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ORIGINAL ARTICLE

The Association Between CYP2C19 Genotype and of In-stent Restenosis Among Patients with Vertebral Artery Stent Treatment

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SUMMARY

Aims: Preventing stroke through endovascular treatment with vertebral artery stent remains a great challenge due to the occurrence of an in-stent restenosis. **Materials & Methods:** In this study, a retrospective analysis was conducted in 90 patients who had been treated with VAS between 2004 and 2011 in Nanjing Drum Tower Hospital. Patients were followed up at 3 months, 6 months,and 1 year after VAS treatment and annually thereafter. For each time point, neurological function tests, vessel ultrasound and computer tomography angiography were performed to preliminarily screen the vessel stenosis. Digital subtraction angiography was used to verify the narrow sign on CTA or ultrasound. Clinical features of each patient including clopido-grel metabolization genes (CYP2C19, CYP3A4, and P2Y12) were recorded with purpose to investigate the possible risk factors for the development of ISR. **Results:** Single factor analysis dem-onstrated that hyperlipidemia ($P < 0.05$) and CYP2C19 ($P < 0.01$) loss-of-function geno-type increased the likelihood of ISR. A multiple logistic cox regression analysis also showed that stroke patients with hyperlipidemia (HR 3.719, 95% CI: 1.094–12.637, $P = 0.035$), and CYP2C19 loss-of-function genotype (HR 2.959, 95% CI: 1.325–6.610, $P = 0.008$) were more likely to suffer from ISR. Furthermore, CYP2C19 alleles were mainly divided into three groups: wt/wt (CYP2C19 *1/*1), wt/m (CYP2C19 *1/*2 and *1/*3), and m/m (CYP2C19 *2/*2,*2/*3 and*3/*3). Recurrent rate of ischemic stroke in m/m and wt/m groups was higher than the wt/wt group (OR: 0.141, 95% CI: 0.016–1.221, $P = 0.042$). **Conclusion:** The study leads to the conclusion that hyperlipidemia and CYP2C19 impotency are possible risk factors for the development of ISR in VAS-treated patients with ischemic. Moreover, VAS-treated patients with CYP2C19 impotency were susceptible to recurrent stroke during our 54-month follow-up.

Introduction

Ischemic stroke is catastrophic disease leading to high mortality and disability. About 25–30% ischemic strokes occur in the vertebral artery (VA) territory [1,2]. With the evolution of endovascular device technology, percutaneous placement of stent has been applied in the management of the VA stenosis. Patients with posterior circulation ischemic symptoms and VA stenosis >50% determined by digital subtraction angiography (DSA) are considered for endovascular therapy [3]. Endovascular treatment of vertebral artery stent (VAS) remains a major challenge for the occurrence of an in-stent restenosis (ISR). Once vessels were injured by high-pressure balloon dilation and deployment of stents, platelets are activated and adhered to the injured site, finally initiating coagulation cascade [4]. Thus, dual antiplatelet therapy (75 mg clopidogrel plus 100 mg aspirin per day) for a minimum of 1 year is recommended for stent-planted patients [3].

Thrombotic complications of stent deployment were classified as subacute thrombosis (occurring within 30 days of stent placement), late stent thrombosis (31 days to 1 year) and very late stent thrombosis (after 1 year) [5]. The mechanisms and risk factors contributing to the development of ISR in each stage are not fully understood. Current literatures demonstrated that patients with advanced age, premature antiplatelet termination, clopidogrel resistance are prone to thrombus formation [6]. Genetic variations of clopidogrel metabolic genes (CYP2C19, CYP3A4, and P2Y12) are indentified to play critical roles in clopidogrel resistance [7]. Especially, in Asian countries, poor metabolize

(CYP2C19*2 and CYP2C19*3) ratio is higher than that in Caucasians [8]. Therefore, it is necessary to evaluate the correlations between genetic polymorphisms of clopidogrel metabolic genes and ISR occurrence in Asian countries. Additionally, it is still uncertain that whether clopidogrel genetic polymorphisms could endanger the long-term efficacy and safety of VAS.

In this retrospective study, we analyzed 90 patients that had been treated with stent between January 2004 and December 2011 in Nanjing Drum Tower Hospital. Data including vertebral artery ultrasound, computer tomography angiography (CTA), and DSA were collected to identify risk factors for the development of ISR during long-term follow-up.

