Association between *IL6* polymorphism and risk of cerebral infarction

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ABSTRACT. We conducted a case-control study to investigate the influence of *IL6* -174G/C (rs1800795) and -572C/G (rs1800796) genetic variants on the development of cerebral thrombosis in a Chinese population. This study included 305 cerebral infarction patients and 326 control subjects enrolled between May 2012 and May 2014. The genotyping of *IL6* -174G/C (rs1800795) and -572C/G (rs1800796) polymorphisms was performed using polymerase chain reaction combined with restriction fragment length polymorphism analysis. By using logistic regression, we found that when compared with the wild-type genotype, CC and GC+CC *IL6* -174G/C (rs1800795) genotypes were associated with an increased risk of cerebral infarction. Odds ratios (and 95% confidence intervals) were calculated to be 3.10 (1.57-6.41) and 1.63 (1.14-2.33) for the CC and GC+CC genotypes, respectively. In conclusion, our study suggests that the CC genotype and C allele of the *IL6* -174G/C (rs1800795) polymorphism are associated with an increased risk of cerebral infarction.

Key words: *IL6*; Polymorphism; Cerebral infarction

INTRODUCTION

Stroke is a complex-trait disease and a leading cause of morbidity and mortality worldwide (Donnan et al., 2008). In addition, it has recently been reported that stroke-related mortality has become the leading cause of death in China (Liu et al., 2011). Cerebral infarction constitutes the most common type of stroke, making up an estimated 43.7 to 78.9% of strokes in the Chinese population (Liu et al., 2007). The etiology of cerebral infarction involves many factors such as hypertension, diabetes, hyperlipidemia, vasculitis, atherosclerosis, and genetic influences (Ionita et al., 2005; Dichgans, 2007; Meschia et al., 2011).

An increasing amount of evidence has shown that inflammation plays a critical role in the pathogenesis of atherosclerosis and cerebral infarction (Stoll and Bendszus, 2006), and that several cytokines are associated with the arterial wall inflammatory process. Variations in genes related to the inflammatory system may alter the pattern of proinflammatory cytokine production, and thus the development of cerebrovascular disease, affecting predisposition and prevalence (Andreotti et al., 2002).

Interleukin 6 (*IL6*) is a cytokine with many important functions and is involved in several contradictory processes. It plays key roles in a wide spectrum of target cells in the immune response, hematopoiesis, neural differentiation, and the acute phase reaction (Schoester et al., 1994). *IL6* is reported to be involved in the inhibition of lipoprotein lipase activity and the stimulation of lipolysis, and thus may affect the pathogenesis of cerebral infarction (Shenhar-Tsarfaty et al., 2010; Saiki et al., 2013). Previous studies have investigated the association between *IL6* polymorphisms and the development of cerebral infarction, but results have been inconsistent (Balding et al., 2004; Yamada et al., 2006; Shenhar-Tsarfaty et al., 2010; Qi et al., 2014). Therefore, we conducted a case-control study to investigate the effect of *IL6* -174G/C (rs1800795) and -572C/G (rs1800796) genetic variants on the development of cerebral thrombosis in a Chinese population.

MATERIAL AND METHODS

Study population

This case-control study included 305 cerebral infarction patients and 326 control subjects enrolled between May 2012 and May 2014. All cerebral infarction patients were diagnosed by computed tomography or magnetic resonance imaging according to the World Health Organization cerebral infarction criteria. The diagnostic criteria were rapidly developing clinical signs of focal or global disturbance of cerebral function lasting more than 24 h with no apparent cause besides that of vascular origin. Patients with intracranial hemorrhage, transient ischemic attacks, peripheral vascular disease, thrombosis-related disease, brain tumors, or brain trauma were excluded from the study.

Cancer-free control subjects were randomly selected from individuals visiting the same hospital for health checkups. Control subjects with a history of strokes, peripheral vascular disease, or cancer were excluded from our study.

Clinical and demographic information regarding cerebral infarction patients and control subjects was collected from medical records. This data included sex; gender; body mass index (BMI); tobacco and alcohol consumption; levels of triglycerides, cholesterol, low-density lipoproteins (LDLs), and high-density lipoproteins (HDLs); and whether subjects had hypertension or diabetes. Written informed consent was obtained from each subject before being enrolled in the study group. This work was approved by the Institute Research Ethics Committee of Xinxiang Central Hospital.

