

## Original Article

# Elevated plasma level of homocysteine is associated with intra/extracranial atherosclerotic stenosis in Chinese patients with large artery atherosclerotic cerebral infarction

Weihua Mai<sup>1</sup>, Hanwei Liu<sup>1</sup>, Xingwei Liu<sup>2</sup>, Xiangqiong Mo<sup>2</sup>, Guangxiao Su<sup>3</sup>

Departments of <sup>1</sup>Neurology, <sup>2</sup>General Surgery, <sup>3</sup>Prevention and Healthcare, The Fifth Affiliated Hospital of Sun Yat-sen University, Zhuhai 519000, Guangdong Province, China

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**Abstract:** Background and aims Hyperhomocysteinemia was recognized as a risk factor for cerebral infarction, especially caused by large-artery atherosclerosis (LAA). But whether high plasma total homocysteine (tHcy) is associated with cervico-cerebral atherosclerotic stenosis (CCAS) remains unclear. The aim of this study is to investigate whether elevated plasma tHcy is a risk factor for CCAS, and identify its effects on the distribution of CCAS in cerebral infarction. Methods: 123 Chinese patients with LAA cerebral infarction were enrolled. Digital subtraction angiography of cervico-cerebral arteries was performed, and plasma tHcy was assessed in all patients. According to the severity of stenosis, the patients were categorized into CCAS and non CCAS (NCCAS). CCAS was furthered classified into intracranial AS (ICAS), extracranial AS (ECAS) and combined intra- and extra-cranial AS (IECAS) subgroups. Results: The concentrations of plasma tHcy were elevated in the patients with CCAS, compared to in NCCAS. After adjusting for other possible confounders, elevated level of plasma tHcy remained an independent risk factor for CCAS (adjusted OR: 1.159). OR of developing CCAS was 3.715 times higher in the highest tHcy quartile, compared to the lowest one. The level of plasma tHcy in the patients with ICAS was higher than that in ECAS. After adjusting for diabetes mellitus, high tHcy remained an independent risk factor for ICAS (adjusted OR: 1.127). Conclusions: The data demonstrate that hyperhomocysteinemia is an independent risk factor for CCAS, and elevated level of plasma tHcy may contribute to the distribution of CCAS in Chinese patients with LAA cerebral infarction.

**Keywords:** Homocysteine, large artery atherosclerosis, cervico-cerebral atherosclerotic stenosis, intracranial atherosclerotic stenosis, extracranial atherosclerotic stenosis, cerebral infarction

## Introduction

Large artery atherosclerosis (LAA), including atherosclerosis of aortic arch and intra-/extracranial large arteries, is the most common etiology of ischemic stroke in China, according to Chinese ischemic stroke subclassification (CISS) system. It is suggested that the distribution of cervico-cerebral atherosclerotic stenosis (CCAS) varies among different races. Intracranial AS (ICAS) is more common in Asians and Africans, whereas extracranial AS (ECAS) is more prevalent in Caucasians [1-3]. Multiple traditional risk factors including age, gender, hypertension, diabetes, dyslipidemia, smoking and metabolic syndrome were implicated in the development of ICAS or ECAS [4-8]. But as to

the factors responsible for the location of atherosclerosis in the EC or IC arteries, conflicting data were reported. Some studies suggested that diabetes, a higher apoB/apoAI ratio and erythrocyte sedimentation rate were more correlated to ICAS than ECAS [9-11], whereas others failed to find any risk factors between ICAS and ECAS, except for a higher frequency of males and higher total cholesterol levels in ECAS [12]. What are the different influences of these factors on ICAS and ECAS in Asian population remain to be elucidated.

High plasma total homocysteine (tHcy) was considered to be a risk factor for ischemic stroke, especially caused by LAA [13]. It was suggested that the deleterious effect of hyperhomocyste-

inemia was mediated primarily via proatherogenic effects [14]. And homocysteine-induced atherothrombosis was formed by several mechanisms [15]. However, whether hyperhomocysteinemia is a risk factor or a marker for atherosclerotic stenosis in cervico-cerebral large arteries remains unidentified.