Materials and methods

Patients

This study was completed in Affiliated Drum Tower Hospital of Nanjing University Medical School and included 90 patients that had been treated with stent. The stenosis was assessed by DSA. Stenosis is defined as symptomatic VA stenosis ≥50% or asymptomatic VA stenosis ≥70% following North American Symptomatic Carotid Endarterectomy Trial criteria [9]. Patients were excluded if they had (1) stenting of dissections; (2) vessel kinks; (3) stroke symptoms not thromboembolic or hemodynamic in nature small-vessel disease of brainstem perforators; (4) previous treatment with a clopidogrel or warfarin for any reasons; (5) history of allergy or any contraindications to aspirin, clopidogrel, or heparin; or (6) cancer or hemodialysis.

Procedures

Vertebral artery stent was carried out by experienced interventional neuroradiologists. All interventions were performed *via* a transfemoral approach. Stent type and the usage of filter-based neuroprotection devices were carefully determined by experienced interventionist. All patients orally take aspirin (300 mg/day) and clopidogrel (75 mg/day) at least 3 days before the procedure. Clopidogrel was continued for a minimum of 1 year after VAS, and aspirin (100 mg/day) was administered chronically.

Follow-up

After treated with VAS, patients were followed up by doctors in the Stroke Center of Jiangsu province at 3 months, 6 months, and 1 year after treatment and annually thereafter. Follow-up examinations were performed by independent neurologists or stroke physicians. For each time point, neurological function tests, vessel ultrasound, and CTA were performed to preliminarily screen the vessel stenosis. DSA was used to verify the narrow sign on CTA or ultrasound. Bad clinical events included ipsilateral posterior circulation ischemic stroke. Ipsilateral posterior circulation ischemic stroke was defined as acute onset of focal neurological deficit lasting longer than 24 h. Events that occurred within 30 days of initial treatment were excluded because they were defined as perioperative complications of treatment. To make sure the natural risk of cerebrovascular events with restenosis, patients who had endovascular treatment for recurrent stenosis after their initial treatment were censored at the time of retreatment.

Genotype Determinations

Whole blood sample (2 mL) was obtained in 90 of the patients, and DNA was extracted for genotyping CYP2C19 (636G>A, 681G>A), CYP3A4 (894C>T), and P2Y12 (34C>T, 52G>T) as described previously [10]. DNA was extracted from 200 μL blood sample using a commercially available DNA extraction Kit (BaiO Technology Co. Ltd., Shanghai, China) according to the manufacturer's instructions. For the genotype of CYP2C19, CYP2C19*2 (681G>A), and CYP2C19*3 (636G>A) mutant alleles were determined using a commercially available Hybrid color kit (BaiO Technology Co. Ltd.). For the genotype of CYP3A4 and P2Y12, ABI 3730XL Sequence Detection System (ABI, Shanghai, China) was involved. Duplicate samples and negative controls were included to ensure the accuracy of genotype.

Statistical Analysis

All statistical analyses were performed with SPSS 17 (Spss Inc., chicago, IL, USA). Nominal variables were expressed as count and percentages; continuous values were expressed as mean ± SD; and non-normal distributed variables were expressed as median values with OR and 95% CI. For univariate comparisons, two-tailed chi-square statistics with Yates' correction and univariate Fisher's exact test were used. Abnormally distributed variables were compared using a Mann–Whitney *U*-test. Overall survival rate during long-term follow-up were determined by Kaplan–Meier curves. A cox regression analysis was used to estimate the potential effect of a variable on the occurrence of an ISR. A value of $P < 0.05$ was considered to indicate a statistically significant difference.

Results

In this study, 90 patients undergoing elective VAS between January 2004 and December 2011 were analyzed (mean age: 66.8 ± 9.6 years, 78.6% men). A total of 60 TIA patients and 30 stroke patients with VAS were treated with stents. Successful dilatation, defined as <30% residual stenosis, was achieved in all cases (100%). Bare metal stents were used in 42 patients and drug-eluting stents were deployed in the remaining 48 (53.3%) patients. A total of 76 (84.4%) patients were placed with the stent in the V1 segment of vertebral artery, and 14 (15.6%) patients were placed in the V4 segment of vertebral arteries. Angiograms after stent placement did not show any sign of arterial wall dissections, rupture, or distal occlusions of major intracranial branches. There were no symptomatic strokes or deaths during the first 30 days after treatment. The detailed patient characteristics are listed in Table 1.

