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ORIGINAL ARTICLES

Association of interleukin-6 gene polymorphism with susceptibility, neurological deficit and recurrence risk of cerebral infarction

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Abstract

Objective: The aim of this study was to explore if the single nucleotide polymorphism (SNP) of IL-6 was related to the susceptibility, severity of neurological deficit and the recurrence risk of cerebral infarction (CI). *Methods:* Three hundred and eighty-two patients with CI and 385 healthy controls were selected for IL-6 gene promotor region-174G /C, -572C/G, -597G/A polymorphism by SNaPshot SNP typing. The National Institute of Health Stroke Scale (NIHSS) and Essen Stroke Risk Score (ESRS) were adopted to evaluate the neurological deficit and stroke relapse risk in CI patients. *Results:* The rs1800796 polymorphism of the IL-6 gene showed a significant correlation with CI, and its GG genotype increased the risk of CI (CG+GG vs CC, P=0. 019). The dominant model of rs1800796 was related to severity of neurological deficit and the recurrence risk of cerebral infarction (CG+GG vs CC. *P* =0. 048 and *P*= 0. 019). No association was observed between rs1800795/rs1800797 and CI. *Conclusion:* IL-6 genetic polymorphism serves as a potential biomarker to determine the susceptibility of CI, neurological deficit and the risk of stroke recurrence.

Keywords: IL-6. Polymorphism, cerebral infarction, neurological deficit, risk of recurrence

INTRODUCTION

Cerebral infarction (CI) is a result of various causes that lead to interruption of regional blood supply disorders in the brain, leading to ischemia and hypoxic necrosis, resulting in neurological deficits. Epidemiology shows that about 16.9 million people experience stroke every year across the world, of which CI accounts for about 80-90%.[1] CI have high Morbidity Rate and mortality. A reported annual recurrence rate of 14-17%, and an early recurrence rate as high as 30%.[2] Conventional risk factors of atherosclerosis (age, hypertension, diabetes, smoking) account for a substantial portion of the risk of CI.[3] More and more clinical and experimental evidences show that genetic factors also increased susceptibility to CI.[4]

The IL-6 single nucleotide polymorphisms(SNPs) can alter transcription and regulate circulating concentrations of the cytokine IL-6, which could promote atherosclerosis via inflammation.[5] Investigators hold different views on the susceptibility of IL-6-572C/G(rs1800796), -174G/C(rs1800795) and-597G/A(rs1800797) in atherosclerosis diseases.[6,7] To the best of our knowledge, there were few data on the association of IL-6 genetic polymorphisms with susceptibility, neurological deficit and the recurrence risk of CI. In this case-control study, we studied the correlation between the SNPs in the promotor areas of IL-6-572C/G, -174G/Cand-597G/A and CI susceptibility, neurological deficit and recurrence risk, to examine whether they can be the basis of CI recurrence.

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METHODS

Patient characteristics

Three hundred and eighty-two CI patients and 385 normal controls were enrolled from May 2019 to October 2020 in the Neurology Department of the Affiliated Hospital of Youjiang National Medical College. The inclusion criteria were as follows: ① The patient was hospitalized for the 1st time or had a history of CI, but no sequelae, whereas this time, there was a new infarct focus. Within 7 days, the patient had focal neurological dysfunction lasting more than 24 hours, and was confirmed as CI by CT and / or MRI. ② The age was 18-80 years old. ③ No anti-inflammatory drugs were used within 2 weeks before admission. The exclusion criteria were as follows: ① Causes such as cardiogenic cerebral embolism, arteritis, drugs and tumors. ② Hemorrhagic stroke. ③ Complications: epilepsy status, severe pneumonia, brain herniation, deep vein thrombosis, post-stroke emotional disorder or failure to complete NIHSS and ESRS score. ④ Patients with severe heart, liver and kidney dysfunction, thyroid disease, pregnancy and drug abuse. ⑤ Patients with incomplete clinical data and those who were lost to follow up. The control group was examined clinically, and stroke was excluded by imaging examination. There was no stroke history and inherited illness. The study was approved by the Affiliated Hospital of Youjiang Medical University for Nationalities, and consent was obtained from all study subjects.

