

An association study between the +781C/T polymorphism in the interleukin-8 gene and the neurological recovery and prognosis of atherosclerotic cerebral infarctions

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Abstract

Background: The aim of this study was to investigate the association between the +781C/T polymorphism in the interleukin-8 (IL-8) gene and the acute neurological recovery and 3-month outcome of atherosclerotic cerebral infarction patients in the Han Chinese population.

Methods: We investigated the +781C/T polymorphism of *IL-8* in 308 consecutive Han Chinese patients who were diagnosed with an atherosclerotic cerebral infarction. The neurological recovery of the patients were evaluated by the difference of National Institute of Health Stroke Scale (NIHSS) score for the acute stage of the stroke and the Barthel Index (BI) score at 3 months after the stroke. Polymorphic genotypes were determined by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP).

Results: We found that the +781C/T genotypes of *IL-8* were significantly associated with neurological recovery after cerebral infarction ($p = 0.045$), and a significant difference in the neurological recovery of the patients was found between patients with the CT genotype and TT genotype (mean difference: 2.934, $p = 0.040$). No statistically significant association was found between the +781C/T polymorphism with the 3-month BI scores obtained after cerebral infarction ($p = 0.416$).

Conclusion: Our results demonstrated that the +781C/T polymorphism of *IL-8* is associated with neurological recovery at the acute stage of atherosclerotic cerebral infarction in the Han Chinese population, and the patients with the CT genotype recovered better than those with other genotypes. However, no association was found between the +781C/T polymorphism and the 3-month outcome of cerebral infarction patients.

Keywords: Interleukin-8; Polymorphism; Cerebral infarction; Neurological recovery; Outcome

Introduction

Stroke is currently the third cause of death and loss of disability-adjusted life-years (DALYs) in the world [1-3], and cerebral infarction is the most common form of stroke. Many factors, including genetic variation, affect neurological recovery after cerebral infarction [4-6]; thus, the effective means of improving neurological recovery and long-term prognosis of survivors of cerebral infarctions are limited. Inflammation, one of the main injury mechanisms after stroke, plays an important role in the neurological recovery and outcome of cerebral

infarction patients [7, 8]. Therefore, the roles of single nucleotide polymorphisms (SNPs) in the genes of some inflammatory cytokines have been investigated in recent years [9].

Interleukin-8 (IL-8) is a typical member of the CXC chemokine subfamily and a strong chemoattractant factor for neutrophils, basophilic leukocytes and T lymphocytes when it binds to its receptors, CXCR1 and CXCR2. As a strong mediator of inflammation, IL-8 plays an important role in promoting the formation of atherosclerosis (AS) and is involved in the process of brain injury after acute cerebral infarction [10-12]. In rabbits, it has been reported that IL-8 levels in the ischemic focus of the brain after cerebral infarction were higher than in healthy areas of the brain and that

brain edema and infarct volume were reduced by anti-IL-8 antibodies [11]. Some animal studies revealed that an inhibitor of the IL-8 receptor, repertaxin, alleviated acute and long-term local inflammation and promoted long-term neurological recovery by reducing polymorphonuclear neutrophil infiltration to the ischemic focus after cerebral infarction [12, 13]. The IL-8 levels in the cerebrospinal fluid of patients with cerebral infarction increased significantly at the acute stage and were still higher than the controls at ninety days after cerebral infarction [14, 15]. The IL-8 mRNA levels in peripheral blood mononuclear cells (PBMC) and the IL-8 concentrations in the plasma of patients with acute cerebral infarction have been shown to increase rapidly; additionally, these increased levels lasted 20 to 31 days after the stroke and were inversely correlated with neurologic impairment scores [14, 16-18]. Taken together, these findings demonstrate that IL-8 affects neurological recovery at the acute stage and the long-term outcome of patients after cerebral infarction. We can suspect that the SNPs of *IL-8* may be associated with neurological recovery and long-term prognosis after cerebral infarction because genetic mutations can affect the mRNA and protein levels of IL-8.

Grau et al. [19] reported that the 250T/A polymorphism in the promoter of *IL-8* did not correlate with IL-8 release in young adults after cerebral infarction. Another two studies revealed that three SNPs, rs4073, rs2227307 and rs2227543, were not associated with a risk for cerebral infarction [20, 21]. However, the associations between the SNPs of *IL-8*, neurological recovery and the prognosis of cerebral infarction patients are unclear.

The +781C/T polymorphism (rs2227306) is one of the common SNPs of *IL-8*, and can increase the level of transcription of IL-8 [22]. Heinzmann et al. [23] revealed that the +781C/T polymorphism was strongly associated with the occurrence asthma attacks and, thus, may play a more important role in inflammatory responses than other SNPs of *IL-8*. The aim of this study was to examine the associations between the +781C/T polymorphism of *IL-8* and the acute neurological recovery and 3-month outcome of atherosclerotic cerebral infarction patients.

