

Impact of Total Protein Level and Dysglycaemia on the Efficacy of Clopidogrel in Patients Underwent Carotid Artery Stenosis with *CYP2C19* Genetic Variants

Sijia Hao

Genetic Diagnosis Center, The First Hospital of Jilin University

Xinyuan Hu

Genetic Diagnosis Center, The First Hospital of Jilin University

Zhongxi Yang

Neurovascular Surgery, The First Hospital of Jilin University

Haoyuan Yin

Neurovascular Surgery, The First Hospital of Jilin University

Yanjiao Li

Department of Clinical Pharmacy, The First Hospital of Jilin University

Xinlu Wang

Department of Clinical Pharmacy, The First Hospital of Jilin University

Yuhao Zhao

Neurovascular Surgery, The First Hospital of Jilin University

Jianmin Piao

Neurovascular Surgery, The First Hospital of Jilin University

Xuan Chen

Neurovascular Surgery, The First Hospital of Jilin University

Yanfang Jiang (✉ jiangyanfang456@126.com)

Genetic Diagnosis Center, The First Hospital of Jilin University

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Abstract

BACKGROUND: *CYP2C19* polymorphisms are associated with the increased risk of major adverse cardiovascular/cerebrovascular events (MACCEs) in cerebral intervention. In this study, we wanted to investigate whether the *CYP2C19* polymorphism and other nongenetic factors can influence the incidence of MACCEs in cerebral artery stenosis disease patients.

METHODS: A total of 164 patients underwent cerebral artery stenting and 138 patients underwent conservative treatment among 1406 who patients underwent *CYP2C19* gene screening and were enrolled in this study. A Cox proportional hazards model and Kaplan–Meier analyses were used to assess the predictive value of *CYP2C19* loss-of-function (LOF) allele (*2, *3) carrier status and other risk factors.

RESULTS: The *CYP2C19* *1/*1 genotype was observed to be the most predominant among the patients (41.96%). The patients who underwent conservative treatment and had glucose levels > 6.5 mmol/L were more likely to experience MACCEs ($p = 0.022$). *CYP2C19* LOF allele variants ($p = 0.032$), total protein < 65 g/L ($p = 0.017$) and glucose > 6.5 mmol/L ($p = 0.028$) were associated with an increased risk of MACCEs in patients who underwent cerebral artery stents in the multivariable Cox analysis.

CONCLUSION: *CYP2C19* polymorphisms, total protein levels and glucose can impact the risk of MACCEs in patients who undergo cerebral artery stents.

Introduction

Cerebral artery stenosis is an important risk factor for ischaemic stroke¹ and can be divided into combined intracranial and extracranial arterial stenosis, intracranial arterial stenosis, and extracranial arterial stenosis². The treatment of cerebral artery stenosis, including conservative treatment, carotid endarterectomy (CEA) and carotid artery stenting (CAS), is most commonly used in cerebral artery stenosis treatment. Meanwhile, dual antiplatelet therapy (DAPT) with aspirin and clopidogrel has been recommended for cerebral artery stenosis to reduce thromboembolic events³.

Clopidogrel, which is an orally administered prodrug, can be converted into an active metabolite by hepatic cytochrome P450 (CYP)⁴, and *CYP2C19* is an important metabolizing enzyme. Among the 25 genetic variants of *CYP2C19*, the wild-type *CYP2C19**1 allele is associated with functional *CYP2C19*-mediated metabolism, while *CYP2C19**2 (c.681G > A; rs4244285) and *3 (c.636G > A; rs4986893) alleles, which are the major *CYP2C19* loss-of-function (LOF) variant alleles, can reduce the level of clopidogrel active metabolites in the blood and induce a decrease in clopidogrel function, resulting in inhibition of platelet aggregation^{4–7}.

It has been reported that *CYP2C19* polymorphism is involved in major adverse cardiovascular/cerebrovascular events (MACCEs) of cerebral intervention⁸. The *CYP2C19* polymorphism influencing the incidence of MACEs has been widely studied in coronary heart disease patients. It has been reported that patients with *CYP2C19* LOF variant alleles who have undergone percutaneous coronary intervention (PCI) are more likely to experience MACCEs⁹, and 5–30% of PCI patients cannot respond to clopidogrel^{10,11}. However, whether the *CYP2C19* polymorphism can influence the incidence of MACCEs in

moderate-severe cerebral artery stenosis disease patients remains unknown. Additionally, many other factors can influence the incidence of MACCEs after cerebral artery stenosis, including patient clinical characteristics such as dysglycaemia¹² and eGFR¹³.

In this study, to assess the risk predictors of the risk of MACCEs after cerebral artery stenosis, we analysed the potential predictive value of risk predictors such as *CYP2C19* polymorphisms, liver function, kidney function and blood glucose level in the conservative treatment group and cerebral artery stent group.

Materials And Methods

Ethics statement

The Ethics Committee of First Hospital of Jilin University approved this project. All of the samples and data were collected after written informed consent was provided by the participants. The management and publication of patient information in this research was strictly in accordance with the Declaration of Helsinki, including confidentiality and anonymity.

