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Original Article

Association between IL-10 gene polymorphisms and the risk of ischemic stroke in a Chinese population

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Abstract: We investigated the possible association between two SNPs of *IL-10* (*IL-10 -1082A/G* and *-819T/C*) and the susceptibility to ischemic stroke. Patients with proven ischemic stroke and control subjects were recruited between March 2013 and May 2015. The *IL-10 -1082A/G* and *-819T/C* polymorphisms were assessed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Conditional logistic regression analyses revealed that the *GA* and the *AA* genotypes were associated with development of ischemic stroke, and the ORs (95% CI) for the *GA* and the *AA* genotypes of *IL-10 -1082A/G* were 1.49 (1.01-2.19) and 1.83 (1.02-3.29) compared with the GG genotype, respectively. In dominant model, the *GA+AA* genotype of *IL-10 -1082G/A* was correlated with increased risk of ischemic stroke compared to the *GG* genotype (OR=1.56, 95% CI=1.08-2.25). The *GA+AA* genotype was associated with moderately increased risk of ischemic stroke in smokers (OR=1.72, 95% CI=1.04-2.84). In conclusion, our study suggests that *IL-10* gene polymorphisms contribute to the development of ischemic stroke, especially in tobacco smokers.

Keywords: *IL-10*, polymorphism, ischemic stroke

Introduction

Ischemic stroke is one of the leading causes of neuronal death in China, and the mortality of this disease shows an increasing trend recently. The development of ischemic stroke is involved in complex vascular and metabolic process, and many factors contribute to its process, such as type 2 diabetes, hypertension, arterial fibrillation, family history of ischemic stroke, sleep apnea syndrome, smoking and etc. [1]. However, not all the individuals who expose to similar risk factors of ischemic stroke would develop this disease, which led us to hypothesize that molecular factors might play a role in the susceptibility to ischemic stroke. Previous studies reported that genetic polymorphisms may influence the development of ischemic stroke, such as interleukin-6 (*IL-6*), *IL-18*, C-reaction protein, *CYP4F2*, *COX-2*, *pre-microRNA-149*, matrix metalloproteinase-9 and *MTHFR* genes [2-9].

Interleukin-10 (*IL-10*) is an immunoregulatory cytokine which is secreted mainly from Th2 cells, a new lineage of T cells, and monocytes. The encoding gene of *IL-10* is located at 1q31-1q32 of chromosome 1. Previous experimental study have reported that *IL-10* is an anti-inflammatory cytokine, and it could play an important role in preventing the synthesis of cytokines, including *IL-6*, *IL-1β*, *IL-1α*, and *TNF-α* in activated macrophage and IFNγ through T cells [10]. Previous studies have reported the association between *IL-10* gene polymorphisms and development of ischemic stroke, but the results are inconclusive [11-14]. Therefore, we investigated the possible association between two SNPs of *IL-10* (*IL-10 -1082A/G* and *-819T/C*) and the susceptibility to ischemic stroke.

Material and methods

Study subjects

Patients with proven ischemic stroke were recruited in our hospital between March 2013 and May 2015. The ischemic stroke was diagnosed by CT or MRI based on the diagnostic criteria of ischemic stroke from world Health

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, year						
<60	149	57.31	146	56.15		
≥60	111	42.69	114	43.85	0.07	0.79
Gender						
Females	188	72.31	188	72.31		
Males	72	27.69	72	27.69	0.00	1.00
Body Mass Index, kg/m^2						
<24	95	36.54	153	58.85		
≥24	165	63.46	107	41.15	25.93	<0.001
Alcohol drinking						
Never	121	46.54	132	50.77		
Ever	139	53.46	128	49.23	0.93	0.33
Tobacco smoking						
Never	85	32.69	141	54.23		
Ever	175	67.31	119	45.77	24.54	<0.001
Hypertension						
No	178	68.46	196	75.38		
Yes	82	31.54	64	24.62	3.09	0.08
Diabetes mellitus						
No	217	83.46	225	86.54		
Yes	43	16.54	35	13.46	0.97	0.33
TC, mmol/L	4.68 ± 1.06		4.51 ± 0.96		1.92	0.03
LDL-c, mmol/L	2.40 ± 0.52		2.18 ± 0.48		9.34	<0.001
HDL-c, mmol/L	1.29 ± 0.28		1.20 ± 0.23		4.01	<0.001
TG, mmol/L	2.51 ± 1.17		2.12 ± 1.08		3.95	<0.001

TC, total cholesterol; LDL-c, low density lipopolysaccharide cholesterol; HDL-c, high density lipopolysaccharide cholesterol; TG, triglyceride.

Organization. Patients who presented transient ischemic attacks, intracranial hemorrhage, brain tumors, brain trauma, severe liver disease and renal failure as well as pregnancy were excluded from the present study. Finally, a total of 260 patients with ischemic stroke were included in our study.

