NEURAL REGENERATION RESEARCH
Volume 8, Issue 16, June 2013
www.nrronline.org

doi:10.3969/j.issn.1673-5374.2013.16.009 [http://www.nrronline.org; http://www.sjzsyj.org]
Mo DH, Xu HW, Zhou WS, Yang QM, Yang JW, Xiao B, Yang QD. Susceptibility gene for stroke or cerebral infarction in the Han population in Hunan Province of China. Neural Regen Res. 2013;8(16):1519-1527.

Susceptibility gene for stroke or cerebral infarction in the Han population in Hunan Province of China★

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Abstract
The scavenger receptor class B type I gene can protect against atherosclerosis; a mononucleotide polymorphism is associated with differences in blood lipid metabolism, postprandial serum lipid levels, insulin resistance, coronary artery disease and familial hyperlipidemia. In this study, the scavenger receptor class B type I gene exon 1 G4A gene polymorphism in atherosclerotic cerebral infarction patients, cerebral hemorrhage patients and normal controls was detected using the polymerase chain reaction-restriction fragment length polymorphism method. The results showed that the GA + AA genotype frequency of scavenger receptor class B type I gene G4A in atherosclerotic cerebral infarction patients was similar to that in cerebral hemorrhage patients and normal controls; however, the A allele frequency was significantly lower than that in normal controls. The serum level of high-density lipoprotein cholesterol in patients with the scavenger receptor class B type I gene G4A GA + AA genotype was significantly higher, while the serum level of low-density lipoprotein cholesterol was significantly lower than that in patients with the GG genotype, in both the atherosclerotic cerebral infarction and cerebral hemorrhage groups. The serum level of high-density lipoprotein cholesterol in patients with the scavenger receptor class B type I gene G4A GA + AA genotype was significantly higher, while the serum levels of low-density lipoprotein cholesterol and total cholesterol were significantly lower than those in normal controls with the GG genotype. Our experimental results suggest that the G4A polymorphism of the scavenger receptor class B type I gene is a possible predisposing risk factor for atherosclerotic cerebral infarction, and that it has no association with cerebral hemorrhage in the Han population in Hunan province of China. The A allele is possibly associated with the metabolism of high-density and low-density lipoprotein cholesterol.

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Received: 2013-01-24
Accepted: 2013-05-20
(N20120915005)

Key Words
neural regeneration; scavenger receptor class B type I; stroke; atherosclerotic cerebral infarction; cerebral hemorrhage; genetic polymorphism; cholesterol; neuroregeneration

Research Highlights
(1) The scavenger receptor class B type I gene exon 1 G4A polymorphism locus may be associated with atherosclerotic cerebral infarction in the Han population from Hunan province of China, while it has no association with cerebral hemorrhage.
(2) When the scavenger receptor class B type I gene exon 1 G4A polymorphism mutates from G to A, leading to amino acid changes, changes in the structure of the protein may affect its functions and accordingly affect scavenger receptor class B type I gene expression. This impacts lipid metabolism and participates in the development of atherosclerosis, ultimately resulting in atherosclerotic cerebral infarction.
(3) Scavenger receptor class B type I gene may influence high-density and low-density lipoprotein metabolism, thus affecting the development of diseases relevant to atherosclerosis.

INTRODUCTION

The scavenger receptor class B type I gene was first identified in 1993, is located at 12q24.2-qter, is about 75 kb in length, and contains 13 exons and 12 introns. The protein it encodes contains 509 amino acids[1-2]. Scavenger receptor class B type I is mainly involved in the initial steps (high-density lipoprotein cholesterol outflow from the arterial wall and its removal) and final steps (selective uptake of high-density lipoprotein cholesterol in liver) of reverse cholesterol transport[3-12]. Furthermore numerous studies using transgenic and knockout animals have shown that scavenger receptor class B type I has a protective effect against atherosclerosis[13-19]. Atherosclerosis is widely recognized as an independent risk factor for stroke[20-22], and its occurrence is closely related to lipid metabolism[23-26]. Structural changes trigger changes in gene functions. Previous studies of scavenger receptor class B type I gene polymorphisms have focused on the exon 1 G4A polymorphism and the exon 8 C1050T polymorphism, and these polymorphic loci impact blood lipid levels and are involved in the dyslipidemia in diabetes patients[27]. Unfortunately there has been no report on the correlation between the scavenger receptor class B type I exon 1 G4A polymorphism (hereinafter scavenger receptor class B type I gene G4A polymorphism) and stroke in China. This study aimed to explore the association of the scavenger receptor class B type I gene G4A polymorphism with lipid metabolism and stroke in the Han population from Hunan province of China.

RESULTS

Quantitative analysis of subjects

One-hundred and fifty patients with atherosclerotic cerebral infarction were included in this study, among which DNA could not be extracted from one owing to peripheral blood hemolysis. Another 150 patients with cerebral hemorrhage were also used, among which nine patients were excluded because of peripheral blood hemolysis in two and low levels of extracted DNA in seven (three of which showed loss of DNA during the PCR amplification process and four of which showed loss of DNA during the enzymatic digestion process). Ultimately 149 patients with atherosclerotic cerebral infarction and 141 patients with cerebral hemorrhage entered the analysis. In addition, 120 normal controls were included.

