

Association and differences in genetic polymorphisms in *PCSK9* gene in subjects with lacunar infarction in the Han and Uygur populations of Xinjiang Uygur Autonomous Region of China

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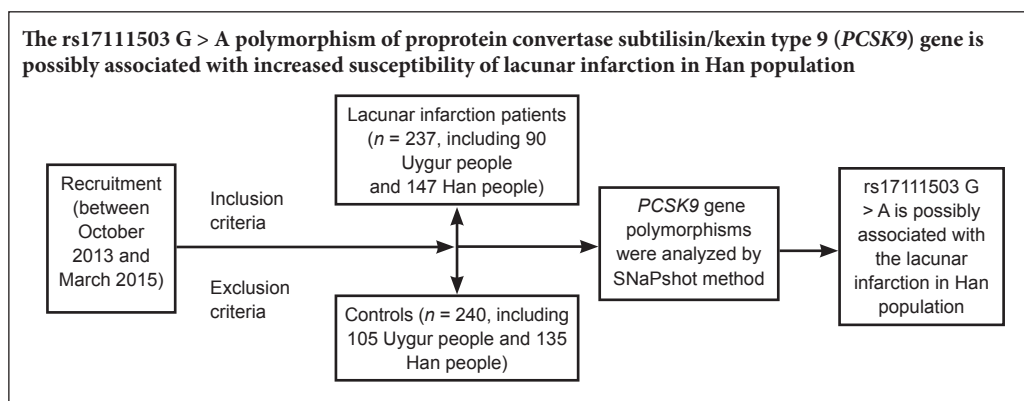
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How to cite this article: Han DF, Ma JH, Hao CG, Tuerhong-Tuerxun, Du L, Zhang XN (2017) Association and differences in genetic polymorphisms in *PCSK9* gene in subjects with lacunar infarction in the Han and Uygur populations of Xinjiang Uygur Autonomous Region of China. *Neural Regen Res* 12(8):1315-1321.

Funding: This work was supported by the National Natural Science Foundation of China, No. 81160145.

Graphical Abstract



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doi: 10.4103/1673-5374.213552

Accepted: 2017-05-13

Abstract

Polymorphisms in the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene are associated with severe hypercholesterolemia and stroke. Here, we investigated the relationship between single nucleotide polymorphisms in *PCSK9* and stroke in 237 patients with lacunar infarction in the Uygur and Han populations in Xinjiang Uygur Autonomous Region of China. Using the SNaPshot single-base terminal extension method, four *PCSK9* gene polymorphisms were analyzed. We found a significantly strong relationship between the *PCSK9* rs17111503 (G > A) polymorphism and increased susceptibility to lacunar infarction by variant homozygote comparison, and using the dominant and recessive models in the Han population but not in the Uygur population. Low triglyceride levels were found in AA carriers (rs17111503, G > A) in the Han population but not in the Uygur population. Association analysis revealed that the rs17111503 (G > A) polymorphism was not significantly associated with smoking, alcohol drinking, history of hypertension or diabetes in the Han or Uygur lacunar infarction patients. rs11583680, rs483462 and rs505151 were not associated with risk of lacunar infarction in the Han or Uygur populations. Our findings suggest that the *PCSK9* rs17111503 (G > A) polymorphism is associated with susceptibility to lacunar infarction in the Han population but not in the Uygur population.

Key Words: nerve regeneration; genetic; proprotein convertase subtilisin/kexin type 9; lacunar infarction; polymorphisms; case control; Uygur populations; Han populations; magnetic resonance imaging; association; susceptibility; neural regeneration

Introduction

Proprotein convertase subtilisin/kexin type 9 (*PCSK9*), also known as neural apoptosis-regulated convertase 1, is the ninth member of the proprotein convertase family (Seidah et al., 2003). The human *PCSK9* gene is located on chromosome 1, p32.3, and consists of 12 exons and encodes a 692 amino acid glycoprotein. *PCSK9* is synthesized as an inactive zymogen, pro-*PCSK9* (73 kDa), consisting of a signal peptide, a prodomain (residues 31–152), a catalytic domain (residues

153–451) and a C-terminal domain (residues 452–692) (Lambert et al., 2009). *PCSK9* acts as a molecular chaperone and serine protease that decreases low-density lipoprotein receptor levels in the hepatic and extrahepatic regions through an endosomal/lysosomal pathway and increases plasma low-density lipoprotein cholesterol (Benjannet et al., 2004; Schmidt et al., 2008). *PCSK9* may also regulate apolipoprotein B-containing lipoprotein synthesis and apolipoprotein B excretion (Ouguerram et al., 2004; Sun et al., 2005).

