Original Article

Association of PCSK9 levels and genetic polymorphisms with stroke recurrence and functional outcome after acute ischemic stroke

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Background: Protein convertase subtilisin/kexin type 9 (PCSK9) is a hepatic protein that participated in the lipid homeostasis. Its high levels and polymorphisms are associated with high low-density lipoprotein cholesterol, increasing the vascular risk potentially. However, the association between PCSK9 levels, genetic polymorphisms, and ischemic stroke remains unclear. We aimed to study the relationship between PCSK9 levels, genetic polymorphisms, and stroke outcomes in patients with ischemic stroke.

Methods: A total of 9,782 acute ischemic stroke patients registered in the China National Stroke Registry-III were included in this prospective study. Circulating PCSK9 concentrations and 11 key single-nucleotide polymorphisms (SNPs) were examined. The clinical outcomes included stroke recurrence, death, and poor functional outcome at 12 months.

Results: The median PCSK9 level was 361.28 ng/mL. After adjusting for confounders, patients in the highest quartile of circulating PCSK9 had a relatively lower risk of 12-month stroke recurrence (HR 0.80, 95% CI: 0.67–0.96). No significant relationship between PCSK9 level and death or poor functional outcome was found. No significant relationship between SNPs and stroke outcomes at 12 months was found.

Conclusions: The high level of PCSK9 was associated with decreased stroke recurrence at 12 months in ischemic stroke patients. There was no significant association between PCSK9 polymorphisms and acute ischemic stroke based on a Chinese registry.

Keywords: Protein convertase subtilisin/kexin type 9 (PCSK9); ischemic stroke; genetic polymorphism

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Introduction

Stroke, as the third leading cause of global disease burden (1), has emerged as a major public health problem (2-4). Ischemic stroke accounts for 60–80% of all strokes (2). As one of the most important risk factors, Low-density lipoprotein cholesterol (LDL-C) and its related biomarkers, as well as genes, attach importance to ischemic stroke (5,6).

Protein convertase subtilisin/kexin type 9 (PCSK9),

the ninth member of the subtilisin family of kexin-like proconvertases, is a serine protease encoded by its gene. It takes part in the proteolytic maturation of several secretory proteins, and modulates the plasma LDL-C levels through a post-transcriptional mechanism (7). The PCSK9 gene of human is located on chromosome 1p32.3 with 22 kb in length which comprises 12 exons and 11 introns. PCSK9 can bind to LDL receptors (LDLRs), causing a conformational change in the receptor. The LDLR-PCSK9 complex can be targeted for lysosomal degradation, which can lead to an increase in serum LDL-C (8). The study suggested that circulating PCSK9 level and a number of single-nucleotide polymorphisms (SNPs) were associated with coronary artery disease outcomes (9). However, the relationship between PCSK9 and stroke is not as clear as the association between PCSK9 and cardiovascular disease (10). In small-sample and meta-analysis studies, the stroke findings have been inconsistent with cardiovascular findings (11-13). The relationship between PCSK9 level, genetic polymorphisms and ischemic stroke needs to be explored further. Therefore, in this prospective study, we investigate the association between PCSK9 levels, genetic polymorphisms and the 12-month outcomes of ischemic stroke. We present the following article in accordance with the STROBE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-870/rc).

Methods

Study design and population

The Third China National Stroke Registry (CNSR-III), is a national, multicenter and prospective transient ischemic attack (TIA) and ischemic stroke registry in China (14). The registry includes 15,166 patients over 18 years of age with ischemic stroke or TIA from symptom to enrolment within 7 days. It covers 22 provinces and 4 municipalities in 201 hospitals between August 2015 and March 2018. We recruited patients with ischemic stroke or TIA enrolled within 7 days of the onset of symptoms. According to the World Health Organization criteria, the acute ischemic stroke diagnosis was confirmed by magnetic resonance imaging or computed tomography (15).

A total of 171 research sites in the registry participated in the biomarker substudy by collecting blood and urine samples. Patients not enrolled in the biomarker substudy, those diagnosed with TIA, and those with incomplete information, missing LDL-C and PCSK9 data or genotyping data were excluded. Finally, the data of 9,782 patients were analyzed in this study. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The ethics committee at Beijing Tiantan Hospital (IRB approval number: KY2015- 001-01) and all study centers approved the CNSR-III study protocol. Written consent was provided by all participants or their legal representatives.

Baseline data collection

The baseline data were collected by face-to-face interviews with trained researchers (neuroscientists from participating hospitals). Baseline data included demographics, risk factors (hypertension, diabetes mellitus, dyslipidemia, known atrial fibrillation, previous ischemic stroke and previous coronary artery disease), pre-hospital medication (antihypertensive drugs, lipid-lowering drugs, antiplatelet drugs and hypoglycemic drugs), laboratory test results, and modified Rankin Scale (mRS) at discharge. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). The National Institutes of Health Stroke Scale (NIHSS) was used to stroke severity.

Blood sample collection and laboratory tests

Blood samples were collected on admission and the plasma specimens were transported to Beijing Tiantan Hospital through a cold chain and stored in a –80 ℃ refrigerator in the central laboratory. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), LDL-C, fasting glucose, white blood cells (WBCs) and neutrophils were measured. The PCSK9 protein level in plasma was measured with an enzyme-linked immunosorbent assay. All the testing was carried out by laboratory personnel blinded to clinical information.