Clinical information on stroke patients

There were no significant differences in age, gender ratio, triglycerides, and smoking history between the atherosclerotic cerebral infarction group and the control group ($P > 0.05$). In atherosclerotic cerebral infarction patients, the body mass index, total cholesterol, low-density lipoprotein cholesterol, and blood glucose levels were higher, while high-density lipoprotein cholesterol levels were lower than those in controls. In addition, a history of hypertension and diabetes mellitus was more prevalent in atherosclerotic cerebral infarction patients than in the control group ($P < 0.05$). There were no significant differences in age, gender ratio, low-density lipoprotein cholesterol, body mass index, blood glucose levels, smoking history, and history of diabetes mellitus between the cerebral hemorrhage group and the control group ($P > 0.05$). In cerebral hemorrhage patients, total cholesterol levels were higher, while high-density lipoprotein cholesterol levels were lower than those in the controls. Previous history of hypertension was more prevalent in the cerebral hemorrhage group than in the control group ($P < 0.05$). No significant difference was found in age, gender ratio, triglycerides, total cholesterol, blood glucose, high-density lipoprotein cholesterol, and smoking history between the atherosclerotic cerebral infarction group and the cerebral hemorrhage group ($P > 0.05$). The atherosclerotic cerebral infarction patients had a higher level of low-density lipoprotein cholesterol and more prevalent complications of diabetes mellitus than did the cerebral hemorrhage patients ($P < 0.05$; Table 1).

Scavenger receptor class B type I G4A genotype analysis

As shown in Figure 1, the scavenger receptor class B type I gene G4A PCR amplification product was 304 bp. After PCR products were digested with the restriction enzyme *Alu*I, three genotypes were observed, one homozygous GG genotype band at 304 bp, three heterozygous GA genotype bands at 304, 188 and 116 bp, and two homozygous AA genotype bands at 188 and 116 bp (Figure 2).

Scavenger receptor class B type I G4A polymorphism distribution in stroke patients

Only the GG and GA genotypes were detected in 149 patients with atherosclerotic cerebral infarction, while the AA genotype was not detected. The GG, GA and AA genotypes were all detected in 141 patients with cerebral hemorrhage and 120 normal controls. The genotype and allele frequencies are shown in Table 2. A

Hardy-Weinberg genetic equilibrium test showed that all genotypes in the three groups met a genetic equilibrium, indicating a population representation ($\chi^2 = 3.92$, $P < 0.05$).

Table 1 Clinical information of stroke patients and normal controls

Item	Atherosclerotic cerebral infarction group (n = 149)	Cerebral hemorrhage group (n = 141)	Control group (n = 120)
Age (year)	59.6±9.3	57.4±12.6	59.0±6.9
Male [n(%)]	94(63.1)	97(68.8)	79(65.8)
Body mass index (kg/m^2)	23.1±2.2[a]	22.6±2.5	22.3±2.8
Number of smoker [n (%)]	44(29.5)	37(26.2)	31(25.8)
History of hypertension [n (%)]	86(57.7)[a]	87(61.7)[a]	24(2.0)
History of diabetes mellitus [n (%)]	31(20.8)[ab]	6(4.1)	4(3.3)
Total cholesterol (mmol/L)	4.72±1.16[a]	4.52±0.98[a]	4.23±1.01
Triglycerides (mmol/L)	1.83±1.14	1.75±1.17	1.68±0.63
High-density lipoprotein cholesterol (mmol/L)	1.25±0.42[a]	1.27±0.41[a]	1.51±0.39
Low-density lipoprotein cholesterol (mmol/L)	2.76±0.97[ab]	2.42±0.89	2.29±0.65
Blood glucose (mmol/L)	6.08±2.34[a]	6.41±2.38	4.76±1.32

Body mass index = body weight (kg)/height^2 (cm^2). Body mass index normal range: 18.5–23.9, total cholesterol normal range: 3.4–5.2 mmol/L, triglyceride normal range: 0.45–1.69 mmol/L, high-density lipoprotein cholesterol normal range: 0.78–1.81 mmol/L, low-density lipoprotein cholesterol normal range: 0–3.36 mmol/L, blood glucose normal range: 3.89–6.11 mmol/L[28].

Measurement data (mean ± SD) between the two groups were compared using a group t-test, and homogeneity test for variance was used to compare among more than two groups in line with the normal distribution, and a completely randomized design analysis of variance was performed. [a]$P < 0.05$, *vs*. control group; [b]$P < 0.05$, *vs*. cerebral hemorrhage group.

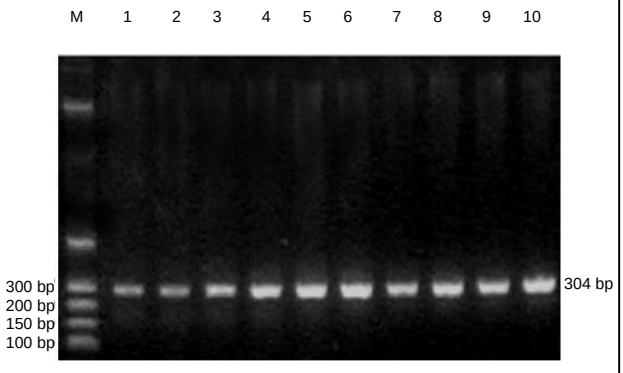

Figure 1 PCR products of scavenger receptor class B type I gene spanning the G4A polymorphic locus.

