Association of intercellular adhesion molecular-1 gene polymorphism in ischemic stroke patients

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Abstract

Background: Ischemic stroke (IS) is a prevalent disease causing a body disability, the third leading cause of death in Taiwan. It shows that the level of intercellular adhesion molecular-1 (*ICAM-1*) in IS patients is higher than control subjects. **Objective:** This study is to investigate the possible association of *ICAM-1* (G1548A) polymorphism in IS patients. **Materials and Methods:** A total of 646 subjects were enrolled in this study, including 312 IS patients, and 334 controls without a history of symptomatic IS. The *ICAM-1* (G1548A) polymorphism was analyzed by polymerase chain reaction and restriction fragment length polymorphism. Clinical factors were also determined. **Results:** The frequencies of the *ICAM-1* (G1548A) polymorphism for G/G, G/A, and A/A were 74.8%, 23.9%, and 0.3%, respectively, in healthy controls, and 62.8%, 32.1%, and 5.1%, respectively, in patients. The frequency of the *ICAM-1* (G1548A) A allele (21.2% versus 13.2%, respectively; P = 0.007) and the carriers of the *ICAM-1* (G1548A) A allele (37.2% versus 25.2%; P = 0.019, OR 1.76, 95% CI 1.1-2.83) are great in IS patients compared with healthy controls. There is a higher risk of IS associated with homozygosity for the *ICAM-1* (G1548A) A allele (AA genotype) compared with the control population (5.1% vs. 0.3%, respectively, P = 0.04; OR 5.1, 95% CI 1.19-21.66). We also observed both hypertension and diabetes has shown a positive association with IS. **Conclusions:** The *ICAM-1* (G1548A) polymorphism was associated with independent risk factor for the development of IS.

Key Words

Allele, intercellular adhesion molecular-1, ischemic stroke, polymorphism

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Introduction

Stroke is a prevalent disease and a leading cause to the risk of body disability and death worldwide. The probability of stroke is estimated to be 0.5-1.6% for the adults who are over 36 year old.[1] More therapeutic methods have been progressed tremendously, but still few treatments are available for stroke. An ischemic stroke (IS) happens because of an inadequate supply of oxygen and glucose flowing to the brain to support cellular homoestasis.[2] The etiology and physiology of IS very complex, and other risk factors, including hypertension, diabetes, smoking, drinking, and inflammation, contribute to the development of IS.[3] Recently, several studies have investigated certain genetic polymorphisms associated with IS, but the results still need to be clearly elucidated.[4,5]

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Intercellular adhesion molecule-1 (*ICAM-1*) is a ligand interacted with a receptor leukocyte function-associated antigen-1 (LFA-1) located on leukocytes.[6,7] The leukocytes could bind to endothelial cells via *ICAM-1*/LFA-1 interaction and then transmigrate into tissues.[8] In the cytokine-mediated immune and inflammatory responses, *ICAM-1* played an important role, especially in the progression of IS.[9,10] Ferrarese *et al*,[11] found the levels of *ICAM-1* in peripheral blood and cerebrospinal fluid were higher in IS patients, comparing with that of controls. Orion *et al*,[12] have also shown that the levels of *ICAM-1* were strictly associated with the risk for IS. Moreover, in knock-out mice experiments, *ICAM-1* deficiency reduced atherosclerotic lesions after null *ICAM-1* mice were fed a high-fat diet. The results has proved that *ICAM-1* might be involved in the pathogenesis of ischemic cardiovascular disorders.[13]

The *ICAM-1* polymorphisms have been examined, and were associated with various inflammatory diseases.[14,15] For an example, Pola *et al*,[16] showed the frequency of the EE

genotype of the *ICAM-1* (E469K) in the IS patients was twofold, comparing with that of controls. Kitawaki *et al*,[17] reported the *ICAM-1* (E469K) and the interleukin-6 (IL-6) (-634C/G) polymorphisms synergistically affected susceptibility to endometriosis. In this study, we are to investigate the association between the occurrence and severity of IS and *ICAM-1* (G1548A) polymorphism in the Taiwanese.

Materials and Methods

Subjects

A total of 646 subjects, including 312 IS patients and 334 healthy controls without a family history of IS, were recruited from the Neurosurgery Division at the Kaohsiung Armed Forces General Hospital, Kaohsiung, Taiwan from March 2009 to March 2011. All subjects agreed and signed an informed consent form to participate in the study. The 312 IS patients included 36 patients with left hemisphere stroke, 140 patients with right hemisphere stroke, and 136 patients with bilateral stroke. The plan was approved by the ethics committee of the Kaohsiung Armed Forces General hospital. Venous blood was taken 2 h after having meal, and plasma and serum sample were either used immediately for analysis or stored frozen at –20°C until DNA extraction.

