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Original Article

Leu125Val polymorphism of platelet endothelial cell adhesion molecule-1 is associated with atherosclerotic cerebral infarction in Chinese Han population

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Abstract: A total of 142 Atherosclerotic cerebral infarction (ACI) patients and 116 controls were enrolled in our study. The Leu125Val polymorphism of PECAM-1 was genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The plasma sPECAM-1 level was measured by enzyme-linked immunosorbent assay (ELISA) method. We found a statistically significant difference in Leu125Val genotypic distribution between cases and controls ($P < 0.05$). The frequencies of the Val allele between ACI group and controls were significantly different ($P < 0.05$). Logistic regression analysis showed that the genotype Val/Val was associated with increased ACI risk (OR = 2.355, 95% CI = 1.153-4.809, $P = 0.019$). In both the ACI group and the control group, the plasma PECAM-1 levels of carriers of the Val/Val genotype were higher than those carrying Leu/Leu and Leu/Val genotypes. The plasma sPECAM-1 level is associated with ACI. Our study showed that Leu125Val polymorphism of PECAM-1 may be associated with ACI risk. Carrying the Val/Val genotype showed increased risk for ACI. The Leu125Val polymorphism of PECAM-1 may be associated with the plasma sPECAM-1 level, which is associated with Chinese ACI also. In conclusions, The Leu125Val polymorphism of the PECAM-1 gene is likely to be related to ACI, and the Val/Val genotype may be an independent risk factor for ACI. The plasma sPECAM-1 level may be associated with ACI risk.

Keywords: Platelet-endothelial cell adhesion molecule-1 (PECAM-1), polymorphism, atherosclerotic cerebral infarction (ACI), restriction fragment length polymorphism (RFLP)

Introduction

China is reported to have the highest incidence rate of stroke in the world [1]. Stroke has become the leading cause of death in China and imposes a great economic burden on families and societies [2, 3]. Cerebral infarction is the most common type of stroke in China, accounting for 43-79% of the whole stroke cases [4]. Atherosclerosis is considered as the most important pathological basis for cerebral infarction, and it has been widely accepted that inflammation involves in the pathophysiological process of atherosclerosis [5, 6]. Current research indicates cell adhesion molecules (CAMs) play important roles in some vital phases in the pathogenesis of atherosclerosis, such as monocyte adhesion to endothelial cells, transendothelial migration, and intimal neovascularization [7-9]. Therefore, genes encoding CAMs have been regarded as a class of important candidate genes for cerebral infarction.

Platelet endothelial cell adhesion molecule-1 (PECAM-1), also called CD31, is an important member of the CAM family. It is expressed on the surface of endothelial cells, circulating platelets, monocytes, neutrophils, and certain T-lymphocyte subsets [10, 11]. The Leu125Val (rs668) polymorphism of PECAM-1 has been found to be associated with coronary artery disease (CAD) [12-14]. Studies have shown significantly elevated plasma soluble PECAM-1 (sPECAM-1) levels in patients with cerebral infarction [15, 16]. Wei YS et al. reported that Leu125Val of PECAM-1 and its sPECAM-1 levels are associated with ischemic stroke [17], to the best of our knowledge, which is the first report on the association of PECAM-1 polymorphism with cerebral infarction. In our study, a case-control

study design was employed to verify the relationship between susceptibility to atherosclerotic cerebral infarction (ACI) and the Leu125Val polymorphism of PECAM-1 and plasma sPECAM-1 levels in a Southern Han population of China.

