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Role of inflammatory parameters in the susceptibility of cerebral thrombosis

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ABSTRACT. We aimed to investigate the association of inflammation-related genes such as IL-10, IL-6 and IL-1B with risk of ischemic stroke. We included 426 cases with ischemic stroke and 426 health controls from Xinxiang, China. Genomic DNA was extracted from the buffy coat layer of collected blood with the TIANamp blood DNA kit. Diabetes, hypertension, obesity, and smoking habits were associated with risk of ischemic stroke. We found that individuals carrying the CC genotype of IL-1B rs1864169 had a higher risk of ischemic stroke when compared with the TT genotype (OR = 1.80, 95%CI = 1.16-2.80). The IL-6 rs1800796 TT genotype was associated with increased risk of ischemic stroke. We found that IL-1B rs1864169 and IL-6 rs1800796 polymorphisms may interact with diabetes, hypertension and obesity. Our study suggests that IL-6 rs1800796 and IL-1B rs1864169

polymorphisms are associated with ischemic stroke risk in the Chinese population.

Key words: Inflammatory parameters; Cerebral thrombosis; Polymorphism

INTRODUCTION

Ischemic stroke is a complex multi-factorial and polygenic disorder, which is influenced by an individual's genetic background and various environmental components. Various factors have been proven to influence ischemic stroke, such as hypertension, smoking, diabetes mellitus, body mass index, age, lipid metabolism, blood pressure regulation, and coagulation. However, the complex etiology of stroke suggests that individual genetic polymorphisms play an important role in the development of ischemic stroke (Dichgans and Markus, 2005). Inflammatory processes have an effect on the development of ischemic cerebrovascular disease and cerebral ischemia (Lindsberg and Grau, 2003). Proinflammatory cytokines are reported to have an effect on cerebral ischemia, and variations in cytokine genes play an important role in altering the transcription profile to induce predisposition and penetrance, changing the pattern of proinflammatory cytokine production (Hollegaard and Bidwell, 2006).

A previous meta-analysis report of six studies investigated the associations of 105 simple deletions and single nucleotide polymorphisms (SNPs) in inflammation- and cardiovascular system-related genes with the risk of ischemic stroke. Therefore, we aimed to conduct a case-control study to investigate the association of inflammation-related genes with risk of ischemic stroke, namely the genes for IL-10, IL-6 and IL-1B, and to determine the potential role of these genes in the etiology of stroke.

MATERIAL AND METHODS

Subjects

We analyzed 426 Chinese patients with the first onset of ischemic stroke, selected from the Third Affiliated Hospital of Xinxiang Medical University, Xinxiang, China. All patients were diagnosed by computed tomography or magnetic resonance imaging, according to the diagnostic criteria of ischemic stroke from the World Health Organization (rapidly developing clinical signs of focal or global disturbance of cerebral function lasting >24 h with no apparent cause but vascular origin). Patients with transient ischemic attacks, intracranial hemorrhage, postseizure palsy and brain tumor or brain trauma were excluded from our study. A total of 336 age- and gender-matched healthy controls were selected from patients who sought a health examination in our study. All patients and controls signed a written informed consent form. Our study was approved by the Ethics Committee of the Third Affiliated Hospital of Xinxiang Medical University.

A structured questionnaire was used to collect the general information from patients and controls, including age, gender, smoking and drinking habits, hypertension, diabetes, and obesity.

Genotyping assays

For genetic analysis, a peripheral venous blood sample was collected from each pa-

tient in a 2-mL EDTA anticoagulant tube. Genomic DNA was extracted from the collected blood using the buffy coat layer with a TIANamp blood DNA kit (Tiangen Biotech, China). Genotyping of IL-1B rs1864169, IL-6 rs1800795, IL-6 rs1800796 and IL-10 rs1800872 were conducted on a 384-well plate on the Sequenom MassARRAY platform, and polymerase chain reaction (PCR) and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry methods were used in our study. The primers of the seven SNPs were designed by the Sequenom Assay Design 3.1 software (Sequenom, San Diego, CA, USA). Each PCR (20 μL) contained 200 ng template DNA, 200 μM dNTP, 1 U Taq DNA polymerase and 200 μM primers, as well as 1.5 mM $MgCl_2$. The amplification cycles consisted of 1 min at 98°C for 3 min to activate Taq polymerase, and 40 cycles of denaturation at 95°C for 20 s, and annealing at 60°C for 60 s. PCR product was examined by 2% agarose gel electrophoresis.