Study population

From October 2016 to December 2019, 1406 patients underwent *CYP2C19* genotype screening at the genetic diagnosis centre of First Hospital of Jilin University. A total of 362 of them were diagnosed with cerebral artery stenosis in the neurosurgery department of our hospital. As shown in Fig. 1, 302 patients were finally recruited in the study on the basis of the inclusion and exclusion criteria.

The inclusion criteria were as follows:

1) Patients were diagnosed with cerebral artery stenosis and underwent conservative treatment or cerebral artery stent; 2) patients received DAPT of clopidogrel (75 mg) and aspirin (100 mg) for at least 6 months.

According to the *CYP2C19* loss-of-function allele (LOF) gene polymorphism, patients were divided into 2 groups according to their *CYP2C19* genotype: a noncarrier group and a carrier group. Patients with wild-type *CYP2C19* (no mutations) (*1/*1) were assigned to the noncarrier group. Patients with *CYP2C19**2 or *3 were assigned to the carrier group.

MACCEs included death, stroke or stent thrombosis. All cerebral artery stenosis patients were diagnosed with moderate-severe cerebral artery stenosis disease. On account of status reasons, such as age factors, fragility of blood vessels and cardiac insufficiency, 143 patients could not tolerate the carotid intervention operation only orally with DAPT.

After cerebral artery stent operation, patients received 75 mg clopidogrel and 100 mg aspirin as dual antiplatelet therapy for at least 6 months. Patients were followed up at 6 months. The *CYP2C19* genotyping results and relevant clinical information were all recorded, including age, sex, liver function tests, kidney function tests and other laboratory test values available in the database. All laboratory tests were

performed at the First Hospital of Jilin University before treatment. Baseline variables are summarized with the use of descriptive statistics.

Genetic Analysis

We used a DNA array to screen the *CYP2C19* genotypes. DNA was extracted from whole-blood samples with nucleic acid extracting reagent (BaiO Technology, Shanghai, China). The PCR program consisted of an initial step at 50 °C for 5 min, 94 °C for 5 min, 35 cycles at 94 °C for 25 s, 48 °C for 40 s and 72 °C for 30 s, and a final extension at 72 °C for 5 min. We obtained images of the hybridization of the amplification products with the gene probes. The images and data were analysed by BaiO Array Doctor Version 2.0 (BaiO Technology, Shanghai, China) software.

Statistical analysis

Data analysis was performed using SPSS 20.0 software (IBM, Armonk, NY, USA) and GraphPad Prism 5. Measurement data are displayed as the mean \pm standard deviation, and categorical variables are expressed as the frequency and percentage. The distribution of categorical variables was compared among the study group using the chi-square test. Differences were considered statistically significant at $p < 0.05$. Kaplan–Meier analyses were used to generate survival plots of time to MACCEs during the 6-month follow-up period, and groups were compared by the log-rank test. Differences between the conservative treatment group and cerebral artery stent group in terms of the rate of MACCEs during the 6-month follow-up were assessed by a Cox proportional hazards regression model. Multivariable Cox regression analyses were adjusted by age, sex, AST, creatinine and BUN as a random effect. The interaction of *CYP2C19* LOF allele carrier status with risk factors such as ALT, total protein and glucose was analysed by the above crude and multivariable Cox models. Two-sided $P < 0.05$ was considered statistically significant.

Results

As shown in Fig. 1, the detailed patient selection process was described. From October 2016 to December 2019, 1406 patients underwent *CYP2C19* genotype screening at the genetic diagnosis centre of First Hospital of Jilin University. A total of 362 of them were diagnosed with cerebral artery stenosis in the neurosurgery department of our hospital. 143 patients only orally took DAPT. Of these, 5 patients could not be followed up and were excluded. Therefore, 138 patients who took DAPT only orally for 6 months were finally recruited as the conservative treatment group. In addition, among the 219 patients who underwent cerebral intervention, 11 patients underwent carotid endarterectomy, 5 patients died in the hospital, 18 patients did not receive 75 mg clopidogrel for economic reasons and adverse effects, and 11 patients could not be followed up. Thus, a total of 55 patients were excluded from this study. Finally, 164 patients who underwent cerebral artery stenting and orally took DAPT for 6 months were recruited as the cerebral artery stent group.

Distribution of the *CYP2C19* genotype in patients

The frequency of the *CYP2C19**2 and *3 alleles did not deviate significantly from Hardy-Weinberg equilibrium. The frequencies of the *CYP2C19**1, *2 and *3 alleles in all patients are shown in Fig. 2. We

found that the *CYP2C19**1/*1 genotype was the most predominant among the patients (41.96%), followed by *CYP2C19**1/*2 (39.69%). The frequency of the *CYP2C19**2 (comprising *1/*2, *2/*2, and *2/*3) allele was 30.75%, and that of the *3 (comprising *1/*3, *2/*3, and *3/*3) allele was 4.77%. According to whether they carried *CYP2C19*LOF alleles, we divided the 1406 patients into 2 groups according to their *CYP2C19* genotype: noncarriers and carriers. Patients with wild-type *CYP2C19* (no mutations) were assigned to the noncarriers. Patients with *CYP2C19**2 or *3 were assigned to the carriers.