Recent studies show that a number of genetic variants of

PCSK9 are associated with plasma cholesterol. There is a tight relationship between gain of function missense mutations in PCSK9 and autosomal dominant hypercholesterolemia. In this form of familial hypercholesterolemia, neither the low-density lipoprotein receptor nor the ligand binding domain of apolipoprotein B100 are mutated (Leren, 2004; Allard et al., 2005). There is also a relationship between loss of function nonsense mutations in PCSK9 and low levels of plasma low-density lipoprotein and reduced morbidity from cardiovascular disease (Hallman et al., 2007; Hooper et al., 2007). Many *in vitro* and *in vivo* overexpression and knock-out/knockdown studies have shown that PCSK9 targets the low-density lipoprotein receptor for degradation (Benjannet et al., 2006; Lagace et al., 2006; Lambert et al., 2006). These studies demonstrate that both rare mutations and common variants affecting the coding regions of PCSK9 impact low-density lipoprotein cholesterol levels and related diseases. However, the single nucleotide polymorphisms (SNPs) located in the PCSK9 promoter, a regulatory region, have not been fully investigated. There is a strong relationship between the SNPs and the enzymatic activity of PCSK9 in the plasma. Furthermore, there is an intimate association between the SNPs and serum lipid levels and vascular disease.

In this study, we selected rs483462 and rs17111503 in the gene promoter region as well as three missense mutations—rs11583680 (p.Ala53Val), rs505151 (p.Gly377Glu) and rs149311926 (p.Gln261Glu)—to evaluate the relationship between human PCSK9 gene polymorphisms and lacunar infarction in the Uygur and Han populations.

Materials and Methods

Study protocol

All participants provided signed informed consent. DNA analyses and collection of relevant clinical data were allowed by all participants. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (approval No. 20120510).

Study population

Subjects were from the Han and Uygur ethnic groups, who lived in the Urumqi region of Xinjiang Uygur Autonomous Region in China. We recruited the lacunar infarction group from the Department of Neurology of the First Affiliated Hospital of Xinjiang Medical University of China between October 2013 and March 2015, and the control group from the same hospital in the same period. The major characteristics of study participants are shown in **Table 1**. The lacunar infarction group and control group were well-matched. The lacunar infarction group contained 237 lacunar infarction patients. The control group contained 240 healthy controls. Study subjects in the control group were recruited from the First Affiliated Hospital of Xinjiang Medical University by posted advertisement in the hospital newspaper asking healthy volunteers to come to the clinic for further screening. There was no statistical difference in the severity of lacunar infarction between the Han and Uygur lacunar infarction groups by magnetic resonance imaging (MRI). **Figure 1** shows representative MRIs of the brain for the Han and Uygur populations.

Inclusion criteria for the lacunar infarction group

Patients meeting all of the following criteria were included: (1) diagnosed in accordance with the standards described previously, with an infarct lesion associated with local small vasculopathy related to hypertension (Macdonell et al., 1987); (2) confirmed by head MRI, with an infarct lesion diameter less than 15 mm.

Exclusion criteria for the lacunar infarction group

Patients meeting any of the following criteria were excluded: (1) coronary heart disease; (2) hemorrhagic cerebrovascular disease confirmed by MRI; (3) refusal to participate in trials.

Inclusion criteria for the control group

Patients meeting all of the following criteria were included: (1) age > 40 years old; (2) no known family history of cerebrovascular disease; (3) no abnormality observed in cardiopulmonary physical examination and nervous system examination; (4) MRI negative for cerebrovascular disease.

Exclusion criteria for the control group

Patients meeting any of the following criteria were excluded: acute or chronic infection, malignant tumor, or autoimmune disease.

