whole blood. TEG is easy to operate and produces accurate data for the comprehensive monitoring of the entire process of coagulation and fibrinolysis and platelet function.

In patients with acute ischemic cerebrovascular disease, the relationship between *CYP2C19* gene polymorphism, clopidogrel high platelet reactivity, and recurrent ischemic events has not been well studied. Hence, this study used TEG to detect clopidogrel high platelet reactivity and *CYP2C19* genotype to detect *CYP2C19* gene polymorphism. Further, we evaluated the relationship between *CYP2C19* gene polymorphism, clopidogrel high platelet reactivity, and recurrent ischemic events. The purpose of this study was to evaluate the effects of *CYP2C19* gene polymorphism and clopidogrel high platelet reactivity on recurrent ischemic events in patients with acute ischemic cerebrovascular disease. We present the following article in accordance with the MDAR reporting checklist (available at https://apm.amegroups.com/article/view/10.21037/apm-21-3775/rc).

Methods

Research objects

A total of 295 patients with acute ischemic cerebrovascular disease hospitalized in the cerebrovascular disease center of Northern Theater General Hospital between January 1, 2020 and February 2, 2021 were selected as subjects. Among them, there were 163 males and 132 females, aged 45–71 years old. Basic clinical data were collected, including age, gender, body mass index (BMI), hypertension, diabetes, smoking, and stroke history. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Research Ethics Committee of The Northern Theater General Hospital [No.: Y (2021)080]. Patients and their families understood the research and signed an informed consent form.

Inclusion criteria were patients who (I) were older than 18 years, (II) took aspirin and clopidogrel, (III) had complete clinical data, and (IV) gave informed consent.

Exclusion criteria were patients with (I) epicardial embolism, (II) a history of clopidogrel allergy, (III) aspirin allergy history; and patients who (IV) took other anticoagulant drugs in March, (V) had hematological diseases, and (VI) received intravenous thrombolysis.
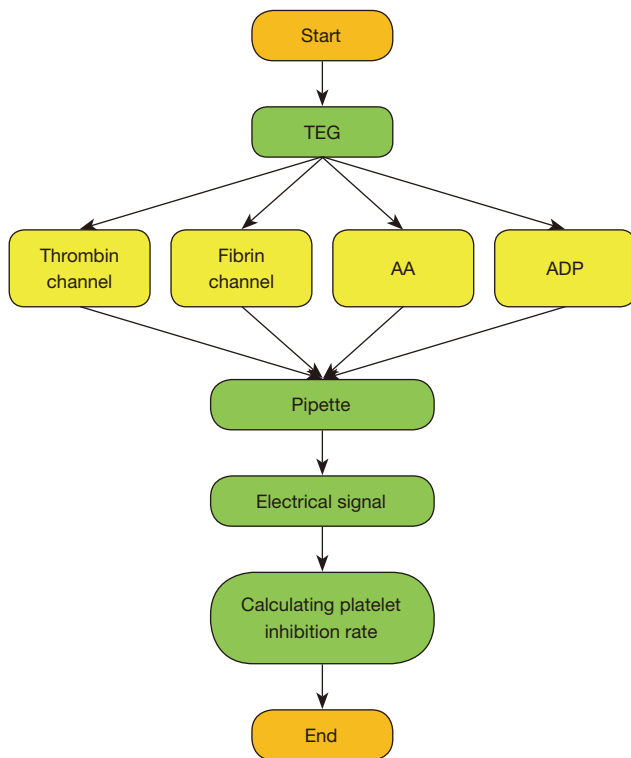
Figure 1 TEG detection process. TEG, thrombelastography; AA, arachidonic acid; ADP, adenosine diphosphate.