M: SSR DNA marker I; 1–10: PCR products. PCR analysis showed that the amplified product was 304 bp.

Blood lipid levels in scavenger receptor class B type I G4A polymorphism with different genotypes

In the control group, high-density lipid protein cholesterol levels were lower, while low-density lipoprotein cholesterol and total cholesterol levels were higher in the scavenger receptor class B type I G4A polymorphism GG genotype subgroup than in the scavenger receptor class B type I G4A GA + AA genotype subgroup ($P < 0.05$; Table 3).

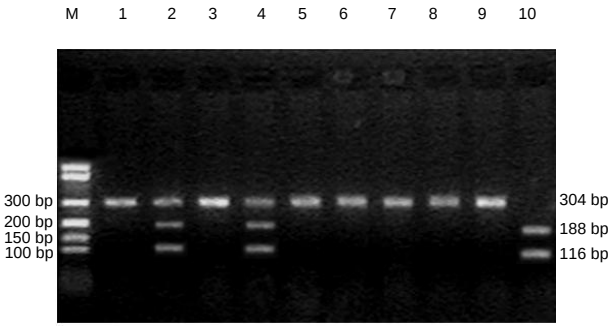

Figure 2 *Alu*I enzymatic digestion of scavenger receptor class B type I PCR products at the G4A polymorphic locus.

M: SSR DNA marker II; 1, 3, 5–9: GG homozygotes; 2, 4: GA heterozygotes; 10: AA homozygote.

Table 2 Scavenger receptor class B type I G4A polymorphism genotype and allele frequency distributions in stroke patients and normal controls

Group	n	Genotype [n(%)]		Allele frequency (%)	
		GG	GA+AA	G	A
Stroke					
Atherosclerotic cerebral infarction	149	127(85.2)	22(14.8)	0.926	0.074[a]
Cerebral hemorrhage	141	116(82.3)	25(17.7)	0.908	0.092
Control	120	91(75.8)	29(24.2)	0.871	0.129

Data were compared using the chi-square test. [a]$P < 0.05$, *vs*. control group.

The serum level of high-density lipoprotein cholesterol in the scavenger receptor class B type I G4A GA + AA genotype subgroup was significantly decreased, while the serum level of low-density lipoprotein cholesterol was significantly increased compared with those in atherosclerotic cerebral infarction and cerebral hemorrhage patients with the GG genotype ($P < 0.05$). However, there was no significant difference in body mass index, and other lipid or lipoprotein levels between the two genotype subgroups ($P > 0.05$; Tables 4, 5).

Table 3 Blood lipid levels in healthy controls with different scavenger receptor class B type I G4A genotypes

Item	Genotype		*P*
	GG (*n* = 91)	GA+AA (*n* = 29)	
Body mass index (kg/m^2)	22.24±2.87	22.48±2.86	0.70
Total cholesterol (mmol/L)	4.79±1.13	4.22±0.97	0.02
Triglycerides (mmol/L)	1.48±0.66	1.45±0.54	0.80
Low-density lipoprotein cholesterol (mmol/L)	2.42±0.62	2.16±0.48	0.04
High-density lipoprotein cholesterol (mmol/L)	1.44±0.37	1.62±0.35	0.03

Body mass index = body weight (kg)/height^2 (cm^2). Measurement data are expressed as mean ± SD and the significance of differences between the two groups was assessed using a group *t*-test. The homogeneity test for variance was used to compare more than two groups in line with the normal distribution, and a completely randomized design analysis of variance was performed.

Table 4 Blood lipid levels in atherosclerotic cerebral infarction patients with different scavenger receptor class B type I G4A genotypes

Item	Genotype		*P*
	GG (*n* = 127)	GA+AA (*n* = 22)	
Body mass index (kg/m^2)	23.07±2.24	23.17±1.74	0.84
Total cholesterol (mmol/L)	4.74±1.20	4.63±0.91	0.67
Triglycerides (mmol/L)	1.78±1.22	1.60±0.80	0.52
Low-density lipoprotein cholesterol (mmol/L)	3.07±0.83	2.46±0.70	0.00
High-density lipoprotein cholesterol (mmol/L)	1.23±0.35	1.39±0.38	0.04

Body mass index = body weight (kg)/height^2 (cm^2). Measurement data are expressed as mean ± SD and the significance of differences between the two groups was assessed using a group *t*-test. The homogeneity test for variance was used to compare more than two groups in line with the normal distribution, and a completely randomized design analysis of variance was performed.

DISCUSSION

Many studies using scavenger receptor class B type I gene transfection and scavenger receptor class B type I gene knockout mouse models have confirmed that high levels of scavenger receptor class B type I can prevent atherosclerosis[2, 13-19, 29-34]. Rodríguez-Esparragón *et al* [35] found that homozygous human scavenger receptor class B type I gene C8C8 was associated with coronary atherosclerotic heart disease in males. No studies to date have focused on the correlation between scavenger receptor class B type I gene G4A polymorphisms and stroke. The results of the present study showed that the scavenger receptor class B type I gene G4A A allele frequency in patients with atherosclerotic cerebral infarction was significantly higher than that in controls, while cerebral hemorrhage patients showed no significant difference in genotype frequency or allele frequency compared with the control group.