It is known that IC and EC arteries have different biological properties. IC arteries have greater antioxidant enzyme activities than EC arteries. However, as age increases, the antioxidant capacity of IC arteries decreases much more than EC arteries, so as to become more prone to oxidative stress, coinciding with a marked acceleration of atherogenesis [16]. In experimental animal models, it has been demonstrated that hyperhomocysteinemia can induce endothelial dysfunction and oxidative stress, manifested by decreased bioavailability of endothelium-derived nitric oxide [13]. Therefore, hyperhomocysteinemia is supposed to be more associated with IC atherosclerosis than EC atherosclerosis. However, with regard to the association of plasma tHcy level with the distribution of CCAS, conflicting results were obtained. It was showed that decreased tHcy was more closely associated with ICAS than with ECAS, but multiple logistic regression analysis indicated that tHcy was not independently associated with ICAS [17]. Another study suggested that hyperhomocysteinemia didn't contribute to ICAS and ECAS in ischemic stroke [18].

In this study, we attempt to investigate whether elevated level of plasma tHcy is an independent risk factor for CCAS defined by digital subtraction angiography (DSA), and identify its effects on the distribution of CCAS in LAA cerebral infarction.

### Material and methods

#### *Subjects*

One hundred and twenty-three consecutive Chinese patients admitted to Department of Neurology in the Fifth Affiliated Hospital of Sun Yat-sen University, from March 2009 to December 2016, were recruited according to the following criteria: 1) 18 to 80 years old, diagnosed as acute ischemic stroke based on clinical manifestation and CT or/and MRI evidence,

within two weeks of onset; 2) categorized as large artery atherosclerotic cerebral infarction according to CISS system; 3) DSA of cervico-cerebral arteries was performed. Exclusion criteria included: 1) cardioembolic stroke; 2) cervico-cerebral artery stenosis caused by other etiologies such as Takayasu arteritis, dissecting aneurysm and cerebrovascular malformation et al.; 3) previous history of vascular stenting or angioplasty in Intra/extracranial arteries; 4) accompanied with other serious diseases such as serious infection, heart disease, thyrotoxicosis, malignant tumor, epilepsy and psychological disease et al., or with unstable medical conditions such as cerebral hernia et al.; 5) pregnant or lactating women. This study was approved by the Local Ethical Committee in the Fifth Affiliated Hospital of Sun Yat-sen University, and all procedures were carried out with the consent of all subjects.

#### *Inquiry of clinical information*

Clinical information including age, gender, previous history of hypertension, diabetes mellitus (DM), ischemic stroke, smoking and heavy drinking were collected from each patient. Hypertension was defined as a high baseline blood pressure (systolic blood pressure  $\geq 140$  mmHg or diastolic blood pressure  $\geq 90$  mmHg) or previous history of antihypertensive medication. DM was diagnosed as a fasting plasma-glucose  $\geq 7.0$  mmol/L, or OGTT 2hPG  $\geq 11.1$  mmol/L, or a previous history of antidiabetic medication. Ischemic stroke was defined as previous neurological symptoms caused by cerebral infarction confirmed by CT/MRI. Smoker and drinkers were described as current smokers/drinkers or ex-smokers/ex-drinkers with a history of smoking/drinking habits.

#### *Assessment of plasma homocysteine and lipid profiles*

The level of plasma tHcy was assessed by AXSYM automatic chemiluminescence analyzer (Abbott i2000, USA), using chemiluminescent immunoassay (CLIA). Blood lipid profiles, including total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL-C) and low-density lipoprotein (LDL-C) were analyzed by automatic biochemical analyzer (Hitachi 7600, Japan). All assessments were conducted in the hospital clinic laboratory.

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**Table 1.** Demographics and clinical characteristics of the patients with CCAS<sup>a</sup> and NCCAS<sup>b</sup>

	NCCAS n=29	CCAS n=94	p-value	F-value	Chi-square
<b>Demographics</b>					
Gender (male %)	15 (51.7%)	72 (76.6%)	0.01*		6.622
Age (years old)	43.03±14.968	51.69±13.264	0.003*	8.879	
<b>Traditional risk factors</b>					
Smoking (%)	8 (27.6%)	27 (28.7%)	0.906		0.014
Drinking (%)	2 (6.9%)	5 (5.3%)	1.000		0.000
Hypertension (%)	20 (69.0%)	81 (86.2%)	0.035*		4.467
Diabetes mellitus (%)	6 (20.7%)	41 (43.6%)	0.026*		4.934
High LDL-C <sup>c</sup> (%)	16 (55.2%)	60 (63.8%)	0.402		0.704
Previous stroke (%)	5 (17.2%)	36 (38.3%)	0.035*		4.422
<b>Laboratory tests</b>					
tHcy <sup>d</sup> , median (IQR <sup>e</sup> ), μmol/L	10.31 (8.30-11.91)	13.01 (11.32-16.67)	0.000*		16.733