TEG detection

TEG was performed within 2 hours after venous blood sampling (16) (*Figure 1*). Platelet reactivity was measured using an improved TEG platelet mapping system (Haemoscope Corp., Braintree, MA, USA) according to the manufacturer's instructions. The improved TEG used 4 channels to detect platelet reactivity through arachidonic acid (AA) and ADP pathways, including: (I) thrombin channel, containing kaolin to induce maximum thrombin activation; (II) fibrin channel, containing activator F (a mixture of reptilase and coagulation factor XIIIa); (III) AA channel containing activator F and AA; and (IV) ADP channel containing activator F and ADP. Whole blood was drawn to each channel by a fluid shifter consisting of an oscillating cup and a suspension needle. Viscoelasticity change occurring during blood clot formation was transmitted to the probe and the resulting torque generated an electrical signal recorded by TEG. The AA- or ADP-induced platelet inhibition rate was calculated by computer software according to the following equation.

$$\text{Suppression ratio} = \frac{MA_{1\,or\,2} - MA_3}{MA_4 - MA_3} \times 100\% \qquad [1]$$

MA_1 represents AA-induced clot strength, MA_2 represents ADP-induced clot strength, MA_3 represents fibrin-induced clot strength, and MA_4 represents thrombin-induced clot strength

Based on the results of cardiovascular disease (17), the boundary value of clopidogrel high platelet reactivity was defined as ADP-induced <30% platelet inhibition rate detected by TEG, and the boundary value of aspirin high platelet reactivity was defined as AA-induced <50% platelet inhibition rate detected by TEG. A platelet inhibition rate higher than the above threshold was defined as normal platelet reactivity.

Genotype detection of CYP2C19

The detection process involved extracting whole blood samples using a QIAGEN blood kit (QIAGEN, Chatsworth, CA, USA) according to the manufacturer's instructions. DNA microarray (gene chip) was used to evaluate *CYP2C19* genotype. *CYP2C19*2* and *CYP2C19*3* alleles were determined using the BaiO BE-2.0 biochip diagnostic analyzer (BaiO Technology Co., Ltd., Shanghai, China). DNA extraction procedures, polymerase chain reaction amplification, hybridization, gene chip detection, and analysis were performed strictly in accordance with the genotype detection gene chip kit manual (BaiO Technology Co., Ltd.).

CYP2C19 genotype grouping (18) was carried out according to the pharmacokinetic characteristics of clopidogrel among different genotypes. Wild-type gene *1/*1(636GG/681GG) was classified as noncarrier. Mutation hybrid *1/*2(636GG/681GA), *1/*3 (636GA/681GG), mutation homozygous *2/*2(636GG/681AA), *2/*3(636GA/681GA), and *3/*3 (636AA/681GG) were classified as having at least 1 functional deletion allele carrier and referred to as carrier.

Endpoint events

Outpatient and telephone follow-up was conducted. The patients were followed up for 3 months and 6 months after discharge for recurrent ischemic events, including recurrent ischemic stroke, transient ischemic attack (TIA), cerebrovascular surgery (angioplasty/stenting, carotid endarterectomy), myocardial infarction, stable angina,
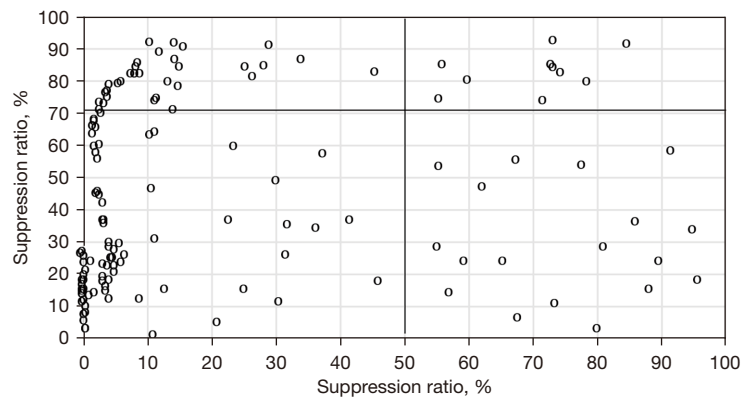
Figure 2 High platelet reactivity distribution in patients. The upper left quadrant is clopidogrel high platelet reactivity. The lower left quadrant is normal platelet response. The upper right quadrant is the high platelet response of clopidogrel and aspirin. The lower right quadrant is aspirin high platelet reactivity.

cardiovascular surgery (coronary artery bypass grafting, angioplasty/stenting), and lower extremity arterial disease or vascular death.