Subjects and methods

Subjects

This study was approved by the Ethics Committee of Xiangya Hospital (Changsha, Hunan Province, China). All participants gave their written informed consent. A total of 142 ACI patients, 86 males and 56 females, who were admitted to the Department of Neurology of Xiangya Hospital between September 2010 and February 2011, were enrolled into this study. These patients, with an average age of 63.7 ± 9.942 years, were all Han residents of Hunan Province without consanguinity. All the ACI patients were diagnosed through clinical observation, CT and/or MRI within 3-7 days of the disease onset. Patients with cerebral infarction caused by cardiogenic factors, arteritis, hematological disorders, tumors, or cerebral vascular malformations were excluded. Patients with hemorrhage after infarction or infarction following hemorrhage were also excluded. Furthermore, patients who took oral anticoagulants or contraceptives, were pregnant, or had liver and kidney diseases or autoimmune diseases were excluded from this study. The control group consisted of 116 healthy volunteers, 64 males and 52 females, who were recruited over the same time period with gender- and age-matching. The subjects in the control group, with an average age of 61.1 ± 9.123 years, had no history of stroke or family history of stroke and no liver or kidney diseases, hematological diseases, or autoimmune diseases.

Biochemical tests, estimation of levels of sPECAM-1and DNA extraction

After fasting for 12 hours, 10 ml of peripheral blood was collected from each subject. A 5 ml portion of the collected blood sample (without anticoagulant) was used for biochemistry tests (blood glucose and blood lipid). Serum sPECAM-1 levels were determined using Human sPECAM-1 Instant ELISA Kit (Bender MedSystems GmbH, Vienna, Austria) according to the manufacturer's instructions. The remaining 5 ml of the blood sample was mixed with EDTA as an anticoagulant and was used for DNA extraction through the phenol-chloroform method.

Primers design and PCR

Primers for the Leu125Val locus were designed using a free online software Prime 3 (simgene.com/Primer3), and sequences were 5'-GCACCACCTCTCACGTCAAG-3' (forward primer) and 5'-CTGTGCTCAGTTCCAA-3' (reverse primer). The length of the amplification product was 225 bp. Primers were synthesized by Sangon Biotech Co., Ltd., (Shanghai, China). Polymerase chain reaction (PCR) conditions were as follows: pre-denaturation at 94°C for 5 minutes; 35 cycles of denaturation at 94°C for 35 seconds, annealing at 55°C for 35 seconds, and extension at 72°C for 35 seconds; and final extension at 72°C for 10 minutes. The PCR product was kept at 4°C until used.

Restriction digestion of amplification product

A 3 μL sample of the PCR product was taken for digestion with restriction enzyme PvuII (Biolabs Co., UK). The enzyme reaction was incubated at 37°C for 5 hours, and 3 μL of the reaction product was taken for electrophoresis (0.5 × TBE, 110 V, 45 minutes) on a 3% agarose gel (containing 0.5 μg/ml ethidium bromide). The results were examined with a gel imaging system (Tanon Science & Technology Co., Shanghai, China). Genotype detection: wild-type homozygotes (Leu/Leu), undigested, with one fragment of 225 bp; heterozygotes (Leu/Val) produced, three fragments with lengths of 225 bp, 188 bp and 37 bp, respectively; two fragments were generated from the mutant homozygotes (Val/Val) with lengths of 188 bp and 37 bp.

Statistical analysis

Statistical analysis was performed using SPSS 18.0 software (IBM SPSS, Armonk, NY, USA). Genotype and allele frequencies were calculated using the direct counting method. χ^2 test was used to examine the Hardy-Weinberg equilibrium and to compare genotype and allele frequencies between groups. Measurement data are expressed as $\bar{x}$ ± s. Data from the groups were compared using the *t* test or analysis of

Table 1. Clinic data of ACI patients and controls

Clinic data	ACI	Controls	*P* values
Mean age (years)	63.7 ± 9.942	61.1 ± 9.123	0.055
Male/female	86/56	64/52	0.447
BMI (kg/m^2)	23.8 ± 3.43	23.9 ± 3.08	0.908
Smoking (N/Y)	59/83	31/85	0.013*
Drinking (N/Y)	23/119	15/101	0.486
History of hypertension (N/Y)	104/38	17/99	0.000*
History of diabetes (N/Y)	32/110	4/112	0.012*
History of hyperlipidemia (N/Y)	72/70	41/75	0.013*
TC (mmol/l)	4.73 ± 0.98	4.49 ± 0.81	0.035*
TG (mmol/l)	1.97 ± 1.24	1.61 ± 0.98	0.012*
HDL(mmol/l)	1.15 ± 0.37	1.27 ± 0.36	0.015*
LDL (mmol/l)	2.76 ± 0.89	2.53 ± 0.72	0.025*

M, male; F, female; N, no; Y, yes; BMI, body mass index; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein. *P < 0.05.