Collection of the clinical characteristics of the study participants

All data listed below were obtained from clinical medical records or questionnaires. Three trained researchers independently collected clinical medical records or questionnaires in the Department of Neurology of the First Affiliated Hospital of Xinjiang Medical University of China. Disagreement was resolved by discussion. All patients completed the standard test registration form, and disclosed the following data:

- (1) General information: Age, sex and ethnic group.
- (2) Personal history: Smoking history (daily average smoking, smoking an average of ≥ 1 cigarette/day or more, time > 1 year, defined as smoking), drinking alcohol (an aver-

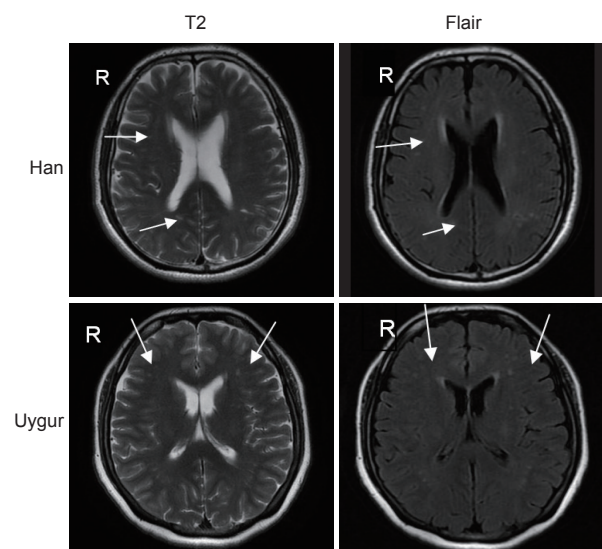


Figure 1 Magnetic resonance imaging of Uygur lacunar infarction patients.

Arrows indicated the infarct area. R: Right.

age of ≥ 3 times per week, more than 50 g each time over a period of > 1 year, defined as drinking), hypertension, diabetes, transient ischemic attack, atrial fibrillation, heart valve disease, heart valve replacement, and peripheral vascular disease. Hypertension: the Seventh WHO/International Society of Hypertension League Conference defined the new standard for the diagnosis of hypertension. In our study, hypertension was diagnosed if patients were treated with anti-hypertensive medication or if the average of three measurements of systolic blood pressure were > 140 mmHg (1 mmHg = 0.133 kPa) or diastolic blood pressure was > 90 mmHg. The diagnosis of diabetes mellitus was based on the standard of the American Diabetes Association (1997). Individuals with daytime random blood glucose ≥ 11.1 mM or fasting glucose ≥ 7.0 mM or glucose in line 2 hours ≥ 11.1 mM or with a history of diabetes or treatment with insulin were identified as diabetic.

(3) Medical history prior to admission: Treatment with antihypertensive drugs, antiplatelet drugs and anticoagulants, diabetes, lipid drug, anti-seizure medication, birth control pills, and hormones.

(4) Family history: Whether grandparents, parents, siblings or children had hypertension, diabetes, cerebral hemorrhage, cerebral infarction, myocardial infarction, coronary heart disease or arrhythmia.

(5) Physical examination: height, weight, blood pressure, pulse, and temperature.

(6) Special tests: electrocardiogram, chest X-ray, heart neck ultrasound, blood routine, blood glucose, and blood lipids.

Single nucleotide polymorphisms selection

Using the HapMap database, with the criterion of a minor allele frequency > 0.05 in Han Chinese in Beijing, we identified four common SNPs (rs483462, rs630431, rs615563 and rs568052) in *PCSK9*, all of which are in high linkage disequilibrium in Han Chinese in Beijing. Therefore, we genotyped only one SNP, rs483462. In addition, we also investigated four functional polymorphisms in *PCSK9*, rs17111503, rs14931192, rs11583680 and rs505151, which were selected because of their role in regulating *PCSK9* gene expression.

DNA isolation and genotyping

Using standard procedures (Promega, Beijing, China), we extracted genomic DNA from peripheral blood leukocytes. Blood samples were collected under fasting conditions at 8:00 a.m. Approximately 5 mL was withdrawn from the basilic vein in the upper arm. We used the single-base terminal extension (SNaPshot) method to genotype the rs17111503 polymorphism. SNaPshot reactions were performed as described in the manufacturer's protocol (Applied Biosystems, Warrington, UK). Briefly, the sample was incubated with 2 U Exonuclease I and 2 U shrimp alkaline phosphatase and incubated at 37°C for 60 minutes. After inactivating the enzymes, 1 μ L of digested reaction product was mixed with 5 μ L of ready reaction premix, 1 μ L of 1.0 μ M primer, and 3 μ L of dH₂O. This mixture was placed in the thermal cycler and underwent 25 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 30 seconds. When completed, 0.5 U of shrimp alkaline phosphatase was

added, and the reaction mixture was incubated for 60 minutes. Prior to loading onto the PRISM 310, 10 μ L of formamide was added to 1 μ L of reaction mixture, and samples were heated to 95°C for 5 minutes. The primary data were analyzed using GeneMapper 4.0 software (Applied Biosystems). Genotypes were determined according to the nucleotide at the SNP site, which was visualized by one or two different color peaks.