Statistical methods

The data in this study were analyzed by SPSS 19.0 statistical software. The measurement data are expressed as mean ± standard deviation ($\bar{x}\pm s$), and count data are expressed as percentage (%). Single factor analysis of variance was used for pairwise comparison. The difference was considered statistically significant with P<0.05. The receiver operating characteristic (ROC) curve was used to evaluate the accuracy of high platelet reactivity, *CYP2C19* gene polymorphism, and *CYP2C19* gene polymorphism combined with high platelet reactivity in predicting recurrent ischemic events in patients. Kaplan-Meier method was used to synthesize cumulative survival curves of patients based on clopidogrel platelet reactivity, *CYP2C19* genotype, and *CYP2C19* genotype combined with clopidogrel platelet reactivity. The logrank test was used to evaluate the statistical difference in survival curves, and least significant difference (LSD) correction was used for comparison between groups. Multivariate Cox regression analysis was used to determine the related variables affecting clinical endpoint events.

Results

Distribution of high platelet reactivity in patients

The distribution of high platelet reactivity in patients is shown in *Figure 2*. In 295 patients, 217 cases (73.88%) had

normal platelet reaction, 10 cases (3.4%) had clopidogrel and aspirin high platelet reaction, 49 cases (5.44%) had clopidogrel high platelet reaction, and 19 cases (6.44%) had aspirin high platelet reaction.

Distribution of CYP2C19 gene polymorphism in patients

The distribution of *CYP2C19* gene polymorphism in patients is shown in *Figure 3*. *Figure 3A* shows that in 295 patients, 118 cases (39.97%) carried wild-type genes, 131 cases (44.56%) carried mutant heterozygous genes, and 46 cases (15.65%) carried mutant homozygous genes. *Figure 3B* shows that among 131 patients carrying mutant heterozygous genes, 115 patients carried *1/*2 and 16 patients carried *1/*3. *Figure 3C* shows that among the 46 patients carrying mutant homozygous genes, 27 patients carried *2/*2, 14 patients carried *2/*3, and 5 patients carried *3/*3.

Relationship between CYP2C19 gene polymorphism and platelet reactivity in patients

Figure 4 shows the comparison of platelet reactivity in noncarriers and carriers. Among the 118 noncarriers, 97 cases (82.2%) showed normal platelet reactivity and 21 cases (17.8%) showed high platelet reactivity. Of the 177 carriers, 120 cases (67.8%) showed normal platelet reactivity and 57 cases (32.2%) showed high platelet reactivity.

Further analysis of different types of gene carriers (*Figure 5*) found that in 131 patients with mutant heterozygous genes, 94 cases showed normal platelet reactivity and 37 cases showed high platelet reactivity.
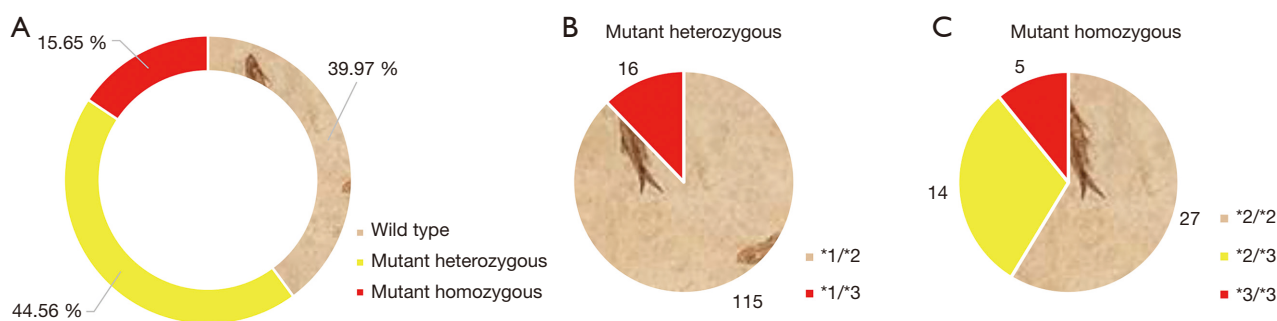
Figure 3 Distribution of *CYP2C19* gene polymorphism in patients. (A) The proportion of wild type, mutant heterozygous, and mutant homozygous genes. (B) The proportion of *1/*2 and *1/*3 in mutant heterozygous genes. (C) The proportion of *2/*2, *2/*3, and *3/*3 in homozygous mutant genes.
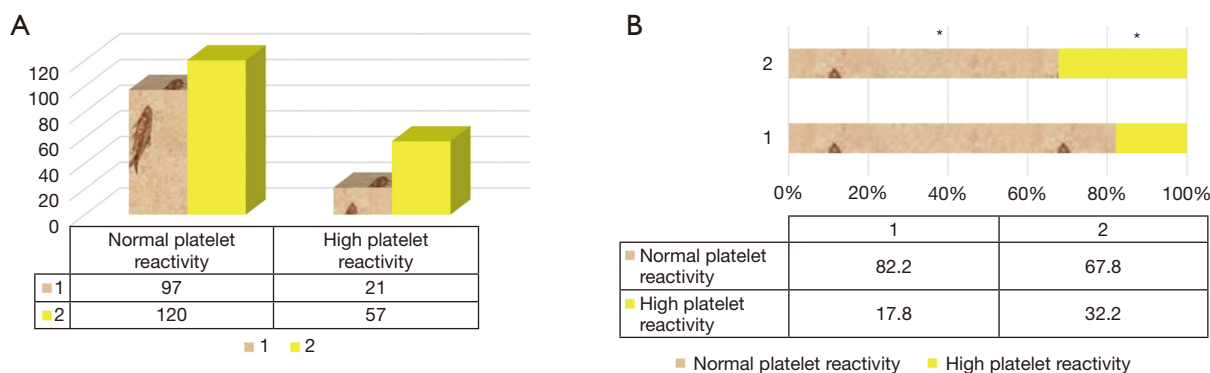