Genotyping

Each patient and control subject provided a 5 mL peripheral venous blood sample after enrollment. The collected blood samples were stored at −20°C until use. Genomic DNA was extracted from peripheral blood using a TIANamp Blood DNA kit (Tiangen Biotech Co., Ltd., Beijing, China). The genotyping of *IL6* -174G/C (rs1800795) and -572C/G (rs1800796) polymorphisms was performed using polymerase chain reaction (PCR) combined with restriction fragment length polymorphism analysis. The forward and reverse primers used to amplify *IL6* -174G/C (rs1800795) were 5'-AGC CTC CGT GAC CAA ATA AG-3' and 5'-GGC GCT GAT TCC AAA GGT TA-3', respectively. Those for *IL6* -572C/G (rs1800796) were 5'-GGA GAC GCC TTG AAG TAA CTG C-3' and 5'-GAG TTT CCT CTG ACT CCA TCG CAG-3', respectively. Amplification was performed using the following cycling program: an initial denaturation step at 95°C for 5 min, then 30 cycles of denaturation at 95°C for 30 s, annealing at 62°C for 45 s, and extension at 72°C for 30 s, followed by a final extension at 72°C for 5 min. PCR products were visualized on a 2% agarose gel stained with ethidium bromide and exposed to ultraviolet light.

Statistical analysis

Statistically significant differences between cases and controls for demographic characteristics were assessed by the chi-square test. The association between *IL6* -174G/C (rs1800795) and -572C/G (rs1800796) polymorphisms and risk of cerebral infarction was analyzed by calculating odds ratios (ORs), 95% confidence intervals (95%CIs), and corresponding P-values. Gene-environment interactions were estimated by stratified analysis of demographic and clinical characteristics. Deviation from Hardy-Weinberg equilibrium for the polymorphisms under investigation was evaluated by comparing expected to observed genotype frequencies using the chi-square test. All P-values were two-sided, and those of less than 0.05 were considered to signify significant differences. All statistical analyses in this study were performed using SPSS software, version 16.0 (SPSS, Chicago, IL, USA) for Windows.

RESULTS

The demographic and clinical characteristics of cerebral infarction patients and control subjects are presented in Table 1. No significant difference was found between the two groups in terms of sex and age ($P > 0.05$).

When compared with control subjects, the cerebral infarction patients were more likely to have a higher BMI, suffer from diabetes mellitus and hypertension, have a habit of tobacco and alcohol consumption, and show higher levels of LDL, HDL, and triglycerides ($P < 0.05$).

IL6 -174G/C (rs1800795) and -572C/G (rs1800796) genotype distributions were found to be consistent with Hardy-Weinberg equilibrium in the control group (Table 2). In the control group, the minor allele frequencies of these two single nucleotide polymorphisms (SNPs) were similar to those given in the National Centre for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/snp).

Using logistic regression analysis, we found that individuals with *IL6* -174G/C (rs1800795) CC and GC+CC genotypes had an increased risk of cerebral infarction when compared with those carrying the wild-type genotype. The ORs (and 95%CIs) were 3.10 (1.57-6.41) and 1.63 (1.14-2.33) for the CC and GC+CC genotypes, respectively (Table 2). However, no significant difference in cerebral infarction risk was found between variants of *IL6* -572C/G (rs1800796).

Table 1. Demographic and clinical characteristics of case and control subjects.

	CI cases N = 305	%	Controls N = 326	%	Chi-square or *t*-test	P value
Mean age, years	60.15 ± 10.70		59.30 ± 10.25		1.02	0.15
<55	151	49.35	175	53.68		
≥55	154	50.33	151	46.32	1.1	0.3
Gender						
Male	182	59.48	185	56.75		
Female	123	40.20	141	43.25	0.55	0.46
BMI, kg/m^2	25.10 ± 2.85		23.50 ± 3.10		6.74	<0.001
Hypertension						
No	163	53.44	223	68.40		
Yes	142	46.56	103	31.60	14.85	<0.001
Diabetes mellitus						
No	218	71.48	291	89.26		
Yes	87	28.52	35	10.74	31.97	<0.001
Tobacco consumption						
Current or ever	118	38.69	79	24.23		
Never	187	61.31	247	75.77	15.33	<0.001
Alcohol consumption						
Current or ever	147	48.20	106	32.52		
Never	158	51.80	220	67.48	16.13	<0.001
Cholesterol	5.25 ± 1.52		5.30 ± 1.36		0.44	0.33
LDL	3.56 ± 1.24		3.05 ± 1.15		5.36	<0.001
HDL	1.25 ± 0.85		1.37 ± 0.67		1.98	0.02
Triglycerides	1.92 ± 1.27		1.60 ± 0.89		3.68	<0.001

CI = cerebral infarction; LDL = low-density lipoprotein; HDL = high-density lipoprotein.