Table 5 Blood lipid levels in cerebral hemorrhage patients with different scavenger receptor class B type I G4A genotypes

Item	Genotype		*P*
	GG (*n* = 116)	GA+AA (*n* = 25)	
Body mass index (kg/m^2)	22.55±2.46	22.96±2.75	0.46
Total cholesterol (mmol/L)	4.51±1.00	4.57±0.91	0.77
Triglycerides (mmol/L)	1.76±1.08	2.15±1.36	0.12
Low-density lipoprotein cholesterol (mmol/L)	2.62±0.77	2.12±0.73	0.00
High-density lipoprotein cholesterol (mmol/L)	1.26±0.36	1.44±0.49	0.04

Body mass index = body weight (kg)/height^2 (cm^2). Measurement data are expressed as mean ± SD and the significance of differences between the two groups was assessed using a group *t*-test. The homogeneity test for variance was used to compare more than two groups in line with the normal distribution, and a completely randomized design analysis of variance was performed.

It can be speculated that the scavenger receptor class B type I gene G4A polymorphism locus may be associated with atherosclerotic cerebral infarction in the Han population from Hunan province of China, but not with cerebral hemorrhage in this population. The A allele may be protective against atherosclerotic cerebral infarction. Yamada *et al* [36] performed a large-scale population screening study for myocardial infarction candidate genes and found that the scavenger receptor class B type I G4A polymorphic locus and G403A (Val135Ile) polymorphic locus had no impact on myocardial infarction. The following reasons explain the observed discrepancies between their and our findings: (1) ethnic differences of the subjects; (2) variations in anatomical structures and cell types lead to different risk factors for myocardial infarction and stroke, although they were both pathologically based on atherosclerosis, and abundant evidence supports the inconsistency of susceptibility genes between the two diseases; (3) the sample size in this study was too small to fully reflect the disease characteristics. Based on mutual support between theoretical research and animal studies of scavenger receptor class B type I, as well as scavenger receptor class B type I gene polymorphism and disease association studies, it can be concluded that, when the scavenger receptor class B type I gene G4A polymorphism mutates from G to A, leading to amino acid changes, change in the structure of the protein

affects its functions and accordingly affects scavenger receptor class B type I gene expression. This impacts lipid metabolism and participates in the development of atherosclerosis, ultimately resulting in atherosclerotic cerebral infarction. At the same time, cerebral hemorrhage, for which the pathological basis is also atherosclerosis, was not associated with the scavenger receptor class B type I gene G4A polymorphism. The reason for this may be that the scavenger receptor class B type I gene polymorphism has less impact on cerebral hemorrhage than on atherosclerotic cerebral infarction.

Both *in vitro* and *in vivo* studies have demonstrated that, scavenger receptor class B type I is involved in the metabolism of plasma lipoproteins, including high-density and non-high-density lipoproteins. Adenovirus-mediated gene transfection induces scavenger receptor class B type I over-expression in rat liver and a transient increase in scavenger receptor class B type I levels on the sinusoid and tubular liver cell surfaces, while plasma levels of high-density lipoprotein cholesterol and apolipoprotein A–I were transiently decreased, and the cholesterol concentration in liver bile was elevated[37]. The role of scavenger receptor class B type I in high-density lipoprotein metabolism in scavenger receptor class B type I knockout mice was also investigated. Compared with wild-type control rats, an increase of 2–2.5 times total cholesterol level was observed in scavenger receptor class B type I knockout rats, and the majority of this was high-density lipoprotein cholesterol. The high-density lipoprotein particles were larger than those in wild-type rats. Despite the increase in the plasma levels of high-density lipoprotein cholesterol, plasma apolipoprotein A-I levels were similar between the two groups, indicating that the increased level of high-density lipoprotein cholesterol contributes to the impairment of selective uptake of high-density lipoprotein cholesterol[38]. Scavenger receptor class B type I binds high-density lipoprotein and selectively uptakes high-density lipoprotein cholesterol. It also binds non-high-density lipoproteins, such as low-density lipoprotein and extremely low-density lipoprotein, and mediates the selective uptake of low-density lipoprotein cholesterol. These findings suggest that scavenger receptor class B type I is involved in non-high-density lipoprotein metabolism[17, 39-46]. Acton *et al* [47] found that the scavenger receptor class B type I gene was associated with lipid metabolism in Southern European Caucasians, and that scavenger receptor class B type I G4A mutations triggered an increase in levels of high-density lipoprotein cholesterol and a decrease in levels of low-density lipoprotein cholesterol in males. Subsequently Osgood *et al* [48] revealed no differences in blood lipid and lipoprotein levels in non-diabetic patients with different scavenger receptor class B type I G4A genotypes; however, the scavenger receptor class B type I G4A A allele causes a reduction in extremely low-density lipoprotein cholesterol levels, and in the diameter of low-density and high-density lipoprotein cholesterol particles in diabetic patients. Pérez-Martínez *et al* [49] detected the responses of different genotypes to diet intervention in healthy population, and the results showed a correlation between scavenger receptor class B type I G4A GA and postprandial low blood lipids. This is evidence that the A allele may increase intrahepatic scavenger receptor class B type I gene expression and accelerate the scavenging of triglyceride-rich small particles. Liao and Xu[27] detected scavenger receptor class B type I gene exon 1 G4A mutated genotypes in 150 patients with type 2 diabetes mellitus and 120 controls with normal glucose tolerance using the PCR-restriction fragment length polymorphism method, and statistical analysis showed that the A allele subgroup had lower levels of low-density lipoprotein cholesterol than the GG genotype subgroup, and that diabetic patients carrying the A allele also had lower levels of high-density lipoprotein cholesterol. In this study, high-density lipoprotein cholesterol levels in the GG genotype subgroup were significantly lower while low-density lipoprotein cholesterol levels were significantly higher than those in the GA + AA genotype subgroup among atherosclerotic cerebral infarction patients, cerebral hemorrhage patients and normal controls. In the control group, total cholesterol levels were significantly higher in the GG genotype subgroup compared with the GA + AA genotype subgroup. We concluded that the A allele may induce the increase in levels of high-density lipoprotein cholesterol and the decrease in levels of low-density lipoprotein cholesterol in normal controls, atherosclerotic cerebral infarction patients and cerebral hemorrhage patients. Our findings are consistent with the results observed by Acton *et al* [50] in a healthy population, but inconsistent with those of Osgood *et al* [48]. Minor inconsistencies may be explained on the basis of racial differences and inclusion criteria of the involved subjects, because the non-diabetic controls that Osgood *et al* mentioned are not necessarily healthy. Our findings are not in agreement with the results of animal experiments, mainly due to differences in the *in vivo* environments of human and animals, and the fact that the major cholesterol carrier in mice and other animals is high-density lipoprotein, but the major carriers in humans are low-density lipoprotein and extremely low-density lipoprotein[51]. Furthermore, all subjects involved in this study showed the same association between scavenger