Genotyping

Based on the known and previously published genetic variants of PCSK9 (16-19), a total of 11 SNPs in PCSK9, including rs505151, rs562556, rs2149041, rs2479394, rs2479415, rs2479409, rs7552841, rs10888897, rs11206510, rs11583680 and rs11591147, were genotyped in this study (20).

Follow-up and outcomes

The patients were interviewed face-to-face at 3 months

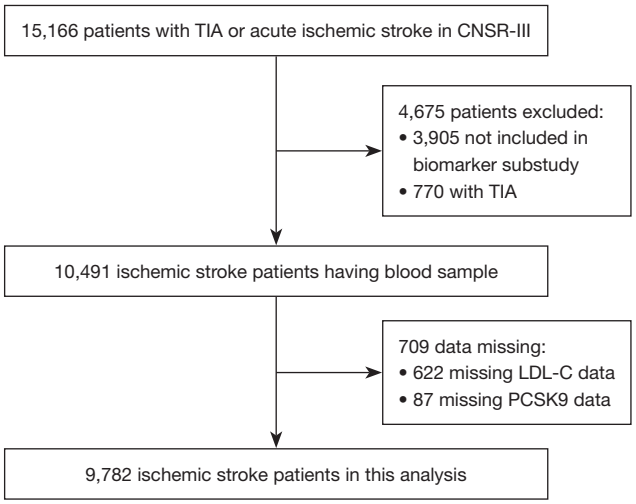

Figure 1 Flowchart of the study. TIA, transient ischemic attack; CNSR-III, The Third China National Stroke Registry; LDL-C, low-density lipoprotein cholesterol; PCSK9, protein convertase subtilisin/kexin type 9.

and over the telephone at 6 months and 1 year. The information, including physical status, cardiovascular and cerebrovascular events, drug use and risk factor control, was collected. Death, stroke recurrence, cardiovascular events and endovascular surgery were recorded during follow-up. The death referred to all-cause death. Stroke recurrence referred to ischemic stroke and hemorrhagic stroke. The poor functional outcome referred to 3–6 scores of mRS. Cerebrovascular events were confirmed by the hospital, and other suspected recurrent cerebrovascular events without hospitalization were judged by the independent endpoint judgment committee. Each death was confirmed by a death certificate from a hospital or local civil registry (14).

Statistical analysis

Categorical variables were expressed as frequencies with percentages, and continuous variables were expressed as the mean ± SD or median (interquartile range, IQR). Baseline characteristics of patients with different PCSK9 levels were analyzed by Chi-square test or Fisher exact test for categorical variables and analysis of variance (ANOVA) or Kruskal-Wallis test for continuous variables.

The relationships between PCSK9 and stroke recurrence and death at 12-month were assessed by Cox proportional hazards models with hazard ratios (HRs) and 95% confidence intervals (95% CIs). The relationship between PCSK9 and poor functional outcome at 12-month was assessed by logistic regression with odds ratios (OR) and 95% CIs. The associations between PCSK9 and stroke recurrence at 12-month were further evaluated for the different etiological subtypes according to TOAST classification. Three different models were used to correct for confounding factors. Age and sex were adjusted in model 1. Age, sex, NIHSS and medical histories (diabetes mellitus, dyslipidemia, known atrial fibrillation, previous ischemic stroke and previous coronary artery disease) were adjusted in model 2. Age, sex, NIHSS, medical histories (diabetes mellitus, dyslipidemia, known atrial fibrillation, previous ischemic stroke and previous coronary artery disease) and pre-hospitalization medication (antihypertensive drugs, lipid-lowering drugs, antiplatelet drugs, hypoglycemic drugs) were adjusted in model 3. The P value of 0.05 was defined as statistically significant(two-sided).

The association of each SNP and stroke outcome was carried out by Cox proportional hazards models and logistic regression. The coefficients for the SNPs of the PCSK9 gene were based on the Global Lipids Genetics Consortium (GLGC) (19), and were used to construct a polygenic score (PGS). The associations between PCSK9 polygenic score and stroke outcome were evaluated by Cox proportional hazards models. To further evaluate the association between PCSK9 and stroke outcome, the PCSK9 genotypes and genetic scores were used as instrumental variables in the regression models. The α was approximately equal to 0.0045 which 0.05 was divided by 11 with Bonferroni correction, and thus P value of 0.0045 was defined as statistically significant(two-sided) in the study of SNPs. All data analyses were conducted with SAS 9.4 (SAS Institute, Cary, NC).

Results

Baseline characteristics of patients

The flowchart of patients included in this study is shown in *Figure 1*. This study included 9,782 patients with acute ischemic stroke, excluding those with TIA, missing data, or those not in the biomarker substudy. Their baseline clinical characteristics, including PCSK9 level, are presented in *Table 1*. The mean age of patients was 62.33±11.31 years (males, 68.9%). The median of PCSK9 level was 361.28 ng/mL (interquartile range, 279.54–452.74 ng/mL). Among the patients, 1,093 (11.2%) received lipid-lowering drugs, among which 1,040 (10.6%) received statins. Compared with the lowest quartile of PCSK9, patients

