Genetics and Molecular Research

Association of the interleukin-10 gene -1082A/G genetic polymorphism with risk of ischemic stroke in a Chinese population

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Genet. Mol. Res. 15 (1): gmr.15017541
Received August 27, 2015
Accepted December 4, 2015
Published March 31, 2016
DOI http://dx.doi.org/10.4238/gmr.15017541

ABSTRACT. We investigated the possible association between two single nucleotide polymorphisms of *IL10* (-1082A/G and -592C/A) and susceptibility to ischemic stroke. In total, 335 patients with proven ischemic stroke and 335 control subjects were recruited from Xinxiang Central Hospital between March 2013 and May 2015. The *IL10* -1082A/G and -529C/A polymorphisms were investigated by polymerase chain reaction-restriction fragment length polymorphism. When compared with the control subjects, patients with ischemic stroke were more likely to be male, have a habit of tobacco smoking, have higher BMI, have hypertension or diabetes mellitus, and have higher levels of TC, LDL-C, HDL-C, and TG. The multivariate logistic regression analyses revealed that the AA genotype of *IL10* -1082A/G was significantly associated with development of ischemic stroke in a Chinese population compared with the GG genotype (OR = 1.93, 95%CI = 1.15-3.25). In the dominant model, the association between GA+AA genotype of *IL10* -1082A/G and risk of ischemic stroke was also significant compared with the GG genotype, and the adjusted OR (95%CI) for the GA+AA genotype was 1.41 (1.02-1.94). Thus, our study suggests that *IL10* gene polymorphisms contribute to the development of ischemic stroke.

Key words: *IL10* -1082A/G; *IL10* -592C/A; Polymorphism; Ischemic stroke

INTRODUCTION

Ischemic stroke is one of the leading causes of morbidity and mortality worldwide, and the mortality rate arising from this disorder has recently increased. The pathological progress of ischemic stroke involves many factors and complex processes, and has many contributory factors such as type 2 diabetes, hypertension, hyperlipidemia, arterial fibrillation, diabetes, family history of ischemic stroke, sleep apnea syndrome, overweight, lack of physicial exercise, and smoking habits (Meschia et al., 2014). However, not all those exposed to potential risk factors for ischemic stroke develop the disease, which suggest that genetic factors may influence the pathological progress of ischemic stroke. It has been reported that certain genes have polymorphisms that may affect the pathological process of ischemic stroke; these genes include the phosphodiesterase 4D gene, matrix metalloproteinase-3 gene, the interleukin-1β gene (*IL1*β), *IL-6*, *IL18*, the receptor gene, and pre-microRNA-146a (Bao et al., 2015; Chehaibi et al., 2015; Kumar et al., 2015; Shi et al., 2015; Tang et al., 2015).

Interleukin-10 (IL-10) is an immunoregulatory cytokine that is secreted mainly from monocytes and type 2 T helper cells, which comprise a recently discovered lineage of T cells. The gene encoding IL-10 (*IL10*) is present on the chromosomal locus 1q31-32, and several genome regions of chromosome 1 (1q21-23, 1q31-32 and 1q42-44) have been observed to be correlated with development of several kinds of diseases, such as diabetes mellitus, peptic ulcer disease, coronary artery disease, leprosy, chronic hepatitis B infection, and oral cancer (Alvarado-Arnez et al., 2015; Ghaleh Baghi et al., 2015; Hsu et al., 2015; Khodaeian et al., 2015; Miftahussurur and Yamaoka, 2015; Yang et al., 2015). The authors of previous researches have reported the correlation of *IL10* gene polymorphisms with the ischemic stroke risk; however, the results are inconsistent (Munshi et al., 2010; Sultana et al., 2011; Xie et al., 2013; Qi et al., 2014). In the present study, we conducted a case-control study to assess the role of two SNPs of *IL10* (*IL10* -1082A/G and -592C/A) in the ischemic stroke risk.

