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RESEARCH ARTICLE

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The SNP43 (G/A) polymorphism in *CAPN10* gene confers an increased risk of cognitive impairment in cerebral small vessel disease

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Background: Cognitive impairment, significantly reducing processing speed and executive function, is the critical consequence of cerebral small vessel disease (SVD), in which genetic variations have been studied. In this study, we explore the role of SNP43 (G/A) and SNP63 (C/T) polymorphism in the *CAPN10* on cognitive impairment process in cerebral SVD.

Methods: Cerebral SVD patients (n = 224) and healthy controls (n = 187) were recruited. The relationship between frequency distribution of SNP43 (G/A) and SNP63 (T/C) genotype and allele in *CAPN10* gene, and cognitive impairment was examined. The independent risk factors for cognitive impairment in SVD were determined by logistic regression analysis.

Results: Accordingly, the frequency distribution of genotype and allele at SNP43 (G/A) was significantly different between cerebral SVD patients and healthy controls. Cerebral SVD patients with GG genotype were more susceptible for cognitive impairment, whereas cerebral SVD patients with GA + AA genotype were less possible to suffer from cognitive impairment, compared with those with GG genotype. And also, cerebral SVD does not include SNP63 (C/T) to associate with cognitive impairment, and SNP43 (G/A), total cholesterol, triglyceride, low-density lipoprotein, and high-density lipoprotein were independent risk factors for cognitive impairment in SVD.

Conclusion: Our study provides evidence that SNP43 (G/A) in the *CAPN10* gene increases the risk of cognitive impairment in SVD patients. Besides it is proven that, patients with G allele are more susceptible to suffer from cerebral SVD with worse cognitive impairment.

KEYWORDS

allele, *CAPN10* gene, cerebral small vessel disease, cognitive impairment, polymorphism, SNP43 (G/A), SNP63 (C/T)

1 | INTRODUCTION

Cerebral small vessel disease (SVD), as one of the most important and common vascular diseases of the brain, is very common in old age and also affects the quality of the connectivity of brain systems.[1,2] Cerebral SVD leads to several different types of clinical presentations, including recurrent lacunar strokes, deep hemorrhagic strokes, vascular dementia, and Parkinsonism.[3] Cerebral SVD is mainly caused by lacunar infarcts, white matter lesions, microbleeds, and intraparenchymal hemorrhage in the cerebral white matter, which are correlated with the reduction of cognitive function.[4,5] Cognitive impairment is the natural result of cerebral SVD and is characterized by prominent impairment of executive function and processing speed, with relative preservation of episodic memory.[6] In

recent days, it is proven that there are other diseases like Parkinson's disease that do associate with genetic polymorphisms and cognitive impairment.[7] Also, gene polymorphisms have been coordinated with cerebrovascular diseases (CVD), such as atherosclerotic cerebral infarction,[8] and the etiological factors of sporadic cerebral hemorrhage in the Chinese population were the polymorphisms such as rs1800790, rs1800787, and rs6050.[9] Our study was performed to investigate at the molecular level, to get the more information on the genetic association of CAPN10 gene polymorphism with cognitive impairment of cerebral SVD in the Chinese population.

Calpains are the calcium-activated neutral proteases of the cytosolic cysteine proteinase family; they can form heterodimers composed of a large 80 kDa catalytic subunit and a common 30 kDa regulatory subunit, and dysfunction of calpains is the cause for certain diseases such as Parkinson's disease, cataract, and Alzheimer's disease.[10] Calpains are activated in response to several classes of cytokines, growth factors, lysophospholipids, and physical stresses,[11] thereby it started participating in degenerative vascular disorders, such as arteriosclerosis.[12,13] Calpain is also involved in physiological and pathophysiological processes, including cytoskeletal reorganization, cell cycle regulation, signal transduction, and apoptosis pathway.[10] rs2736100 of *CAPN10* from GG genotype works as independent risk factor which is specially associated with the presence of arteriosclerosis.[14] In addition, upregulation of *CAPN10* exhibits its role in neurodegenerative diseases such as Alzheimer-type disease, by enhancing the accumulation of β-amyloid peptide, inducing the hyperphosphorylation in the central nervous system where it degenerates the neurologic functions.[15] Moreover, it was proved that *CAPN10* plays an important role in intellectual disability and cognitive impairment processes, from which we got inspired, although it is from a congenital disease perspective and the mechanism how *CAPN10* works remains unclear.[16] In addition, there is a study that demonstrates the effect of *CAPN10* on cerebral impairment from genetic and chromosomal perspectives.[17] Therefore, more exhaustive examination is required to explain the exact function regarding the role of *CAPN10*.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

