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Correlation between *interleukin-18* promoter -607C/A polymorphism and susceptibility to ischemic stroke

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Abstract

Single nucleotide polymorphisms in the promoter region of *interleukin-18* (*IL-18*), an inflammatory cytokine, have been linked to susceptibility to many diseases, including cancer and immune dysfunction. Here, we explored the potential association between the *IL-18* -607C/A (rs1946518) promoter region polymorphism and susceptibility to ischemic stroke (IS). This locus was amplified from peripheral blood samples of 386 IS patients (cases) and 364 healthy individuals (controls) by the polymerase chain reaction with sequence-specific primers. Significant differences were observed by the χ^2 test in the -607C/A (rs1946518) genotype and allele frequencies between cases and controls ($P < 0.05$). Furthermore, after excluding for age, gender, smoking status, and hypertension, logistic regression indicated that IS susceptibility of -607C carriers increased 1.6 times (OR = 1.601, 95%CI = 1.148-2.233, P = 0.006) compared to -607A carriers. Additionally, similar increases in IS risk were noted for male patients or patients less than 65 years old. In conclusion, *IL-18* -607C/A (rs1946518) promoter polymorphism is associated with IS susceptibility, and the C allele may confer increased IS risk.

Key words: IL-18; Polymorphism; Ischemic stroke; Correlation

Introduction

Ischemic stroke (IS) is characterized by local brain tissue disintegration or destruction resulting from sudden reduced arterial perfusion of the local blood supply, completely interrupted blood flow, or inadequate oxygen or sugar supply (1). Risk factors for IS include diabetes, high cholesterol, and high blood pressure (1). Indeed, IS is a major cause of morbidity and mortality worldwide. While the etiology of stroke has not been completely defined, pathological studies have demonstrated the roles of atherosclerosis, particularly the inflammatory cytokines and the responses involved in arterial injury, in the pathogenesis of ischemic events (2,3).

Interestingly, susceptibility to IS has been associated with polymorphisms, particularly single nucleotide polymorphisms (SNPs), in the genes producing cytokines like IL-6, TGF-β_1, and TNF-α (4-6). IL-18, a member of the interleukin-1 family, is a pleiotropic pro-inflammatory cytokine (7) that functions in the inflammatory response (8). IL-18 stimulates cell-mediated immunity and induces natural killer and T cells to release interferons (7). It can also induce severe inflammatory reactions, leading to disease processes. In patients experiencing IS, peripheral blood levels of IL-18 have been found to be significantly higher compared to healthy individuals (9,10). Specifically, an SNP in the IL-18 promoter (designated -607C/A) has been associated with the development of cardiovascular disease (11,12), which can include vascular endothelial damage and formation of atherosclerosis, processes that can induce stroke. This polymorphism affects the expression and activity of IL-18 (13-15), thereby offering a potential mechanistic basis leading to increased susceptibility to cardiovascular disease. To determine whether alteration in IL-18 may also affect susceptibility to IS, we investigated whether the IL-18 promoter -607C/A (rs1946518) SNP is associated with increased incidence of IS.

Material and Methods

Subjects

This prospective clinical study identified 386 IS patients who received treatment in the Neurology

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Department, the First Hospital of Yancheng City (Yancheng, China). IS was confirmed in all patients by clinical diagnosis, radiological examination, cardiac function test, and supersonic examination and met WHO Diagnostic Criteria for Stroke (16). Patients with the following diseases were excluded: diabetes, liver or kidney dysfunction, myocardial infarction, asthma, cancer, peripheral vascular disease, or a history of cardiovascular surgery. An additional 364 healthy individuals who received physical examination in our hospital during the same period of time were selected as the control group. These individuals were ⩾40 years old and exhibited no history of diabetes, liver or kidney disease, asthma, or cerebrovascular disease. Smoking history was obtained and was defined as at least 1 cigarette daily for more than 6 months. The study protocols were approved by the Ethics Committee of Yancheng Health Vocational and Technical College and all participants gave written informed consent to participate.