DNA extraction

Total genomic DNA was extracted with the DNeasyTM Kit (QIAGEN Group) according to the manufacturer's instructions. Briefly, the blood was digested with 0.5 mg/mL proteinase K in 400 μL cell lysis solution for 24 h at 55°C until the blood was completely lysed. After adding 200 μL of absolute ethanol to the lysed sample, the mixture was transferred into the DNeasy mini column and centrifuged for 1 min at 8000 rpm. The DNeasy mini column was washed with 500 μL washing buffer and centrifuged for 1 min at 8000 rpm. Finally, the DNA was eluted into a clean 1.5-mL microcentrifuged tube. Quantification of the DNA was performed using a spectrophotometer (GeneQuant), and the sample was stored at −20°C until polymerase chain reaction (PCR) amplification.

Determination of *ICAM-1* polymorphism

The G/A subatitution located at position + 1548 in the structural region of the *ICAM-1* gene. This region was amplified by PCR, using the primers 5′-CCATCGGGGAATCAGTG-3′ (sense) and 5′-ACAGAGCACATTCACGGTC-3′ (antisense). The amplifications were performed as follows: 1 cycle at 95°C for 4 min, 50 cycles at 95°C for 1 min, 61°C for 1 min, 72°C for 1 min and a final extention at 72°C for 3 min in an automated PCR cycler (GeneAmp PCR system 2400; Perkin-Elmer, Darmstadt, Germany). The amplified product was identified by digestion with BstUI (Promega) in a final volume of 10 μL at 37°C overnight. The digested products were separated in a 3% agarose gel followed by staining with ethidium bromide, and the genotypes were determined by analyzing the different bands.

Statistical analysis

Demographic and clinical data between groups were compared by analysis of variance. Genotype and allele frequencies between the control and IS groups were compared by the Chi-square test. The *P* values, odds ratios (ORs), and 95% confidence intervals were calculated. A *P* value of less than 0.05 was considered as significant for all analyses.

Results

We recruited 646 subjects, including 312 IS patients, mean age and standard deviation (SD), 68.3 (4.5) years, and 334 controls, mean age and SD, 67.8 (5.3) years [Table 1]. Differences in hypertension and diabetes were significantly different, but there were no significant differences in male : female ratio and hypercholesterolemia.

The distribution of *ICAM-1* (G1548A) genotypes and alleles is shown in Table 2. In IS patients, the A/A genotype in *ICAM-1* (G1548A) is more frequent than that of controls (5.1% vs. 0.3%; $P = 0.04$). In addition, the G/A genotype is also slightly overrepresented in the IS patients compared to controls (32.1% vs. 23.9%; $P = 0.051$). The frequency of A allele in *ICAM-1* (G1548A) is significantly increased in IS patients (21.2% vs. 13.2%, $P = 0.007$). In comparison with controls there are significantly more A allele carriers of the *ICAM-1* (G1548A) among IS patients (37.2% versus 25%; $P = 0.019$), with the OR of 1.76 (95% CI 1.1-2.83), is shown in Table 3.

Table 1: Demographic and clinical data for subjects

	IS patients (*n*=312)	Controls (*n*=334)	*P*
Age (years±SD)	68.3±4.5	67.8±5.3	NS
Male : female ratio	153:158	161:173	NS
Hypertension	162 (52.2)	105 (31.9)	<0.001
Diabetes	145 (46.5)	90 (27)	<0.001
Hypercholesterolemia	96 (31)	86 (26)	NS

IS=Ischemic stroke, NS=Not statistically significant, SD=Standard deviation

Table 2: Genotype and allele frequencies of *ICAM-1* (G1548A) in subjects

ICAM-1 (G1548A)	IS patients (*n*=312)	Controls (*n*=334)	Odds ratio (95% CI)	*P*
Genotype				
GG	196 (62.8)	250 (74.8)	1 (0.90-1.10)	NA
GA	100 (32.1)	80 (23.9)	1.63 (0.99-2.00)	0.051
AA	16 (5.1)	2 (0.3)	5.1 (1.19-21.66)	0.04
Allele			1.77 (1.77-2.68)	0.007
A	132 (21.2)	88 (13.2)		
G	492 (78.8)	580 (86.8)		

IS=Ischemic stroke <0.001, OR=Odds ratio, CI=Confidence interval, *ICAM-1*=Intercellular adhesion molecular-1, NA=Not available, GG=*ICAM-1* GG genotype, GA=*ICAM-1* GA genotype, AA=*ICAM-1* AA genotype