Table 1 Baseline characteristics of patients by quartiles of PCSK9 level

Characteristics	Overall	PCSK9 level				P
		Q1	Q2	Q3	Q4	
Patients (n)	9,782	2,445	2,446	2,445	2,446	
Sex, male (%)	6,737 (68.9)	1,787 (73.1)	1,795 (73.4)	1,656 (67.7)	1,499 (61.3)	<0.001
Age, years	62.33±11.31	62.93±11.89	62.50±11.34	62.04±11.20	61.88±10.77	0.005
BMI, kg/m^2	24.69±3.33	24.64±3.35	24.74±3.33	24.80±3.36	24.57±3.26	0.07
Admission NIHSS score	3 (2.00–6.00)	4 (2.00–7.00)	3 (2.00–6.00)	3 (2.00–6.00)	3 (2.00–6.00)	<0.001
Risk factors, n (%)						
Hypertension	6,162 (63.0)	1,546 (63.2)	1,535 (62.8)	1,551 (63.4)	1,530 (62.6)	0.91
Diabetes mellitus	2,364 (24.2)	594 (24.3)	618 (25.3)	578 (23.6)	574 (23.5)	0.45
Dyslipidemia	780 (8.0)	176 (7.2)	178 (7.3)	222 (9.1)	204 (8.3)	0.04
Known atrial fibrillation	710 (7.3)	205 (8.4)	190 (7.8)	158 (6.5)	157 (6.4)	0.02
Previous ischemic stroke	2,077 (21.2)	528 (21.6)	500 (20.4)	529 (21.6)	520 (21.3)	0.72
Previous coronary artery disease	1,042 (10.7)	263 (10.8)	283 (11.6)	262 (10.7)	234 (9.6)	0.16
Pre-hospital medication, n (%)						
Antihypertensive drugs	4,422 (45.2)	1,100 (45.0)	1,122 (45.9)	1,100 (45.0)	1,100 (45.0)	0.90
Lipid-lowering drugs	1,093 (11.2)	218 (8.9)	276 (11.3)	293 (12.0)	306 (12.5)	<0.001
Statin	1,040 (10.6)	209 (8.6)	260 (10.6)	281 (11.5)	290 (11.9)	<0.001
Antiplatelet drugs	1,695 (17.3)	385 (15.8)	432 (17.7)	428 (17.5)	450 (18.4)	0.09
Hypoglycemic drugs	1,840 (18.8)	444 (18.2)	494 (20.2)	464 (19.0)	438 (17.9)	0.16
Laboratory test results						
LDL-C, mmol/L	2.31 (1.72–2.97)	2.41 (1.77–3.06)	2.37 (1.78–3.01)	2.26 (1.70–2.94)	2.18 (1.64–2.83)	<0.001
HDL-C, mmol/L	0.93 (0.77–2.97)	0.93 (0.77–1.12)	0.92 (0.77–1.10)	0.94 (0.78–1.12)	0.94 (0.78–1.11)	0.10
TC, mmol/L	3.97 (3.31–4.72)	4.09 (3.38–4.83)	3.99 (3.33–4.72)	3.90 (3.30–4.69)	3.87 (3.23–4.63)	0.004
Fasting glucose, mmol/L	5.56 (4.90–6.99)	5.54 (4.89–7.00)	5.59 (4.93–7.16)	5.57 (4.90–6.97)	5.52 (4.90–6.77)	0.69
WBC, /L	6.92 (5.72–8.43)	6.95 (5.72–8.43)	6.85 (5.70–8.40)	6.94 (5.79–8.44)	6.97 (5.70–8.50)	0.36
Neutrophil, /L	4.48 (3.50–5.80)	4.50 (3.52–5.92)	4.41 (3.50–5.72)	4.48 (3.52–5.74)	4.50 (3.41–5.80)	0.35
mRS at admission						
0–2	9,347 (95.6)	2,338 (95.6)	2,327 (95.1)	2,332 (95.4)	2,350 (96.1)	0.43
3–5	435 (4.5)	107 (4.4)	119 (4.9)	113 (4.6)	96 (3.9)	

Continuous variables were expressed as the mean ± SD or median (interquartile range, IQR). Q1: <279.54 ng/mL; Q2: 279.54–361.27 ng/mL; Q3: 361.28–452.73 ng/mL; Q4: >452.74 ng/mL. PCSK9, protein convertase subtilisin/kexin type 9; BMI, body mass index; NIHSS, National Institutes of Health Stroke Scale; mRS, Modified Rankin Scale; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; WBC, white blood cells.

