

Asn563Ser polymorphism of *CD31/PECAM-1* is associated with atherosclerotic cerebral infarction in a southern Han population

Yanmin Song
Qunfang Li
Lili Long
Ning Zhang
Yunhai Liu

Department of Neurology, Xiangya Hospital, Central South University, Changsha, Hunan Province, People's Republic of China

Background: CD31, also called platelet endothelial cell adhesion molecule-1 (PECAM-1), is thought to play a role in the pathological mechanisms of atherosclerosis. Leu125Val polymorphism and elevated plasma levels of soluble PECAM-1 (sPECAM-1) were found to be associated with cerebral infarction. Our aim was to investigate the association between the Asn563Ser polymorphism of *CD31/PECAM-1*, plasma level of sPECAM-1, and the risk of atherosclerotic cerebral infarction (ACI) in the southern Han population of the People's Republic of China.

Subjects and methods: A total of 147 subjects with ACI and 114 controls were enrolled in the study. The Asn563Ser *CD31/PECAM-1* polymorphism was detected using the polymerase chain reaction–restriction fragment length polymorphism method. The plasma sPECAM-1 level was measured using the enzyme-linked immunosorbent assay method.

Results: In this study, statistically significant differences in Asn563Ser genotype and allele distribution were found between the cases and controls ($P < 0.05$). Furthermore, logistic regression analysis showed that the GG genotype is associated with increase in ACI risk (odds ratio = 4.862, $P < 0.001$). The plasma level of sPECAM-1 was associated with ACI (odds ratio = 1.431, $P = 0.038$). In both the ACI and the control groups, the plasma sPECAM-1 level in subjects with the GG genotype was higher than that in subjects carrying the AA or GA genotype ($P < 0.05$).

Conclusion: Our study showed that the Asn563Ser polymorphism of *CD31/PECAM-1* gene and elevated plasma sPECAM-1 level are related to ACI risk in the southern Han population of People's Republic of China.

Keywords: genetic polymorphism, CD31, platelet endothelial cell adhesion molecule-1 (PECAM-1)

Introduction

Atherosclerosis is the principal pathological basis of cerebral infarction. The inflammatory response may play a vital role in the pathological mechanisms of atherosclerosis and cerebral vascular disease.^{1,2} Cell adhesion molecules mediate inflammatory cell aggregation and adhesion to endothelial cells, which are important initial steps in the development of atherosclerosis.^{3,4} CD31, also called platelet endothelial cell adhesion molecule-1 (PECAM-1), as a cell adhesion molecule, is mainly expressed on the surface of endothelial cells, circulating platelets, monocytes, neutrophils, and certain T-cell subsets.^{5,6} CD31/PECAM-1 has been shown to play dual roles (proinflammatory or anti-inflammatory) in the inflammatory response. It can mediate cell adhesion through the upregulation of integrin and plays important roles in the transendothelial migration of leukocytes, the inhibition of T-cell-mediated signal transduction, and the regulation of platelet function.^{7–10} On the other hand, it can

Correspondence: Yunhai Liu
Department of Neurology, Xiangya Hospital, Central South University, 87 Xiangya Road, Changsha, Hunan 410008, People's Republic of China
Tel +86 731 8432 7216
Email csuliuyunhai@163.com

exert anti-inflammatory effects by increasing the threshold for leukocyte activation, suppressing the generation of proinflammatory cytokines, and maintaining the integrity of the vascular barrier.¹⁰

Three common polymorphisms (Leu125Val, Asn563Ser, and Gly670Arg) are located on the *CD31/PECAM-1* gene.¹¹ These *CD31/PECAM-1* polymorphisms have been deemed to be associated with myocardial infarction or coronary artery disease (CAD).^{12–15} However, only one study¹⁶ to date has focused on the relationship between *CD31/PECAM-1* polymorphisms and cerebral infarction, and it found that Leu125Val polymorphism and plasma sPECAM-1 levels are associated with cerebral infarction.

We conducted a case–control study by genotyping the Asn563Ser polymorphism and testing the plasma sPECAM-1 levels in southern Han patients with ACI from the People's Republic of China to determine whether the Asn563Ser genotype is associated with elevated plasma sPECAM-1 levels and ACI risk.