Patient Baseline Characteristic

The baseline characteristics among individuals who orally took DAPT and underwent cerebral artery stenting by experiencing MACCEs are shown in Table 1. Patient demographics, medical history, *CYP2C19* genotyping results and laboratory values were included in the analysis. In the conservative treatment group and cerebral artery stent group, there were no significant differences between the patients with and without MACCEs in age, sex, hypertension, diabetes mellitus, smoking or drinking. In the conservative treatment group, the glucose of the patients who experienced MACCEs was significantly higher than that of the non-MACCE patients ($p = 0.007$). There was no significant difference in the frequency of *CYP2C19* LOF alleles ($p = 0.536$) in the conservative treatment group regardless of whether the patients experienced MACCEs. In addition, in the cerebral artery stent group, the frequency of *CYP2C19* LOF alleles ($p = 0.049$) and the ALT ($p = 0.019$) of the patients who experienced MACCEs was significantly higher than that of the non-MACCE patients, and the total protein was significantly lower ($p = 0.002$).

Table 1 . Baseline Characteristics Among Individuals underwent conservative treatment and carotid artery stent by experience MACCEs or not.

	Conservative treatment group (n = 138)			Cerebral artery stent group (n = 164)		
	MACCEs (n = 20)	Non-MACCEs (n = 118)	P	MACCEs (n = 15)	Non-MACCEs (n = 149)	P
Age, (yrs)	59.15±9.67	61.80±10.16	0.263	58.87±9.33	61.05±9.62	0.402
Male, n (%)	15 (75.00)	87 (73.73)	0.905	12 (80.00)	123 (82.55)	0.805
Medical history, n (%)						
Hypertension	12 (60.00)	49 (41.53)	0.124	8 (57.14)	88 (58.67)	0.912
Diabetes mellitus	3	14	0.693	2	35	0.439
Smoking	6	24	0.333	7	59	0.402
Drinking	4	11	0.156	5	48	0.526
CYP2C19 genotyping results						
*1/*1	7 (35.00)	51 (43.22)	0.705	2 (13.33)	66 (44.29)	0.097
*1/*2	9 (45.00)	44 (37.29)		8 (53.33)	52 (34.89)	
*1/*3	1 (5.00)	10 (8.47)		1 (6.67)	9 (6.04)	
*2/*2	1 (5.00)	8 (6.78)		4 (5.24)	16 (10.74)	
*2/*3	1 (5.00)	4 (3.39)		0 (0.00)	6 (4.04)	
*3/*3	1 (5.00)	1 (0.85)		0 (0.00)	0 (0.00)	
Noncarriers	7 (35.00)	51 (43.22)	0.536	2 (13.33)	66 (44.29)	0.049
Carriers	13 (65.00)	67 (56.78)		13 (86.67)	83 (52.71)	
Laboratory values						
ALT (U/L)	19.94±8.54	26.66±20.73	0.156	31.59±21.12	24.03±10.10	0.019
AST (U/L)	20.29±13.20	25.57±18.10	0.215	35.67±35.98	26.18±19.63	0.107
ALP (U/L)	61.56±25.49	73.57±30.49	0.106	90.74±30.35	78.14±25.60	0.076
Total Protein (g/L)	64.79±5.70	65.69±6.98	0.590	60.41±7.39	65.33±5.54	0.002
Albumin (g/L)	41.48±8.44	40.36±7.92	0.567	36.61±4.49	38.36±3.60	0.081
Total Bilirubin (µmol/L)	14.11±10.85	13.88±8.70	0.921	9.33±5.09	12.66±6.83	0.068
Indirect Bilirubin	8.23±4.18	8.91±4.49	0.554	6.25±4.00	9.51±7.21	0.087

($\mu\text{mol/L}$)						
Glucose (mmol/L)	6.98 \pm 3.13	5.70 \pm 1.63	0.007	6.83 \pm 1.96	6.24 \pm 2.32	0.346
Creatinine ($\mu\text{mol/L}$)	64.1 \pm 25.38	68.16 \pm 24.76	0.526	69.3 \pm 17.38	73.8 \pm 15.83	0.316
BUN (mmol/L)	13.4 \pm 22.49	9.76 \pm 15.80	0.395	5.50 \pm 1.71	5.66 \pm 1.46	0.692

CYP2C19 LOF allele variants, ALT levels, total protein levels and blood glucose levels are associated with an increased risk of MACCEs in patients who underwent cerebral artery stents

To identify the influencing factors of the clinical endpoint after cerebral artery stenosis, we performed log-rank tests. As shown in Table 2, in the conservative treatment group, we found that the patients whose glucose level was higher than 6.5 mmol/L were more likely to experience MACCEs ($p = 0.022$). In the cerebral artery stent group, we found that patients carrying *CYP2C19* LOF alleles (*2 and *3) were more likely to experience MACCEs than noncarriers ($p = 0.020$). We also found that the rates of MACCEs were significantly higher in patients whose ALT was higher than 35 U/L ($p = 0.022$), total protein was lower than 65 g/L ($p = 0.020$) or glucose was higher than 6.5 mmol/L ($p = 0.019$).