Statistical analyses

The Statistical Package for Social Sciences 22.0 software (IBM, Armonk, IL, USA) was utilized for all statistical analyses. All continuous variables are expressed as the mean \pm SD (such as age, body mass index, and cholesterol levels). The difference between the lacunar infarction and control groups was analyzed using an independent-sample *t*-test and unpaired *t*-test with Welch's correction, as appropriate. The chi-square test was used for analyzing the Hardy-Weinberg equilibrium and the differences in general characteristics between lacunar infarction patients and matched controls, such as sex, hypertension, diabetes mellitus, smoking, drinking and genotype. The latent relationship of genotypic frequencies of the *PCSK9* polymorphisms with the risk of lacunar infarction was assessed by the odds ratios (ORs) with their 95% confidence intervals (CIs) from logistic regression models. A value of $P < 0.05$ was considered statistically significant.

Results

Comparison of clinical data between the lacunar infarction and control groups

A total of 477 subjects were registered, including 237 lacunar infarction patients and 240 healthy controls, in this case-control study. The clinical characteristics of the lacunar infarction patients and matched participants are shown in **Table 1**. For all Han and Uygur subjects, there were no significant differences in sex or age between the lacunar infarction patients and matched subjects. We observed a few differences between the lacunar infarction group and the control group. Between these two groups, several common risk factors for lacunar infarction were significantly different: fasting plasma glucose, systolic blood pressure, diastolic blood pressure (except Uygur subjects), total cholesterol (except Uygur subjects), and low-density lipoprotein cholesterol (except Han subjects) ($P < 0.05$). Other lacunar infarction risk factors, such as apolipoprotein A1, apolipoprotein B, triglyceride levels and body mass index, were not significantly different.

Hardy-Weinberg equilibrium test

The Hardy-Weinberg equilibrium test of the observed and expected genotype values suggested the lacunar infarction and control groups were in Hardy-Weinberg equilibrium at rs17111503, rs11583680, rs483462 and rs505151. The characteristics of the populations in these groups are shown in **Table 2**. Only the CC genotype was found for rs149311926 (data not shown).

Association analysis

For rs17111503, the dominant model (GG vs. GA + AA: OR = 1.70, 95%CI = 1.06–2.73, $P = 0.03$) and the recessive

Table 1 Major clinical characteristics of study participants

	Han			Uygur		
	Lacunar infarction group (n = 147)	Control group (n = 135)	P	Lacunar infarction group (n = 90)	Control group (n = 105)	P
Gender (male/female, n)	81/66	72/63	0.811 [*]	50/40	55/50	0.669 [*]
Age (year)	62.52±11.37	61.64±11.59	0.524 [#]	59.44±11.16	61.13±11.52	0.117 [#]
Body mass index (kg/m ²)	24.30±2.33	24.20±3.13	0.728 [#]	25.23±3.37	24.93±2.60	0.386 [#]
Systolic blood pressure (mmHg)	142.3±21.34	128.80±19.02	< 0.001 [#]	142.5±24.10	131.3±23.54	0.001 [#]
Diastolic blood pressure (mmHg)	83.78±14.97	77.98±11.29	< 0.001 [#]	84.76±15.25	80.75±14.24	0.06 [#]
Fasting plasma glucose (mM)	6.36±1.66	5.36±1.66	< 0.001 [#]	5.63±2.29	7.31±4.15	< 0.001 [#]
Triglyceride (mM)	1.91±1.21	1.81±1.17	0.483 [#]	2.08±1.23	2.31±1.50	0.241 [#]
Total cholesterol (mM)	4.37±1.03	4.27±0.96	0.421 [#]	4.40±1.05	3.92±1.32	0.007 [#]
High density lipoprotein (mM)	1.09±0.30	1.15±0.48	0.211 [#]	1.03±0.29	1.54±1.11	< 0.001 [#]
Low density lipoprotein (mM)	2.56±0.81	2.68±0.89	0.255 [#]	2.82±0.93	2.39±0.80	< 0.001 [#]
Apolipoprotein A1 (g/L)	1.24±0.23	1.28±0.23	0.138 [#]	1.20±0.21	1.17±0.35	0.448 [#]
Apolipoprotein B (g/L)	0.94±0.93	0.89±0.93	0.655 [#]	0.90±0.27	0.89±0.24	0.822 [#]

Data are presented as the mean ± SD with the exception of gender. #P values of continuous variables were calculated with the independent *t*-test. *P values of categorical variables were calculated with the chi-square test.