MATERIAL AND METHODS

Study subjects

Between January 2013 and January 2015, 335 ischemic stroke patients were collected from Xinxiang Central Hospital. Ischemic stroke was confirmed by computed tomography or magnetic resonance imaging according to the World Health Organization's diagnostic criteria for ischemic stroke.

In total, 335 randomly selected individuals who had undergone a regular health examination in Xinxiang Central Hospital during the same period were used as controls. The control subjects had no history of ischemic stroke. The exclusion criteria of this study were individuals who were diagnosed with transient ischemic attacks, intracranial hemorrhage, brain tumors, brain trauma, severe liver disease, or renal failure.

The demographic characteristics of the included subjects were collected using an *ad hoc* questionnaire, and comprised gender, age, BMI, alcohol consumption, tobacco consumption, history of type 2 diabetes, and history of hypertension. Fasting blood sample (5 mL) was collected from each subject for obtaining the clinical characteristics. The clinical variables of the included subjects comprised TC, TG, LDL-C, and HDL-C levels.

Both patients and control subjects provided and signed a written informed consent before participating into this study. This study has been approved by the Institutional Research Ethics Committee of Xinxiang Central Hospital.

DNA extraction and genotyping

Peripheral venous blood (5 mL) was collected from each study subject using ethylenediaminetetraacetic acid (EDTA)-coated tubes to avoid coagulation and stored at -20°C until analyzed. Isolation of DNA for genotyping was carried out using a TIANamp Blood DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer instructions. Amplification and identification of *IL10* -1082A/G and -592C/A polymorphisms were carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The forward and reverse primers for *IL10* -1082A/G were 5'-CTACTAAGGCTTCTTTGGGAA-3' and 5'-CAGTG CCAACTGAGAATTTGG-3', respectively; and the forward and reverse primers for *IL10* -592C/A were 5'-GTGAGCACTACCTGACTAGC-3' and 5'-CCTAGGTCACAGTGACGTGG-3', respectively. The PCR was conducted in a 25-μL reaction solution with 25 mM $MgCl_2$, each primer and 2 mM deoxynucleotide triphosphates, 1 mM $MgCl_2$, 1.25 U Taq polymerase and 5X PCR buffer. The PCR conditions were as follows: 95°C for 5 min, 35 cycles of 95°C for 40 s, 63°C for 60 s, and 72°C for 40 s, and a final extension step of 72°C for 10 min. The restriction enzymes for *IL10* -1082A/G and -592C/A were *Bse*RI and *Rsa*I, respectively. The products of PCR were confirmed on 2% agarose gel stained with ethidium bromide under ultraviolet light. The A allele of *IL10* -1082A/G showed a product size of 139 bp, and the G allele showed 106 and 33 bp. The C allele of *IL10* -592C/A presented product size of 412 bp, and the A allele showed 175 and 237 bp.

Statistical analysis

Differences in the demographic and clinical characteristics between patients with ischemic stroke and the controls were analyzed by the Student *t*-test and the chi-square (χ^2) test. The Hardy-Weinberg Equilibrium (HWE) for any deviation from expected allele frequencies in the control subjects were tested by using goodness-of-fit χ^2 test. Association between *IL10* -1082A/G and -592C/A polymorphisms and ischemic stroke risk was assessed by computing the odds ratios (ORs) and 95% confidence intervals (95%CI), using multiple-logistic regression analyses.

Univariate and multivariate logistic regression analyses were done by using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). A P value <0.05 at 95%CI was taken as statistically significant.

RESULTS

Using the chi-square test, no significant difference was observed between patients with ischemic stroke and the control subjects in terms of age or alcohol consumption ($P < 0.05$; Table 1). When compared with the control subjects, patients with ischemic stroke were more likely to be male, have higher BMI, have a habit of smoking tobacco, suffer from hypertension or diabetes mellitus, and have higher levels of TC, LDL-C, HDL-C, and TG (all P values <0.05).