Table 2

Association between baseline characteristics and risk of MACCEs at 6 months in conservative treatment group and cerebral artery stent group

Characteristics	Group	The MACCEs rate of conservative treatment group	P value (log-rank)	The MACCEs rate of cerebral artery stent group	P value (log-rank)
Age	< 65 yrs	14/101 (1.27)	0.168	12/117 (10.26)	0.427
	> 65 yrs	6/37 (16.22)		3/47 (6.38)	
Sex	Male	15/102 (14.70)	0.682	12/135 (8.89)	0.828
	Female	5/36 (13.89)		3/29 (10.34)	
<i>CYP2C19</i> genotypes	Noncarriers	7/58 (12.07)	0.453	2/68 (2.94)	0.020
	Carriers	13/80 (16.25)		13/96 (13.54)	
AST	< 40 U/L	18/120 (15.00)	0.750	12/145 (8.28)	0.286
	> 40 U/L	2/16 (12.50)		3/19 (15.79)	
ALT	< 35 U/L	19/118 (16.10)	0.239	11/146 (7.53)	0.032
	> 35 U/L	1/18 (5.56)		4/18 (2.22)	
Total Protein	< 65 g/L	10/67 (14.93)	0.947	12/84 (14.29)	0.020
	> 65 g/L	10/68 (14.70)		3/80 (3.75)	
Albumin	< 40 g/L	13/90 (14.44)	0.938	12/118 (10.17)	0.493
	> 40 g/L	7/45 (15.56)		3/45 (6.67)	
Glucose	< 6.5 mmol/L	11/104 (10.58)	0.022	6/109 (5.50)	0.019
	> 6.5 mmol/L	9/34 (26.47)		9/55 (16.36)	
Creatinine	< 60 μ mol/L	6/39 (15.38)	0.862	5/34 (14.71)	0.175
	> 60 μ mol/L	12/87 (13.79)		10/129 (7.75)	
BUN	< 6.5 mmol/L	9/85 (10.59)	0.086	10/116 (8.62)	0.709
	> 6.5 mmol/L	9/41 (21.95)		5/47 (10.64)	

Dysglycaemia may influence the predictive value of *CYP2C19* loss-of-function alleles in patients who underwent cerebral artery stents

To further investigate how the influencing factors impact the predictive value of *CYP2C19* LOF alleles, we generated Kaplan-Meier curves to assess the association of *CYP2C19* LOF alleles and the incidence of MACCEs stratified by patient status. We found that the *CYP2C19* LOF alleles were identified as a predictor of MACCEs at 6 months after carotid artery stenting only when the patients had glucose levels > 6.5 mmol/L ($p = 0.0260$) (Fig. 3).

***CYP2C19* LOF allele variant, total protein level and dysglycaemiasynergically impact the risk of MACCEs in patients who underwent cerebral artery stents**

To further investigate the relationship between *CYP2C19* LOF allele carriers and patient status with MACCE risk, the Cox proportional hazards regression model was used to assess the association.

As shown in Table 3, the univariable analysis revealed that glucose > 6.5 mmol/L ($p = 0.033$) was significantly associated with MACCEs at 6 months in the conservative treatment group. Similarly, only glucose > 6.5 mmol/L ($p = 0.010$) remained significant in the multivariable Cox analysis. Nevertheless, the univariable analysis revealed that *CYP2C19* genotypes ($p = 0.037$), ALT > 35 U/L ($p = 0.033$), total protein < 65 g/L ($p = 0.030$) and glucose > 6.5 mmol/L ($p = 0.028$) were significantly associated with MACCEs at 6 months in the cerebral artery stent group (Table 4). We found that *CYP2C19* genotypes ($p = 0.032$), total protein < 65 g/L ($p = 0.017$) and ($p = 0.028$) remained significant in the multivariable Cox analysis (Table 4). Collectively, these results indicated that *CYP2C19* LOF allele variants, total protein levels and dysglycaemiasynergically increase the risk of MACCEs.

Table 3
Association between baseline characteristics and risk of MACCEs at 6 months in 138 patients only take DAPT medicine

Characteristics	Conservative treatment group (n = 138)			
	MACCEs at 6 months (Bivariable)		MACCEs at 6 months (Multivariable)	
	HR (95% CI)	P	HR (95% CI)	P
Age > 65 yrs	1.214 (0.466, 3.158)	0.692	1.453 (0.517–4.083)	0.479
Male	0.894 (0.325, 2.459)	0.828	1.727 (0.498–5.991)	0.389
<i>CYP2C19</i> genotypes	1.404 (0.56, 3.519)	0.469	1.026(0.379–2.780)	0.959
AST > 40	0.795(0.185, 3.427)	0.758	0.944(0.192–4.635)	0.944
ALT > 35 U/L	0.329 (0.044, 2.457)	0.279	0.196(0.023–1.704)	0.140
Total protein > 65 g/L	0.972 (0.404, 2.335)	0.972	1.082 (0.379–3.084)	0.883
Albumin < 40 g/L	1.036 (0.413, 2.597)	0.940	0.947 (0.302–2.966)	0.925
Glucose > 6.5	2.604 (1.079, 6.284)	0.033	4.077 (1.396–11.912)	0.010
Creatinine > 60 µmol/L	0.919 (0.345, 2.449)	0.866	1.997 (0.561–7.105)	0.285
BUN > 6.5 MMOL/L	2.149 (0.853, 5.414)	0.105	2.144 (0.772–5.954)	0.143