Statistical analysis

Continuous variables are reported as means ± SD and analyzed using an independent sample *t*-test. Categorical variables are reported as number of subjects (%) and analyzed using a χ^2 test. The Hardy-Weinberg equilibrium evaluation and between-group comparison of genotype distribution were carried out using a χ^2 test. An odds ratio (OR) and 95% confidence interval (95%CI) were determined to compare the genotypes of seven SNPs in cases and control subjects. Multivariate logistic regression models were used to assess the role of seven SNP polymorphisms in ischemic stroke after adjusting for potential confounding factors. Two-sided probability was used for all statistical tests, and $P < 0.05$ was regarded as significant.

RESULTS

Distributions of the demographics for the cases and controls are summarized in Table 1. The mean age for the 426 cases and 426 controls was 46.4 ± 10.5 and 43.7 ± 9.7 years, respectively (Table 1). Diabetes, hypertension, obesity, and smoking habits were associated with risk of ischemic stroke, with ORs (95%CI) of 4.90 (2.47-10.60), 4.04 (2.74-6.03), 4.92 (2.66-9.67), and 1.44 (1.04-1.99), respectively.

The genotype distribution of seven SNPs involved in inflammatory processes in the control subjects did not significantly deviate from Hardy-Weinberg equilibrium. For the IL-1B rs1864169 polymorphism, we found a significant difference in the genotypic distributions between cases and controls ($P < 0.05$; Table 2). We found that individuals carrying the CC genotype of IL-1B rs1864169 had a higher risk of ischemic stroke when compared with the TT genotype (OR = 1.80, 95%CI = 1.16-2.80). Similarly, there was also a significant difference in the frequencies of IL-6 rs1800796 genotypes ($P < 0.05$), where those with the TT genotype of this gene were more likely to have a greatly increased risk of ischemic stroke (OR = 5.74, 95%CI = 2.59-14.6).

For further analysis, IL-1B rs1864169 and IL-6 rs1800796 were classified into three subgroups on the basis of the analysis of the demographics that could influence the risk of ischemic stroke (Table 3). Among patients with diabetes and obesity, we found the frequencies of TT, TC and CC genotypes of the IL-1B rs1864169 polymorphism were significantly different between cases and controls. Similarly, the distributions of GG, GT and TT genotypes of IL-6 rs1800796 were found to be significantly different between patients with diabetes, hypertension and obesity.

Table 1. Distributions of the demographic characteristics.

Variable	Cases (N = 426)	%	Controls (N = 426)	%	OR (95%CI)	P value
Age (means ± SD)	46.4 ± 10.5		43.7 ± 9.7			
Gender						
Men	250	58.7	236	55.5	-	-
Women	176	41.3	190	44.5	0.87 (066-1.16)	0.33
Diabetes						
No	377	88.5	415	95.1	-	-
Yes	49	11.5	11	4.9	4.90 (2.47-10.60)	<0.001
Hypertension						
No	293	68.7	383	89.9	-	-
Yes	133	31.3	43	10.1	4.04 (2.74-6.03)	<0.001
Obesity						
No	365	86.7	412	96.7	-	-
Yes	61	13.3	14	3.3	4.92 (2.66-9.67)	<0.001
Smoking						
Non-smoker	304	71.3	325	76.2	-	-
Current or ex-smoker	122	28.7	101	23.8	1.44 (1.04-1.99)	0.02
Drinking						
No	360	84.5	377	98.4	-	-
Yes	66	15.5	49	11.6	1.41 (0.93-2.14)	0.08

Table 2. Genotype frequencies of polymorphisms of seven genes among cases and controls and OR (95%CI) in patients.

SNP		Cases (N = 426)	%	Controls (N = 426)	%	OR (95%CI)	P value
IL-18	GG	194	45.5	202	47.4	-	
rs187238	GC	188	44.2	186	43.6	1.05 (0.78-1.41)	0.72
	CC	44	10.3	38	9	1.21 (0.73-2.01)	0.45
IL-18	CC	130	30.5	141	33.1	-	
rs1946518	CA	227	53.3	224	52.6	1.10 (0.80-1.50)	0.54
	AA	69	16.2	61	14.3	1.23 (0.79-1.91)	0.34
IL-1B	TT	144	33.7	176	41.3	-	
rs1864169	TC	207	48.6	199	46.7	1.27 (0.94-1.72)	0.11
	CC	75	17.7	51	12	1.80 (1.16-2.80)	0.005
IL-1B	TT	278	65.3	296	69.5	-	
rs3136558	TC	116	27.2	108	25.4	1.15 (0.83-1.58)	0.39
	CC	32	7.5	22	5.1	1.56 (0.85-2.87)	0.13
IL-6	TT	215	50.5	233	54.7	-	
rs1800795	TC	163	38.3	161	37.8	1.09 (0.82-1.48)	0.53
	CC	48	11.2	32	7.5	1.63 (0.97-2.73)	0.05
IL-6	GG	265	62.1	304	71.3	-	-
rs1800796	GT	121	28.4	114	26.8	1.22 (0.89-1.67)	0.20
	TT	40	9.5	8	1.9	5.74 (2.59-14.6)	<0.001
IL-10	AA	199	46.8	193	45.4	-	-
rs1800872	AC	172	40.3	167	39.2	1.0 (0.74-1.35)	0.99
	CC	55	12.9	66	15.4	0.81 (0.52-1.24)	0.31