receptor class B type I and blood lipid levels, indicating that the scavenger receptor class B type I gene may influence high-density and low-density lipoprotein metabolism, thus affecting the development of diseases relevant to atherosclerosis.

The scavenger receptor class B type I gene has been comprehensively studied in animal models, and its expression and polymorphisms in the human body remain elusive. The results of the few existing studies are controversial. Therefore, more studies are needed, especially studies focused on scavenger receptor class B type I gene polymorphisms in different races and geographical populations, in a broader attempt to analyze the role of the scavenger receptor class B type I gene in humans.

SUBJECTS AND METHODS

Design

A case-control study.

Time and setting

Experiments were performed from October 2005 to February 2007 in the Department of Neurology, Xiangya Hospital of Central South University, China.

Subjects

Three-hundred hospitalized stroke patients were selected from the Department of Neurology, Xiangya Hospital of Central South University, China between October 2005 and February 2007. All patients were diagnosed according to the diagnostic criteria of the Fourth Academic Conference of National Cerebral Vascular Disease, as revised in 1995[52], using head CT and (or) MRI. Patients with stroke caused by cardiogenic lesions, arteritis, trauma, blood diseases, drugs, tumor, cerebral vascular malformations or aneurysms were excluded; patients presenting bleeding after infarction and a history of coronary heart disease were also excluded. The involved 300 patients were divided into an atherosclerotic cerebral infarction group (n = 150) and a cerebral hemorrhage group (n = 150) according to their disease pathogenesis.

One hundred and twenty healthy controls were recruited from the Medical Examination Center of Xiangya Hospital, Central South University, China with no familial history of coronary heart disease, cerebrovascular disease, or cardiovascular disease. None of the involved subjects had liver or kidney diseases, autoimmune diseases, pregnancy, lipid-lowering therapy within the previous 6 months, and all were unrelated Han from Hunan province of China. They were well matched in terms of age and gender. The participants and their families gave informed consent.

Methods

Determination of blood lipid levels

Blood lipid levels were obtained through medical records from the Department of Clinical Laboratory, Xiangya Hospital, China. In brief, serum levels of triglycerides and total cholesterol were assayed using the oxidase method. Serum high-density lipoprotein cholesterol was precipitated and then the level of cholesterol in the supernatant was determined as follows: low-density lipoprotein cholesterol = total cholesterol – high-density lipoprotein cholesterol – (triglycerides/5). Serum apolipoprotein A–I and B levels were assayed using the immune turbidimetric method.

Preparation of DNA

Genomic DNA was extracted by the conventional phenol/chloroform extraction method. Briefly, blood from the cubital vein was placed in a 50 mL centrifugation tube and mixed with 5 volumes (25 mL) of 1 × erythrocyte lysis buffer, placed on ice for 30 minutes until the mixture became transparent, and then centrifuged at 4°C at 3 000 r/min for 10 minutes. The supernatant was discarded, and the leukocytic cream in the tube bottom was reserved. Then, 3 mL of nuclei lysing solution, 150 μL of 20% sodium dodecyl sulfate solution and 20 μL of Proteinase K (20 mg/mL) solution were successively added, and the mixture was digested overnight at 37°C. The next day, 3 mL of saturated redistilled phenol was added and the tube was well shaken for 10 minutes, after which the mixture was centrifuged at 4°C, 3 000 r/min for 10 minutes and the upper aqueous phase was transferred to another 15 mL sterile centrifugation tube. Next, 3 mL of phenol/chloroform (v/v) was added and well shaken for 10 minutes, the mixture was centrifuged at 4°C, 3 000 r/min for 10 minutes, and the upper aqueous phase was transferred to another 15 mL sterile centrifugation tube. After adding two volumes of absolute ethanol, a white flocculent precipitate from the peripheral blood extracts was visible, that is, genomic DNA. Using a clean glass hooked needle, white flocculent precipitates were obtained and rinsed twice with 70% ethanol, and dried at room temperature for 2–3 minutes. Then, the genomic DNA was dissolved in 500 μL of 1 × TE buffer and maintained overnight at room temperature, then preserved at –70°C for further use.