Single factor analyses demonstrated that hyperlipidemia and CYP2C19 loss-of-function genotype were risk factors for patients to develop ISR. There were no significant interactions among the effects of hypertension ($P = 0.051$), hypercholesterolemia ($P = 0.016$), hypertriglyceridemia ($P = 0.125$), high LDL cholesterol ($P = 0.914$), and low HDL cholesterol ($P = 0.257$) (Table 1).

Table 1 Clinical, demographic, aboratorial, radiological, surgical variables and the in-stent restenosis of vertebral artery

	No ISR (n = 75)	ISR ≥ 50% (n = 15)	*P* value
Age	67.30 ± 9.57	66.73 ± 8.00	0.878
Sex			
Male	60	14	0.218
Female	15	1	
Median duration of clinical follow-up (months)	55.9 ± 0.58	56.07 ± 18.03	0.169
Symptoms			
TIA	51	9	0.584
Stroke	24	6	
Hypertension	58	12	0.051
History of diabetes	22	5	0.758
Hyperlipidemia	21	9	0.016*, †
Hypercholesterolemia	17	6	0.160
Hypertriglyceridemia	16	6	0.125
High LDL cholesterol	19	4	0.914
Low HDL cholesterol	15	5	0.257
Hyperuricemia	12	3	0.704
Smoking (past or present)	24	6	0.549
History of coronary heart disease	13	1	0.298
History of peripheral vascular disease	2	1	0.431
Coexistent other cerebral arteries stenosis	36	8	0.706
Vessel size (mm)	4.11 ± 0.65	4.03 ± 0.67	0.690
Before stenting‡			
Minimal luminal diameter (mm)	0.72 ± 0.51	0.71 ± 0.50	0.974
Diameter stenosis (%)	70.8 ± 14.9	72.1 ± 13.2	0.324
Stenosis length (mm)	12.9 ± 5.1	13.7 ± 4.7	0.536
After stenting‡			
Minimal luminal diameter (mm)	2.16 ± 0.46	2.49 ± 0.21	0.847
Diameter stenosis (%)	8.1 ± 5.1	9.2 ± 4.7	0.747
Stented length (mm)	16.8 ± 6.2	16.1 ± 5.3	0.605
Contralateral vessels severe stenosis or occlusion	8	2	0.764
Kinds of stents			
BMS	35	7	1.000
DES	40	8	0.603
Location of stenosis or stents			
V1	64	12	
V4	11	3	
Drug therapy during follow-up (%)			
PPI	14	2	0.668
CYP3A4-metabolized statin	58	11	0.883
Insulin	28	6	0.949
Genotype			
P2Y12			
C34T			
CC	48	10	0.583
CT	22	5	
TT	5	0	
G52T			
GG	50	12	0.491
GT	21	3	
TT	4	0	
CYP3A4			
894 site			
CC	59	11	0.775
CT	15	4	
TT	1	0	

(continued)

Table 1 (Continued)

	No ISR (n = 75)	ISR ≥ 50% (n = 15)	*P* value
CYP2C19			
wt/wt	44	2	0.001*, †
wt/m	25	9	
m/m	6	4	

Data are presented as mean ± SD, number, or median (OR 95% CI) at the time of two subgroups. ISR, in-stent restenosis; TIA, transient ischemic attack; DES, drug-eluting stents; BMS, bare metal stents; PPI, proton pump inhibitors. *Numbers are included in multiple regression analysis ($P < 0.1$, univariate analysis). †Numbers are remained significant after cox regression analysis. ‡Degree of stenosis measured on angiography at study entry according to NASCET (North American Symptomatic Carotid Endarterectomy Trial) method.