Data collection

All subjects' personal information, clinical data and medical history were recorded by a neurologist. Hypertension was defined by a SBP ≥ 140 mmHg (12.8 KPa) and a DBP ≥ 90 mmHg (12.0 KPa) on ≥ 3 individual occasions or patients on antihypertension medications. The diagnostic criteria for diabetis mellitus were RBG ≥ 11.1 mmol / L, FBG ≥ 7.0 mmol / L, history of diabetes and taking hypoglycemic drugs or insulin. The diagnostic criteria for hyperlipidemia were fasting sera TG ≥ 1.7 mmol / L, or previous hyperlipidemia and taking lipid-lowering drugs. Hyperhomocysteinemia was defined as fasting serum homocysteine (Hcy) ≥15 umol/L. According to the National Institute of Health Stroke Scale (NIHSS) score on admission: mild stroke with NIHSS < 6, moderate and severe stroke with NIHSS ≥ 6.[8] According to the Essen Stroke Risk Score (ESRS), high relapse risk is ≥ 3, while low relapse risk is ESRS < 3.[9]

Genotyping

The fasting venous blood were obtained from all participants. We completed the extraction of blood genomic DNA as per the specification of the DNA abstraction kit of Tiangen Biochemical Technology Company (Tiangen Biotech, Beijing, China). An ultramicro spectrophotometer was used to determine the thickness and purity of DNA and it was subsequently stored at -80°C in a freezer. Primer 3 was used to design PCR primers and extension primers for IL-6 gene (Table 1). Genotype was realized via the SNaPshot method with the technological support from Genesky Biotechnologies (PRC). The Genemarker V3. 0.1 (Demo) was used to determine the genotype and record the results.

Statistical analysis

The entire data were studied via SPSS 25.0 The continuous variable was described as average ± S. D. Student's *t* test was adopted to compare the measurement data. To compare counting data χ^2 inspection, the Hardy -Weinberg equilibrium method was used to test the group representativeness. We adopted the Chi-square test of four grid table data and RxC list to analyze the difference of genotyping and the allelic frequency distribution between the patient group and the control. The modified odds ratio (OR) and their 95% confidence interval were computed via the multivariable logical regressive assay. The $P < 0.05$ was deemed as statistically significant.

RESULTS

Demographic features

The baseline data were compared between the patient group and the control (Table 2). There were significant differences in the high blood pressure, diabetes, smoking and alcohol consumption between the cases and controls ($P < 0. 05$). Hence, these variables were adjusted in multivariate logistic regression analysis for estimating the main effects of IL-6 polymorphisms on the risk of CI.

Association of IL-6 polymorphisms with risk of CI

According to the sequencing results, only the GG genotype was found at -174G/C and -597G/A loci. Additionally, CC, CG and GG genotypes were detected at -572C/G loci. Therefore, we only analyzed the genotyping and allelic frequency of

Table 1: SNP loci, primer sequences and extended primers

SNPs	Primer sequence	Extension primer sequence
rs1800795	F: 5′-GCGCTAGCCTCAATGACGACCT-3′ R: 5′-TGAGCCTCAGACATCTCCAGTCCT-3′	SF: 5′-TCCCCCTAGTTGTGTCTTGC-3′
rs1800796	F: 5′-CCACCTGGAGACGCCTTGAAGTA-3′ R: 5′-GAACTGAGTTTCCTCTGACTCCATCG-3′	SR: 5′-TTTTTTTTTTGTTCTGGCTCTCCCTGTGAG-3′
rs1800797	F: 5′-CCACCTGGAGACGCCTTGAAGTA-3′ R: 5′-GAACTGAGTTTCCTCTGACTCCATCG-3′	SR: 5′-TTTTTTTTTTTTTTTTTTTTGTAGAACTGCCTGGCCA-3′

IL-6-572C/G loci in the case and control groups (Table 3). The genotype distributions in the controls were not deviated from Hardy-Weinberg equilibrium ($P>0.05$). After the adjustment for hypertension, diabetes, smoking and spirits dose in the multivariable logical regressive analysis, the dominant model of -572C/G loci significantly increased the risk of CI (modified OR=1.428, 95%CI = 1.060-1.923, P=0.019).