Materials and methods

Study populations

We enrolled acute ACI patients who were hospitalized in the Department of Neurology, Xiangya Hospital, Central South University, People's Republic of China, continuously between September 2007 and February 2008. A total of 147 cases (87 males and 60 females; mean age: 62.61±8.51 years) were enrolled in this study. All subjects were examined by computed tomography (CT) and/or magnetic resonance imaging (MRI). The ACI diagnostic criteria were based on the criteria of the Fourth National Cerebrovascular Disease Conference of China.¹⁷ The exclusion criteria were as follows: cerebral infarction caused by cardiogenic factors; arthritis, blood diseases, tumor, or cerebrovascular malformation; hemorrhage after cerebral infarction; the use of contraceptives; severe liver and kidney disease; disseminated intravascular coagulation; autoimmune diseases; and pregnancy.

A total of 114 healthy controls (66 males and 48 females; average age: 61.04±7.75 years) were recruited from the Health Examination Center of the Xiangya Hospital during the same period. CT and/or MRI was conducted to exclude cerebral infarction. The exclusion criteria were as follows: stroke history or family history of stroke; thrombosis history; the use of oral contraceptives or anticoagulants; severe liver and kidney disease; disseminated intravascular coagulation; autoimmune diseases; and pregnancy.

All subjects were nonconsanguineous. This study was approved by the Ethics Committee of the Xiangya Hospital, and all subjects signed a written informed consent.

DNA extractions and blood biochemistry tests

A total of 10 mL of peripheral venous blood was collected into ethylenediamine tetraacetic acid (EDTA)-containing tubes for each subject; 5 mL of peripheral blood was used to extract genomic DNA using the traditional phenol–chloroform method, and another 5 mL was used for biochemistry tests (fasting blood sugar and blood lipids) and the measurement of the plasma levels of sPECAM-1. The Human sPECAM-1 Instant ELISA Kit (Bender Med-Systems GmbH, Vienna, Austria) was used for determination of the plasma level of sPECAM-1 by enzyme-linked immunosorbent assay according to the manufacturer's instructions.

Primer designs and polymerase chain reaction–restriction fragment length polymorphism

The primers for Asn563Ser were designed according to Wenzel et al¹⁸ and were synthesized by Invitrogen (Shanghai, People's Republic of China). The primer sequences were as follows: 5'–TGTGAATCCTTTTCCTGCTTTTC–3' (upstream primer); and 5'–GCTTGCTATGGAGACCCTGAC–3' (downstream primer). The polymerase chain reaction (PCR) was performed as follows: predenaturation at 94°C for 5 minutes; followed by 35 cycles of amplification comprising denaturation at 94°C for 20 seconds, annealing at 55°C for 35 seconds, and then extension at 72°C for 35 seconds; with a final extension at 72°C for 10 minutes.

A variant of G→A (Asn→Ser) can lead to the appearance of the Nhe I restriction site. PCR amplification products were digested by restriction endonuclease Nhe I (Thermo Fisher Scientific, Waltham, MA, USA) and detected by electrophoresis on 2% agarose gel with 0.5× Tris–borate–EDTA buffer (115 V, 30–40 minutes). After electrophoresis, an ultraviolet light–based gel-imaging system (Tanon Science & Technology Co, Shanghai, People's Republic of China) was used to observe and photograph the bands. The detection of Asn563Ser genotype was as follows: wild-type homozygotes had only one 81-bp band; heterozygotes had three fragments with lengths of 338 bp, 217 bp, and 121 bp; and the mutant homozygotes had only one fragment with a length of 180 bp.

Table 1 Clinical data of the ACI and control groups

Clinical data	ACI group	Control group	P-values
Male/female	87/60	66/48	0.834
Mean age (years)	62.61±8.51	61.07±7.75	0.134
BMI (kg/m ²)	23.73±2.93	23.32±2.68	0.248
Smoking history	62/85	42/72	0.383
Drinking history	81/66	58/56	0.497
History of hypertension	115/32	29/35	<0.001*
History of T2DM	36/111	9/105	<0.001*
History of CAD	37/110	6/108	<0.001*
History of hyperlipidemia	51/96	19/95	0.001*
TC (mmol/L)	4.81±1.24	4.56±1.00	0.078
TG (mmol/L)	2.04±1.28	1.63±1.17	0.008*
HDL-C (mmol/L)	1.16±0.35	1.32±0.34	<0.001*
LDL-C (mmol/L)	3.07±0.94	2.85±0.75	0.038*

Note: * $P < 0.05$.

Abbreviations: ACI, atherosclerotic cerebral infarction; BMI, body mass index; CAD, coronary artery disease; HDL-C, high-density-lipoprotein cholesterol; LDL-C, low-density-lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; T2DM, type 2 diabetes mellitus.