This study was approved by the Ethics Committee of Xiangya Hospital, Central South University and in conformity with the Helsinki declaration. And also, the informed consent was signed by all the patients and respective guardians.

2.2 | Study subjects

From March 2012 to May 2016, a total number of 224 imaging-confirmed cerebral SVD patients in Xiangya Hospital, Central South University were selected as the cerebral SVD group. The patients were included in this study, if any: (1) patients suffered from risk factors for cardio-cerebral vascular diseases, such as high blood pressure, diabetes, hyperlipemia and etc.; (2) patients had one of the imaging features as follows: lacunar infarction (LI), leukoaraiosis, cerebral microhemorrhage, or enlarged perivascular space; (3) patients were not detected with cortical or watershed infarction and had no subcortical focus of infection greater than 15 mm in diameter. Another 187 healthy volunteers who took physical examination in Xiangya Hospital, Central South University during the same period were selected as the control group. A comprehensive inspection of all the patients' medical history and physical examinations were carried out to take a record of metabolic disease history (including hypertension, diabetes, and hyperlipemia), age, gender, body mass index (BMI), education, smoking, and drinking history. The levels of total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) of the patients were determined by automated biochemical analyzer (AU5800, Beckman Coulter Inc, CA, USA). On the day of blood sampling collection, their systolic blood pressure (SBP) and diastolic blood pressure (DBP) were also measured.

According to the level of cognitive function, the cerebral SVD group was further divided into non-cognitive impairment group (NCI group, including 103 cases) and vascular cognitive impairment group (VCI group, including 121 cases). When the patients had no complaint about cognitive impairment, the cerebral SVD patients were considered as normal cognitive impairment; this also included patients who scored equal to or higher than 23 points by Montreal cognitive function assessment (MoCA); patients who were estimated with 26 points or lower considered by their activities of daily living (ADL).[18] In the same order, cerebral SVD patients were classified with cognitive impairment if patients had complaints of cognitive function impairment; patients scored lower than 23 points by MoCA; not like above in here, patients were assessed to be normal in terms of ADL; patients who met the diagnostic criteria of Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR).[19] The exclusion criteria included patients who suffered from diseases occurred by pre-stroke dementia like Alzheimer's disease, and also cerebral infarction caused by cardiac disease were not considered; patients who were diagnosed with diseases that had adverse impact on cognitive function such as encephalitis, psychosis, alcoholism, or drug abuse; patients who had a history of severe head trauma, cerebral apoplexy or cerebral hemorrhage, or patients who suffered from schizophrenia, diabetes complications, or dysfunction in heart, liver, and kidney; also patients with cognitive function impairment caused by non-cerebral-small-vascular factors such as encephalitis or Lewy body dementia.

2.3 | Cognitive function assessment

The cognitive function assessment was performed based on the measurement of the Mini-Mental State Exam (MMSE) score, orientation ability, memory, attention, and calculation ability, recalling memory, language ability, word sense discrimination, recitation of numbers, image free recall, meaningless graphic recognition, portrait

TABLE 1 Primer sequence of SNP43 (G/A) and SNP63 (T/C) loci

Loci	Primer sequence	
SNP43 (G/A)	Forward	5′-CACGCTTGCTGTGAAGTAATG-3′
	Reverse	5′-CTCTGATTCCCATGGTCTGTAG-3′
SNP63 (T/C)	Forward	5′-AAGGGGGGCCAGGGCCTGACGGGGGT GGCG-3′
	Reverse	5′-AGCACTCCCAGCTCCTGATC-3′

associating recall, and trail making test A and B. All tests were carried out under the double-blind circumstances.