Table 2 Association between PCSK9 and 12-month outcomes

	PCSK9 level	Events, n (%)	Model 1			Model 2			Model 3		
			HR/OR*	95% CI	P	HR/OR	95% CI	P	HR/OR	95% CI	P
Stroke recurrence	Q1	275 (11.3)	Ref.	Ref.		Ref.	Ref.		Ref.	Ref.	
	Q2	261 (10.7)	0.95	0.80–1.12	0.52	0.95	0.80–1.13	0.55	0.95	0.80–1.12	0.52
	Q3	222 (9.1)	0.79	0.67–0.95	0.01	0.81	0.68–0.97	0.02	0.81	0.68–0.97	0.02
	Q4	218 (8.9)	0.78	0.65–0.93	0.006	0.80	0.67–0.96	0.02	0.80	0.67–0.96	0.015
	Per 1 ng/mL		0.97	0.95–0.995	0.02	0.98	0.95–1.00	0.047	0.98	0.95–0.999	0.04
	Per 1-SD		0.93	0.87–0.99	0.02	0.94	0.88–0.999	0.047	0.94	0.88–0.998	0.04
All-cause mortality	Q1	106 (4.3)	Ref.	Ref.		Ref.	Ref.		Ref.	Ref.	
	Q2	87 (3.6)	0.85	0.64–1.13	0.27	0.83	0.63–1.11	0.21	0.84	0.63–1.11	0.22
	Q3	74 (3.0)	0.74	0.55–0.99	0.04	0.81	0.59–1.10	0.17	0.83	0.61–1.11	0.21
	Q4	74 (3.0)	0.77	0.57–1.03	0.08	0.80	0.60–1.07	0.13	0.81	0.60–1.09	0.16
	Per 1 ng/mL		0.99	0.95–1.03	0.68	0.996	0.96–1.04	0.82	0.997	0.96–1.04	0.89
	Per 1-SD		0.98	0.88–1.09	0.68	0.99	0.89–1.10	0.82	0.99	0.90–1.10	0.89
Poor functional outcome	Q1	368 (15.5)	Ref.	Ref.		Ref.	Ref.		Ref.	Ref.	
	Q2	325 (13.6)	0.89	0.77–1.05	0.17	0.92	0.77–1.10	0.38	0.92	0.77–1.10	0.37
	Q3	309 (12.9)	0.85	0.72–1.00	0.05	0.95	0.79–1.14	0.56	0.95	0.79–1.14	0.57
	Q4	315 (13.2)	0.89	0.75–1.05	0.16	0.96	0.80–1.16	0.69	0.97	0.81–1.16	0.71
	Per 1 ng/mL		0.999	0.98–1.02	0.90	1.01	0.99–1.03	0.47	1.01	0.99–1.03	0.48
	Per 1-SD		0.996	0.94–1.06	0.90	1.02	0.96–1.09	0.47	1.02	0.96–1.09	0.48

*, the relationship between PCSK9 level and stroke recurrence, all-cause mortality at 12 months was assessed with hazard ratios. The relationship between PCSK9 level and poor functional outcome at 12 months was assessed with odds ratios. Model 1: adjusted for age and sex; Model 2: adjusted for age, sex, NIHSS and medical histories (diabetes mellitus, dyslipidemia, known atrial fibrillation, previous ischemic stroke and previous coronary artery disease); Model 3: adjusted for age, sex, NIHSS, medical histories (diabetes mellitus, dyslipidemia, known atrial fibrillation, previous ischemic stroke and previous coronary artery disease) and pre-hospitalization medication (antihypertensive drugs, lipid-lowering drugs, antiplatelet drugs, hypoglycemic drugs). Q1: <279.54 ng/mL; Q2: 279.54–361.27 ng/mL; Q3: 361.28–452.73 ng/mL; Q4: >452.74 ng/mL. PCSK9, protein convertase subtilisin/kexin type 9; HR, hazard ratio; OR, odds ratio; CI, confidence interval.

with the highest quartile of PCSK9 had a lower proportion of men, were younger, and had relatively lower NIHSS, greater dyslipidemia, less previous atrial fibrillation occurrence, lower LDL-C and lower TC.

Associations between PCSK9 level and stroke outcome at 12 months

The rates of stroke recurrence at 12 months in the four quartiles (low to high) of PCSK9 levels were 11.25%, 10.67%, 9.08% and 8.91%. After adjusting for age, gender, NIHSS, other risk factors (diabetes, dyslipidemia, known atrial fibrillation, previous ischemic stroke and previous coronary artery disease) and pre-hospital drugs (antihypertensive drugs, lipid-lowering drugs, antiplatelet drugs, hypoglycemic agents), the risk of stroke recurrence at 12 months decreased with increasing PCSK9 level (Q3 *vs.* Q1: HR =0.81, 95% CI: 0.68–0.97; Q4 *vs.* Q1: HR =0.80, 95% CI: 0.67–0.96) (*Table 2*). For every 1 ng/mL increment in PCSK9 level, the HR for stroke recurrence was 0.98 (95% CI: 0.95–0.999, P=0.04). For each 1 SD increment in PCSK9 level, the HR for stroke recurrence was 0.94 (95% CI: 0.88–0.998, P=0.04). There was no significant association between PCSK9 level and poor functional outcome or death at 12 months. Furthermore, PCSK9 level was associated with stroke recurrence at 12 months

Table 3 Associations between PCSK9 polygenic score and stroke outcomes

	PCSK9 level reduction		LDL-C level reduction		Stroke recurrence at 12 months		
	ng/mL (95% CI)	P value	mmol/L (95% CI)	P value	Events, n (%)	HR (95% CI)	P value
PCSK9-PGS*		<0.001		0.011			
Q1	388.03 (380.14, 395.91)		2.47 (2.41, 2.54)		100 (12.06)	Ref.	
Q2	381.33 (375.79, 386.88)		2.46 (2.42, 2.51)		203 (24.49)	1.001 (0.79, 1.27)	0.99
Q3	374.23 (368.24, 380.22)		2.48 (2.43, 2.53)		190 (22.92)	1.09 (0.86, 1.39)	0.47
Q4	371.09 (364.32, 377.87)		2.47 (2.42, 2.53)		170 (20.51)	1.29 (1.01, 1.65)	0.04
Q5	351.60 (345.22, 357.97)		2.37 (2.32, 2.42)		166 (20.02)	1.10 (0.86, 1.41)	0.46
Per 0.1 score		<0.001		0.008		1.04 (0.99, 1.09)	0.09