\*P<0.05. <sup>a</sup>CCAS: cervico-cerebral atherosclerotic stenosis; <sup>b</sup>NCCAS: non cervico-cerebral atherosclerotic stenosis; <sup>c</sup>LDL-C≥2.59 mmol/L; <sup>d</sup>tHcy: total homocysteine; <sup>e</sup>IQR: inter-quartile range.

## Digital subtraction angiography (DSA)

DSA of cervico-cerebral arteries was performed in all patients (PHILIPS INTEGRIS CV 12. System). The severity of stenosis was analyzed by AVA software. According to the severity of stenosis defined by NASCET criteria [19], the patients were categorized into two groups: CCAS, with 50-100% diameter stenosis in cervico-cerebral arteries and non-CCAS (NCCAS), with 0-49% diameter stenosis in cervico-cerebral arteries. The CCAS group was furthered classified into ICAS, ECAS and combined intra- and extra-cranial AS (IECAS) subgroups. Intracranial arteries include the proximal portion of middle cerebral artery (MCA:M1), anterior cerebral artery (ACA:A1), posterior cerebral artery (PCA:P1), intracranial portion of internal carotid artery (ICA) or vertebral artery (VA) and basilar artery (BA). Extracranial arteries consist of extracranial portion of ICA or VA, common carotid artery (CCA) and the proximal portion of subclavian artery (SCA).

## Statistical analysis

Differences among groups were compared by Chi-square test, one way analysis of variance (ANOVA) and Kruskal-Wallis test as appropriate. To evaluate the risk factors for atherosclerotic stenosis, a stepwise multivariate logistic regression analysis was performed. Variables with P<0.05 by univariate analysis were selected for entry into binary logistic regression analysis. Odds ratios (ORs) and 95% confidence intervals (95% CI) were used to estimate the relative

risks of different variables for atherosclerotic stenosis. Plasma tHcy were further stratified into four levels according to the quartile ranges. ORs of the four quartiles were evaluated by binary logistic regression analysis. All statistical analyses were performed with SPSS 16.0. p values less than 0.05 were considered statistically significant.

## Results

### CCAS and NCCAS

**Demographics and clinical characteristics of the patients with CCAS and NCCAS:** Based on the severity of stenosis determined by DSA, 94 enrolled patients (76.4%) were classified into CCAS group, and 29 ones (23.6%) were into NCCAS group. Demographics and clinical characteristics of the patients were summarized in **Table 1**. The age and the percentage of male in the patients with CCAS were significantly higher than that of NCCAS. In addition, compared to NCCAS, the patients with CCAS had higher frequencies of hypertension, DM, and previous stroke. The concentrations of plasma tHcy in the patients with CCAS were significantly higher than that in NCCAS.

**Risk factors for CCAS:** By univariate analysis, it was showed that male sex, old age, hypertension, DM, previous stroke and high level of plasma tHcy were potential risk factors for CCAS (**Table 2**). After adjusting for gender, age, hypertension, DM and previous stroke by binary

**Table 2.** Risk factors for CCAS by Binary logistic regression analysis

Independent variables <sup>g</sup>	CCAS <sup>a</sup> (vs NCCAS <sup>b</sup> )					
	Crude OR <sup>c</sup> (95% CI <sup>d</sup> )	Beta-value	p-value	Adjusted OR (95% CI)	Beta-value	p-value
Gender (male)	3.055 (1.279-7.297)	1.117	0.012*	4.173 (1.232-14.133)	1.429	0.022*
Age	1.045 (1.013-1.078)	0.044	0.005*	1.046 (1.009-1.086)	0.045	0.015*
Smoking	1.058 (0.418-2.678)	0.056	0.906			
Drinking	0.758 (0.139-4.133)	-0.277	0.749			
Hypertension	2.804 (1.052-7.476)	1.031	0.039*	1.571 (0.470-5.249)	0.452	0.463
Diabetes mellitus	2.956 (1.106-7.954)	1.087	0.031*	3.228 (0.877-11.884)	1.172	0.078
High LDL-C <sup>e</sup>	1.434 (0.616-3.335)	0.360	0.403			
Previous stroke	2.979 (1.043-8.509)	1.092	0.041*	2.536 (0.753-8.540)	0.931	0.133
tHcy <sup>f</sup> μmol/L	1.238 (1.070-1.433)	0.214	0.004*	1.159 (1.000-1.343)	0.148	0.049*