A total of 260 controls were randomly selected from individuals who underwent a regular health examination in our hospital during the same period. The control subjects were age- and sex-matched with the patients with ischemic stroke. Control subjects were free of ischemic stroke.

The demographic characteristics of patients with ischemic stroke and control subjects were collected from a self-designed questionnaire, including sex, age, alcohol drinking, tobacco smoking, body mass indexes, type 2 diabetes, and hypertension. The clinical characteristics of patients with ischemic stroke and control subjects were collected from medical records, including total cholesterol (TC), triglyceride (TG), low density lipopolysaccharide cholesterol (LDL-c), and high density lipopolysaccharide cholesterol (HDL-c) levels.

Prior to participating into our study, each patient and control subjects signed a written informed consent. The protocol of study was previously approved by the Institutional Research Ethics Committee of our hospital.

DNA extraction and genotyping

For DNA extraction, 5 ml peripheral venous blood was collected from each participant, collected in ethylene diamine tetra-acetic acid (EDTA)-coated tubes, and stored at -20°C in non-anticoagulant. The DNA was extracted with the TIANamp Blood DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer

Table 2. Association between the *IL-10 -1082A/G* and *-819T/C* gene polymorphisms and the risk of ischemic stroke

IL-10 gene	Patients	%	Controls	%	χ^2-test	*P* value	P for HWE	OR (95% CI)[1]	*P* value
-1082G/A									
Codominant									
GG	95	36.54	123	47.31				1.0 (Ref.)	-
GA	124	47.69	108	41.54				1.49 (1.01-2.19)	0.04
AA	41	15.77	29	11.15	6.76	0.03	0.47	1.83 (1.02-3.29)	0.03
Dominant									
GG	95	36.54	123	47.31				1.0 (Ref.)	-
GA + AA	165	63.46	137	52.69	6.19	0.01		1.56 (1.08-2.25)	0.01
Recessive									
GG+GA	219	84.23	231	88.85				1.0 (Ref.)	-
AA	41	15.77	29	11.15	2.38	0.12		1.49 (0.87-2.58)	0.12
-819T/C									
Codominant									
TT	104	40.00	116	44.62				1.0 (Ref.)	-
TC	113	43.46	111	42.69				1.14 (0.77-1.68)	0.5
CC	43	16.54	33	12.69	1.99	0.37	0.42	1.45 (0.83-2.55)	0.16
Dominant									
TT	104	40.00	116	44.62				1.0 (Ref.)	-
TC+CC	156	60.00	144	55.38	1.13	0.28		1.21 (0.84-1.74)	0.29
Recessive									
TT+TC	217	83.46	227	87.31				1.0 (Ref.)	-
CC	43	16.54	33	12.69	1.54	0.21		1.36 (0.81-2.30)	0.21

[1]Adjusted for sex, age, BMI, tobacco smoking, TC, LDL-c, HDL-c and TG. HWE, Hardy-Weinberg equilibrium; OR, Odd's ratio; CI, confidence interval.

instructions. The *IL-10 -1082A/G* and *-819T/C* polymorphisms were assessed by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). The forward and reverse primers for *IL-10 -1082A/G* were 5'-TCATTCTATGTGCTATATGG-3' and 5'-TGGTAAGTGAATAAGAGT-3', respectively; and the forward and reverse primers for *IL-10 -819T/C* were 5'-TGTGCACTAGGTGACTAGC-3' and 5'-CTACCTCACAGTGACGTCC-3', respectively. The PCR conditions were set as follows: 95°C for 5 min, 30 cycles of 95°C for 30s, 63°C for 30 s, and 72°C for 30 s and a final extension step of 72°C for 10 min. The restriction enzymes for *IL-10 -1082A/G* and *-819T/C* were *Bse*RI and *Ms*II, respectively. The products of PCR were confirmed by a 2% agarose gel stained with ethidium bromide and ultraviolet light.

Statistical analysis

Demographic and clinical characteristics between patients with ischemic stroke and controls were analyzed by student *t*-test and χ^2 test. The goodness-of-fit χ^2-test was tested for deviation from the Hardy-Weinberg equilibrium (HWE) in control subjects. Association between *IL-10 -1082A/G* and -819T/C polymorphisms and ischemic stroke was calculated by computing the odds ratio (OR) and 95% confidence intervals (95% CI) from logistic regression analyses. A *p*-value less than 0.05 was considered statistically significant. SPSS software version 17.0 (SPSS, Chicago, IL, USA) was used to conduct the analyses.