*, the β coefficients of PCSK9 gene were estimated from the Global Lipids Genetics Consortium (GLGC) to construct a PGS of 11 single-nucleotide polymorphisms. Q, quintile; Q1 (<0.039), Q2 (0.039, 0.12), Q3 (0.13, 0.21), Q4 (0.22, 0.31), Q5 (≥0.32). PCSK9, protein convertase subtilisin/kexin type 9; LDL-C, low-density lipoprotein cholesterol; HR, hazard ratio; CI, confidence interval; PGS, polygenic score.

for stroke of other determined etiology in the TOAST classification system (HR per 1 ng/mL increment of PCSK9 level =1.32, 95% CI: 0.08–1.62, P=0.01; HR per 1 SD increment of PCSK9 level =2.12, 95% CI: 1.24–3.63, P=0.01) after adjusting for confounding factors (Table S1). There was no significant association between PCSK9 level and stroke recurrence, death, or poor functional outcomes at 12 months for the other TOAST types (Table S1).

Associations between PCSK9 genetic polymorphisms and stroke outcome

All the SNPs (rs2479394, rs11206510, rs2479415, rs2149041, rs2479409, rs11591147, rs11583680, rs10888897, rs7552841, rs562556, rs505151) met the Hardy-Weinberg equilibrium (Table S2). The concentrations of PCSK9 among the various SNP genotypes differed significantly, including rs11206510 (P<0.001), rs2149041 (P<0.001), rs2479409 (P<0.001), rs11583680 (P<0.001) and rs10888897 (P<0.001) (Table S3).

With every increase of 0.1 score in the PGS, the PCSK9 and LDL-C levels decreased significantly (P<0.001 and P=0.008, respectively), but no significant associations between PCSK9 genetic polymorphisms and 12-month stroke recurrence. Similarly, a higher PCSK9 PGS was associated with decreased PCSK9 and LDL-C levels (P<0.001 and P=0.011, respectively), but not with 12-month stroke recurrence (*Table 3*).

Among the 11 SNPs, no significant association was found between SNPs and stroke recurrence, death or disability (*Table 4*).

Discussion

The results showed high PCSK9 levels were associated with low stroke recurrence at 12 months in acute ischemic stroke patients. No significant difference was found between PCSK9 levels and death or poor functional outcome at 12 months. The 11 SNPs were not associated with stroke outcomes at 12 months.

The prognostic value of PCSK9 in cerebrovascular disease is still controversial (21). Higher circulating PCSK9 levels have been independently associated with cardiovascular events, death and future risk (22,23). Moreover, PCSK9 could be a potential biomarker of the severity of coronary artery disease (24). However, high PCSK9 levels do not predict mortality or severity and recurrence based on a prospective cohort study of cardiovascular disease (25,26). At present, there are few reports on PCSK9 levels and the prognosis of stroke. After adjusting for confounding factors, there was no significant association between baseline PCSK9 level and stroke outcome in a study of more than 4,000 patients with type 2 diabetes (27). More than 300 patients with familial hypercholesterolemia showed a negative relationship between high PCSK9 levels and stroke outcome, but no statistically significant correlation (28). In addition, decreased PCSK9 in serum was associated with poor outcomes and significant adverse cardiovascular events at 90 days, which was totally different from in stark contrast to a study of cardiovascular disease (13). Our study is also based on the registered follow-up cohort, but the sample size was larger than in previous studies. Our results are inconsistent

Table 4 Association of SNPs, the unweighted genetic score and weighted genetic score, with the risk of stroke outcomes