Table 4

Association between baseline and treatment characteristics and risk of MACCEs at 6 months in 164 patients underwent cerebral artery stent group

Characteristics	Cerebral artery stent group (n = 164)			
	MACCEs at 6 months (Bivariable)		MACCEs at 6 months (Multivariable)	
	HR (95% CI)	P	HR (95% CI)	P
Age > 65 yrs	0.604(0.171–2.142)	0.436	0.703(0.191–2.587)	0.596
Male	1.149(0.324–4.071)	0.830	0.972(0.241–3.913)	0.968
<i>CYP2C19</i> genotypes	4.863(1.097–21.551)	0.037	5.295(1.157–24.226)	0.032
AST > 40 U/L	1.956(0.552–6.933)	0.299	2.403(0.613–9.416)	0.208
ALT > 35 U/L	3.235(1.030-10.161)	0.033	3.193(0.790-12.901)	0.103
Total protein < 65 g/L	0.253(0.071–0.895)	0.030	0.127(0.023–0.691)	0.017
Albumin < 40 g/L	0.647(0.183–2.293)	0.500	0.535(0.146–1.953)	0.344
Glucose > 6.5	3.181(1.132–8.939)	0.028	3.283(1.139–9.464)	0.028
Creatinine > 60 µmol/L	0.486(0.166–1.422)	0.188	0.383(0.113–1.294)	0.122
BUN > 6.5 mmol/L	1.224(0.418–3.581)	0.712	1.530(0.501–4.667)	0.455

Discussion

We analysed the *CYP2C19* genotype screening results of 1406 patients genotyped at the genetic diagnosis centre of the First Hospital of Jilin University from October 2016 to December 2019, and the frequency of *CYP2C19*LOF variant allele carriers was 58.04%. Furthermore, the allelic frequencies of *CYP2C19* variants display significant interethnic differences⁷. In Asian countries, *CYP2C19*LOF variant allele carriers are observed at a relatively high frequency^{14,15}, and the frequencies of the *CYP2C19**2 and *3 alleles in Chinese Han populations are significantly higher than those in other racial groups⁷. A previous study reported that the allele frequency of *CYP2C19*LOF variant allele carriers is 62.38% in China¹⁶. The results of our study are similar to the frequencies of the variant alleles *CYP2C19**2 and *3 reported in previous studies.

The rate of MACCEs in the conservative treatment group was 14.49%, and that in the cerebral artery stent group was 9.14% (**Table S1**). A 403-patient study showed that MACCEs were observed in 8.19% of patients after treatment with CAS¹⁷, which is in line with our study. Considering the limited number of patients, the rate of MACCEs in this study may not reflect the overall situation. This could also be driven by the patients who underwent conservative treatment, and carotid artery stents were almost all moderate-severe cerebral artery stenosis disease patients. Because conservative treatment patients whose own condition is not good enough to undergo surgery, they cannot tolerate carotid intervention operations, such as age factors, fragility of blood vessels and cardiac insufficiency, only orally. In this way, the rate of MACCEs in the conservative treatment group was higher than that in the cerebral artery stent group.

As a commonly prescribed antiplatelet, clopidogrel is usually used to prevent secondary ischaemia in patients treated by endovascular techniques. Composite MACCE outcomes, such as death, stroke, and stent thrombosis, limit the long-term success rate of interventions through the recurrence of symptoms. Although intervention has been associated with minimal complications, previous studies have reported that *CYP2C19* loss-of-function polymorphisms may be a significant risk factor for in-stent restenosis¹⁸. Clopidogrel plus aspirin is used to reduce the risk of recurrent stroke. As one of the most prominent genetic polymorphisms, *CYP2C19* polymorphisms can be used to explain a poor response to clopidogrel, and *CYP2C19*2* is the strongest predictor of high residual platelet reactivity¹⁹. However, few studies have reported the significance of *CYP2C19* polymorphisms in affecting the incidence of MACCEs after carotid artery stenosis, especially carotid artery stents.