2.4 | Polymorphism detection

In this experiment from the patients, around 3 ml of anticoagulant venous blood was taken from which the leukocytes were separated using lymphocyte separation solution, and the extraction of genomic DNA from leukocytes was made using the conventional phenol-chloroform method. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to detect the genotype of the SNP43 (G/A) and SNP63 (T/C) loci of the *CAPN10* gene. The primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. (Table 1). The PCR system included 2.5 μL 10 × buffer, 0.9 μL of Dntp Mix (2.5 mmol/L), 0.75 μL of forward primer (10 umol/L), 0.75 μL of reverse primer (10 umol/L), 0.25 μL of template, 0.4 μL of polymerase (5 U/μL), and 17.2 μL of dd H_2O. Then, it was calculated that the total volume of the PCR system was 20 μL. The PCR conditions of SNP43 were as follows: predenaturation at 96°C for 5 minutes, 35 cycles of denaturation at 94.5°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 10 minutes. The PCR conditions of SNP63 were as follows: predenaturation at 94°C for 2 minutes, 35 cycles of denaturation at 94°C for 40 seconds, annealing at 60°C for 40 seconds, extension at 72°C for 30 seconds, overall elongation at 72°C for 10 minutes.

Next, 5 μL of PCR products was added with 1 μL spotting solution. The PCR products were identified by the help of 2% agarose gel electrophoresis and visualized by gel imaging apparatus. Under UV light, the corresponding PCR amplification of various DNA was observed. Then using the restriction endonuclease Nsi I (R6531, Promrga corporation, Madison, WI, USA) and Hha I (R0139V, NEB, Beverly, MA, USA), digestion of PCR products was performed and the SNP43 (G/A) loci and SNP63C/T could be recognized by endonuclease Nsi I and Hha I, respectively. The restriction enzyme digestion volume was 12 μL, including 1 μL Nsi I/Hha I (10 units/μL), PCR product of 6 μL, Buffer of 2 μL (10 ×), dd H2O of 3 μL. The mixture was shaken gently and then briefly centrifuged. It was bathed in water at 37°C for 16 hours; later, the restriction enzyme digestion was ended. Finally, 2% agarose gel electrophoresis was used to treat the restriction enzyme digestion and then, it was envisaged using gel imaging apparatus, where three copies of each genotype were chained and examined using Basic Local Alignment Search Tool (BLAST).

2.5 | PCR-RFLP amplification products of SNP43 (G/A) and SNP63 (T/C) loci

After restriction enzyme digestion, 121 bp and 23 bp fragments appeared at SNP43 (G/A) loci, and the sample of strip appearing at 121 bp fragment after electrophoresis was AA genotype, while the adjacent sample of strip by 144 bp was GG genotype. The common sample of strip of 144 bp and 121 bp was GA genotype. 162 bp and 30 bp fragments were detected at SNP63C/T loci after restriction enzyme digestion. The sample of strip that appeared at 162 bp after electrophoresis was CC genotype; the adjacent sample of strip that appeared at 192 bp was TT genotype. The common sample of strip showed at 192 bp and 162 bp was CT genotype (Figure S1).

2.6 | Statistical analysis

Statistical analysis was conducted using SPSS 19.0 (IBM Corp. Armonk, NY, USA). The measurement data were expressed by mean ± standard deviation, and categorical data were expressed as percentage. *t* test and chi-square test were used to conduct comparison of measurement data and comparison of categorical data, respectively. The genotype distribution frequency was analyzed by PLINK (http://pngu.mgh.harvard.edu/purcell/plink) and verified by Hardy-Weinberg equilibrium. Logistic regression was used to assess odd ratios (OR) of cerebral SVD and also its 95% confidence interval (CI). The two-tailed test was considered significant if $P < .05$.