SNPs	Stroke recurrence			Death			Disability (mRS 3–6)		
	Events, n (%)	HR (95% CI)	P	Events, n (%)	HR (95% CI)	P	Events, n (%)	OR (95% CI)	P
rs2479394									
CC	324 (10.0)	Ref.		117 (3.6)	Ref.		437 (13.8)	Ref.	
CT	418 (10.3)	1.04 (0.90, 1.20)	0.60	143 (3.5)	0.99 (0.77, 1.26)	0.91	565 (14.3)	1.05 (0.91, 1.22)	0.49
TT	122 (8.9)	0.91 (0.74, 1.12)	0.38	44 (3.2)	0.94 (0.67, 1.34)	0.75	183 (13.7)	1.04 (0.85, 1.28)	0.69
rs11206510									
TT	780 (10.1)	Ref.		271 (3.5)	Ref.		1,058 (14.0)	Ref.	
TC	80 (8.9)	0.88 (0.70, 1.10)	0.26	32 (3.6)	1.08 (0.74, 1.55)	0.70	128 (14.6)	1.11 (0.89, 1.38)	0.35
CC	4 (11.8)	1.26 (0.47, 3.37)	0.65	0 (0.0)	0	0.96	1 (2.9)	0.23 (0.03, 1.75)	0.16
rs2479415									
CC	605 (9.7)	Ref.		222 (3.6)	Ref.		870 (14.3)	Ref.	
CT	243 (10.8)	1.11 (0.96, 1.29)	0.17	74 (3.3)	0.88 (0.67, 1.14)	0.33	294 (13.4)	0.87 (0.74, 1.02)	0.08
TT	16 (8.3)	0.87 (0.53, 1.43)	0.59	7 (3.7)	1.19 (0.56, 2.52)	0.66	22 (11.8)	0.82 (0.50, 1.35)	0.43
rs2149041									
CC	345 (9.3)	Ref.		122 (3.3)	Ref.		514 (14.2)	Ref.	
CG	403 (10.4)	1.136 (0.98, 1.31)	0.08	148 (3.8)	1.17 (0.92, 1.48)	0.21	537 (14.3)	1.02 (0.88, 1.18)	0.79
GG	120 (11.1)	1.222 (0.99, 1.51)	0.06	32 (3.0)	0.90 (0.61, 1.33)	0.59	137 (13.0)	0.93 (0.75, 1.17)	0.54
rs2479409									
GG	385 (9.5)	Ref.		134 (3.3)	Ref.		564 (14.3)	Ref.	
GA	379 (10.0)	1.06 (0.92, 1.23)	0.40	138 (3.7)	1.05 (0.83, 1.34)	0.67	507 (13.7)	0.95 (0.83, 1.10)	0.50
AA	97 (11.8)	1.27 (1.01, 1.58)	0.04	28 (3.4)	0.97 (0.64, 1.46)	0.88	109 (13.6)	0.996 (0.78, 1.28)	0.97
rs11591147									
GG	870 (10.0)	Ref.		303 (3.5)	Ref.		1,189 (14.0)	Ref.	
GT	0 (0.0)	0	0.95	0 (0.0)	0	0.97	0 (0.0)	0	0.96
rs11583680									
CC	672 (9.8)	Ref.		228 (3.3)	Ref.		935 (13.9)	Ref.	
CT	181 (10.7)	1.10 (0.93, 1.29)	0.27	72 (4.2)	1.24 (0.95, 1.62)	0.11	242 (14.6)	1.06 (0.89, 1.25)	0.52
TT	15 (13.6)	1.46 (0.87, 2.44)	0.15	4 (3.6)	1.22 (0.45, 3.30)	0.70	12 (11.0)	0.93 (0.49, 1.77)	0.83
rs10888897									
CC	546 (9.7)	Ref.		186 (3.3)	Ref.		772 (14.1)	Ref.	
TC	278 (10.4)	1.08 (0.93, 1.25)	0.31	103 (3.9)	1.15 (0.90, 1.46)	0.26	365 (14.1)	1.02 (0.88, 1.19)	0.76
TT	34 (10.1)	1.04 (0.74, 1.47)	0.83	14 (4.1)	1.15 (0.67, 1.99)	0.62	46 (13.8)	0.999 (0.70, 1.42)	0.99

Table 4 (*continued*)

Table 4 (*continued*)

SNPs	Stroke recurrence			Death			Disability (mRS 3–6)		
	Events (n)	HR (95% CI)	P	Events (n)	HR (95% CI)	P	Events (n)	OR (95% CI)	P
rs7552841									
CC	594 (9.7)	Ref.		209 (3.4)	Ref.		847 (14.1)	Ref.	
CT	238 (10.3)	1.10 (0.94, 1.28)	0.23	82 (3.6)	1.08 (0.835, 1.39)	0.56	311 (13.9)	0.967 (0.83, 1.13)	0.67
TT	32 (14.5)	1.57 (1.10, 2.24)	0.01	12 (5.4)	1.75 (0.977, 3.15)	0.06	29 (13.3)	0.982 (0.63, 1.52)	0.94
rs562556									
AA	855 (10.0)	Ref.		298 (3.5)	Ref.		1,168 (14.0)	Ref.	
AG	11 (9.1)	0.897 (0.50, 1.6)	0.72	5 (4.1)	1.23 (0.51, 2.97)	0.65	18 (15.4)	1.12 (0.64, 1.95)	0.70
rs505151									
AA	755 (9.9)	Ref.		267 (3.5)	Ref.		1,049 (14.0)	Ref.	
AG	101 (10.6)	1.06 (0.86, 1.31)	0.57	35 (3.7)	0.99 (0.70, 1.41)	0.97	127 (13.7)	0.93 (0.75, 1.16)	0.52
GG	3 (13.6)	1.40 (0.45, 4.35)	0.56	0 (0.0)	–	–	3 (13.6)	1.25 (0.34, 4.60)	0.74

SNPs, single-nucleotide polymorphisms; mRS, modified Rankin Scale; HR, hazard ratio; CI, confidence interval.

with studies on diabetes and familial hypercholesterolemia patients, but are consistent with findings in small-sample stroke cohorts (13,27,28), which may be because the latter focused on ischemic stroke patients, despite race and follow-up time differences.

We speculate that the association between increased PCSK9 levels and reduced stroke recurrence may be multifaceted. First, when acute ischemic stroke occurs, the cause and changes of PCSK9 in circulating may vary. Circulating PCSK9 may not fully reflect the complex regulation of hepatic PCSK9 (29-31). After brain injury, the expression of PCSK9 increases in the brain. Under physiological conditions, PCSK9 cannot cross the blood-brain barrier (BBB). However, after stroke, serum PCSK9 may directly cause brain injury because of BBB damage (13,32). Second, PCSK9 may regulate local physiological stress via paracrine mechanisms and by affecting LDLR levels in various organs (33). PCSK9 also regulates a large number of immune responses and genes related to apoptosis, such as LDP-1 and LDP-6 (13,34), and inflammation. PCSK9 in plasma enhances platelet activation and thrombosis by binding to platelet CD36 to activate downstream signaling pathways (35), and it is involved in many immune responses involved in stroke injury (34,36). Third, differences in population, race and associated factors may affect the relationship between circulating PCSK9 and ischemic events (37).