\*P<0.05; <sup>a</sup>CCAS: cervico-cerebral atherosclerotic stenosis; <sup>b</sup>NCCAS: non cervico-cerebral atherosclerotic stenosis; <sup>c</sup>OR: odds ratios; 95% <sup>d</sup>CI: 95% confidence intervals; <sup>e</sup>LDL-C≥2.59 mmol/L; <sup>f</sup>tHcy: total homocysteine. <sup>g</sup>Reference group for every independent variable: Female, no smoking, no drinking, no hypertension, no diabetes mellitus, normal LDL-C (<2.59 mmol/L), no previous stroke.

**Table 3.** Risks of different tHcy levels for CCAS by Binary logistic regression analysis

tHcy <sup>c</sup> quartile (μmol/L)	CCAS <sup>a</sup> (vs NCCAS <sup>b</sup> )		
	Adjusted OR <sup>d</sup> (95% CI <sup>e</sup> )	Beta-value	p-value
1 (<10.44) <sup>f</sup>	Reference		
2 (10.44~12.45)	1.974 (0.601-6.479)	0.680	0.262
3 (12.45~15.42)	2.454 (0.413-14.602)	0.898	0.324
4 (≥15.42)	4.715 (1.105-20.115)	1.551	0.036*

\*P<0.05; <sup>a</sup>CCAS: cervico-cerebral atherosclerotic stenosis; <sup>b</sup>NCCAS: non cervico-cerebral atherosclerotic stenosis; <sup>c</sup>tHcy: total homocysteine; <sup>d</sup>OR: odds ratios; 95% <sup>e</sup>CI: 95% confidence intervals; <sup>f</sup>the reference category.

logistic regression analysis, high level of plasma tHcy remained an independent risk factor for CCAS (adjusted OR: 1.159; 95% CI: 1.000-1.343, P=0.049) (Table 2).

*Risks of different tHcy levels for CCAS:* To further define the association of plasma tHcy with CCAS, the concentrations of plasma tHcy were stratified into four levels according to the quartile ranges. With the lowest quartile (<10.44 μmol/L) as a reference, ORs of developing CCAS in the highest (≥15.42 μmol/L) and middle quartiles (10.44~12.45 μmol/L and 12.45~15.42 μmol/L) were evaluated by binary logistic regression analysis. As presented in Table 3, the risk of developing CCAS increased across the quartiles. After adjusting for gender, age, hypertension, DM and previous stroke, OR of developing CCAS was 3.715 times higher in the highest tHcy quartile, compared to the lowest one (adjusted OR: 4.715; 95% CI: 1.105-20.115; P=0.036) (Table 3).

*ICAS and ECAS*

*Demographics and clinical characteristics of the patients with ICAS and ECAS:*

Among the patients with CCAS, 48 ones (51.1%) were classified into ICAS, 22 ones (23.4%) were ECAS and 24 ones (25.5%) were IECAS. To define the differences between ICAS and ECAS, demographics and clinical characteristics of the patients with ICAS and ECAS were summarized in Table 4. Compared to ECAS, the patients of ICAS had higher frequencies of DM. And the concentrations of plasma tHcy in the patients with ICAS were significantly higher than that in ECAS.

*Risk factors for ICAS:* By univariate analysis, it was suggested that only DM and high level of plasma tHcy were potential risk factors for ICAS (Table 5). After adjusting for DM by binary logistic regression analysis, high level of plasma tHcy remained an independent risk factor for ICAS (adjusted OR: 1.127; 95% CI: 1.029-1.233, P=0.010) (Table 5).