Table 3. Subgroup analysis of IL-1B rs1864169 and IL-6 rs1800796 polymorphisms with risk of ischemic stroke.

Subgroup		IL-1B rs1864169				IL-6 rs1800796			
		TT	TC	CC	P value[1]	GG	GT	TT	P value[1]
Gender	Men	82	124	45	-	156	70	24	-
	Women	63	83	31	0.37	109	51	16	0.43
Diabetes	No	138	184	56	-	253	101	23	-
	Yes	6	23	19	0.01	12	20	17	0.02
Hypertension	No	96	144	53	-	193	79	21	-
	Yes	48	63	22	0.007	72	42	19	0.004
Obesity	No	133	173	59	-	178	68	19	-
	Yes	11	34	16	0.005	87	53	21	0.002
Smoking	Non-smoker	103	148	53	-	166	73	26	-
	Current or ex-smoker	41	59	22	0.23	99	48	14	0.16

[1]Compared with controls.

DISCUSSION

To our knowledge, we provide the first report of an association between variants in the seven inflammation-related genes with risk of cerebral thrombosis, and report that common variations of IL-6 and IL-1B were associated with risk of ischemic stroke. The genotype frequencies in our study are very consistent with the ones reported in controls from the Chinese population of previous studies (Tong et al., 2010; Ye et al., 2012). Our results showed that the polymorphisms of IL-6 rs1800796 and IL-1B rs1864169 were significantly associated with the risk of ischemic stroke. The findings suggest the functional promoter polymorphisms of IL-6 and IL-1B genes may be useful as genetic susceptibility markers for ischemic stroke.

The IL-6 gene, which is located on chromosome 7p21 in humans, consists of 5 exons, 4 introns and a proximal promoter region (Georges et al., 2001). Previous studies have reported that rs1800795 and rs1800796, which are two promoter polymorphisms, are associated with various diseases, such as ischemic stroke and systemic-onset juvenile chronic arthritis (Fishman et al., 1998; Terry et al., 2000; Tong et al., 2010). We found that the IL-6 rs1800796 TT genotype was greatly associated with risk of ischemic stroke, and that the IL-6 rs1800795 CC genotype was marginally associated with risk of ischemic stroke. Our finding is consistent with previous studies conducted in Chinese, Japanese, American, and British populations (Humphries et al., 2001; Yamada et al., 2006; Podgoreanu et al., 2006; Tong et al., 2010). This finding suggests that in individuals carrying the TT genotype of IL-6 rs1800796, IL-6 transcription can be influenced through a complex interaction, and a previous study indicated that the T allele of rs1800796 was associated with increased transcription efficiency of IL-6 (Terry et al., 2000). However, some studies have reported conflicting results, even in the same population (Nauck et al., 2002; Basso et al., 2002). The inconsistency of these results may be due to ethnicities, sample size and case selection.

The interleukin-1B gene, which is located on chromosome 2q14, is approximately 7 kb long with 7 exons, and plays a key role in acute and chronic inflammation involved in atherosclerosis and the development and prognosis of ischemic stroke (Jander et al., 1998). Our study found that the IL-1B rs1864169 polymorphsim was associated with slight increased risk of ischemic stroke. Previous evidence supports our findings. IL-1B rs1864169 and rs3136558 have been indicated in various cerebrovascular phenotypes, such as stroke, ischemic injury and aneurysmal rupture (Boutin et al., 2001; Iacoviello et al., 2005; Slowik et al., 2006). These findings suggest that the IL-1B polymorphism is involved in the development of ischemic stroke.

In conclusion, this study suggests that the polymorphisms in IL-6, rs1800796, and IL-1B, rs1864169, are associated with ischemic stroke risk in the Chinese population. However, we did not find that the variations in IL-18 and IL-10 were associated with risk of ischemic stroke. It is difficult to draw a solid conclusion due to the relatively small sample size, and a further large sample size study is greatly warranted.

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