It has been reported that patients with *CYP2C19* LOF variant alleles have a 3.58 times higher risk for death and stroke than patients with the *CYP2C19* wild-type genotype²⁰. A recent systematic review also demonstrated that *CYP2C19* loss-of-function alleles were associated with clinical outcomes for ischaemic stroke²¹. In addition, a previous study reported that *CYP2C19*2* was an independent risk factor for the primary outcomes of clopidogrel treatment in patients with acute ischaemic stroke²². In our study, the incidence of MACCEs in *CYP2C19* LOF allele noncarriers (n = 343) was 7.14% (9/126), and in carriers, it was 14.69% (26/177). Patients with *CYP2C19* LOF variant alleles had a 2.05 times higher risk of MACCEs than patients with the *CYP2C19* wild-type genotype. Table 2 shows that there was no significant difference in the frequency of *CYP2C19* LOF alleles in the conservative treatment group regardless of whether the patients experienced MACCEs. The frequency of *CYP2C19* LOF alleles of the patients who experienced MACCEs was significantly higher than that of the non-MACCE patients. In the log-rank tests and multivariable Cox analysis, we found that *CYP2C19* LOF allele variants were associated with an increased risk of MACCEs in patients who underwent cerebral artery stents. In this way, we infer that *CYP2C19* LOF allele carriers are related to MACCE incidence in the cerebral artery stent group. These results could be driven by the fact that within conservative treatment patients, the patient's condition is so poor that the *CYP2C19* gene does not work.

In addition, the baseline characteristics showed that in the conservative treatment group, the glucose of the patients who experienced MACCEs was significantly higher. In addition, in the cerebral artery stent group, the frequency of *CYP2C19* LOF alleles and the ALT of the patients who experienced MACCEs was significantly higher, and the total protein was significantly lower. To identify the influencing factors of the clinical endpoint after cerebral artery stenosis, we first performed log-rank tests. We found that *CYP2C19* LOF allele variants, high ALT levels, low total protein levels and high blood glucose levels were associated with an increased risk of MACCEs in patients who underwent cerebral artery stents. However, only a high blood glucose level is associated with an increased risk of MACCEs in patients who underwent conservative treatment. These findings suggested that glycaemic control was key to MACCE prevention in all carotid artery stenosis patients. In addition, we generated Kaplan-Meier curves to assess the association of *CYP2C19* LOF alleles and the incidence of MACCEs stratified by patient status. We found that the *CYP2C19* LOF allele carriers were identified as a predictor of the incidence of MACCEs at 6 months after carotid artery stenting only when the patients had high levels of glucose (glucose > 6.5 mmol/L).

A Danish cohort of nearly 60 000 patients with myocardial infarction demonstrated that the clinical efficacy of clopidogrel in diabetes mellitus (DM) was impaired²³. It has also been reported that DM significantly increases the risk of stroke recurrence and poor outcome in the small-artery occlusion subtype²². In our study, the rate of MACCEs in *CYP2C19* LOF allele carriers was higher than that in noncarriers in patients with high blood glucose levels who underwent carotid artery stents, while in patients with normal blood glucose levels, it was not. This could be ascribed to high blood glucose levels decreasing the active metabolite of clopidogrel²⁴, especially impairing the clopidogrel clinical efficacy of *CYP2C19* LOF allele carriers with genetically poor clopidogrel metabolism. The mechanism of this has been reported: the active metabolite of clopidogrel could increase platelet reactivity by upregulating platelet P-selectin²⁵ and protein kinase C²⁶ via the glycation of platelet surface proteins and osmotic effects of glucose²⁷. These findings remind us that it is necessary to perform *CYP2C19* genotype tests in patients who underwent cerebral artery stents with dysglycaemia.

Next, we used the Cox proportional hazards regression model to assess the association between *CYP2C19* LOF allele carriers and patient status with MACCE risk. As shown in Table 3, the univariable analysis and multivariable Cox analysis revealed that glucose > 6.5 mmol/L was significantly associated with MACCEs at 6 months in the conservative treatment group. Nevertheless, the univariable analysis revealed that *CYP2C19* genotypes, ALT > 35 U/L, total protein < 65 g/L and glucose > 6.5 mmol/L were significantly associated with MACCEs at 6 months in the cerebral artery stent group (Table 4). We found that *CYP2C19* genotypes, total protein < 65 g/L and glucose > 6.5 mmol/L remained significant in the multivariable Cox analysis. Collectively, these results indicated that *CYP2C19* LOF allele variants, total protein levels and dysglycaemia synergistically impact the risk of MACCEs. On the one hand, malnutrition has an important prognostic value in patients who undergo cerebral artery stents²⁸, and total protein < 65 g/L may reflect the malnourished status of the patient. On the other hand, it could be due to poor kidney function, as the protein is lost in the urinary system, leading to a decrease in total protein. Treatment resistance for clopidogrel. It has been reported that chronic kidney disease is associated with impaired drug absorption and transport and platelet abnormalities, which are thought to decrease the response to clopidogrel^{11,29}. In addition, *CYP2C19* LOF was reported to be associated with an increased risk of adverse cerebrovascular outcomes in patients in the lowest quintile of eGFR. In this way, low total protein levels may reflect poor kidney function, and the response to clopidogrel is decreased.

In this way, we conclude that *CYP2C19* LOF allele variants, total protein levels and dysglycaemia synergistically impact the risk of MACCEs in patients who underwent cerebral artery stents. In addition, it is necessary to perform *CYP2C19* genotype tests in patients who underwent cerebral artery stents with dysglycaemia and low total protein levels.