Materials and Methods

Cases

The subjects in this study were chosen as previously described [24]. In brief, a total of 308 unrelated consecutive Han Chinese patients who were hospitalized for their first atherosclerotic cerebral infarction from 2010 to 2011 at the Fifth Affiliated Hospital of Sun Yat-sen University were enrolled in this study. The subjects met the WHO diagnostic criteria for stroke [25]. The patient diagnoses were also verified with either a CT or an MRI. Those patients with non-atherosclerotic cerebral infarctions, hemorrhagic stroke, blood diseases, malignant tumors, autoimmune diseases, inflammatory diseases and a history of ischemic cerebrovascular disease were excluded from the study. All participants gave written informed consent. This study was also approved by the Independent Ethics Committee of the Fifth Affiliated Hospital of Sun Yat-sen University.

Clinical evaluation

The medical history of the patients, including demographic characteristics, history of smoking, drinking, hypertension and diabetes, were recorded. Examinations, including routine blood tests; the determination of erythrocyte sedimentation rate, blood glucose level, blood lipid level and homocysteic acid level; electrocardiogram; chest direct digital radiography; fundus examination; color Doppler ultrasonography of the carotid arteries and heart; head CT/CTA and head MRI/MRA, were performed. The patients were scored according to the National Institute of Health Stroke Scale (NIHSS) [26] on the first and fourteenth day after stroke, and the modified Barthel Index (BI) [27] was given at 3 months after the stroke. The patients' 3-month outcomes were designated as severe disability (BI score \leq 40), moderate disability ($40 <$ BI score \leq 65) or mild disability (BI score $>$ 65).

Genetic analyses

Genomic DNA was prepared for genetic analysis from EDTA. K₂ anticoagulated peripheral blood with the E.Z.N.A.[™] SQ DNA Kit II (Omega Bio-Tek, Inc. Norcross, GA, USA). To analyze the +781C/T polymorphism of *IL-8*, PCR-RFLP was performed as previously described [28]. Briefly, amplification of a 203-bp fragment of *IL-8* was performed in a total volume of 25 μ l with 300ng of extracted genomic DNA, 12.5 μ l of 2X Taq Master Mix (Omega Bio-Tek, Inc. Norcross, GA, USA) and two primers for the region of *IL-8* including the polymorphism. The forward primer was 5'-CTCTAACTCTT-TATATAGGAATT-3' and the reverse primer was 5'-GATTGATTTTATCAACAGGCA-3' [23]. The PCR consisted of 1 cycle of 5 min at 94 $^{\circ}$ C; 35 cycles of 30s at 94 $^{\circ}$ C, 30 s at 50 $^{\circ}$ C and 60 s at 72 $^{\circ}$ C; and 1 cycle of 10min at 72 $^{\circ}$ C in a DNA Engine Option[™] 2 PCR (MJ Research Corporation, Watertown, Mass., USA). The PCR product (10 μ l) was cleaved at 37 C with a 5 U EcoRI restriction enzyme (Fermentas, Canada) for approximately 4 hours. To analyze the +781C/T polymorphism, gel electrophoresis was performed on the EcoRI digested PCR products; the CC homozygotes yielded bands of 184bp and 19bp, the TT homozygotes yielded one band of 203 bp and the CT heterozygotes yielded three bands (203 bp, 184 bp and 19 bp).

Statistical analysis

All statistical analyses were performed with the SPSS 13.0 statistical package (SPSS Inc., Chicago, IL, USA). The differences among groups for continuous values were calculated by analysis of variance. For dichotomous variables, chi-squared (χ^2) analysis was applied. The criterion for statistical significance was a *p-value* of $<$ 0.05.

Results

The patients' characteristics are shown in Table 1. The +781C/T genotypes of IL-8 were successfully determined for all patients. The genotypes found in this study were CC, CT and TT, with frequencies of 0.454, 0.416 and 0.130. The genotype distribution was consistent with those expected from Hardy-Weinberg equilibrium ($p = 0.213$).

The recovery of neurological function in cerebral infarction patients can be indicated by the difference of the first and final

NIHSS scores in the acute stage of the cerebral infarction. We found that the +781C/T genotypes of IL-8 were significantly associated with neurological recovery of cerebral infarction ($p = 0.045$). By a further multiple comparison using the Bonferroni correction, we found that the rate of neurological recovery was not significantly different between the patients with the CC genotype and CT genotype ($p = 0.814$) or TT genotype ($p = 0.237$); however, a significant difference in neurological recovery was found between the patients with the CT and TT genotype ($p = 0.040$), which indicated that the patients with the CT genotype had a better neurological recovery at the acute stage of cerebral infarction (Table 2).

The +781C/T polymorphism of *IL-8* did not show a significant association with the 3-month outcome after cerebral infarction (Table 3).

Discussion

In this study, we attempted to examine the association between the +781C/T polymorphism in *IL-8* with the acute neurological recovery and 3-month outcome of atherosclerotic cerebral infarction patients in the Han Chinese population. We found that the CT genotype of the +781C/T polymorphism may aid in neurological recovery after cerebral infarction. However, we found no association of the +781C/T polymorphism with the 3-month outcome of cerebral infarction patients.