The relationship between PCSK9 genetic polymorphisms and stroke still remains unclear, and further research is needed. PCSK9 variants, including rs11591147, rs505151, rs11206510, rs2479409, rs562556 and rs11583680, are associated with coronary heart disease, but their link with ischemic stroke is weak (12,16). Genetic polymorphisms differ among populations. For example, PCSK9 loss-of-function variants are associated with differential reductions in LDL-C in blacks and whites, but not with stroke events in either blacks or whites (38,39). Our study population was Chinese stroke patients, and the distribution of the 11 SNPs differs from a previous study (40). However, the relationship between PCSK9 genetic polymorphisms and 12-month stroke outcomes was roughly similar to the previous study (12). The 11 SNPs were not associated with 12-month clinical outcomes. Some SNPs, such as rs505151(E670G), with the risk of coronary artery disease and large-artery atherosclerosis (LAA) stroke, had no significant impact on stroke outcome (41). Thus, the relationship between the PCSK9 gene and stroke outcome is not clear. An underlying cause of the lack of associations may be that the selected SNPs may mainly reflect variation in European and American cohorts. Second, not just lipid pathways are likely affected in stroke. Ischemic stroke also involves phenotypic heterogeneity, and different TOAST genotypes may have different biological drivers, compared with the more homogeneous cardiovascular disease phenotypes (11). It is

therefore necessary to clarify the differences in the PCSK9 gene among different populations, and investigate their pathogenetic involvement in stroke. Third, genes represent long-term effects, while the outcomes in research are short-term.

This study has several limitations. First, the level of PCSK9 was detected only at baseline and not again at follow-up. Moreover, the source of circulating PCSK9 was not determined. The dynamic changes in its concentration and origin may affect the long-term prognosis. Second, although our analysis adjusted for some factors, the results may be affected by other unmeasured or residual factors. Finally, all patients in the present study were Chinese, while most of the SNPs examined were initially reported in European and American populations. Thus, further research is needed to clarify whether the results can be extrapolated to other populations.

Conclusions

In the population with acute ischemic stroke, the levels of elevated circulating PCSK9 were associated with decreased stroke recurrence at 12 months. There was no significant relationship between PCSK9 gene polymorphisms and the outcomes of acute ischemic stroke based on a Chinese registry. Future studies on different ethnicities are warranted to elucidate further the complex relationship between the PCSK9 and stroke outcomes.

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Footnote

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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 ethics committee at Beijing Tiantan Hospital and all study centers approved the CNSR-III study protocol (IRB approval number: KY2015-001-01). Written consent was provided by all participants or their legal representatives.

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Supplementary

Table S1 Associations between PCSK9 and stroke recurrence at 12 months according to TOAST etiological subtype

	PCSK9 level	Events (%)	Model 1			Model 2			Model 3		
			HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
LAA	Q1	104 (15.0)	Ref.	Ref.		Ref.	Ref.		Ref.	Ref.	
	Q2	87 (13.2)	0.87	0.66–1.16	0.35	0.87	0.65–1.15	0.32	0.87	0.65–1.15	0.32
	Q3	62 (10.5)	0.68	0.50–0.93	0.02	0.67	0.49–0.92	0.01	0.67	0.48–0.91	0.01
	Q4	68 (11.9)	0.78	0.57–1.06	0.11	0.77	0.57–1.05	0.10	0.78	0.57–1.06	0.12
	Per 1 ng/mL		0.97	0.93–1.01	0.15	0.97	0.93–1.01	0.13	0.97	0.93–1.01	0.14
	Per 1-SD		0.92	0.82–1.03	0.15	0.92	0.82–1.03	0.13	0.92	0.82–1.03	0.14
CE	Q1	25 (13.1)	Ref.	Ref.		Ref.	Ref.		Ref.	Ref.	
	Q2	23 (14.7)	1.12	0.63–1.97	0.71	1.09	0.62–1.92	0.77	1.10	0.62–1.94	0.75
	Q3	23 (15.2)	1.14	0.65–2.01	0.64	1.16	0.65–2.05	0.62	1.16	0.65–2.06	0.61
	Q4	10 (6.9)	0.52	0.25–1.07	0.08	0.52	0.25–1.10	0.09	0.53	0.25–1.11	0.09
	Per 1 ng/mL		0.93	0.85–1.01	0.09	0.93	0.85–1.01	0.09	0.93	0.85–1.01	0.10
	Per 1-SD		0.82	0.66–1.03	0.09	0.82	0.66–1.03	0.09	0.83	0.66–1.04	0.10
SAO	Q1	48 (8.7)	Ref.	Ref.		Ref.	Ref.		Ref.	Ref.	
	Q2	36 (6.8)	0.79	0.51–1.21	0.27	0.79	0.51–1.21	0.29	0.79	0.51–1.21	0.28
	Q3	31 (5.9)	0.67	0.43–1.05	0.08	0.66	0.42–1.04	0.08	0.66	0.42–1.04	0.08
	Q4	30 (5.3)	0.59	0.38–0.94	0.03	0.61	0.38–0.96	0.03	0.61	0.38–0.96	0.03
	Per 1 ng/mL		0.95	0.89–1.01	0.13	0.96	0.90–1.02	0.17	0.96	0.90–1.02	0.17
	Per 1-SD		0.88	0.74–1.04	0.13	0.89	0.75–1.05	0.17	0.89	0.75–1.05	0.17
SOE	Q1	2 (8.7)	Ref.	Ref.		Ref.	Ref.		Ref.	Ref.	
	Q2	1 (3.7)	0.42	0.03–4.65	0.48	0.40	0.04–4.69	0.47	0.60	0.04–8.17	0.70
	Q3	0 (0.0)	–	–	–	–	–	–	–	–	–
	Q4	9 (28.1)	3.79	0.81–17.64	0.10	4.06	0.76–21.79	0.10	7.12	0.99–51.43	0.051
	Per 1 ng/mL		1.24	1.06–1.44	0.0058	1.29	1.07–1.57	0.01	1.32	1.08–1.62	0.01
	Per 1-SD		1.77	1.18–2.66	0.0058	1.99	1.18–3.34	0.01	2.12	1.24–3.63	0.01
SUE	Q1	96 (9.7)	Ref.	Ref.		Ref.	Ref.		Ref.	Ref.	
	Q2	114 (10.6)	1.08	0.83–1.42	0.56	1.09	0.83–1.42	0.56	1.07	0.82–1.41	0.61
	Q3	106 (9.2)	0.93	0.70–1.23	0.60	0.97	0.74–1.28	0.83	0.96	0.73–1.27	0.80
	Q4	101 (8.9)	0.89	0.67–1.17	0.40	0.93	0.70–1.23	0.59	0.92	0.69–1.21	0.54
	Per 1 ng/mL		0.98	0.95–1.02	0.35	0.99	0.95–1.03	0.55	0.99	0.95–1.02	0.51
	Per 1-SD		0.96	0.87–1.05	0.35	0.97	0.88–1.07	0.55	0.97	0.88–1.07	0.51