Figure 4 Comparison of platelet reactivity between noncarriers and carriers (1 is noncarriers, 2 is carriers). (A) The number of cases, and (B) the proportion. *, represents statistical differences versus 1 (P<0.05).

Among the 46 patients carrying mutant homozygous genes, 32 showed normal platelet reactivity and 14 showed high platelet reactivity. To further analyze the relationship between *CYP2C19* gene polymorphism and platelet reactivity, one-way ANOVA (*Figure 4B*) showed that the proportion of carriers with high platelet reactivity was significantly higher than that of non-carriers (P<0.05), and that the proportion of normal platelet reaction in carriers was significantly lower than that in non-carriers (P<0.05). Spearmam correlation analysis showed that there was an extremely significant positive correlation between high platelet reactivity and *CYP2C19* gene polymorphism (R=0.751, P<0.001).

ROC curve analysis of high platelet reactivity and CYP2C19 gene polymorphism in predicting recurrent ischemic events in patients

Figure 6 shows the ROC curve of high platelet reactivity and *CYP2C19* gene polymorphism for predicting recurrent ischemic events in patients. The AUC of recurrent ischemic events predicted by high platelet reactivity was 0.57, and the AUC of recurrent ischemic events predicted by *CYP2C19* gene polymorphism was 0.66.

Figure 7 shows the ROC curve of high platelet reactivity and *CYP2C19* gene polymorphism in predicting recurrent ischemic events in patients. The AUC of high platelet reactivity and *CYP2C19* gene polymorphism in predicting recurrent ischemic events in patients was 0.71.

Survival analysis of high platelet reactivity, CYP2C19 gene polymorphism, and recurrent ischemic events