Table 2 Genotype frequencies (n) of the four polymorphisms in the studied subjects

Polymorphism	Ethnicity	Genotype	Lacunar infarction group (n = 237)	Control group (n = 240)	P1 of HWE	P2 of HWE
rs17111503	Han	GG/GA/AA	55/67/25	68/56/11	0.55	0.91
	Uygur	AA/GA/GG	27/38/25	29/52/24	0.14	0.94
rs11583680	Han	CC/CT/TT	113/34/0	109/24/2	0.22	0.63
	Uygur	CC/CT/TT	76/13/1	86/17/2	0.47	0.26
rs483462	Han	AA/GA/GG	103/39/5	90/39/6	0.56	0.57
	Uygur	AA/GA/GG	55/30/5	68/33/4	0.76	1
rs505151	Han	AA/GA/GG	129/18/1	121/14/0	0.46	1
	Uygur	AA/GA/GG	81/8/1	89/15/1	0.23	0.51

P1 of HWE: Hardy–Weinberg equilibrium for lacunar infarction. P2 of HWE: Hardy–Weinberg equilibrium for control.

model (GG + GA vs. AA: OR = 2.31, 95%CI = 1.09–4.90, *P* = 0.02) suggested a significant difference between the lacunar infarction and matched controls in the total and Han groups but not in the Uygur group. In addition, the AA genotype of rs17111503 was significantly higher in frequency in the lacunar infarction patients than in matched controls in the Han group (Han: 17.01% vs. 8.15%, AA vs. GG: OR = 2.81, 95%CI = 1.27–6.21, *P* = 0.02) but not in the Uygur group. When the analysis was adjusted for gender, age and body mass index, similar results were obtained (Table 3). However, logistic regression analyses showed that the relationships between the three polymorphisms (rs11583680, rs483462, and rs505151) and the risk of lacunar infarction were weak (Table 3).

Genotype of rs17111503 and the clinical characteristics of patients

The rs17111503 polymorphism in the *PCSK9* gene promoter is present as three genotypes (GG/GA/AA). The genotype and genetic models contributed to group assignment, and the clinical characteristics were calculated for each genotype. The dominant model was GA + AA compared to GG; the recessive model was AA compared to GG + GA. In the Han population,

triglyceride levels were lower in the AA group than in the GG + GA group or the GG or GA groups, by unpaired *t*-test with Welch's correction, and the differences were statistically significant. However, there was no significant difference in the other indicators among the different groups (*P* > 0.05; Table 4).

In addition, from the chi-square analysis for smoking, alcohol drinking, history of hypertension and diabetes, the results were not significant in the Han and Uygur populations, as the *P*-values were greater than 0.05 (Table 5).

Discussion

Cerebral ischemic stroke is a primary cause of morbidity and mortality, and is expected to remain so until at least 2030 (Mathers and Loncar, 2006). Cerebral ischemic stroke and coronary heart disease are major manifestations of atherosclerotic processes. Lacunar infarction is the most common type of ischemic stroke, and is associated with sustained hypertension and cerebrovascular atherosclerosis. There is a strong relationship between improved living conditions and increased morbidity from lacunar infarction (Wang et al., 2014). High levels of low-density lipoprotein cholesterol are a risk factor for atherosclerosis (Hobbs et al., 1990). Activi-

Table 3 Association between the risk of lacunar infarction and the four polymorphisms