DNA extraction

Venous blood (3 mL) was collected in the early morning from each fasting patient and placed in a 2.5% EDTA anticoagulant tube. DNA was extracted using a genomic DNA extraction kit (Takara, Japan) according to manufacturer instructions. Extracted DNA samples were preserved at -70°C.

Detection of *IL-18* promoter polymorphism

PCR primers used to amplify the promoter region of the *IL-18* gene were designed with the Primer-5 software according to a previous study (17) and were synthesized by Sangon Biological Engineering (China). Specific upstream primers were used to detect the C allele (5′-GTTGCAGAAAGTGTAAAAATTATTAC-3′) or the A allele (5′-GTTGCAGAAAGTGTAAAAATTATTAA-3′). Other primers included a forward primer for internal reference (5′-CTTTGCTATCATTCCAGGAA-3′) and a downstream primer (5′-TAACCTCATTCAGGACTTCC-3′). The reaction mix (total volume, 50 μL) contained 50 ng template DNA, 5 μL 10X PCR buffer, 2.5 U Taq enzyme, 4 μL dNTP mixture, 5 μL $MgCl_2$, 0.3 μM each of the two specific primers, and 0.6 μM forward and downstream primers. PCR cycling conditions were 94°C for 4 min; 8 cycles of 94°C for 40 s, 64°C for 40 s, and extension at 72°C for 40 s; 30 cycles of 94°C for 40 s, 56°C for 40 s, and 72°C for 40 s; 72°C for 5 min. Amplified products were detected by separation on 2% agarose gel with ethidium bromide staining.

Statistical analysis

Hardy-Weinberg equilibrium analysis was used to detect group representativeness of *IL-18* alleles in patient and control groups. The SPSS17.0 statistical software was applied for statistical analysis. The two-sample *t*-test was used to compare difference in age between subjects younger than 65 years old and older than 65 years; the χ^2 test was used to compare age, gender, smoking status, high blood pressure, and genotype and allele frequencies between the two groups. The relationship of gene polymorphism and IS was analyzed by the odds ratio (OR) and its 95% confidence interval (95%CI) was obtained by logistic regression. All analyses were two-sided and $P < 0.05$ was considered to be statistically significant. The statistical power analysis was performed using the QUANTO software.

Results

Study population

The clinical and demographic information about the study subjects, specifically age, gender, smoking status, and blood pressure, is shown in Table 1. No statistical difference was observed in age or gender distribution between the control and patient groups. However, significantly higher proportions of IS patients smoked or had hypertension compared to the control group (28.8 and 74.6% *vs* 12.9 and 36.3%, respectively; $P < 0.05$). Our data showed statistical power to detect association (86.33%) in this sample.

IL-18 gene promoter -607C/A (rs1946518) polymorphism

PCR amplification of the -607C/A (rs1946518) locus in the *IL-18* gene promoter produced both a locus-specific product of 196 bp and an internal reference product of 301 bp. The two alleles, C and A, of this locus were amplified using primers specific for these alleles (Figure 1), allowing us to distinguish CC, CA, and AA genotypes.

Hardy-Weinberg analysis indicated that genotype distributions for this locus were in equilibrium for both the control and patient groups ($P > 0.05$). Statistically significant differences in genotype and allele frequencies were detected for the *IL-18* promoter -607C/A (rs1946518) locus between the control and patient groups (both $P < 0.05$, Table 2). Additionally, genotype and allele frequencies were significantly different between male patients and male controls as well as between patients and controls <65 years old (both $P < 0.05$). No differences were noted for female patients versus female controls or for patients versus controls ⩾65 years old (both $P > 0.05$).

Relationship between genotype and IS

Further analysis of this SNP by logistic regression showed that, after excluding the impact of age, gender, smoking status, and high blood pressure, IS risk was increased 1.6 times in patients carrying the -607C allele compared to patients carrying the -607A allele (OR = 1.601, 95%CI = 1.148-2.233, $P < 0.01$; Table 3). Similarly, increased risk was noted for male patients and patients <65 years old ($P < 0.05$).