Survival analysis of high platelet reactivity and recurrent ischemic events is shown in *Figure 8*. The survival rate of patients with high platelet reactivity was significantly lower than that of patients with normal platelet reactivity, and
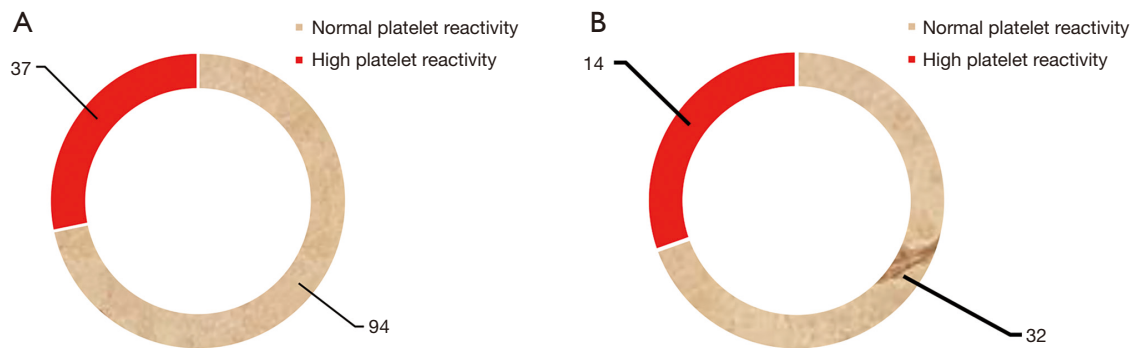
Figure 5 Platelet reactivity of different types of gene carriers. (A) Patients carrying mutant hybrid genes, and (B) patients carrying mutant homozygous genes.
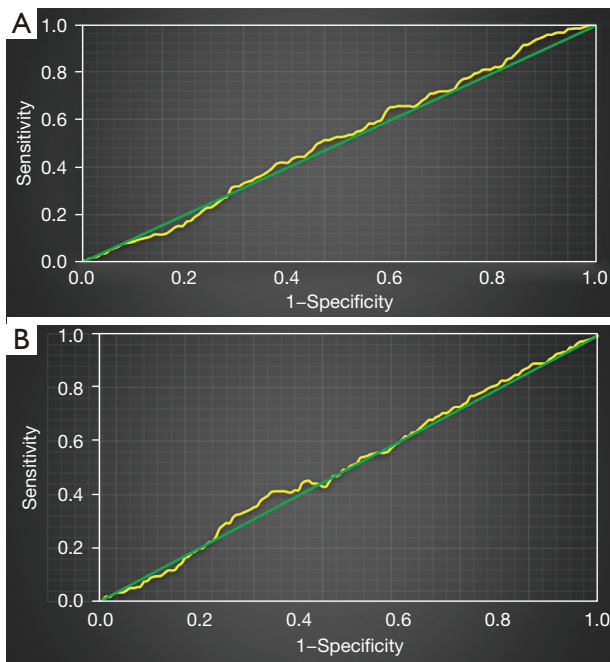

Figure 6 ROC curve of high platelet reactivity and *CYP2C19* gene polymorphism in predicting recurrent ischemic events in patients. (A) *CYP2C19* gene polymorphism, and (B) high platelet reactivity. ROC, receiver operating characteristic

Figure 7 ROC curve of high platelet reactivity and *CYP2C19* gene polymorphism in predicting recurrent ischemic events in patients.

there was a high risk of recurrent ischemic events.

Figure 9 shows survival analysis of *CYP2C19* gene polymorphism and recurrent ischemic events. The survival rate of carriers was significantly lower than that of noncarriers, and there was a high risk of recurrent ischemic events.

Figure 10 shows survival analysis results of combined application of high platelet reactivity, *CYP2C19* gene
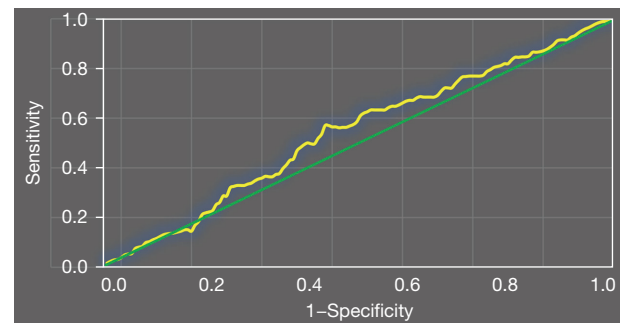
polymorphism, and recurrent ischemic events. Survival rates of patients with carrier + high platelet reactivity were significantly lower than those of patients with carrier + normal platelet reactivity, noncarrier + normal platelet reactivity, and noncarrier + high platelet reactivity.