Statistical analysis

Statistical analyses were performed with SPSS version 18.0 (SPSS Inc, Chicago, IL, USA). The direct counting method was used to calculate the allele and genotype frequencies. The chi-square test was used to examine Hardy–Weinberg equilibrium and the differences in genotype and allele frequencies between the two groups. Comparison of the measurement data between the two groups was performed using the independent samples *t*-test. The odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated, and a *P*-value < 0.05 was considered statistically significant.

Results

Clinical data of subjects

As shown in Table 1, there were no significant differences in sex, age, body mass index, smoking history, drinking history, and plasma level of total cholesterol between the ACI and the control groups ($P > 0.05$). Common risk factors for stroke were higher in the ACI group than in the control group ($P < 0.05$); these include a history of hypertension, type 2 diabetes mellitus (T2DM), CAD, and hyperlipidemia, as well as

the plasma levels of triglycerides and low-density-lipoprotein cholesterol. The plasma levels of high-density-lipoprotein cholesterol in the ACI group were lower, than in the control group ($P < 0.05$).

Genotyping of Asn563Ser

The PCR-amplified product was 338 bp in length, which was presented by the three genotypes deduced by agarose gel electrophoresis after being digested by restriction endonuclease Nhe I. The genotype and allele frequencies of Asn563Ser in the ACI and the control groups are shown in Table 2. These data were consistent with Hardy–Weinberg equilibrium. The genotypes and allele frequencies of Asn563Ser were significantly different between the ACI and the control groups ($P = 0.018$ and 0.05 , respectively).

Logistic regression analysis

We built a logistic regression model for ACI risk and the eight traditional risk factors of cerebrovascular disease. We selected the “Backward: LR” method and set 0.10 and 0.15, respectively, as the significance levels of the entered variables and eliminated variables. As shown in Table 3, the results indicated that the GG genotype, a history of hypertension, CAD, T2DM, and hyperlipidemia were major risk factors for ACI. After adjustment to exclude the interference of confounding factors, the GG genotype still showed a significant correlation with ACI (OR=4.862, 95% confidence interval [CI]=2.059–11.479, $P < 0.001$).

Relation between sPECAM-1 levels and the Asn563Ser genotype

As shown in Table 4, plasma sPECAM-1 levels in the ACI group were higher than those of the control group ($P = 0.004$). After adjustment by logistic regression analysis to eliminate the effects of confounding factors, the plasma level of sPECAM-1 was still significantly associated with ACI (OR=1.431, 95% CI=1.020–2.006, $P = 0.038$). Plasma sPECAM-1 levels of GG, GA, and AA genotypes in the ACI and the control groups are shown in Table 4.

Table 2 Comparison of genotypes and alleles of Asn563Ser between the ACI and the control groups

Group	N	Genotypes, n (%)			χ^2 ; P	Alleles, %		
		GG	GA	AA		G	A	χ^2 ; P
ACI	147	35 (23.8)	81 (55.1)	31 (21.1)	$\chi^2=8.698$; $P=0.013^*$	51.4	48.6	$\chi^2=7.854$; $P=0.005^*$
Control	114	14 (12.3)	61 (53.5)	39 (34.2)		39.0	61.0	

Note: * $P < 0.05$.

Abbreviation: ACI, atherosclerotic cerebral infarction.

Table 3 Unconditional logistic regression analysis for ACI

Variables	β	SE	Wald statistic	P	OR	95% CI
GG genotype	1.581	0.438	13.014	<0.001*	4.862	2.059–11.479
Age	0.284	0.217	1.706	0.191	1.328	0.868–2.032
History of smoking	0.074	0.355	0.043	0.835	1.077	0.537–2.160
History of drinking	0.145	0.316	0.209	0.647	1.156	0.622–2.148
History of hypertension	2.487	0.333	55.622	<0.001*	12.026	6.255–23.119
History of CAD	1.395	0.534	6.815	0.009*	4.035	1.416–11.503
History of T2DM	0.717	0.459	2.436	0.119	2.047	0.832–5.035
History of hyperlipidemia	0.553	0.377	2.154	0.142	1.738	0.831–3.637
Constant	-1.758	0.289	36.917	0.000	0.172	

Note: * $P < 0.05$.