Table 2. Association between *IL6* -174G/C (rs1800795) and -572C/G (rs1800796) polymorphisms and risk of cerebral infarction.

Genotype	Patients N = 305	%	Controls N = 326	%	OR (95%CI)[1]	P value
rs1800795						
GG	196	64.26	243	74.54	1.0 (Ref)	-
GC	74	24.26	69	21.17	1.33 (0.89-1.98)	0.14
CC	35	11.48	14	4.29	3.10 (1.57-6.41)	<0.001
GC+CC	109	35.74	83	25.46	1.63 (1.14-2.33)	0.005
rs1800796						
GG	117	38.36	139	42.64	1.0 (Ref)	-
GC	149	48.85	151	46.32	1.17 (0.83-1.66)	0.35
CC	39	12.79	36	11.04	1.29 (0.74-2.23)	0.34
GC+CC	188	61.64	187	57.36	1.19 (0.86-1.66)	0.27

[1]Adjusted for age, gender, body mass index, hypertension, diabetes mellitus, tobacco consumption, alcohol consumption, low-density lipoproteins, high-density lipoproteins, and triglycerides. OR = odds ratio; 95%CI = 95% confidence interval; Ref = reference.

DISCUSSION

It is well known that *IL6* is an important mediator of *in vivo* inflammatory reactions and the inflammatory response to cerebral infarction, and is associated with atherosclerotic disease (Pola et al., 2003). In addition, a previous study reported that serum levels of this cytokine significantly increase in cerebral infarction (Acalovschi et al., 2003). Experimental studies have reported that SNPs in the non-coding promoter sequence of the *IL6* gene can significantly affect its expression, and our study has indicated that the -174G/C (rs1800795) polymorphism, also present in its promoter, might influence the development of cerebral infarction.

Previous investigations have demonstrated a significant association between the -174G/C

(rs1800795) polymorphism and risk of ischemic stroke, but their results have been inconsistent (Lalouschek et al., 2006; Strand et al., 2007; Yang et al., 2014; Bazina et al., 2015). Bazina et al. (2015) conducted a study with 144 ischemic stroke patients and 187 healthy controls, and identified a significant link between this polymorphism and an increased risk of ischemic stroke. Moreover, Yang et al. (2014) reported a case-control study in a Chinese population, from which they concluded that both -174G/C (rs1800795) and -572C/G (rs1800796) SNPs were associated with an elevated risk of ischemic stroke in young patients, and that these polymorphisms interact with other factors including hypertension, obesity, and etiologic subtype. In the present work, we found that the *IL6* -174G/C (rs1800795) CC genotype and C allele are associated with an increased risk of cerebral infarction, a result that corresponds with the above-mentioned findings. However, Lalouschek et al. (2006) previously failed to establish a significant relationship between this polymorphism and the risk of ischemic cerebrovascular events. Likewise, Strand et al. (2007) reported no significant association between the -174G/C (rs1800795) polymorphism and first-ever stroke incidence. Such discrepancy between the results of previous studies may have been caused by differences in populations, study designs, and sample sizes.

There are several limitations to our study. First, all patients and control subjects were selected from one hospital, therefore selection bias may not have been avoided in this study. Second, in addition to *IL6* -174G/C (rs1800795) and -572C/G (rs1800796), other polymorphisms may influence the development of cerebral infarction. Third, the small numbers of cases and controls enrolled may have limited the statistical power of this investigation. Therefore, further studies incorporating greater numbers of subjects are needed to clarify the association between *IL6* -174G/C (rs1800795) and -572C/G (rs1800796) SNPs and the risk of cerebral infarction.

In conclusion, our study suggests that the CC genotype and C allele of the -174G/C (rs1800795) polymorphism are associated with an increased risk of cerebral infarction. Further well-designed research efforts involving large sample sizes are greatly needed to confirm our results.

Conflicts of interest

The authors declare no conflict of interest.

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