Analysis of related factors of recurrent ischemic events in patients

Single factor logistic regression analysis was performed with age, gender, BMI, hypertension, diabetes, smoking, stroke history, carriers, and high platelet reactivity as independent variables, and recurrent ischemic events as the dependent variable. As shown in *Table 1*, the P values of age, gender, BMI, smoking, and recurrent ischemic events were all greater than 0.05, indicating that there was no significant correlation. The regression coefficients of hypertension, diabetes, stroke history, carriers, and high platelet reactivity with recurrent ischemic events were 0.441, 0.386, 0.457,
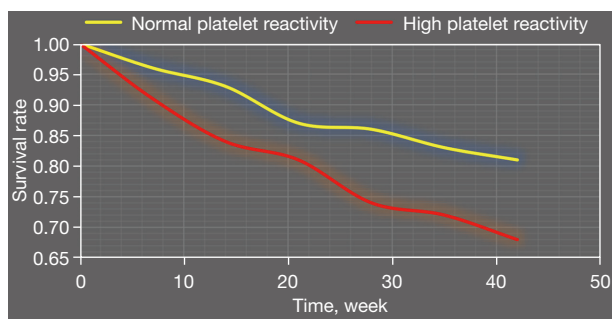
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Figure 8 Survival analysis of high platelet reactivity and recurrent ischemic events.
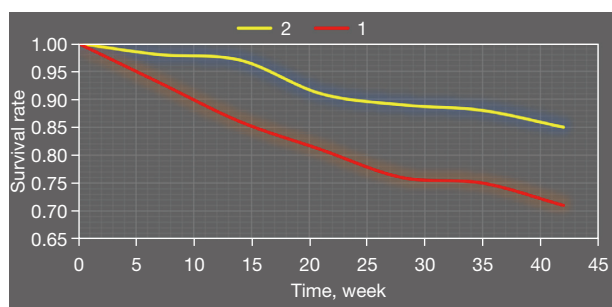
Figure 9 Survival analysis of *CYP2C19* gene polymorphism and recurrent ischemic events (1 is carriers, 2 is noncarriers).

Figure 10 Combined application of high platelet reactivity and *CYP2C19* gene polymorphism and survival analysis of recurrent ischemic events (1 is carriers, 2 is noncarriers).

0.422, and 0.407, respectively, and there was a significant positive correlation (P<0.05).

Multivariate logistic regression analysis was performed with hypertension, diabetes, stroke history, carriers, and high platelet reactivity as independent variables and recurrent ischemic events as the dependent variable. As shown in *Table 2*, the regression coefficients of hypertension, stroke history, carriers, high platelet reactivity with recurrent ischemic events were 0.341, 0.402, 0.358, and 0.281, respectively, with significant positive correlation (P<0.05).

Discussion

The recurrence of ischemic events is a major concern in clinical practice, and an effective prevention and treatment method is necessary (19,20). Our study included 295 patients with acute ischemic cerebrovascular disease who were hospitalized in the cerebrovascular disease center of Northern Theater General Hospital between January 1, 2020 and February 2, 2021. Basic clinical data such as age,
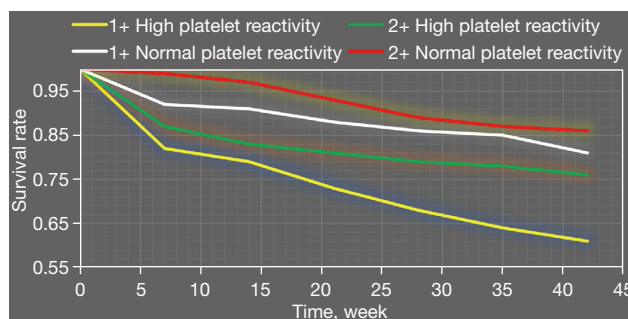
gender, BMI, hypertension, diabetes, smoking, and stroke history of the patients were collected, and patient platelet reactivity and *CYP2C19* gene polymorphism were detected by TEG (21). The results showed that in 295 patients, 217 cases (73.88%) had normal platelet reaction, 10 cases (3.4%) had clopidogrel and aspirin high platelet reaction, 49 cases (5.44%) had clopidogrel high platelet reaction, and 19 cases (6.44%) had aspirin high platelet reaction. Our results were lower than those of Kagami *et al.* (22), due to the use of different criteria and platelet reaction test methods. *CYP2C19* gene polymorphism in 118 noncarriers was analyzed, and 97 cases of normal platelet reactivity and 21 cases of high platelet reactivity were found. Among 177 carriers, 120 showed normal platelet reactivity and 57 showed high platelet reactivity, indicating that the *CYP2C19* gene had a high mutation rate in cerebrovascular disease (23). According to one-way ANOVA for the proportion of high platelet reactivity, the proportion of carriers was significantly higher than that of non-carriers, while the proportion of normal platelet reactivity was significantly lower than that of non-carriers (P<0.05), indicating that high platelet reactivity may be associated with *CYP2C19* gene polymorphism. Spearmam correlation analysis further showed that high platelet reactivity was significantly positively correlated with *CYP2C19* gene polymorphism (R=0.751, P<0.001). Further analysis of different types of gene carriers showed that there were 94 cases of normal platelet reactivity and 37 cases of high platelet reactivity in 131 patients with mutant heterozygous genes. Among the 46 patients carrying mutant homozygous genes, 32 cases presented normal platelet reactivity and 14 presented high platelet reactivity. The results were similar to those of Jukić *et al.* (24), indicating that *CYP2C19*