Abbreviations: ACI, atherosclerotic cerebral infarction; CAD, coronary artery disease; T2DM, type 2 diabetes mellitus; SE, standard error; OR, odds ratio; CI, confidence interval.

Single-factor variance analysis showed that in both the ACI and the control groups, the plasma sPECAM-1 levels among the various Asn563Ser genotypes were significantly different ($P = 0.003$ and 0.049 , respectively). Multiple comparison results showed that the plasma sPECAM-1 level in subjects carrying the GG genotype was significantly higher than the level in those carrying the GA or AA genotypes ($P < 0.05$).

Discussion

CD31/PECAM-1, a 130-kDa member of the immunoglobulin (Ig) superfamily, is thought to play a role in the pathogenesis of atherosclerosis and the underlying mechanism may involve inflammation. CD31/PECAM-1 mediates cell migration by regulating integrins and stimulates integrin activation.^{19,20} Some studies have found that polymorphisms of *CD31/PECAM-1* are associated with several diseases such as myocardial infarction or acute coronary syndrome, bronchial asthma, and severe malaria;^{12–15,21–23} however, studies to date seldom focus on cerebral infarction risk. Asn563Ser is a polymorphism that is located on exon 8 at codon 563 of the *CD31/PECAM-1* gene and it leads to a change from an asparagine to a serine. Our study showed a significant difference in the genotypes and alleles of *CD31/PECAM-1* Asn563Ser between the ACI and the control

groups. These findings suggest a possible correlation between the Asn563Ser polymorphism and ACI risk. The logistic regression analysis showed that the GG genotype is an independent risk factor for ACI. This result was consistent with the report by Wei et al.¹⁶ Zaremba et al²⁴ and Marquardt et al²⁵ also reported that significantly increased sPECAM-1 levels were found in the early stages of acute cerebral infarction. Our study also indicated that plasma sPECAM-1 levels in the ACI group were significantly higher than in controls; the sPECAM level was also associated with the risk of ACI after adjustment. The plasma sPECAM-1 level of subjects carrying the GG genotype was significantly higher than the level in those carrying the GA or AA genotype.

The underlying mechanism of how Asn563Ser polymorphism affects ACI risk and leads to the upregulated levels of plasma sPECAM-1 remains unclear. Asn563Ser is located on the sixth Ig-like homology domain of CD31 and plays an important role in cation binding. Substituting an amino acid may influence the binding affinity with integrins by altering the structure of CD31 or by affecting Ca^{2+} -dependent cell interactions; this may influence signaling pathways that involve cell migration, differentiation, or cell apoptosis.^{26–28} More research is needed to elucidate the underlying mechanism.

Table 4 Plasma sPECAM-1 levels among different genotypes of Asn563Ser

Groups	sPECAM-1 levels	P	P_{adj}	OR _{adj}	95% CI _{adj}	Plasma sPECAM-1 levels among various genotypes			F	P
						GG	GA	AA		
ACI group	40.20±14.26	0.004*	0.038*	1.431	1.020–2.006	48.06±14.86	38.82±13.52	34.93±12.11	8.629	0.003*
Control group	35.58±11.31					42.43±13.63	34.91±10.86	34.15±10.50	3.103	0.049*

Note: * $P < 0.05$.

Abbreviations: ACI, atherosclerotic cerebral infarction; sPECAM-1, soluble platelet endothelial cell adhesion molecule-1; F, F-value for analysis of variance; P_{adj} , adjusted P-value; OR_{adj}, adjusted odds ratio; 95% CI_{adj}, adjusted 95% confidence interval.

The Leu125Val, Asn563Ser, and Gly670Arg polymorphisms in the *CD31/PECAM-1* gene are found in close genetic linkages, although these single-nucleotide polymorphisms (SNPs) show population diversity.^{29,30} A limitation of our study is the lack of genotyping and haplotype analysis for these three SNPs together. It is necessary to expand the sample sizes in the southern Han population and verify this correlation in other populations.

In summary, the *CD31/PECAM-1* Asn563Ser polymorphism is associated with genetic susceptibility to ACI in the southern Han Chinese population, independent of traditional risk factors. The Asn563Ser polymorphism may influence the plasma level of sPECAM-1 and play an important role in the pathogenesis of ACI.

Conclusion

Our study showed that the Asn563Ser polymorphism of the *CD31/PECAM-1* gene and elevated soluble PECAM-1 levels are related to ACI risk in the southern Han population of the People's Republic of China.

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Disclosure

The authors report no conflicts of interest in this work.

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