3 | RESULTS

3.1 | Baseline characteristics of cerebral SVD patients and healthy controls

Initially, the number of cerebral SVD patients with hypertension, diabetes, and also hyperlipidemia and the cerebral SVD patients with the level of TG, TC, LDL, HDL, gender, mean age, mean BMI, and number of patients taking a sedentary life style, smoking, and drinking, and the education were evaluated. In comparison with the control group, the number of hypertension, diabetes, and also hyperlipidemia and the level of TG, TC, LDL, and HDL of the cerebral SVD patients were noticeably higher ($P < .05$), while there was no obvious difference between the two groups in terms of the gender, mean age, mean BMI, number of patients taking a inactive life style, smoking and drinking, and the education ($P > .05$). Compared with the NCI group, the level of TG, TC, LDL, and HDL of the VCI group was much higher in cerebral

TABLE 2 Baseline characteristics of cerebral SVD patients and healthy controls

Characteristics	Cerebral SVD			Control (n = 187)
	Total (n = 224)	NCI (n = 103)	VCI (n = 121)	
Male/female (n)	120/104	54/49	66/55	103/84
Age (y)	72.82 ± 3.62	72.46 ± 3.69	73.12 ± 3.55	73.42 ± 3.37
MoCA score	22.52 ± 3.00*	25.42 ± 1.43	20.06 ± 1.78#	26.47 ± 1.33
BMI (kg/m^2)	23.31 ± 2.91	23.07 ± 3.00	23.52 ± 2.82	22.79 ± 2.74
Sedentary life style (n)	137	62	75	104
Hypertension (n)	130*	59	71	83
Diabetes (n)	120*	52	68	71
Hyperlipidemia (n)	123*	54	69	75
Smoking (n)	110	51	59	85
Alcohol (n)	87	40	47	78
Education year (y)	11.08 ± 3.75	11.05 ± 3.82	11.10 ± 3.70	11.71 ± 3.46
TC (mmol/L)	5.13 ± 1.08*	4.31 ± 0.92	5.82 ± 0.63#	4.16 ± 0.81
TG (mmol/L)	1.75 ± 0.33*	1.67 ± 0.17	1.82 ± 0.41#	1.65 ± 0.51
LDL (mmol/L)	2.89 ± 0.94*	2.39 ± 0.71	3.32 ± 0.91#	2.23 ± 0.83
HDL (mmol/L)	1.42 ± 0.41*	1.21 ± 0.32	1.60 ± 0.40#	1.16 ± 0.38
SBP (mm Hg)	142.65 ± 18.05*	143.53 ± 17.48	141.90 ± 18.56	138.70 ± 13.46
DBP (mm Hg)	92.86 ± 8.35*	92.89 ± 8.85	92.83 ± 7.94	89.37 ± 6.78

BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; SVD, small vessel disease; TC, total cholesterol; TG, triglyceride; VCI: vascular cognitive impairment.
*$P < .05$ vs the control group.
#$P < .05$ vs the NCI group.

SVD patients ($P < .05$) (Table 2). All results above verified that hypertension, diabetes, hyperlipidemia, TG, TC, LDL, and HDL may be associated with cerebral SVD.

3.2 | Frequency distribution of GG, GA, and AA genotype and G and A allele in the VCI and NCI groups

The distribution of genotypes and allele frequency of *CAPN10* gene SNP43 (G/A) and SNP63 (C/T) loci is shown in Table 3. The actual values and theoretical values were all in line with Hardy-Weinberg equilibrium, and the result of goodness of fit test is favorable ($P > .05$).

SNP43 (G/A) loci: the distribution frequency of GA + AA genotype in the cerebral SVD group decreased, where it suggests that the G-allele at the SNP43 (G/A) loci is predisposing gene. If it is compared with the NCI group, obvious differences were found in the distribution frequency of GG, GA + AA genotype in the VCI group as well as in the distribution frequency of the G and A allele.

SNP63 (C/T) loci: distribution frequency of genotype and allele of each group was compared, respectively, being uncorrelated.