Polymorphism	VR Ho vs. WT Ho		Ht vs. WT Ho		Dominant model		Recessive model	
	<i>P</i>	OR (95%CI)	<i>P</i>	OR (95%CI)	<i>P</i>	OR (95%CI)	<i>P</i>	OR (95%CI)
Han population								
rs17111503	0.02*	2.81*(1.27–6.21)	0.13	1.48(0.90–2.44)	0.03*	1.70*(1.06–2.73)	0.02*	2.31*(1.09–4.90)
rs11583680		NA	0.13	1.37(0.76–2.45)	0.43	1.26(0.71–2.24)		NA
rs483462	0.61	0.73(0.21–2.47)	0.80	0.87(0.52–1.48)	0.54	0.85(0.52–1.41)	0.65	0.76(0.23–2.54)
rs505151		NA	0.49	1.14(0.54–2.41)	0.62	1.21(0.57–2.53)		NA
Uygur population								
rs17111503	0.77	1.12(0.52–2.41)	0.57	0.78(0.40–1.53)	0.71	0.89(0.48–1.66)	0.43	1.30(0.68–2.48)
rs11583680	0.65	0.57(0.05–6.36)	0.84	0.87(0.39–1.90)	0.64	0.83(0.39–1.78)	0.65	0.58(0.05–6.49)
rs483462	0.53	1.55(0.40–6.03)	0.79	1.12(0.61–2.07)	0.60	1.17(0.65–2.10)	0.56	1.49(0.39–5.71)
rs505151	0.95	1.10(0.07–17.86)	0.50	0.59(0.24–1.45)	0.27	0.62(0.26–1.48)	0.91	1.17(0.07–18.95)
Han population (adjusted by gender, age and body mass index)								
rs17111503	0.02*	2.89*(1.30–6.43)	0.13	1.47(0.89–2.43)	0.03*	1.70*(1.05–2.73)	0.02*	2.39*(1.12–5.11)
rs11583680		NA	0.12	1.37(0.76–2.46)	0.42	1.26(0.71–2.25)		NA
rs483462	0.60	0.75(0.22–2.56)	0.79	0.86(0.51–1.46)	0.52	0.85(0.51–1.40)	0.69	0.78(0.23–2.65)
rs505151		NA	0.47	1.14(0.54–2.43)	0.81	1.21(0.58–2.55)		NA
Uygur population (adjusted by gender, age and body mass index)								
rs17111503	0.81	1.22(0.56–2.66)	0.60	0.85(0.43–1.67)	0.90	0.96(0.51–1.81)	0.37	1.35(0.70–2.61)
rs11583680	0.71	0.90(0.08–10.50)	0.89	0.82(0.37–1.83)	0.64	0.83(0.39–1.79)	0.95	0.93(0.08–10.75)
rs483462	0.65	1.63(0.41–6.46)	0.71	1.19(0.64–2.21)	0.49	1.24(0.68–2.24)	0.53	1.54(0.39–6.00)
rs505151	0.96	1.13(0.07–18.52)	0.30	0.49(0.19–1.24)	0.15	0.52(0.21–1.28)	0.89	1.23(0.08–20.05)

*The numbers in bold indicated statistically significant values. *P* values, OR and 95%CI were calculated by logistic regression analyses. OR: Odds ratio; CI: confidence interval; VR Ho: variant homozygote; WT Ho: wide-type homozygote; Ht: heterozygote; NA: not available.

Table 4 Genotype of rs17111503 and the clinical characteristics of the patients

Ethnicity	Variables	GG	GA	AA	<i>P</i> value**		<i>P</i> value***	
					Dominant	Recessive	Dominant	Recessive
Han	No.	55	67	25				
	SBP (mmHg)	143.20±21.74	143.60±22.57	137.00±16.41	0.703	0.170	0.705	0.101
	DBP (mmHg)	82.24±14.31	85.39±16.18	82.84±12.98	0.337	0.733	0.329	0.704
	FPG (mM)	6.49±2.13	6.38±2.49	5.99±2.36	0.587	0.414	0.575	0.424
	TG (mM)	1.97±1.21	2.01±1.36	1.49±0.58*	0.639	0.062	0.638	0.003#
	TC (mM)	4.43±1.05	4.44±1.01	4.00±1.03	0.601	0.078	0.603	0.089
	HDL (mM)	1.05±0.28	1.11±0.26	1.13±0.41	0.191	0.510	0.183	0.618
	LDL (mM)	2.77±0.90	2.72±0.88	2.35±0.83	0.328	0.054	0.331	0.051
	ApoA1 (g/L)	1.27±0.21	1.28±0.23	1.32±0.27	0.674	0.406	0.665	0.480
	ApoB (g/L)	0.86±0.23	1.07±1.34	0.78±0.23	0.384	0.372	0.278	0.066
Uygur	No.	25	38	27				
	SBP (mmHg)	140.60±25.66	138.40±20.95	150.00±25.88	0.054	0.654	0.070	0.667
	DBP (mmHg)	87.64±14.51	80.63±15.81	87.89±14.25	0.204	0.190	0.268	0.257
	FPG (mM)	7.74±4.41	6.99±4.28	7.37±3.78	0.556	0.934	0.572	0.930
	TG (mM)	2.25±1.31	2.05±1.35	1.93±0.93	0.400	0.486	0.421	0.419
	TC (mM)	4.34±1.02	4.35±0.96	4.53±1.21	0.739	0.471	0.736	0.512
	HDL (mM)	0.98±0.24	1.06±0.24	1.02±0.40	0.357	0.870	0.306	0.895
	LDL (mM)	2.74±0.93	2.88±0.90	2.79±0.99	0.636	0.868	0.638	0.873
	ApoA1 (g/L)	1.20±0.20	1.19±0.20	1.21±0.22	0.994	0.729	0.994	0.740
	ApoB (g/L)	0.86±0.28	0.87±0.24	0.97±0.30	0.464	0.117	0.473	0.151