Table 3: A allele frequencies of *ICAM-1* (G1548A) in subjects

ICAM-1 (G1548A)	IS patients (*n*=312)	Controls (*n*=334)	Odds ratio (95% CI)	*P*
GA+AA	116 (37.2)	84 (25.2)	1.76 (1.10-2.83)	0.019
GG	196 (62.8)	250 (74.8)		

IS=Ischemic stroke, OR=Odds ratio, CI=Confidence interval, *ICAM-1*=Intercellular adhesion molecular-1, AA=*ICAM-1* AA genotype, GA=*ICAM-1* GA genotype, GG=*ICAM-1* GG genotype

Discussion

The leading causes of IS are associated with many clinical disorders, including myocardial infarction, circulatory shock, lacunar infarction, and inflammatory cytokines. Among these risk factors, cytokines are thought to play a vital role in the regulation of the immune response. Previous studies have reported the levels of cytokines in IS patients, such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), IL-6, and *ICAM-1*, were higher than that of healthy controls.[17-19] In addition, genetic risk factors might have important contributions in the pathogenesis of IS. Likewise, several studies have shown that the synergistic effect of the IL-6 promoter and *ICAM-1* polymorphisms could be the lead cause of serious immune diseases IS or endometriosis in the world.

ICAM-1, induced by IL-1 and TNFα, is a cellular adhesion protein located at the cell membrane of leukocytes and endothelial cells. The expressed *ICAM-1* has led to various inflammatory and cardiovascular disorders. Gaetani *et al*,[18] first demonstrated that the *ICAM-1* (G1548A) polymorphism was associated with the peripheral arterial occlusive disease. Kamiuchi *et al*,[19] found the *ICAM-1* polymorphism was associated with diabetic retinopathy in Type 2 diabetes mellitus. Longoni *et al*,[20] also reported the *ICAM-1* (K469E) polymorphism was highly represented in a spontaneous cervical artery dissection subgroup. Other studies have analyzed the relationship between the *ICAM-1* variation and the risk of IS. In Italian patients, Pola *et al*,[16] showed the existence of a synergistic effect of the IL-6 (−174G/C) polymorphism and the *ICAM-1* (469 E/K) polymorphism in patients with a history of IS. *ICAM-1* possesses an amino-terminal extracellular domain, a single transmembrane domain, and a carboxy-terminal cytoplasmic domain. Staunton *et al*,[21] described how the *ICAM-1* (K469E) could affect the arrangement of the immunoglobulin-like domain 5 of the protein's structure. The domain 5 of *ICAM-1* bond to the Macrophage Adhesion Ligand-1 (Mac-1), LFA-1, and fibrinogen.[22,23] In addition, in the Japanese population, Yamada *et al*,[24] reported that the *ICAM-1* (K469E) polymorphism was more predictive of IS. This study found that this genotypic variant in the IS patients could differentiate between Western and Asian populations. However, more clinical results are needed for confirmation of these results.

Campanella *et al*,[25] showed that cerebral ischemia was associated with the infiltration of inflammatory cells into the ischemic region. The recruitment of inflammatory cells seemed to increase ischemic brain injury and the pathogenesis of IS. Leukocyte-endothelial cell adhesion was an important step in the recruitment of leukocytes into post-ischemic brain tissue. In this inflammatory environment, cerebral endothelial cells increased their expression of cell surface adhesion molecules, such as *ICAM-1*, that mediate the recruitment of leukocytes and platelets to the ischemic region. Several studies have reported that the expression of *ICAM-1* is induced by IL-1, IL-6, and TNF-α in IS patients.[26-29] Experiments have also shown that *ICAM-1* ligation produced inflammatory leukocyte recruitment by signaling though signaling cascades involving a number of kinases, including the Src tyrosine kinase,[30] Raf-1,[31] and the mitogen-activated protein kinases (MAPKs).[32]

In this study, we noted that the frequency of IS was positively associated with the level of plasma glucose. The results were consistent with previous studies by Tanne *et al*,[33] they found a J-shaped association between fasting plasma glucose and the incidence of ischemic cerebrovascular events in patients with plasma glucose levels (>100 mg/dL). However, Targher *et al*,[34] reported that in diabetic subjects, the plasma *ICAM-1* and E-selectin were negatively associated with total glucose disposal during the insulin clamp. In these patients, acute hyperinsulinemia did not contribute any significant effect on plasma adhesion molecules. The possibility was proposed that adipose tissue releases one or more factors that adversely affect endothelial function.

In summary, the *ICAM-1* (G1548A) polymorphism was a genetic factor that could influence the severity of IS in Taiwanese patients. Furthermore, both plasma glucose and hypertension was association with the risk of IS. In conclusion, this study indicated that the *ICAM-1* (G1548A) polymorphism was significantly associated with IS, and the result suggested this polymorphism was an independent risk factor for IS.

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