*Risks of different tHcy levels for ICAS:* As described above, the concentrations of plasma tHcy were further stratified into four levels according to the quartile ranges. With the lowest quartile as a reference, ORs of developing ICAS in the highest and middle quartiles were evaluated by binary logistic regression analysis. Compared to the lowest quartile and after adjusting for DM, the risk of developing ICAS increased across the quartiles, but with no significant differences (Table 6).

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**Table 4.** Demographics and clinical characteristics of the patients with ICAS and ECAS

	ICAS <sup>a</sup> n=48	ECAS <sup>b</sup> n=22	p-value	F value	Chi-square
Demographics					
Gender (male,%)	35 (72.9%)	16 (72.7%)	0.987		0.000
Age (years old)	51.45±14.815	51.56±14.174	0.977	0.001	
Traditional risk factors					
Smoking (%)	15 (31.2%)	6 (27.3%)	0.736		0.114
Drinking (%)	2 (4.2%)	1 (4.5%)	0.942		0.005
Hypertension (%)	41 (85.4%)	19 (86.4%)	1.000		0.000
Diabetes mellitus (%)	31 (64.6%)	8 (36.4%)	0.027*		4.869
High LDL-C <sup>c</sup>	31 (64.6%)	14 (63.6%)	0.939		0.006
Previous stroke (%)	15 (31.2%)	8 (36.4%)	0.672		0.179
Laboratory tests					
tHcy <sup>d</sup> , median (IQR <sup>e</sup> ), μmol/L	14.35 (12.72-23.85)	12.12 (10.55-13.91)	0.004*		8.140

\*P<0.05. <sup>a</sup>ICAS: intracranial atherosclerotic stenosis; <sup>b</sup>ECAS: extracranial atherosclerotic stenosis; <sup>c</sup>LDL-C≥2.59 mmol/L; <sup>d</sup>tHcy: total homocysteine; <sup>e</sup>IQR: inter-quartile range.

**Table 5.** Risk factors for ICAS by Binary Logistic regression analysis

Independent variables <sup>g</sup>	ICAS <sup>a</sup> (vs ECAS <sup>b</sup> )					
	Crude OR <sup>c</sup> (95% CI <sup>d</sup> )	Beta-value	p-value	Adjusted OR (95% CI)	Beta-value	p-value
Gender (male)	0.889 (0.283-2.789)	-0.118	0.840			
Age	0.999 (0.965-1.036)	0.000	0.976			
Smoking	1.256 (0.418-3.775)	0.228	0.684			
Drinking	1.095 (0.094-12.76)	0.091	0.942			
Hypertension	1.081 (0.252-4.646)	0.078	0.916			
Diabetes mellitus	3.191 (1.116-9.128)	1.160	0.030*	2.827 (0.901-8.869)	1.039	0.075
High LDL-C <sup>e</sup>	1.050 (0.369-2.991)	0.049	0.927			
Previous stroke	0.778 (0.267-2.269)	-0.251	0.645			
tHcy <sup>f</sup> μmol/L	1.141 (1.037-1.254)	0.132	0.007*	1.127 (1.029-1.233)	0.119	0.010*

\*P<0.05; <sup>a</sup>ICAS: intracranial atherosclerotic stenosis; <sup>b</sup>ECAS: extracranial atherosclerotic stenosis; <sup>c</sup>OR: odds ratios; 95% CI: 95% confidence intervals; <sup>e</sup>LDL-C≥2.59 mmol/L; <sup>f</sup>tHcy: total homocysteine. <sup>g</sup>Reference group for every independent variable: Female, no smoking, no drinking, no hypertension, no diabetes mellitus, normal LDL-C (<2.59 mmol/L), no previous stroke.

**Table 6.** Risk of different homocysteine levels for ICAS by Binary logistic regression analysis

tHcy <sup>c</sup> quartile (μmol/L)	ICAS <sup>a</sup> (vs ECAS <sup>b</sup> )		
	Adjusted OR <sup>d</sup> (95% CI <sup>e</sup> )	Beta-value	p-value
1 (<10.44) <sup>f</sup>	Reference		
2 (10.44-12.45)	1.118 (0.209-5.975)	0.112	0.896
3 (12.45-15.42)	2.658 (0.401-17.632)	0.978	0.311
4 (≥15.42)	4.146 (0.897-19.157)	1.422	0.069

<sup>a</sup>ICAS: intracranial atherosclerotic stenosis; <sup>b</sup>ECAS: extracranial atherosclerotic stenosis; <sup>c</sup>tHcy: total homocysteine; <sup>d</sup>OR: odds ratios; 95% CI: 95% confidence intervals; <sup>f</sup>the reference category.