Table 1 Single factor logistic regression analysis of related factors of recurrent ischemic events in patients

Index	Regression coefficient	t	P
Age	0.205	2.414	0.051
Gender	0.188	1.964	0.078
BMI	0.197	2.681	0.074
Hypertension	0.441	4.271	0.035
Diabetes	0.386	3.953	0.046
Smoking	0.185	2.116	0.052
Stroke history	0.457	4.684	0.016
Carriers	0.422	4.229	0.023
High platelet reactivity	0.407	3.975	0.018

BMI, body mass index.

Table 2 Multivariate logistic regression analysis of related factors of recurrent ischemic events in patients

Index	Regression coefficient	t	P
Hypertension	0.341	3.861	0.039
Diabetes	0.318	3.175	0.050
Stroke history	0.402	4.025	0.031
Carriers	0.358	4.113	0.029
High platelet reactivity	0.281	3.673	0.021

gene polymorphism may be related to platelet reactivity.

ROC curve analysis revealed that the AUC of recurrent ischemic events predicted by high platelet reactivity was 0.57, and the AUC of recurrent ischemic events predicted by *CYP2C19* gene polymorphism was 0.66, indicating that *CYP2C19* gene polymorphism was correlated with recurrent ischemic events (25). Survival analysis showed that the survival rate of patients with high platelet reactivity was significantly lower than that of patients with normal platelet reactivity. Survival analysis of *CYP2C19* gene polymorphism and recurrent ischemic events suggested that the survival rate of carriers was significantly lower than that of noncarriers, which indicated that patients with high platelet reactivity and *CYP2C19* gene carriers had higher risk of recurrent ischemic events. Age, gender, BMI, hypertension, diabetes, smoking, stroke history, carriers, and high platelet reactivity of patients were used as independent variables, and recurrent ischemic events was used as a dependent variable for regression analysis. Regression coefficients between hypertension, stroke history, carriers, and high platelet reactivity with recurrent ischemic events were

0.341, 0.402, 0.358, and 0.281, respectively, with significant positive correlation (P<0.05) (26). The results showed that hypertension, stroke history, carriers, and high platelet reactivity were all independent risk factors for recurrent ischemic events, and thus *CYP2C19* gene polymorphism and high platelet reactivity could be used to predict recurrent ischemic events in clinical cerebrovascular disease.

Conclusions

In this study, 295 patients with acute ischemic cerebrovascular disease who were hospitalized in the cerebrovascular disease center of Northern Theater General Hospital between January 1, 2020 and February 2, 2021 were selected. Clinical data, including age, gender, BMI, hypertension, diabetes, smoking, and stroke history, were collected. TEG was used to detect platelet reactivity and *CYP2C19* gene polymorphism. The results showed that hypertension, stroke history, carriers, and high platelet reactivity were all independent risk factors for recurrent ischemic events. *CYP2C19* gene polymorphism and high

platelet reactivity could be used as effective predictors of recurrent ischemic events in clinical cerebrovascular disease. However, there was no intensive antiplatelet therapy for patients with high platelet response in this study, and a larger number of patients is needed for further discussion on the relationship between treatment regimens and prognosis. In conclusion, this study provides data support for the diagnosis and treatment of cerebrovascular diseases and the prediction and evaluation of recurrent ischemic events by discussing the relationship between hyper-platelet reactivity and recurrent ischemic events with *CYP2C19* gene polymorphism, as well as the predictive value of efficacy and outcome in patients with hyper-platelet reactivity combined with *CYP2C19* gene polymorphism.

Acknowledgments

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://apm.amegroups.com/article/view/10.21037/apm-21-3775/rc

Data Sharing Statement: https://apm.amegroups.com/article/view/10.21037/apm-21-3775/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://apm.amegroups.com/article/view/10.21037/apm-21-3775/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Research Ethics Committee of The Northern Theater General Hospital [No.: Y (2021)080]. Patients and their families understood the research and signed an informed consent form.

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