Other clinical characteristics were similar between the two endovascular treatment subgroups (Table 1). To further identify independent predictors for ISR, diverse clinical parameters were analyzed by a multiple cox regression. The strongest statistically significant predictors for the development of a subsequent ISR by multiple cox regression analysis were hyperlipidemia (HR 3.719, 95% CI: 1.094–12.637, $P = 0.035$) and CYP2C19 (HR 2.959, 95% CI: 1.325–6.610, $P = 0.008$) (Table 2).

Genetic data were available in all the 90 patients enrolled in our study. Of the 90 patients (90 arteries), 46 were wild-type homozygous (*1/*1) (wt/wt group), 34 were heterozygotes (30 for *1/*2 and 4 for * 1/*3) (wt/m group), and 10 were homozygous (8 for *2/*2, 1 for *2/*3, and 1 for *3/*3) (m/m group). Consequently, 44 patients (48.9%) were carriers of at least one *2 or *3 allele. During the first 30-day periprocedural follow-up time, no patients (0%) suffered from stroke or death, whereas the rate rose in the long-term follow-up period (>30 days after VAS). Correlation between clinical complications (ipsilateral posterior circulation ischemic stroke) and CYP2C19 genotype during our follow-up was represented by Kaplan–Meier curve as shown in Fig. 1. In contrast with wt/wt group, clinical complication incidence of wt/m and m/m group was much higher by Kaplan–Meier analysis (OR: 0.141, 95% CI: 0.016–1.221, $P = 0.042$).

Discussion

In this study, we found that the strongest statistically significant predictors for the development of a subsequent ISR were hyperlipidemia and CYP2C19 loss-of-function genotype by single factor analysis and *cox* regression analysis. Furthermore, patients in CYP2C19 m/m and wt/m groups are more prone to ischemic stroke than that in wt/wt group during a median of 54-month follow-up time.

Table 2 Final multiple logistic regression analysis showing the independent variables associated with in-stent restenosis of vertebral artery

Variable	HR (95% CI)	*P* value
Hypertension	3.524 (0.378–6.770)	0.524
Hyperlipidemia	3.719 (1.094–12.637)	0.035*
CYP2C19	2.959 (1.325–6.610)	0.008*

*$P < 0.05$

Currently, around one-fifth of posterior circulation strokes resulted from vertebral artery stenosis [1,2]. VA stenosis may occur either extra- or intracranially, but it is frequently localized at the origin of VA where it arises from the subclavian artery, and atherosclerosis is the most common cause of VA stenosis [11]. The treatment of asymptomatic patients with significant stenosis of VA origin is controversial. Although most asymptomatic patients do not require endovascular treatment, some investigators believe that high-grade stenosis (>70%) affecting the origin of a dominant or single VA should be treated in case of the occurrence of ischemic stroke [12]. In our study, symptomatic VA stenosis ≥50% or asymptomatic VA stenosis ≥70% received the deployment of stent and dual antiplatelet drugs in the following 1 year. However, the best treatment option of VAS is still uncertain, which calls for further studies.

Previous studies have reported associations between different clinical characteristics and ISR, and identified a certain number of risk factors that may or may not be related with restenosis [13,14]. In our study, ISR showed significant positive correlations with the

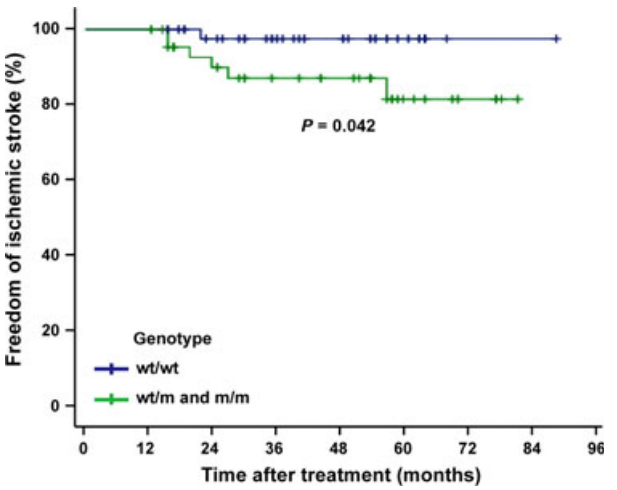

Figure 1 Kaplan–Meier curve represented the correlation between clinical complications (ipsilateral posterior circulation ischemic stroke) and CYP2C19 genotype during our follow-up. CYP2C19 loss-of-function alleles were divided into three groups: wt/wt (CYP2C19 *1/*1), wt/m (CYP2C19 *1/*2 and *1/*3), m/m (CYP2C19 *2/*2, *2/*3, and *3/*3). Blue line represented patient percentage exempted from ischemic stroke in wt/wt group. Green one represented that in wt/m and m/m group.