PCR analysis of the scavenger receptor class B type I gene G4A polymorphism

Scavenger receptor class B type I gene G4A polymorphic locus primers were designed using Primer3 software: upstream 5'-CGA TGG GGC ATA AAA CCA C-3', downstream 5'-GGA CCT GCT GCT TGA TGA G-3'. The total volume of the PCR reaction system was 25 μL, including 16 μL of ddH_2O, 2.5 μL of 10 × PCR Buffer, 0.5 μL of 10 mmol/L dNTPs, 1.5 μL of 25 mmol/L $MgCl_2$, 1 μL of each primer at 10 pmol/μL, 1.5 μL of Taq enzyme (1 U/μL), and 1 μL of 100 ng/μL template (genomic DNA). The steps were 30 cycles of pre-denaturation at 94°C for 5 minutes, denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 45 seconds, followed by a 10-minute extension at 72°C.

Genotype analysis

The genotype analysis was performed using restriction fragment length polymorphism analysis. The enzymatic digestion system consisted of 8 μL of PCR products, 1 μL of restriction enzyme *Alu*I (Biolabs, UK), 2 μL of 10 × buffer, and 9 μL of double distilled water at 37°C overnight (12 hours). Then, 5 μL of the amplification product was mixed with 1 μL of 6 × neutral loading buffer and loaded onto a 2.5% agarose gel (containing 0.5 μg/mL ethidium bromide), and electrophoresed in 0.5 × TBE electrophoresis buffer at 110 V for 30 minutes. The electrophoresis results were observed using a gel image analysis system (Tanon Science & Technology Co., Ltd., Shanghai, China) and the genotype was determined.

Statistical analysis

Measurement data were expressed as mean ± SD and statistically analyzed using SPSS 11.50 software (SPSS, Chicago, IL, USA). The representation of samples was assayed according to the Hardy-Weinberg equilibrium. The genotype and allele frequencies of stroke patients and normal controls were calculated using the gene counting method. Count data were compared using the chi-square test or Fisher's exact probability test. Measurement data from the two groups were compared using a group *t*-test, and comparisons among groups were performed using a homogeneity test for variance in line with the normal distribution, and a completely randomized design analysis of variance was used. A *P* value < 0.05 was considered significantly different.

Acknowledgments: We would like to thank all staff from the Department of Neurology, Xiangya Hospital of Central South University for their great help and support, and Mawangdui Hospital of Hunan Province, China.

Author contributions: Danheng Mo was responsible for the study concept and design, provided experimental data, performed data integration and analysis, and wrote the manuscript. Bo Xiao and Qidong Yang supervised the study. Wensheng Zhou, Qiming Yang and Jianwen Yang provided technical and academic support. Hongwei Xu guided the research. All authors approved the final version of the paper.

Conflicts of interest: None declared.

Ethical approval: This study was approved by Medical Ethics Committee, Xiangya Hospital of Central South University, China.

Author statements: The manuscript is original, has not been submitted to or is not under consideration by any other publications, has not been previously published in any language or any form, including electronic, and contains no disclosure of confidential information or authorship/patent application disputations.

REFERENCES

[1] Calvo D, Vega MA. Identification, primary structure, and distribution of CLA-1, a novel member of the CD36/LIMPII gene family. J Biol Chem. 1993;268(25):18929-18935.

[2] Cao G, Garcia CK, Wyne KL, et al. Structure and localization of the human gene encoding SR-BI/CLA-1. Evidence for transcriptional control by steroidogenic factor 1. J Biol Chem. 1997;272(52):33068-33076.

[3] Van Eck M, Twisk J, Hoekstra M, et al. Differential effects of scavenger receptor BI deficiency on lipid metabolism in cells of the arterial wall and in the liver. J Biol Chem. 2003; 278(26):23699-23705.

[4] Krieger M. Charting the fate of the "good cholesterol": identification and characterization of the high-density lipoprotein receptor SR-BI. Annu Rev Biochem. 1999;68: 523-558.

[5] Braun A, Zhang S, Miettinen HE, et al. Probucol prevents early coronary heart disease and death in the high-density lipoprotein receptor SR-BI/apolipoprotein E double knockout mouse. Proc Natl Acad Sci U S A. 2003;100(12): 7283-7288.

[6] Fluiter K, Sattler W, De Beer MC, et al. Scavenger receptor BI mediates the selective uptake of oxidized cholesterol esters by rat liver. J Biol Chem. 1999;274(13): 8893-8899.

[7] Imachi H, Murao K. HDL metabolism in lifestyle-related illnesses. Rinsho Byori. 2012;60(11):1081-1086.