### Discussion

It was suggested that the deleterious effect of hyperhomocysteinemia on ischemic stroke was

mediated primarily via proatherogenic effects [15]. But as to the association between hyperhomocysteinemia and carotid atherosclerosis, conflicting results were obtained. Some studies suggested that plasma level of tHcy was correlated to carotid plaque area [20] and carotid atherosclerotic plaque score [21]. Association of plasma tHcy concentrations with carotid artery intima-media thickness was also found in certain sub-population [22], such as middle-aged eastern Finnish men [23] and non-hypertensive Japanese [24]. But another study failed to find correlations between the level of plasma tHcy and intima-media thickness of the carotid artery [25]. Due to differences in study popula-



tion, imaging modalities and compounding effects of other risk factors, it was difficult to compare these various findings.

It was indicated that moderate hyperhomocysteinemia was associated with the number of stenosed vessels [26], and elevated level of plasma tHcy was correlated to the internal carotid artery occlusion and stenosis [27, 28]. But whether hyperhomocysteinemia is a risk factor for atherosclerotic stenosis in cervico-cerebral large arteries remains unclear. In this study, we found that the concentrations of plasma tHcy were elevated in the patients with CCAS. After adjusting for other possible confounders, elevated level of plasma tHcy remained an independent risk factor for CCAS. OR of developing CCAS was 3.715 times higher in the highest tHcy quartile, compared to the lowest one. Our findings demonstrate that elevated level of plasma tHcy is an independent risk factor for atherosclerotic stenosis in cervico-cerebral large arteries. Previous studies suggested that hyperhomocysteinemia was implicated in the development of atherosclerosis, probably due to endothelial dysfunction and oxidative stress [13], increased cerebral arterial resistance [29], accelerated neointimal hyperplasia [30], and enhanced susceptibility to arterial thrombosis [31]. These may be related to the mechanisms of homocysteine-induced atherosclerotic stenosis in cervico-cerebral large arteries.

Owing to the distinct biological properties of IC and EC arteries, hyperhomocysteinemia is supposed to be more associated with IC atherosclerosis than EC atherosclerosis. However, previous studies failed to identify the effects of elevated plasma tHcy level on the distribution of CCAS [17, 18]. Our data revealed that the levels of plasma tHcy in the patients with ICAS were higher than that in ECAS. After adjusting for DM, elevated level of plasma tHcy remained an independent risk factor for ICAS, but the risks for ICAS in different tHcy quartiles were not distinct. This suggests that elevated level of plasma tHcy may be associated with the distribution of CCAS in LAA ischemic stroke.

In this study, the severity of artery stenosis was evaluated by DSA, the gold standard for diagnosis, which was more sensitive and accurate than other imaging techniques including transcranial doppler, computed tomography

angiography and magnetic resonance angiography et al. In addition, both anterior and posterior cerebral circulatory systems, as well as carotid artery system were assessed at the same time. These ensure our results more accurate and comprehensive. However, since DSA was an invasive assessment and might cause vascular complications, healthy controls and patients accompanied with unstable medical conditions were not included.

In summary, the data demonstrate that hyperhomocysteinemia is a risk factor for CCAS, independent of other traditional vascular risk factors. Elevated level of plasma tHcy is more correlated to ICAS than ECAS, probably contribute to the distribution of CCAS in Chinese patients with LAA cerebral infarction. Our study provides new insights into the pathogenesis of CCAS, identifying potential targets for prevention and therapy. Further researches are essential to investigate the mechanisms of hyperhomocysteinemia in CCAS, and to evaluate the therapeutic effects of tHcy-lowering treatment on the prevention of cervico-cerebral atherosclerotic stenosis-occlusion.

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### Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Weihua Mai, Department of Neurology, The Fifth Affiliated Hospital of Sun Yat-sen University, NO. 52 East Meihua Road, Zhuhai 519000, Guangdong Province, China. Tel: (86)13926932385; Fax: (86)7562528058; E-mail: weihuamai2004@163.com (EHM); maiwh@mail.sysu.edu.cn (MW)

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