Table 1. Demographic and clinical characteristics of patients with ischemic stroke and controls.

Variable	Cases (n = 386)	Controls (n = 364)	t/χ^{2}*	P
Average age (years)	65.7 ± 8.8	64.6 ± 9.9	1.604	0.109
Age [n (%)]				
<65	159 (41.2)	163 (44.8)	0.985	0.321
⩾65	227 (58.8)	201 (55.2)		
Gender [n (%)]				
Male	270 (69.9)	233 (64.0)	2.990	0.084
Female	116 (30.1)	131 (36.0)		
Smoking status [n (%)]				
Smokers	111 (28.8)	47 (12.9)	28.283	0.001
Nonsmokers	275 (71.2)	317 (87.1)		
Hypertension [n (%)]				
Yes	288 (74.6)	132 (36.3)	111.806	0.001
No	98 (25.4)	232 (63.7)		

Data are reported as means ± SD. *t value for average age and χ^{2} value for other variables. The two-sample t-test was used to compare the average ages between cases and controls. The χ^{2} test was used to compare the difference in age between subjects younger than 65 years and older than 65 years, gender, smoking status, and high blood pressure between the two groups.

Discussion

SNPs are commonly found in humans, with an incidence of 1% in the whole population (18). These genetic variations are influenced by many factors, including race and environment. Identifying relationships between SNPs and disease pathology is critical for the development of novel treatment and preventive measures for a variety of human diseases.

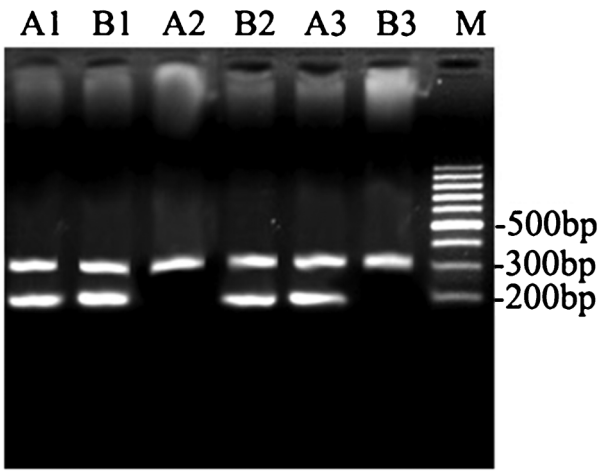

Figure 1. PCR amplification with specific primers for the identification of *IL-18* promoter -607C/A (rs1946518) polymorphisms in ischemic stroke patients and healthy controls. Samples 1, 2, and 3 were amplified with common downstream primers and internal reference upstream primers; samples A1, A2, and A3 were amplified with specific upstream primers (for the C allele); samples B1, B2, and B3 were amplified with specific upstream primers (for the A allele). The genotypes of samples 1, 2, and 3 were CA, AA, and CC, respectively. *Lane M* = DNA molecular weight marker.

The contribution of SNPs in *IL-18* to variations in expression and activity of this inflammatory cytokine has become important for the understanding of diseases related to immune function. Many studies have cloned and analyzed the promoter region of *IL-18* to characterize gene expression and regulation (13). By means of various analyses, several SNPs have been identified in the promoter region of *IL-18* exon 2, at -137, -607, and -656 loci (19). The -607C/A (rs1946518) variation is located in the binding region of nuclear factor cAMP-response element binding protein and histone H4 transcription factor (8). The SNP affects biological functions of IL-18, and, thus, this locus is currently the most widely studied IL-18 polymorphism (8). Recent studies of the *IL-18* polymorphism have focused on allergic diseases, viral infections, autoimmune diseases, and cancers. Results indicate that the -607C/A (rs1946518) locus is correlated with allergic asthma, allergic rhinitis, nasopharyngeal cancer, chronic hepatitis B virus, and human immunodeficiency virus infection, among other diseases (20-24). Previously, the association of two functional polymorphisms in the IL-18 promoter, -607C/A (rs1946518) and -137G/C (rs187238), with the risk of IS was investigated in a Han Chinese population, and the results revealed that the -607C allele was associated with an increased risk of IS and the presence of the -137G allele was correlated with an increased risk of IS in the subtype of patients with large artery atherosclerosis (25). However, the study did not present results indicating an effect of age or gender.