There have been a few studies regarding the association be-

	Patients
N	308
Age, y (mean \pm SD)	63.63 \pm 13.02
Male (n)	205
Smoking (n)	122
Drinking (n)	50
Hypertension (n)	233
Diabetes mellitus (n)	99
Hyperlipidemia (n)	159
High homocysteine (n)	84
AS in ICA (n)	224

Table 1: Patients' characteristics

geno- types	n	difference of NIHSS scores (mean \pm SD)	P	Bonferroni comparisons among genotypes	mean difference of the differ- ence of NIHSS scores	P
CC	140	1.857 \pm 6.267		CC versus CT	-0.877	0.814
CT	128	2.734 \pm 6.075		CC versus TT	2.057	0.237
TT	40	-0.200 \pm 8.431	0.045	CT versus TT	2.934	0.04

Table 2: Association of +781C/T genotypes with neurological recovery.

Analysis of variance was used to compare values of all parameters in the genotypes.

genotypes	mild disability n = 243	moderate disability n = 27	severe disability n = 38	χ^2	P
CC	116	10	14		
CT	94	14	20		
TT	33	3	4	3.929	0.416

Table 3: Association of +781C/T genotypes with the 3 month outcome after cerebral infarction.

Chi-square test was used to compare values of all parameters in the 3 month outcomes.

tween SNPs of chemokines and the neurological recovery and prognosis of cerebral infarction patients. Giannakopoulou et al. [29] were unable to demonstrate a significant association of the monocyte chemotactic protein-1-2518A>G gene polymorphism with ischemic stroke occurrence, severity or functional outcome in a Greek Caucasian population. In another study, we could not find an association between polymorphisms of the Fractalkine receptor gene, *CX3CR1*, and the neurological recovery or outcome of cerebral infarction patients [24]. This study indicated that the +781C/T polymorphism of *IL-8* was significantly associated with the acute neurological recovery of cerebral infarction patients, and the patients with the CT genotype at the acute stage of cerebral infarction recovered better than the patients with the other genotypes. This result indicates that the +781C/T polymorphism is involved in neurological function repair and may explain the individual differences in neurological recovery at the acute stage of cerebral infarction. We speculate that the CT genotype of +781C/T might reduce the expression level of IL-8 in the plasma, cerebral spinal fluid and damaged brain tissue, which relieves the brain injury caused by inflammation. In another study, we revealed that the CT genotype of +781C/T might be a protective factor for AS of the ICA [28]. Thus, we propose that the CT genotype also provides cerebrovascular protection, which benefits neurological recovery.

We found that the +781C/T polymorphism of *IL-8* was not associated with the 3-month outcome of cerebral infarction patients, which might be due to the IL-8 reduction during the convalescence of ischemic stroke patients. Many studies revealed that the IL-8 levels in the CSF and plasma of the patients with ischemic stroke at the sub acute stage and during convalescence were down regulated significantly relative to those at the acute stage; however, the levels were still higher at the subacute stage and during convalescence than in the controls [14, 15, 17, 18]. These results indicated that the inflammatory activity was very weak at these stages. It is well known that interleukin-1 β (IL-1 β) and tumor necrosis factor alpha (TNF- α) are strong stimulators of IL-8 expression. The expression of IL-1 β mRNA by blood cells was decreased back to the normal level in 20 to 31 days after ischemic stroke [14]. The high level of TNF- α being released by blood cells can last more

than 90 days after stroke; however, this level of TNF- α does not have enough intensity to stimulate IL-8, presumably because it is inhibited by the anti platelet drugs given to patients during the therapy period [30-32]. At the same time, the expression of IL-8 was also inhibited by anti platelet drugs [32, 33]. In addition, the transcription and expression of IL-8 were regulated by some signaling pathways, such as NF- κ B, JNK, P38 MAPK and ERK. The coordination of these signaling pathways might determine the expression level of IL-8, and the regulation of those signaling pathways during convalescence might be different than that at the acute stage of ischemic stroke [34]. The combined effect of these factors might decrease the IL-8 expression during the convalescence of cerebral infarction patients. Of course, the effect caused by the +781C/T polymorphism was limited because of the strong genetic heterogeneity of stroke.

We could not analyze the interaction of the +781C/T polymorphism and other SNPs of *IL-8* because we did not detect other SNPs of *IL-8* in this study. We also did not detect the IL-8 concentrations in the CSF and plasma of patients in this study. All these factors might affect the accurate interpretation of our results. In addition, this study only represented the Han Chinese population and not other ethnic groups. Thus, additional future studies are necessary.

To summarize, our findings indicated that the +781C/T polymorphism of IL-8 is associated with neurological recovery at the acute stage of atherosclerotic cerebral infarction in the Han Chinese population and that patients with CT genotype recover better than those with other genotypes. However, the +781C/T polymorphism was not found to be associated with the 3-month outcome of cerebral infarction patients. Our results may be beneficial for the treatment of cerebral infarction.

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