presence of hyperlipidemia, consistent with other bivariate correlation analysis in which hyperlipidemia emerged as a univariate predictor of clinical restenosis [15,16]. Given the substantial risk of high triglycerides and LDH [17], it seems that hyperlipidemia were independent predictors of clinically adverse cardiovascular events owing to the clinical plaque progression.

Nowadays, patients with ischemic stroke or stent therapy are conventionally treated with clopidogrel to inhibit the platelet aggregation. Clopidogrel is a kind of prodrugs that had to be metabolized into active derivate, which owns the ability to inhibit the platelet aggregation *in vivo*. Multiple G-protein-coupled receptors and enzymes are involved in the process of clopidogrel metabolization [18,19]. Recently, much attention has focused on genetic variations influencing clopidogrel metabolization and the risk of restenosis.

In this study, we investigated P2Y12, CYP3A4, and CYP2C19 genes, in which only CYP2C19 was demonstrated to be related with stent restenosis. CYP2C19 is located on chromosome 10q24.1-q24.3 site and encodes a member of the cytochrome P450 enzymes, participating in clopidogrel metabolism and lipid reactions. Several independent clinical studies have demonstrated that CYP2C19 polymorphism was closely related to stent thrombosis in cardiovascular diseases [20,21]. Carriage of the CYP2C19*2 or CYP2C19*3 allele has been repeatedly reported to be associated with a diminished pharmacokinetic and pharmacodynamic response to clopidogrel in coronary diseases [22–24]. At the meantime, patients with high residual platelet response to clopidogrel were thought to have increased risk of stent thrombosis [20,25,26]. Consequently, the CYP2C19 loss-of-function allele was significantly associated with on-clopidogrel platelet reactivity, resulting in an early coronary stent thrombosis [25,26].

Additionally, patients carrying CYP2C19*2 or CYP2C19*3 allele had a higher rate of subsequent cardiovascular events than those carrying CYP2C19*1 when they received stent and clopidogrel therapy. In the Impact of Extent of Clopidogrel-Induced Platelet Inhibition During Elective Stent Implantation on Clinical Event Rate (EXCELSIOR) trial, patients with on-clopidogrel residual platelet aggregation >14% at predischarge had a 3-fold increase in the 1-year incidence of death and myocardial infarction after stent placement [26]. In a word, there is a strong link among CYP2C19 genetic polymorphisms, decreased clopidogrel responsiveness, and clinical outcomes including ISR [27,28]. Carriers of CYP2C19*2 allele generates less amounts of the active metabolite of clopidogrel, resulting in a decreased antiplatelet effect and therefore carry an increased risk of major adverse cardiac events after stent placement. However, some studies suggested that genotyping alone or in combination with clinical factors could not guide antiplatelet therapy in patients undergoing stent [29]. And further studies are warranted to evaluate the genetic polymorphism to guide antiplatelet therapy.

Overall, hyperlipidemia and CYP2C19 loss-of-function genotype were risk factors for patients placed with stent to develop ISR. With respect to the clinical relevance of ISR and the lack of a commonly accepted treatment strategy, all efforts should be made to carefully follow up especially patients with the presence of hyperlipidemia or genotype of CYP2C19. However, there are also several shortages in this study. For instance, it is an observational and retrospective study. Although we detected most of factors that have been probably associated with ISR, we cannot exclude the possibility that unmeasured clinical parameters may explain some of our data. Additionally, due to small sample size in m/m group, we only evaluated recurrent stroke rate and death, and other complications including transient ischemic attack, myocardial infarction could be further evaluated in this special group. More data from ongoing randomized trials are needed to assess whether modern stenting techniques are as effective as surgical or medical tools for preventing restenosis in the long term and to determine accurately the relation between restenosis and recurrent stroke over time.

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Disclosures

None.

Conflict of Interest

The authors declare no conflict of interest.

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