3.3 | G allele at SNP43 (G/A) is a risk factor for cognitive impairment in cerebral SVD patients

Mini-Mental State Exam is the best way to find the cognitive assessment and analysis in cerebral SVD patients, and the results are shown in Table 4. Patients with GG genotype scored lower than patients with GA + AA genotype at SNP43 (G/A) loci in terms of the aggregate scores of MMSE, orientation ability, memory, attention and calculation ability, recall, language ability, word sense discrimination, recitation of numbers, image free recall, meaningless graphic recognition, and portrait associating recall (all $P < 05$), while they were scored higher in terms of trail making test A and B, and the differences were statistically significant (all $P < .05$). It was suggested that SNP43 (G/A) locus is an influential factor for the cognitive function of cerebral SVD patients and patients with G allele may perform relatively poor performance in cognitive function. There was no perceptible difference observed in the score of MMSE assessment and other tests attained by patients with CC genotype and patients with CT + TT genotype at SNP63 (C/T) ($P > .05$).

3.4 | SNP43 (G/A), TG, TC, LDL, and HDL are independent risk factors for cognitive impairment in cerebral SVD patients

With the cognitive impairment of cerebral SVD patients, and SNP43 (G/A) loci, SNP63 (C/T) loci, history of diabetes, hypertension, and hyperlipidemia, and level of TG, TC, LDL, and HDL as dependent and independent variables, respectively, a bivariate logistic regression analysis was practiced. The occurrence of cognitive impairment in cerebral SVD patients is closely related to the SNP43 (G/A) loci and

TABLE 3 The distribution frequency of genotype and allele of *CAPN10* gene SNP43 and SNP63 loci [n (%)]

Genotype	Cerebral SVD (n = 224)	Control (n = 187)	*P*	OR	95% CI	NCI (n = 103)	VCI (n = 121)	*P*	OR	95% CI
SNP43 (G/A)										
GG	185 (82.6)	129 (69.0)		1		73 (70.9)	112 (92.6)		1	
GA	35 (15.6)	48 (25.7)	.006	0.51	0.31-0.83	27 (26.2)	8 (6.6)	<.001	0.19	0.08-0.45
AA	4 (1.8)	10 (5.3)	.025	0.28	0.09-0.91	3 (2.9)	1 (0.8)	.152	0.22	0.02-2.13
GA + AA	39 (17.4)	58 (31.0)	.001	0.47	0.29-0.75	30 (29.1)	8 (7.4)	<.001	0.17	0.07-0.40
G	405 (90.4)	306 (81.8)		1		173 (84.0)	232 (95.9)		1	
A	43 (9.6)	68 (18.2)	<.001	0.48	0.32-0.72	33 (16.0)	10 (4.1)	<.001	0.23	0.11-0.47
SNP63 (C/T)										
CC	134 (59.8)	112 (59.9)		1		61 (59.2)	73 (60.3)		1	
CT	77 (29.9)	60 (32.1)	.744	1.07	0.70-1.63	36 (34.9)	41 (33.9)	.863	0.95	0.54-1.67
TT	13 (10.3)	15 (8.0)	.419	0.72	0.33-1.59	6 (5.9)	7 (5.8)	.965	0.97	0.31-3.06
CT + TT	90 (40.2)	75 (40.1)	.988	1	0.67-7.49	42 (41.8)	48 (39.7)	.866	0.96	0.56-1.63
C	345 (77.0)	160 (75.9)		1		160 (77.7)	185 (76.4)		1	
T	103 (23.0)	40 (24.1)	.718	0.94	0.68-1.30	46 (22.3)	57 (23.6)	.886	0.97	0.62-1.50

CI, confidence interval; NCI, no cognitive impairment; OR, odds ratio; SVD, small vessel disease; VCI, vascular cognitive impairment.

the level of TG, TC, LDL, and HDL (all $P < .05$), which are considered as the independent risk factors for cognitive impairment in cerebral SVD patients. SNP63 (C/T) loci, hypertension history, diabetes history, and hyperlipidemia history were not considerably related to cognitive impairment in cerebral SVD patients ($P > .05$) (Table 5). These results indicated that SNP43 (G/A), TG, TC, LDL, and HDL might be independent risk factors for cognitive impairment in cerebral SVD patients.