[8] Pan B, Ma Y, Ren H, et al. Diabetic HDL is dysfunctional in stimulating endothelial cell migration and proliferation due to down regulation of SR-BI expression. PLoS One. 2012; 7(11):e48530.

[9] Leiva A, Verdejo H, Benítez ML, et al. Mechanisms regulating hepatic SR-BI expression and their impact on HDL metabolism. Atherosclerosis. 2011;217(2):299-307.

[10] Iatan I, Palmyre A, Alrasheed S, et al. Genetics of cholesterol efflux. Curr Atheroscler Rep. 2012;14(3):235-246.
[11] Saddar S, Mineo C, Shaul PW. Signaling by the high-affinity HDL receptor scavenger receptor B type I. Arterioscler Thromb Vasc Biol. 2010;30(2):144-150.
[12] Röhrl C, Fruhwürth S, Schreier SM, et al. Scavenger receptor, Class B, Type I provides an alternative means for beta-VLDL uptake independent of the LDL receptor in tissue culture. Biochim Biophys Acta. 2010;1801(2):198-204.
[13] El Bouhassani M, Gilibert S, Moreau M, et al. Cholesteryl ester transfer protein expression partially attenuates the adverse effects of SR-BI receptor deficiency on cholesterol metabolism and atherosclerosis. J Biol Chem. 2011;286(19):17227-17238.
[14] Mineo C, Shaul PW. Functions of scavenger receptor class B, type I in atherosclerosis. Curr Opin Lipidol. 2012; 23(5):487-493.
[15] Zhao JF, Ching LC, Huang YC, et al. Molecular mechanism of curcumin on the suppression of cholesterol accumulation in macrophage foam cells and atherosclerosis. Mol Nutr Food Res. 2012;56(5):691-701.
[16] Zhao Y, Pennings M, Vrins CL, et al. Hypocholesterolemia, foam cell accumulation, but no atherosclerosis in mice lacking ABC-transporter A1 and scavenger receptor BI. Atherosclerosis. 2011;218(2):314-322.
[17] Kozarsky KF, Donahee MH, Glick JM, et al. Gene transfer and hepatic overexpression of the HDL receptor SR-BI reduces atherosclerosis in the cholesterol-fed LDL receptor-deficient mouse. Arterioscler Thromb Vasc Biol. 2000;20(3):721-727.
[18] Arai T, Wang N, Bezouevski M, et al. Decreased atherosclerosis in heterozygous low density lipoprotein receptor-deficient mice expressing the scavenger receptor BI transgene. J Biol Chem. 1999;274(4):2366-2371.
[19] Covey SD, Krieger M, Wang W, et al. Scavenger receptor class B type I-mediated protection against atherosclerosis in LDL receptor-negative mice involves its expression in bone marrow-derived cells. Arterioscler Thromb Vasc Biol. 2003;23(9):1589-1594.
[20] Yu M, Sun XJ. Study progress of cranial artery atherosclerotic stenosis and ischemic stroke. Zhongguo Laonian Xue Zazhi. 2009;29(4):896-899.
[21] Tang YD, Dong Q. Risk factors and etiology of stroke in young. Zhonghua Naoxueguan Zazhi: Dianzi Ban. 2010; 4(3):182-190.
[22] Fu WG. Interpretation of “treatment guidelines of extracranial carotid stenosis”. Zhongguo Linchuang Yisheng. 2009;37(2):63-66.
[23] Chen CL, Liu IH, Fliesler SJ, et al. Cholesterol suppresses cellular TGF-beta responsiveness: implications in atherogenesis. J Cell Sci. 2007;120(Pt 20):3509-3521.
[24] Kleemann R, Verschuren L, van Erk MJ, et al. Atherosclerosis and liver inflammation induced by increased dietary cholesterol intake: a combined transcriptomics and metabolomics analysis. Genome Biol. 2007;8(9):R200.
[25] Szabó KJ, Adány R, Balla J, et al. Advances in the prevention, diagnosis and therapy of vascular diseases. Orv Hetil. 2012;153(13):483-498.
[26] Mashimo Y, Maeda T, Teramoto T. Dyslipidemia. Nihon Rinsho. 2012;70(5):852-856.
[27] Liao LY, Xu YC. The influence of Gly4Ser variant of the scavenger receptor class B type I gene exon 1 polymorphism on the serum lipid of type 2 diabetes mellitus. Yixue Xinzhi Zazhi. 2005;15(1):27-29.
[28] Lu ZY, Zhong NS. Internal Medicine. 7th ed. Beijing: People’s Medical Publishing House. 2008.
[29] Goti D, Hrzenjak A, Levak-Frank S, et al. Scavenger receptor class B, type I is expressed in porcine brain capillary endothelial cells and contributes to selective uptake of HDL-associated vitamin E. J Neurochem. 2001; 76(2):498-508.
[30] Mardones P, Strobel P, Miranda S, et al. Alpha-tocopherol metabolism is abnormal in scavenger receptor class B type I (SR-BI)-deficient mice. J Nutr. 2002;132(3): 443-449.
[31] Yuhanna IS, Zhu Y, Cox BE, et al. High-density lipoprotein binding to scavenger receptor-BI activates endothelial nitric oxide synthase. Nat Med. 2001;7(7):853-857.
[32] Li XA, Titlow WB, Jackson BA, et al. High density lipoprotein binding to scavenger receptor, Class B, type I activates endothelial nitric-oxide synthase in a ceramide-dependent manner. J Biol Chem. 2002;277(13): 11058-11063.
[33] Gong M, Wilson M, Kelly T, et al. HDL-associated estradiol stimulates endothelial NO synthase and vasodilation in an SR-BI-dependent manner. J Clin Invest. 2003;111(10):1579-1587.
[34] Zhang W, Yancey PG, Su YR, et al. Inactivation of macrophage scavenger receptor class B type I promotes atherosclerotic lesion development in apolipoprotein E-deficient mice. Circulation. 2003;108(18):2258-2263.
[35] Rodríguez-Esparragón F, Rodríguez-Pérez JC, Hernández-Trujillo Y, et al. Allelic variants of the human scavenger receptor class B type 1 and paraoxonase 1 on coronary heart disease: genotype-phenotype correlations. Arterioscler Thromb Vasc Biol. 2005;25(4):854-860.
[36] Yamada Y, Izawa H, Ichihara S, et al. Prediction of the risk of myocardial infarction from polymorphisms in candidate genes. N Engl J Med. 2002;347(24):1916-1923.
[37] Connelly MA, Williams DL. Scavenger receptor BI: a scavenger receptor with a mission to transport high density lipoprotein lipids. Curr Opin Lipidol. 2004;15(3): 287-295.
[38] Swarnakar S, Temel RE, Connelly MA, et al. Scavenger receptor class B, type I, mediates selective uptake of low density lipoprotein cholesteryl ester. J Biol Chem. 1999; 274(42):29733-29739.
[39] Ueda Y, Gong E, Royer L, et al. Relationship between expression levels and atherogenesis in scavenger receptor class B, type I transgenics. J Biol Chem. 2000; 275(27):20368-20373.