Table 2. Genotype and allelic frequencies of Leu-125Val in ACI patients and controls

SNP	ACI (n = 142)	Controls (n = 116)
Genotype n (%)		
Leu/Leu	29 (20.4)*	28 (24.1)
Val/Val + Leu/Val	113 (79.6)*	88 (75.9)
Allele (%)		
Leu	(40.5)	(52.1)
Val	(59.5)*	(47.9)

*P < 0.05.

variance. The threshold for statistical significance was $P < 0.05$. Non-conditional Logistic regression analysis was used to analyses the relationship between the genotype and atherosclerotic cerebral infarction, then calculate the P value, the odds ratios (OR) and 95% confidence intervals (95% CI). Partial analysis was conducted between Leu125Val polymorphism, plasma sPECAM-1 level and ACI risk.

Results

Clinic data of patients and the control subjects

Clinic data of subjects were shown in **Table 1**. The two groups showed no significant difference in gender, age, body mass index (BMI), or smoking and drinking history ($P > 0.05$), indicating that the ACI group and the control group were comparable. Common risk factors for stroke, such as histories of hypertension, diabetes, CAD, and hyperlipidemia were significantly higher ($P < 0.05$) in the ACI group than in the control group. The plasma triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) levels were significantly higher ($P < 0.05$) in the ACI group than in the control group, while the plasma HDL-C level was significantly lower in the ACI group than in the control group ($P < 0.05$). No significant difference in plasma TC level was found between the two groups ($P > 0.05$). The sPECAM-l plasma concentration was significantly higher in the ACI group than in the control group ($P < 0.05$).

Comparison of PECAM-1 (Leu-125Val) genotype distribution between the patient group and controls

As shown in **Table 2**, there were 29 cases of Leu/Leu, 57 cases of Leu/Val, and 56 cases of Val/Val in the ACI group, and there were 28 cases of Leu/Leu, 65 cases of Leu/Val, and 23 cases of Val/Val in the control group, both compatible with the Hardy-Weinberg equilibrium. The frequencies of the Leu/Leu, Leu/Val, and Val/Val genotypes were 20.4%, 40.2%, and 39.4% in the ACI group and 24.1%, 56.0%, and 19.9% in the control group. The distribution of the Leu125Val genotype was significantly different in the ACI group and the control group ($P < 0.05$). The Val allele frequency was 0.595 in the ACI group and 0.479 in the control group, showing a significant difference ($P < 0.05$).

Non-conditional logistic regression analysis

In this study, ACI incidence and 8 factors were analyzed by logistic regression analysis. Under these conditions, the significance level of the included variables was 0.10, and the significance level of the excluded variables was 0.15; the "Backward-LR" method was applied to determine significant risk factors for ACI. The results showed that the GG genotype, histories of hypertension, CAD, diabetes, and hyperlipidemia were major risk factors for ACI. After excluding the effect of confounding factors, the results indicated that the Val/Val genotype was an independent risk factor for ACI (OR = 2.355, 95% CI = 1.153-4.809, $P = 0.019$) (**Table 3**).

Table 3. Logistic regression analysis about ACI

Risk factors	β	S.E.	Wald	*P*	OR	95% CI
Val/Val	0.857	0.364	5.529	0.019*	2.355	1.153-4.809
smoking	0.683	0.344	3.932	0.047*	1.979	1.008-3.886
drinking	0.369	0.470	0.617	0.432	1.446	0.576-3.634
History of hypertension	2.530	0.341	55.095	0.000*	12.551	6.435-24.480
History of diabetes	1.815	0.627	8.392	0.004*	6.141	1.799-20.968
History of hyperlipidemia	0.710	0.333	4.532	0.033*	2.034	1.058-3.910
Constant	-1.890	0.325	33.794	0.000	0.151	-

*$P < 0.05$.