Here, we further investigated the correlation between variants of *IL-18* and risk of IS. Both the C and A alleles of the *IL-18* promoter region -607 locus were detected in IS patients and healthy controls, and statistically significant

Table 2. Genotype and allele frequencies for the *IL-18* -607C/A (rs1946518) locus in patients with ischemic stroke and controls.

Variable	n	Genotype					Allele			
		CC	CA	AA	χ^2	P	C	A	χ^2	P
All										
Cases	386	116 (30.1)	188 (48.7)	82 (21.2)	7.945	0.019	420 (54.4)	352 (45.6)	6.267	0.012
Controls	364	77 (21.2)	195 (53.6)	92 (25.3)			349 (47.9)	379 (52.1)		
Age <65 years										
Cases	159	93 (58.5)	49 (30.8)	17 (10.7)	16.064	0.001	235 (73.9)	83 (26.1)	13.803	0.001
Controls	163	59 (36.2)	78 (47.9)	26 (16.0)			196 (60.1)	130 (39.9)		
Age ⩾65 years										
Cases	227	23 (10.1)	139 (61.2)	65 (28.6)	0.932	0.628	185 (40.7)	269 (59.3)	0.645	0.422
Controls	201	18 (9.0)	117 (58.2)	66 (32.8)			153 (38.1)	249 (61.9)		
Male										
Cases	270	97 (35.9)	142 (52.8)	31 (11.3)	6.207	0.045	336 (62.2)	204 (37.8)	5.484	0.019
Controls	233	63 (27.0)	130 (55.8)	40 (17.2)			256 (54.9)	210 (45.1)		
Female										
Cases	116	19 (16.4)	46 (39.7)	51 (44.0)	3.120	0.210	84 (36.2)	148 (63.8)	0.027	0.869
Controls	131	14 (10.7)	65 (49.6)	52 (39.7)			93 (35.5)	169 (64.5)		

Data are reported as n with the percent in parentheses. The χ^2 test was used to compare genotype and allele frequencies between the two groups.

Table 3. Association between the *IL-18* promoter -607C/A (rs1946518) polymorphism and ischemic stroke (IS).

Variable	B	SE	Wald	OR	95%CI	P
All	0.471	0.170	7.699	1.601	1.148-2.233	0.006
Age <65 years	0.910	0.229	15.776	2.484	1.585-3.891	0.001
Age ⩾65 years	0.136	0.331	0.170	1.146	0.599-2.192	0.680
Male	0.414	0.195	4.531	1.513	1.033-2.215	0.033
Female	0.493	0.378	1.700	1.637	0.780-3.434	0.192

The relationship of gene polymorphism and IS was analyzed by odds ratio (OR) and its 95% confidence interval (CI) was obtained by logistic regression.

differences in genotype and allele frequency were detected between these populations. Additionally, genotype and allele frequencies differed between patients and controls for males and for those <65 years of age. IS risk was increased 1.6 times in patients carrying -607C compared to patients carrying -607A after excluding for age, gender, smoking status, and hypertension. These results indicate an association between the -607C/A (rs1946518) polymorphism and the occurrence of cerebral infarction; individuals carrying the A allele have a higher risk of IS. The C allele may confer increased risk by increasing the expression of IL-18, and highly expressed IL-18 can increase the risk of IS. Whether a difference in protein expression is present in stroke patients with the C allele versus those with the A allele requires further investigation.

In summary, these results show that the *IL-18* promoter polymorphism is correlated with susceptibility to IS and that the C allele at the -607 locus confers increased risk of IS. These findings add to the body of evidence demonstrating a role for IL-18 in disease susceptibility and pathology. Additional research will provide a detailed understanding of the pathological mechanisms linking IL-18 to IS.

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