There are several limitations in our study. Considering that this is a single-centre study, further multicentre and large sample studies are needed to expand upon our findings. Glucose levels were assessed only at baseline, and a dynamic evaluation of glucose levels could be more informative. In addition, glucose levels can only reflect the current status, and glycated albumin and haemoglobin A1c can more accurately reflect

the actual status of glycaemic control. Moreover, we did not assess the status of insulin resistance, which could lead to abnormal platelet P2Y₁₂ receptor signalling in diabetic patients.

Declarations

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Contributors

Xinyuan Hu and Sijia Hao analysed the data and performed the experiments. Sijia Hao drafted paper. Haoyuan Yin, Xinlu Wang and Zhongxi Yang, Jianmin Piao, and Yuhao Zhao selected the data. Yanjiao Li followed the patients' statement. Yanfang Jiang designed and fund the study. Xuan Chen designed and selected data.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval Research aspects of this study were approved by Ethics Committee of the First Hospital of Jilin University 2016-151

Patient consent for publication Not required

Conflict of interest statement The authors declare that they have no conflicts of interest.

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Figures

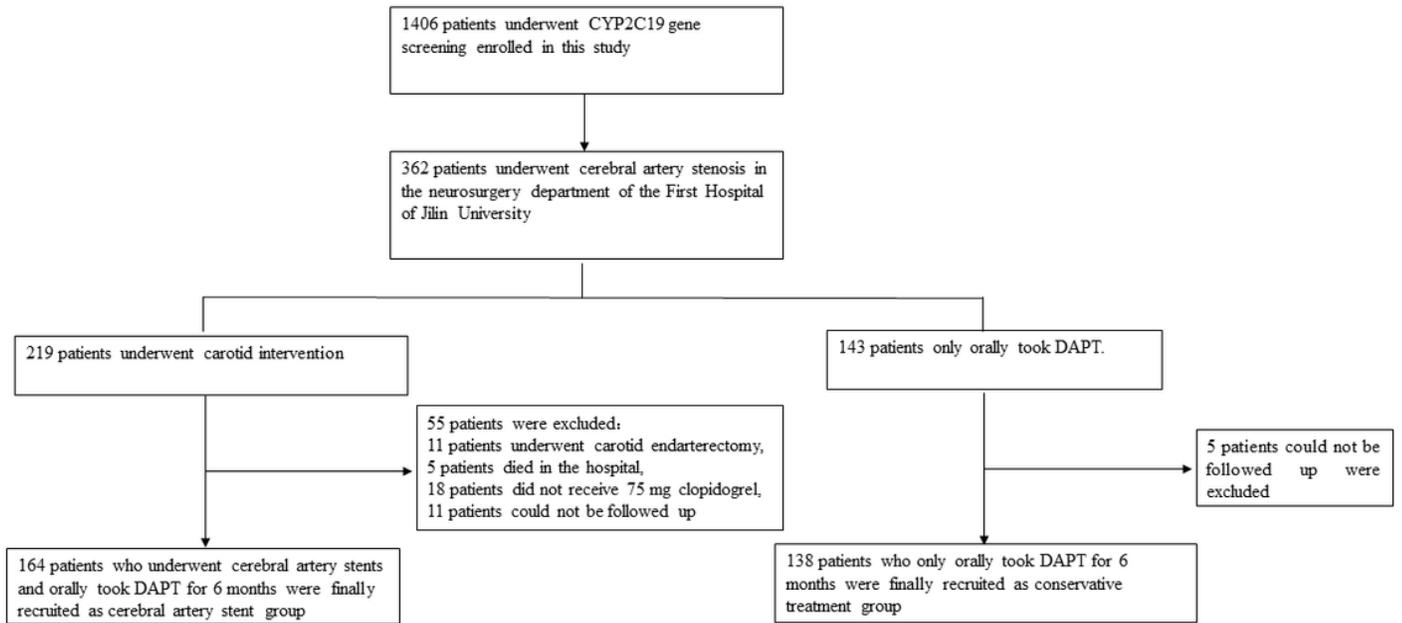


Figure 1

Flow diagram of the study population recruitment process.

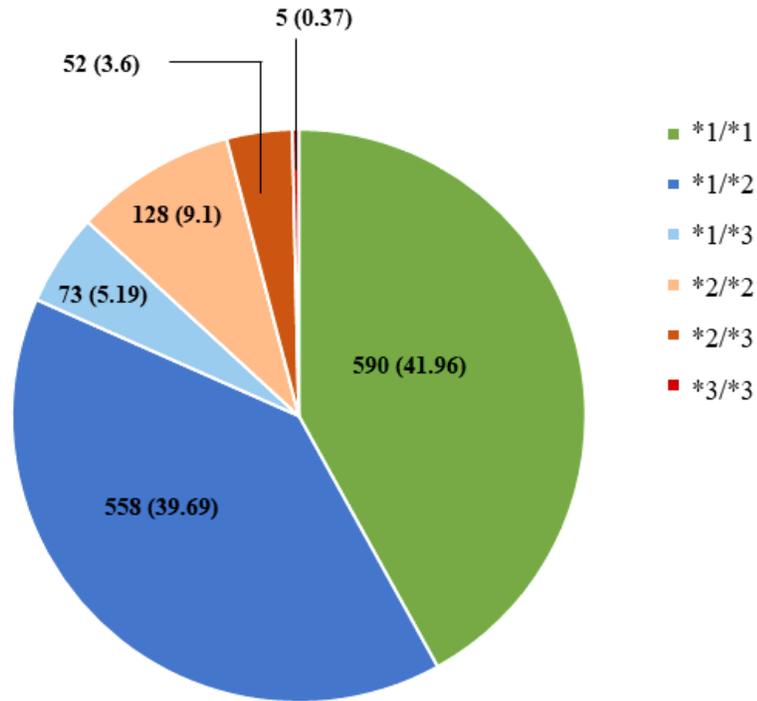


Figure 2

Distribution of the CYP2C19 genotype in patients.