4 | DISCUSSION

Cerebral SVD constitutes a major source of cognitive decline and neurological disorders in the elderly, and it occurs in relation multiple inherited and genetic disorders.[20,21] Accumulating studies have reported the link between genetic polymorphism of specific genes and cerebrovascular disease, such as, the correlation of Arg972 insulin receptor substrate-1 polymorphism with risk and severity of Alzheimer's disease.[22]Also, Calpain was examined in cognitive impairment following cerebrovascular diseases because it comes to light in the early stages of the disease,[23] thus, it suggests its crucial role in the occurrence of neurodegenerative disease. In the current study, we provided evidence that *CAPN10* gene polymorphism was closely associated with cerebral SVD. SNP43 (G/A) GG genotype in *CAPN10* gene increased the risk of cerebral SVD, and G allele of SNP43 (G/A) in *CAPN10* gene was associated with an increased risk of cognitive impairment in cerebral SVD.

Our results displayed that SNP43 (G/A) GG genotype of *CAPN10* was considered as the risk factor for cerebral SVD. Calpains, as very common family of the calcium-dependent cysteine proteases, were always involved in a large range of differentiation processes and cell regulation.[24] It was reported in a prior study that Calpains played a crucial role in neurodegeneration, cell motility, and also in synaptic plasticity.[25] *CAPN10* protein worked on regulating pancreatic β-cell function, thermogenesis, and glucose metabolism, and several sites of *CAPN10* polymorphism were undergone exhaustive research for their potential markers for the metabolic syndrome and type 2 diabetes.[26] The GG genotype of *CAPN10* was associated with increased susceptibility which leads to colorectal cancer.[27] In a recent study, SNP 43 and SNP 44 polymorphisms of *CAPN10* gene were proved to have effect on the variance in *CAPN10* mRNA level, in which way to increase the risk of cardiovascular diseases.[28] In addition, SNP43 has been proved for its potential role in the occurrence of type 2 diabetes where this leads to higher risk of cardiovascular disease.[29] Orho-Melander et al[30] has demonstrated the SNP-43 allele 1 was associated with elevated fasting serum insulin and homeostasis model assessment index. Notably, it has been proved that insulin resistance is a key risk factor for the increased risk of cognitive impairment.[31] Meanwhile, we also found that the allele G of SNP43 of *CAPN10* would increase the risk of cognitive impairment in cerebral SVD. A study of Liebetrau et al[32] revealed the importance of intracellular calpain levels in focal cerebral ischemia, where it is elevated,

TABLE 4 Analysis for the relationship between the SNP43 (G/A) and SNP63 (C/T) loci and the cognitive impairment in patients with cerebral small vessel disease