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

Variables	Patients	%	Controls	%	χ^2 test or *t*-test	P value
Age (years)						
<60	176	52.54	178	53.13		
≥60	159	47.46	157	46.87	0.02	0.88
Gender						
Female	230	68.66	199	59.40		
Male	105	31.34	136	40.60	6.23	0.01
BMI						
<24	209	62.39	135	40.30		
≥24	126	37.61	200	59.70	32.72	<0.001
Alcohol consumption						
Never	151	45.07	173	51.64		
Sometimes	184	54.93	162	48.36	2.89	0.09
Tobacco consumption						
Never	110	32.84	176	52.54		
Sometimes	225	67.16	159	47.46	26.57	<0.001
Hypertension						
No	210	62.69	247	73.73		
Yes	125	37.31	88	26.27	9.42	0.002
Diabetes mellitus						
No	242	72.24	300	89.55		
Yes	93	27.76	35	10.45	32.49	<0.001
TC (mM)	4.65 ± 1.02		4.43 ± 0.99		2.83	0.002
LDL-C (mM)	2.36 ± 0.47		2.15 ± 0.45		5.91	<0.001
HDL-C (mM)	1.27 ± 0.25		1.16 ± 0.21		6.17	<0.001
TG (mM)	2.40 ± 1.12		2.08 ± 1.10		3.73	<0.001

TC = total cholesterol; LDL-C = low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG = triglycerides.

Before carrying out the association analysis, the genotype distributions of controls were tested for HWE. The genotype distributions of *IL10* -1082A/G and -592C/A did not deviate from the HWE (P values were 0.84 and 0.43, respectively; Table 2). The multivariate logistic regression analyses revealed that the AA genotype of *IL10* -1082A/G was significantly associated with development of ischemic stroke in a Chinese population compared with the GG genotype (OR = 1.93, 95%CI = 1.15-3.25). In the dominant model, the association between GA+AA genotype of *IL10* -1082A/G and risk of ischemic stroke was also significant compared with the GG genotype, and the adjusted OR (95%CI) for the GA+AA genotype was 1.41 (1.02-1.94). However, no significant association was observed between the *IL10* -592C/A gene polymorphism and ischemic stroke risk.

Table 2. Association between *IL10* -1082A/G and -592C/A genetic polymorphisms and the risk of ischemic stroke.

IL10 gene	Patients	%	Controls	%	P value for HWE	OR (95%CI)[1]	P value
-1082A/G							
Codominant							
GG	130	38.81	158	47.16		1.0 (Ref.)	-
GA	151	45.07	143	42.69		1.28 (0.91-1.80)	0.13
AA	54	16.12	34	10.15	0.84	1.93 (1.15-3.25)	0.01
Dominant							
GG	130	38.81	158	47.16		1.0 (Ref.)	-
GA+AA	205	61.19	177	52.84		1.41 (1.02-1.94)	0.03
-592C/A							
Codominant							
CC	104	31.04	116	34.63		1.0 (Ref.)	-
CA	113	33.73	111	33.13		1.14 (0.77-1.68)	0.5
AA	43	12.84	33	32.24	0.43	1.45 (0.83-2.55)	0.16
Dominant							
CC	104	31.04	116	34.63		1.0 (Ref.)	-
CA+AA	156	68.96	144	65.37		1.21 (0.84-1.74)	0.29

[1]Adjusted for gender, age, BMI, hypertension, diabetes mellitus, tobacco consumption, TC, LDL-C, HDL-C, and TG. HWE = Hardy-Weinberg equilibrium; OR = odds ratio; CI = confidence interval.

The interaction between the *IL10* -1082A/G gene polymorphism and the environmental factors for the ischemic stroke risk was shown in Table 3. However, no significant association was found between the *IL10* -1082A/G gene polymorphism and the demographic characteristics of ischemic stroke risk.

Table 3. Association between the *IL10* -1082A/G polymorphism and the development of ischemic stroke stratified by demographic characteristics.