Association between IL-6 polymorphisms and stroke severity of CI

As shown in Table 4, according to the NIHSS score on admission, 162 patients (42.4%) were categorized as severe stroke (NIHSS score≥ 6) and 220 patients (57.6%) as minor stroke (NIHSS score < 6). The group of severe stroke registered a greater percentage of hypertension versus the minor stroke group (59.3% vs. 47.7%, P=0.026). Posterior to the modification for hypertension in the multivariable logical regressive assay, the dominant model of -572C/G was related to severe stroke (CG+GG vs. CC, OR=1.521, 95%CI=1.004-2.303, P=0.048).

Correlation between IL-6 polymorphisms and risk of stroke relapse

As shown in Table 5, each patient's risk of stroke relapse was evaluated as per ESRS. Of these patients, 209 patients (54.7%) in the $risk_{low}$ group (ESRS score< 3) and 173 patients (45. 3%) in the $risk_{high}$ group (ESRS score≥ 3). There were significant differences in the seniority, high blood pressure, diabetes and smoking between the $risk_{low}$ group and $risk_{high}$ group ($P<0.05$). Posterior to the modification of seniority, hypertension, diabetes and smoking by the multivariate logical regressive assay, it was found that the dominant model of -572C/G loci was related to the risk of recurrence in CI sufferers (CG+GG vs. CC, *OR* =1.665, *95% CI*:1.089-2.545, P = 0.019).

DISCUSSION

In this study, we analyzed the association between the IL-6 genetic polymorphic status and CI susceptibility, neurological deficit and the recurrence risk. Our findings showed that -174G/C and -597G/A loci did not show polymorphism. The IL-6-572C/G genetic polymorphic status is remarkably related to the susceptibility of CI. In addition, it is also found that - 572C/G gene mutation can aggravate the level of neurological deficit and increase the risk of recurrence.

Table 2: The baseline data of patients in the patient group and the control

Variable	Control n=385 (%)	Case group n=382 (%)	*P*-value
Sex (male/female)	250/135	270/112	0.089
Age (years, average±S.D.)	60.66±9.08	61.91±9.85	0.068
BMI(kg/m^2, average±S.D.)	23.07±3.10	23.18±3.54	0.639
Hypertension, n (%)	168 (43.6)	201 (52.6)	0.013*
Diabetes, n (%)	56 (14.5)	79 (20.7)	0.026*
Hyperlipemia, n (%)	98 (25.5)	109 (28.5)	0.337
Hyperhomocysteinemia, n (%)	115 (29.9)	135 (35.3)	0.106
Smoking, n (%)	110 (28.6)	137 (35.9)	0.031*
Alcohol consumption, n (%)	113 (29.4)	142 (37.2)	0.021*

Note: *P<0.05

Table 3: The correlation of IL-6-572C/G polymorphic status with the risk of cerebral infarction

Variable	Case group n=382 (%)	Control n=385 (%)	*P*-value	*OR (95% CI)*
CC	222 (58.1)	253 (65.7)		1.0(Ref.)
CG	132 (34.6)	115 (29.9)	0.061[a]	1.350 (0.986-1.848)[a]
GG	28 (7.3)	17 (4.4)	0.040[a]	1.952 (1.032-3.689)[a]
Alleles				
C	576 (75.4)	621 (80.6)		
G	188 (24.6)	149 (19.4)	0.013	0.735 (0.577-0.937)
Dominant model				
CC	222 (58.1)	253 (65.7)		1.0(Ref.)
CG+GG	160 (41.9)	132 (34.3)	0.01^{9}a	1.428 (1.060-1.92^3)[a]
Recessive model				
CG+CC	354 (92.7)	368 (95.6)		1.0(Ref.)
GG	28 (7.3)	17 (4.4)	0.077[a]	1.761 (0.940-3.298)[a]