Data are expressed as the mean ± SD. **P* < 0.05, compared with the GG group by unpaired *t*-test with Welch's correction. ***P* values were calculated with unpaired *t*-test. ****P* values were calculated by unpaired *t*-test with Welch's correction. #The numbers in bold indicate statistically significant differences. 1 mmHg = 0.133 kPa. SBP: Systolic blood pressure; DBP: diastolic blood pressure; FPG: fasting plasma glucose; TG: triglyceride; TC: total cholesterol; HDL: high density lipoprotein; LDL: low density lipoprotein; ApoA1: apolipoprotein A1; ApoB: apolipoprotein B.

Table 5 Impact of the rs17111503 polymorphism on hypertension, diabetes, smoking and alcohol drinking in patients

Ethnicity	Variables	WT Ho/Ht/VR Ho [n(%)]	P
Han (n = 147)	H.T.(+)	37(34.3)/52(48.1)/19(17.6)	0.416
	H.T.(−)	18(46.2)/15(38.5)/6(15.4)	
	Diabetes(+)	13(30.9)/19(45.2)/10(23.8)	0.323
	Diabetes(−)	42(40)/48(45.7)/15(14.3)	
	Smoking(+)	16(33.3)/21(43.8)/11(22.9)	0.400
	Smoking(−)	39(39.4)/46(46.5)/14(14.1)	
	Alcohol drinking(+)	8 (30.8)/12(46.1)/6(23.1)	0.589
	Alcohol drinking(−)	47(38.8)/55(45.5)/19(15.7)	
Uygur (n = 90)	H.T.(+)	15(26.3)/27(47.4)/15(26.3)	0.407
	H.T.(−)	12(36.4)/11(33.3)/10(30.3)	
	Diabetes(+)	9(32.1)/13(46.4)/6(21.4)	0.663
	Diabetes(−)	18(29.0)/25(40.3)/19(30.6)	
	Smoking(+)	5(22.7)/10(45.5)/7(31.8)	0.685
	Smoking(−)	22(32.4)/28(41.2)/18(26.5)	
	Alcohol drinking(+)	3(23.1)/6(46.1)/4(30.8)	0.841
	Alcohol drinking(−)	24(31.2)/32(41.6)/21(27.3)	

H.T.(+): Hypertension (+); H.T.(−): hypertension (−); WT Ho: wide-type homozygote; Ht: heterozygote; VR Ho: variant homozygote. P values were calculated with the chi-square test.

ty of the low-density lipoprotein receptor in the liver is the primary factor that determines the serum concentrations of low-density lipoprotein cholesterol. PCSK9 was recently discovered to play a key role in cholesterol homeostasis through the enhanced degradation of low-density lipoprotein receptor (Benjannet et al., 2004; Park et al., 2004; Peterson et al., 2008; Horton et al., 2009), and possibly in neural development. In addition, both rare mutations and common variants in the coding regions of PCSK9 can affect low-density lipoprotein cholesterol levels and stroke risk. Recent studies have identified several PCSK9 variants influencing circulating low-density lipoprotein cholesterol levels (Chen et al., 2005; Yue et al., 2006; Horton et al., 2007; Miyake et al., 2008; Folsom et al., 2009). However, polymorphisms in the promoter region have not been well investigated. Our previous study found that the rs17111503 G > A polymorphism is associated with cerebral ischemic stroke in the Han population of China (Han et al., 2014). However, the study further revealed the relationship between the PCSK9 rs17111503 G > A polymorphism and lacunar infarction in the Uygur and Han populations. Therefore, we investigated the association between the five polymorphisms and the risk of lacunar infarction in Chinese Han and Uygur populations in this case-control study of lacunar infarction. Finally, these preliminary findings show that the PCSK9 rs17111503 G > A polymorphism is possibly associated with the susceptibility to lacunar infarction in Han population, but not in Uygur population.