	Patients		Controls		OR (95%CI)	P value
Variables	GG	GA+AA	GG	GA+AA		
Gender						
Female	89	141	92	107	1.36 (0.91-2.04)	0.12
Male	41	64	66	70	1.47 (0.85-2.55)	0.14
Hypertension						
No	87	123	121	126	1.36 (0.92-2.00)	0.11
Yes	43	82	37	51	1.38 (0.76-2.52)	0.26
Diabetes mellitus						
No	94	148	139	161	1.36 (0.95-1.95)	0.08
Yes	36	57	19	16	1.88 (0.80-4.45)	0.11
Tobacco consumption						
Never	42	68	81	95	1.38 (0.83-2.31)	0.19
Sometimes	88	137	77	82	1.46 (0.95-2.25)	0.07

OR = odds ratio; CI = confidence interval.

DISCUSSION

Genetic susceptibility to disease has generated interest in the gene polymorphisms involved in several kinds of disorders. IL-10 is a pleiotropic cytokine, and a wealth of evidence supports its regulatory role in the pathology process of several kinds of diseases (Alvarado-Arnez et al., 2015; Ghaleh Baghi et al., 2015; Hsu et al., 2015; Khodaeian et al., 2015). In addition, experimental studies indicated that IL-10 expression could maintain the balance between pro-inflammatory and anti-inflammatory stimuli in the process of cerebrovascular disease. It is therefore rational to consider the *IL10* gene as a susceptibility candidate for cerebrovascular disease. In our study, we observed that the AA genotype of *IL10* -1082A/G is associated with the risk of ischemic stroke in a Chinese population.

The *IL10* gene is reported to be association with the regulation of the complex network of reactions involved in cerebral ischemia. The level of *IL10* gene expression changes with neurological deterioration, and functional polymorphisms might alter the anti-inflammatory process. The authors of previous experimental study have reported that acute phase *IL10* concentration was significantly higher in patients with high risk of cardiogenic stroke (Arponen et al., 2015). Liang et al. (2015) reported that transduction of the *IL10* gene in the cerebral artery before ischemia attenuates brain injury induced by ischemia in rats. Tukhovskaya et al. (2014) indicated that the anti-inflammatory cytokine *IL10* plays an important role in exerting the rapid neuroprotective effects by transcription-independent modulation of ischemia-induced intracellular Ca^{2+} responses. Thus, the expression of *IL10* gene is associated with the pathologic process of ischemic stroke.

The authors of previous studies have reported an association between *IL10* gene polymorphisms and the development of cerebrovascular diseases; however, the results are inconsistent (Balding et al., 2004; Trompet et al., 2007; Munshi et al., 2010; Sultana et al., 2011; Xie et al., 2013; Chao et al., 2014). Balding et al. (2004) found that *IL10* gene polymorphisms were not associated with ischemic stroke. Munshi et al. (2010) conducted a study in Caucasians and revealed that individuals bearing A allele of *IL10* -1082A/G gene were more predisposed to

stroke. Xie et al. (2013) reported that the individuals carring *IL10* rs1800872 and rs3021094 gene variant exhibited a significantly association with risk of ischemic stroke in a Chinese population. A recent meta-analysis pooled 16 studies and revealed that the AA genotype of *IL10* -1082A/G is associated with the risk of ischemic stroke (Chao et al., 2014). In the present study, we found that the AA genotype of *IL10* -1082A/G is associated with the risk of ischemic stroke, which is in line with previous studies.

Two limitations must be noted. First, to some extent due to hospital-based controls, selection bias cannot be ruled out. To limit the potential selection bias, we recruited the samples by matching the controls to the cases based on age and sex. Two, the sample size of our study was relatively small, which may have limited the statistical power to determine differences between groups and could explain our failure to find an association with the IL10 -819T/C polymorphism.

In conclusion, our research provides evidence that that the IL10 -1082A/G gene polymorphism contributes to the development of ischemic stroke. These findings need to be validated in larger, preferably population-based, studies including different ethnic groups.

Conflicts of interest

The authors declare no conflict of interest.

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