[a]Adjusted for high blood pressure, diabetic diseases, smoking, and alcohol consumption

Table 4: The association of IL-6-572C/G polymorphic status with the apoplexy severity of CI

Variable	NIHSS,6 n=220 (%)	NIHSS>6 n=162 (%)	*P*-value	*OR (95%CI)*[a]
Age≥60 years, n (%)	119 (54.1)	89 (54.9)	0.869	
Female, n (%)	70 (31.8)	42 (25.9)	0.211	
Hypertension, n (%)	105 (47.7)	96 (59.3)	0.026	
Diabetes, n (%)	44 (20.0)	35 (21.6)	0.702	
Smoking, n (%)	73 (33.2)	64 (39.5)	0.203	
Alcohol consumption, n (%)	78 (35.5)	64 (39.5)	0.418	
rs1800796				
CC	137 (62.3)	85 (52.5)		1.0 (Ref.)
CG	69 (31.3)	63 (38.9)	0.066	1.511 (0.974-2.346)
GG	14 (6.4)	14 (8.6)	0.267	1.568 (0.708-3.469)
Dominance model				
CC	137 (62.3)	85 (52.5)		1.0 (Ref.)
CG+GG	83 (37.7)	77 (47.5)	0.048	1.521 (1.004-2.303)
Recessiveness model				
CG+CC	206 (93.6)	148 (91.4)		1.0 (Ref.)
GG	14 (6.4)	14 (8.6)	0.457	1.342 (0.618-2.916)

[a]Adjusted for hypertension

Table 5: The correlation of IL-6-572C/G polymorphic status with the recurrent risk of CI

Variable	ESRS<3 n=209 (%)	ESRS≥3 n=173 (%)	*P*-value	*OR (95%CI)*[a]
Age≥60 years, n (%)	103 (49.3)	105 (60.7)	0.026	
female, n(%)	65 (31.1)	47 (27.2)	0.401	
Hypertension, n (%)	99 (47.4)	102 (59.0)	0.024	
Diabetes, n (%)	34 (16.3)	45 (26.0)	0.019	
Smoking, n (%)	65 (31.1)	72 (41.6)	0.033	
Alcohol consumption, n (%)	72 (34.4)	70 (40.5)	0.226	
rs1800796				
CC	135 (64.6)	87 (50.3)		1.0(Ref.)
CG	63 (30.1)	69 (39.9)	0.055	1.553 (0.991-2.436)
GG	11 (5.3)	17 (9.8)	0.047	2.298 (1.010-5.227)
Dominant model				
CC	135 (64.6)	87 (50.3)		10(Ref.)
CG+GG	74 (35.4)	86 (49.7)	0.019	1.665 (1.089-2.545)
Recessive model				
CG+CC	198 (94.7)	156 (90.2)		1.0(Ref.)
GG	11 (5.3)	17 (9.8)	0.093	1.990 (0.893-4.438)

ESRS Essen Stroke Risk Score

[a]Adjusted for seniority, high blood pressure, diabetic diseases and smoking

The occurrence of CI is induced by various risk factors, which atherosclerosis is the most common.[10] Atherosclerosis is a slowly progressive inflammatory vascular disease, the inflammatory cell factor IL-6 is important for its development.[11] There are eight cytokine members in the classic IL-6 family, including: IL-6, IL-11, IL-27, OSM, LIF, CNTF, CT-1 and CLCF1, with IL-6 being a basic member of the family.[12,13] In the acute phase of CI, there were obvious pathophysiological changes in the cerebral ischemic tissue. Elevated levels of IL-6 in the cerebrospinal fluid and circulation, and the infiltration of IL-6 in glial cells and vascular endothelial cells in the ischemic area.[14] Researches have revealed that the IL-6 levels and the infarct size are positively correlated with neurological deficit, that is, the larger the infarct size, the more obvious the inflammatory reaction.[15] The disorder of IL-6 level leads to a complex inflammatory mechanism, which is pivotal for the CI etiopathogenesis.