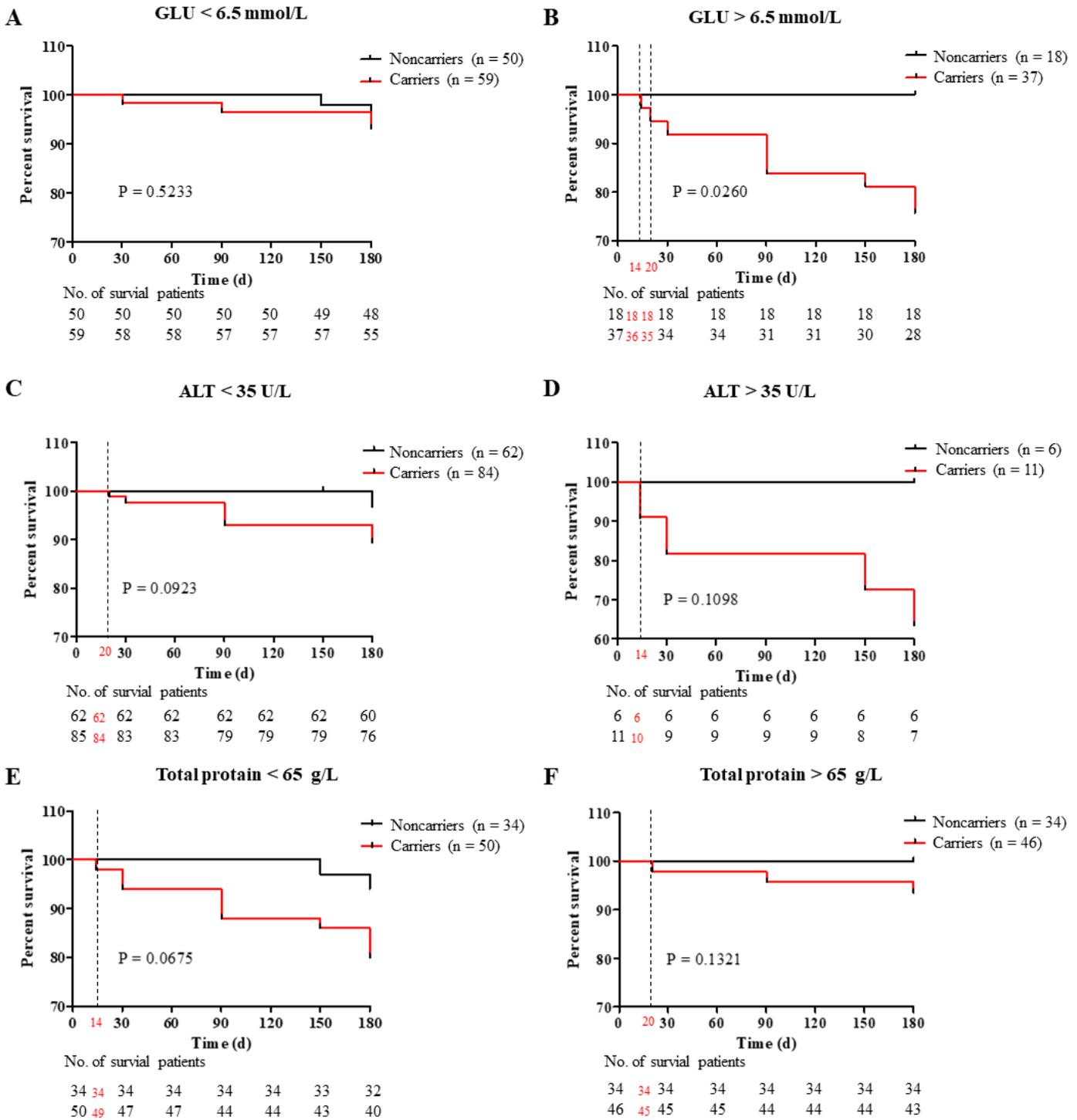


Figure 3

Rates of MACCEs over 6 months of follow-up in patients underwent cerebral artery stent. CYP2C19 LOF alleles carriers including the genotypes *1/*2, *1/*3, *2/*2, *2/*3 and *3/*3. The genotype of non-carriers is CYP2C19 *1/*1. The curves represent the percentage of patients surviving at the endpoints. The numbers below the survival curves are the numbers of patients in each group who survived at the endpoints and were still at risk over the follow-up period.

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