To our knowledge, the present study is the first to demonstrate an association between the polymorphisms and the susceptibility to lacunar infarction.

We found that the PCSK9 rs17111503 G > A polymorphism has a statistically significant impact on the susceptibility to lacunar infarction in the Han population but not in the Uygur population. PCSK9 was synthesized by liver, kidney

and small intestine of vertebrates, primarily (Gupta et al., 2010). It was primarily expressed in liver and small intestine of vertebrates. To the best of our knowledge, little is reported regarding whether the rs17111503 polymorphism affected PCSK9 enzyme activity (does it increase or decrease?) and the activity of rs17111503 in blood was ambiguous. This needs further investigation.

The PCSK9 rs11583680, rs483462, rs505151 and rs149311926 polymorphisms were not associated with the risk of lacunar infarction in the Han or Uygur population in China. However, we found a strong relationship between the rs17111503 G > A SNP in the PCSK9 gene promoter and the morbidity of lacunar infarction in the Han population but not in the Uygur population, suggesting that ethnicity contributes to the differences. In particular, there was a significantly strong relationship between the AA genotype and morbidity from lacunar infarction, suggesting that the AA genotype may have an important pathogenetic role in lacunar infarction. In the Han population, there was a strong relationship between the polymorphism at this locus and abnormal lipid metabolism, as the AA genotype was associated with lowered levels of triglycerides. Low-density lipoprotein cholesterol levels displayed borderline causative properties in the recessive model ($P = 0.051$, calculated by unpaired *t*-test with Welch's correction). However, no significant association was observed for the other clinical characteristics in the Han and Uygur populations. In addition, subgroup analysis of the Han and Uygur lacunar infarction patients by hypertension, diabetes mellitus, smoking and drinking showed no significant association with the rs17111503 G > A polymorphism.

Taken together, our findings suggest that the A allele at this locus is causative for lacunar infarction by increasing the risk for ischemic stroke by affecting low-density lipoprotein cholesterol levels and other serum components in the Chinese Han population. However, there are a number of limitations to this study. The patients and healthy controls were registered from the First Affiliated Hospital of Xinjiang Medical University of China and may not adequately represent the general population. Furthermore, a comprehensive assessment of the impact of genetic variability in PCSK9 may not have been given by the polymorphisms investigated in our study. Further fine mapping needs to be performed to better understand the impact of susceptibility regions on stroke. In addition, in this study, the moderate sample size also limited the statistical power of the analyses. Further studies are required to validate our findings, and gene-environment interaction studies should also help elucidate the genetic mechanisms of lacunar infarction.

Despite the limitations of this study, it is the first to investigate the effect of PCSK9 polymorphisms on the susceptibility to lacunar infarction in the Chinese Han and Uygur ethnic groups. The major finding is that the rs17111503 G > A polymorphism has a statistically significant impact on the susceptibility to lacunar infarction in the Han population but not the Uygur population.

Acknowledgments: We are very grateful to director of Li-xin Ying and Tuerxun-Shabier from the Department of Neurology of First Affiliated Hospital of Xinjiang Medical University of China for their good proposals.

Meanwhile, we are very grateful to head nurse of Jian Jiang and nurses in the Department of Neurology of First Affiliated Hospital of Xinjiang Medical University for their help in blood sample collection.

Author contributions: XNZ designed this study, performed experiments and analyzed data. DFH performed experiments, analyzed data and wrote the paper. JHM performed experiments and analyzed data. CGH, Tuerhong•Tuerxun and LD performed data collection. All authors approved the final version of the paper.

Conflicts of interest: None declared.

Research ethics: The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (approval No. 20120510). The study followed the Declaration of Helsinki and relevant ethical principles.

Declaration of patient consent: The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Plagiarism check: Checked twice by iThenticate.

Peer review: Externally peer reviewed.

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Copyedited by Patel B, Norman C, Wang J, Li CH, Qiu Y, Song LP, Zhao M