Table 4. Partial association between Leu-125Val genotype, plasma sPECAM-1 level and ACI risk

		Val/Val	SPECAM-1	ACI
Val/Val	R	1.000	0.454	0.133
	P	-	0.000	0.036
SPECAM-1	R	0.454	1.000	0.223
	P	0.000	-	0.000
ACI	R	0.133	0.223	1.000
	P	0.036	0.000	-

Correlation analysis of Leu125Val gene polymorphism, plasma sPECAM-1 level in ACI

After correction of gender, body mass index, and other confounding factors, the Val/Val genotype and the plasma sPECAM-1 level were significantly correlated ($P < 0.001$). The plasma sPECAM-1 concentration exhibited a significant correlation with atherosclerotic cerebral infarction ($P < 0.001$), and the Val/Val genotype was significantly correlated with ACI ($P = 0.036$). The results are illustrated in the **Table 4**.

Discussion

In this study, PCR-restriction fragment length polymorphism (PCR-RFLP) was used to identify the Leu125Val genotype of PECAM-1 in a Hunan Han population of China. Leu/Val was found to be the most common genotype at Leu125Val in the Hunan Han population, which is consistent with previous results [18-21]. In addition, the general information revealed a larger proportion of subjects with histories of smoking, hypertension, diabetes, and hyperlipidemia in the ACI group than in the control group. Logistic regression analysis further confirmed that histories of smoking, hypertension, diabetes, and hyperlipidemia were predisposing factors for ACI incidence, which is also consistent with previous studies [22-24].

We analyzed the relationship between the plasma sPECAM-1 level and ACI. The results showed a higher plasma sPECAM-1 level in the ACI group than in the control group ($P < 0.001$), and plasma sPECAM-1 was still correlated with ACI after correction for age, gender, and hyperlipidemia via partial correlation analysis ($P < 0.001$). In the experiment, we hypothesized that PECAM-1 gene polymorphism was related to susceptibility to ACI, which was confirmed in the study by showing that the PECAM-1 Val/Val genotype and Val allele frequency were significantly higher in the ACI group than in the control group. The results of the logistic regression analysis showed that the Leu125Val genotype polymorphism of PECAM-1 was an independent risk factor for ACI, suggesting that single nucleotide polymorphisms of the PECAM-1 Leu125Val locus were correlated with the risk of ACI. We also analyzed the relationship between Leu125Val polymorphism of PECAM-1 and plasma sPECAM-1 level. The results showed that the plasma sPECAM-1 level was relatively higher in patients with the Val/Val genotype in both the ACI and the control group. The results of our study were consistent with the results of Wei YS et al. in Singapore [17].

Leu125Val is located in the first loop of the extracellular immunoglobulin domain, which has been reported to play roles in blocking the transendothelial migration of leukocytes [25]. Therefore, we speculated that Leu125Val might lead to the elevated function of the PECAM-1-mediated leukocyte transendothelial migration, making the Val/Val carriers more susceptible to ACI. Some studies have also found that PECAM-1 inhibits the interaction between platelets and fibrinogen and prevents further expansion of

the platelet thrombus on the fibrinogen surface [26, 27]. The Leu125Val mutation may cause loss of this function and then promote the occurrence of ACI. In addition, PECAM-1 and angiotensin-converting enzyme (ACE) gene are co-localized at 17q23 within an extremely short distance. ACE-D allele is a pro-atherosclerosis factor [28]. Japanese researchers have found the linkage disequilibrium among multiple site mutations of PECAM-1 (including Leu125Val, Ser563Asn and Arg670Gly), so the correlation of Leu125Val mutation with ACI pathogenesis may be a result of the gene linkage disequilibrium [19].

In summary, ACI is a disease related to multiple genetic and environmental factors. The Leu125Val polymorphism of the PECAM-1 gene is likely to be related to ACI, and the Val/Val genotype may be an independent risk factor for ACI. The plasma sPECAM-1 level may be associated with ACI risk. Leu125Val may stimulate ACI pathogenesis by changing the plasma sPECAM-1 level, but the underlying mechanisms are worth further investigation.

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Disclosure of conflict of interest

None.

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