[40] Acton SL, Scherer PE, Lodish HF, et al. Expression cloning of SR-BI, a CD36-related class B scavenger receptor. J Biol Chem. 1994;269(33):21003-21009.
[41] Calvo D, Gómez-Coronado D, Lasunción MA, et al. CLA-1 is an 85-kD plasma membrane glycoprotein that acts as a high-affinity receptor for both native (HDL, LDL, and VLDL) and modified (OxLDL and AcLDL) lipoproteins. Arterioscler Thromb Vasc Biol. 1997;17(11):2341-2349.
[42] Williams DL, Connelly MA, Temel RE, et al. Scavenger receptor BI and cholesterol trafficking. Curr Opin Lipidol. 1999;10(4):329-339.
[43] Wang DQ, Carey MC. Susceptibility to murine cholesterol gallstone formation is not affected by partial disruption of the HDL receptor SR-BI. Biochim Biophys Acta. 2002; 1583(2):141-150.
[44] Wiersma H, Nijstad N, Gautier T, et al. Scavenger receptor BI facilitates hepatic very low density lipoprotein production in mice. J Lipid Res. 2010;51(3):544-553.
[45] Ji A, Meyer JM, Cai L, et al. Scavenger receptor SR-BI in macrophage lipid metabolism. Atherosclerosis. 2011; 217(1):106-112.
[46] Hildebrand RB, Lammers B, Meurs I, et al. Restoration of high-density lipoprotein levels by cholesteryl ester transfer protein expression in scavenger receptor class B type I (SR-BI) knockout mice does not normalize pathologies associated with SR-BI deficiency. Arterioscler Thromb Vasc Biol. 2010;30(7):1439-1445.
[47] Acton S, Osgood D, Donoghue M, et al. Association of polymorphisms at the SR-BI gene locus with plasma lipid levels and body mass index in a white population. Arterioscler Thromb Vasc Biol. 1999;19(7):1734-1743.
[48] Osgood D, Corella D, Demissie S, et al. Genetic variation at the scavenger receptor class B type I gene locus determines plasma lipoprotein concentrations and particle size and interacts with type 2 diabetes: the framingham study. J Clin Endocrinol Metab. 2003;88(6):2869-2879.
[49] Pérez-Martínez P, Pérez-Jiménez F, Bellido C, et al. A polymorphism exon 1 variant at the locus of the scavenger receptor class B type I (SCARB1) gene is associated with differences in insulin sensitivity in healthy people during the consumption of an olive oil-rich diet. J Clin Endocrinol Metab. 2005;90(4):2297-2300.
[50] Acton S, Rigotti A, Landschulz KT, et al. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. Science. 1996;271(5248):518-520.
[51] Higashijima M, Kato K, Nawata H, et al. Studies on lipoprotein and adrenal steroidogenesis: II. Utilization of low density lipoprotein- and high density lipoprotein-cholesterol for steroid production in functioning human adrenocortical adenoma cells in culture. Endocrinol Jpn. 1987;34(5):647-657.
[52] Wang XD. Various types diagnostic points of cerebrovascular disease. Zhonghua Shenjing Ke Zazhi. 1996;29(6):379-380.

(Reviewed by McGowan D, Norman C, Sui RB, Li X)
(Edited by Yu J, Yang Y, Li CH, Song LP)