Model 1: adjusted for age and sex; Model 2: adjusted for age, sex, NIHSS and medical histories (diabetes mellitus, dyslipidemia, known atrial fibrillation, previous ischemic stroke and previous coronary artery disease); Model 3: adjusted for age, sex, NIHSS, medical histories (diabetes mellitus, dyslipidemia, known atrial fibrillation, previous ischemic stroke and previous coronary artery disease) and pre-hospitalization medication (antihypertensive drugs, lipid-lowering drugs, antiplatelet drugs, hypoglycemic drugs). PCSK9, protein convertase subtilisin/kexin type 9; LAA, large artery atherosclerosis; CE, cardioembolism; SAO, small artery occlusion; SOE, stroke of other determined etiology; SUE, stroke of undetermined etiology; HR, hazard ratio; OR, odds ratio; CI, confidence interval.

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Table S2 Allele frequency and Hardy-Weinberg equilibrium

PCSK9 SNP	Minor allele	Minor allele frequency-N (%)	HWE-P value
rs2479394	T	39	0.1285
rs11206510	C	6	0.299
rs2479415	T	15	0.3051
rs2149041	G	34	0.2274
rs2479409	A	31	0.06619
rs11591147	T	0	1.000
rs11583680	T	11	0.3397
rs10888897	T	19	0.3309
rs7552841	T	16	0.8653
rs562556	G	1	1.000
rs505151	G	6	0.1508

PCSK9, protein convertase subtilisin/kexin type 9; SNP, single-nucleotide polymorphism.

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Table S3 Concentrations of PCSK9 according to genotypes and genetic scores used as instrumental variables in genetic analyses

	Genotype	Patients (n)	PCSK9 level (95%CI)	P	F test	R^2
rs2479394	CC	3,247	(372.84, 382.01)	0.12	4.04	0.000931
	CT	4,051	(367.92, 376.12)			
	TT	1,365	(358.48, 372.62)			
rs11206510	TT	7,726	(372.80, 378.74)	<0.001	14.56	0.003353
	TC	901	(342.83, 360.21)			
	CC	34	(293.43, 382.92)			
rs2479415	CC	6,224	(371.09, 377.72)	1.71	0.1816	0.000394
	CT	2,250	(363.30, 374.33)			
	TT	192	(361.05, 398.81)			
rs2149041	CC	3,716	(380.78, 389.32)	<0.001	32.95	0.007553
	CG	3,867	(363.50, 371.87)			
	GG	1,079	(343.28, 359.12)			
rs2479409	GG	4,049	(375.31, 383.50)	<0.001	20.32	0.004679
	GA	3,781	(367.90, 376.38)			
	AA	819	(337.95, 356.18)			
rs11591147	GG	8,701	(370.38, 375.99)	0.36	0.84	0.000097
	GT	3	(292.94, 594.90)			
rs11583680	CC	6,885	(376.83, 383.09)	<0.001	51.14	0.011629
	CT	1,700	(343.13, 355.73)			
	TT	110	(279.66, 329.19)			
rs10888897	CC	5,629	(377.93, 384.86)	<0.001	42.39	0.009723
	CT	2,670	(355.86, 365.92)			
	TT	338	(313.26, 341.54)			
rs7552841	CC	6,153	(369.60, 376.27)	0.58	0.54	0.000124
	CT	2,302	(366.65, 377.55)			
	TT	221	(364.25, 399.42)			
rs562556	AA	8,560	(369.98, 375.63)	0.13	2.35	0.000271
	GA	121	(367.77, 415.30)			
rs505151	AA	7,668	(369.20, 375.17)	0.19	1.68	0.000388
	AG	950	(370.93, 387.89)			
	GG	22	(343.86, 455.30)			

PCSK9, protein convertase subtilisin/kexin type 9; CI, confidence interval.

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