The location of IL-6 lies at chromatosome 7p21, with a total length of 5kb, consisting of 5 extrons, 4 introns and a near promotor region. It is a precursor protein composed of 232 amino acids.[16] The promoter region of a gene is a DNA sequence located in the upstream region of the 5 'end of a structural gene. It can activate RNA polymerase to bind to template DNA accurately and has the specificity of transcription initiation. The change of promotors will affect the affinity with RNA polymerase, thus affecting the level of gene expression. There are many SNPs in the promotor area of human IL-6, which can regulate the transcription of IL-6 and lead to the change of IL-6 plasma concentration. Finally affecting the disease process and clinical outcomes. At present, about 50 SNPs have been recognized in the promoter region of IL-6 gene.[17] -174G/C, -597G/A polymorphisms have been studied more in Western countries, but the data in Asia are still scarce.[18] Previous studies have shown that -174G/C polymorphic status is related to diabetes, CHD and obesity.[7,19] There are some controversial results about the association between -174G/C and - 597G/A polymorphism and CI susceptibility.[20] In our study, results showed that the -174G/C and - 597G/A loci did not have gene polymorphism. Such difference may be related to race, region and sample size. There are many studies on IL-6-572C/G loci polymorphism in China and abroad, and the conclusions obtained from these studies are different. Previous studies have shown that GG genotype carriers at -572C/G loci are not only more likely to suffer from diabetes and coronary heart disease[21,22], but also participate in hypertension through gene mutation the regulation of IL-6, the stimulation of vascular remotensis and vasoconstriction, and increase angiotensin II concentration.[23] The relationship between -572C/G polymorphism and CI is controversial. There was a meta-analysis by Wang *et al*.[24] with 2,547 patients and 3,958 controls, the conclusion of which is that the -572C/G polymorphic status

is related to CI. However, another meta-analysis reported that the -572C/G polymorphic status may not be associated with change in CI risk.[25]

The result of this study showed that the mutated IL-6-572C/G was not only associated with CI susceptibility, but also with higher disease severity and the risk of recurrence. The reasons of which may be explained by: (a) IL-6 level disorder induces acute-phase proteins, the activation of VSM proliferative activities, the activated thrombocytes, and the ability to activate endothelial cells to participate in adipose activities and plaque generation. (b) IL-6 also impacts the endocranium -- it activates the hypothalamic-pituitary-adrenal axis. The stimulation of which is related to visceral obesity, high blood pressure.[26] It is directly or indirectly involved in the cerebral thrombosis. (c) In the acute phase of CI, many inflammatory cytokines including IL-6 are released. Continuous inflammatory reaction leads to the enlargement of infarct size and the aggravation of neurological impairment.[27] Finding a solution to cerebral infarction is still a challenge. Our study identified that the expressing of IL-6-572C/G could forecast greater CI relapse risk. The possible explanation of these results might be that -572C/G realized the upregulation of the level of proinflammation cell factors through the stimulation of local or systemic inflammatory reaction and immunoreaction to promote the progression and recurrence of CI.[28]

The limitations of this study are: First, inadequate number of patients. Second, the geographical area of the patient group was limited. Third, only three IL-6 gene polymorphisms were investigated, which failed to reflect the entire gene polymorphic status of IL-6. To further clarify the function of IL-6 genetic variations in the etiopathogenesis of CI, a larger sample size involving different ethnic groups, a wider geographical area, and large-scale prospective studies of IL-6 polymorphism are warranted to confirm these findings.

In conclusion, our findings showed that the IL-6-572C/G genetic polymorphism is correlated with CI susceptibility, acute neurological deficit and the risk of recurrence.

DISCLOSURE

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Conflict of interest: None

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