	SNP43 (G/A)				SNP63 (C/T)			
Index	GG(n = 185)	GA + AA(n = 39)	*P*	*t*	CC(n = 134)	CT + TT(n = 90)	*P*	*t*
Aggregate MMSE score	20.43 ± 1.70	23.59 ± 1.76	<.001	10.49	20.94 ± 2.17	21.04 ± 1.97	.726	0.35
Orientation ability	6.53 ± 1.06	7.18 ± 0.94	<.001	3.55	6.66 ± 1.07	6.61 ± 1.07	.732	0.34
Memory	1.66 ± 0.47	2.08 ± 0.35	<.001	5.28	1.69 ± 0.51	1.81 ± 0.42	.066	1.85
Attention and calculation ability	2.99 ± 0.52	4.05 ± 0.69	<.001	10.88	3.21 ± 0.68	3.13 ± 0.67	.386	0.87
Recall	1.98 ± 0.13	2.21 ± 0.41	<.001	6.31	2.03 ± 0.21	2.01 ± 0.24	.510	0.66
Language ability	7.26 ± 1.14	8.08 ± 1.13	<.001	4.09	7.35 ± 1.20	7.48 ± 1.13	.417	0.81
Word sense discrimination	7.90 ± 1.10	9.03 ± 1.46	<.001	5.48	8.10 ± 1.34	8.10 ± 1.09	>.999	0.00
Recitation of numbers	9.12 ± 1.62	10.87 ± 1.73	<.001	6.06	9.54 ± 1.72	9.24 ± 1.84	.215	1.24
Image free recall	10.55 ± 1.86	16.36 ± 2.16	<.001	17.22	11.68 ± 3.01	11.38 ± 2.79	.452	0.75
Meaningless graphic recognition	10.38 ± 1.75	12.03 ± 2.03	<.001	5.20	10.67 ± 1.90	10.66 ± 1.91	.969	0.04
Portrait associating recall	3.36 ± 0.56	4.90 ± 0.88	<.001	13.95	3.69 ± 0.93	3.53 ± 0.74	.173	1.37
Trail making test A	147.08 ± 23.71	125.26 ± 19.84	<.001	5.36	141.81 ± 24.53	145.47 ± 24.39	.274	1.10
Trail making test B	92.68 ± 15.12	87.10 ± 13.66	.034	1.94	91.98 ± 15.52	91.31 ± 14.25	.744	0.03

MMSE, Mini-Mental State Exam.

TABLE 5 Logistic regression analysis for the risk factors of cognitive impairment in patients with cerebral small vessel disease

						95% CI of EXP (B)	
Variants	B	*SE*	Wald	*P*	Exp (B)	Lower limit	Upper limit
SNP43 (G/A)	2.506	0.876	8.191	.004	12.261	2.203	68.230
SNP63 (C/T)	−3.347	2.913	1.320	.251	0.035	0.000	10.611
Hypertension (n)	1.124	3.547	0.100	.751	3.078	0.003	3216.424
Diabetes (n)	0.488	1.958	0.062	.803	1.629	0.035	75.628
Hyperlipidemia (n)	0.493	2.869	0.030	.863	1.638	0.006	453.222
TC (mmol/L)	2.247	0.399	31.790	<.001	9.464	4.333	20.672
TG (mmol/L)	2.27	1.041	4.752	.029	9.681	1.257	74.546
LDL (mmol/L)	1.233	0.335	13.564	<.001	3.432	1.781	6.615
HDL (mmol/L)	3.505	0.892	15.459	<.001	33.295	5.801	191.099

HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglyceride.

with longer reperfusion in the areas of cerebral ischemia leading to an increase in calpain positivity.

In our study, we found that TG, TC, LDL, and HDL were closely correlated to cognitive impairment in cerebral SVD patients. Prospective and cross-sectional observational researches have studied the association between cognitive impairment and the level of serum lipids/lipoproteins, such as TC, TG, HDL, and LDL, but these studies have demonstrated inconsistent and conflicting results.[33] A study showed that high TC and LDL-C may contribute to Alzheimer's disease, dementia, or cognitive deficits of elderly people when compared with sex- and age-matched nondemented peers, which was consistent with our findings here.[34] HDL acts as an antioxidant and represents as the most important anti atherosclerotic factors which implicates the endothelial dysfunction.[35] Alzheimer's disease, as a neurodegenerative disease, it is occurred when the level of TC levels is high, so is the cognitive impairment.[36] In addition, recent finding showed that TG level was involved and played an important role in cognitive impairment of MDD, especially in late memory.[37]

Based on the results obtained in this current study, *CAPN10* gene polymorphism was associated with cerebral SVD and the

allele G of SNP43 (G/A) which increased the risk of cognitive impairment in cerebral SVD, which makes it a potential molecular target for CSVD treatment. As most studies focused on cognitive impairment in Alzheimer's disease rather than in cerebral SVD, and were mostly demonstrated using the macro perspective, the efforts made in this study may provide some novel perspective. However, the mechanism of SNP43 in cerebral SVD analyzed with larger sample sizes is required to be manifested in a more detailed way in the future.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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