Results

Demographic and clinical characteristics of the study subjects

The demographic and clinical characteristics of the study subjects are shown in **Table 1**. No significant difference was identified between the patients with ischemic stroke and control subjects in terms of sex, age, alcohol drinking, hypertension and diabetes (P<0.05). When compared with the control subjects, patients

Table 3. Association between *IL-10 -1082A/G* polymorphism and development of ischemic stroke stratified by demographic characteristics

Variables	Patients		Controls		OR (95% CI)	*P* value
	GG	*GA+AA*	*GG*	*GA+AA*		
Age, year						
<60	55	94	69	77	1.53 (0.94-2.51)	0.07
≥60	40	71	54	60	1.60 (0.90-2.82)	0.08
Gender						
Females	72	116	89	99	1.45 (0.94-2.23)	0.03
Males	23	49	34	38	1.91 (0.92-3.97)	0.08
Body Mass Index, kg/m^2						
<24	35	60	73	80	1.56 (0.90-2.74)	0.06
≥24	60	105	50	57	1.54 (0.91-2.59)	0.09
Tobacco smoking						
Never	34	51	66	75	1.32 (0.74-2.37)	0.09
Ever	61	114	57	62	1.72 (1.04-2.84)	0.03

with ischemic stroke were more likely to have a higher BMI (χ^2=25.93, P<0.001), have a habit of tobacco smoking (χ^2=24.54, P<0.001), and have higher level of TC (t=1.92, P=0.03), LDL-c (t=9.34, P<0.001), HDL-c (t=4.01, P<0.001) and TG (t=3.95, P<0.001).

Association between IL-10 gene polymorphisms and development of ischemic stroke

The genotype distributions of *IL-10 -1082G/A* and *-819T/C* confirmed with the HWE in the control subjects, and the *P*-values were 0.47 and 0.42, respectively (**Table 2**). By χ^2-test, there was significant difference between genotype distributions of *IL-10 -1082G/A* (χ^2=0.03, *P* value=0.47). Conditional logistic regression analyses revealed that the *GA* and the *AA* genotypes were associated with development of ischemic stroke, and the ORs (95% CI) for the *GA* and the *AA* genotypes of *IL-10 -1082A/G* were 1.49 (1.01-2.19) and 1.83 (1.02-3.29) compared with the *GG* genotype, respectively. In dominant model, the *GA+AA* genotype of *IL-10 -1082G/A* was correlated with increased risk of ischemic stroke compared to the *GG* genotype (OR=1.56, 95% CI=1.08-2.25). However, we did not find any significant association between the *IL-10 -819T/C* gene polymorphism and risk of ischemic stroke.

Stratification analysis by sex, age, BMI and tobacco smoking

The association between *IL-10 -1082G/A* gene polymorphisms and the development of ischemic stroke was stratified based on sex, age, BMI and tobacco smoking (**Table 3**). The *GA+AA* genotype was associated with moderately increased risk of ischemic stroke in smokers (OR=1.72, 95% CI=1.04-2.84). Moreover, we did not find significant interaction between *IL-10 -1082A/G* polymorphism and TC, LDL-c, HDL-c and TG in the risk of ischemic stroke.

Discussion

Genetic susceptibility to diseases has attracted growing attention to the study of gene polymorphisms involved in several kinds of diseases. Previous studies have reported that cytokines could maintain the balance between pro-inflammatory and anti-inflammatory stimuli in the process of cerebrovascular disease [14, 15]. In our study, we investigated the influence of *IL-10 -1082A/G* and *-819T/C* gene polymorphisms on the development of ischemic stroke. We found that the GA and the AA genotypes of *IL-10 -1082A/G* were associated with the risk of ischemic stroke, and had interaction with tobacco smoking.

The *IL-10* gene plays an important role in regulate the complex network of reactions in the process of cerebrial ischemia. The level of *IL-10* gene production with neurological deterioration and functional polymorphisms could alter the anti-infolammatory process. Previous studies have reported the association between *IL-10* gene polymorphisms and development of stroke [12-15]. Xie et al. conducted a study in a Chinese population, and they found that rs-

1800872 and rs1554286 were associated with the development of ischemic stroke [14]. Park et al. conducted a study in a Korean population, and they found that *IL-10* gene polymorphism would contribute to the development of ischemic stroke with hypertension [15]. Another study reported that *IL-10 -1082A/G* gene variant was significant associated with ischemic stroke in the south Indian population, and hypertensive and diabetic individuals had interaction with *IL-10* gene polymorphism in the stroke risk [12]. However, several previous studies reported inconsistent results [16, 17]. Two previous studies did not find an association between *IL-10 -1082A/G* gene polymorphisms and risk of ischemic stroke [16, 17]. One previous meta-analysis pooled with five case-control studies, and it showed that *IL-10 -1082A/G* gene polymorphism was associated with the risk of ischemic stroke [18]. In our study, we found that the GA and the AA genotypes of *IL-10 -1082A/G* were associated with the risk of ischemic stroke. Such discrepancy between above mentioned studies may be attributed to ethnic variations, differences in the source of patients and sample size.

Our study found that *IL-10 -1082A/G* gene polymorphism had interaction with tobacco smoking in the risk of ischemic stroke. Previous studies have reported that *IL-10 -1082A/G* have associated with tobacco smoking related diseases [19, 20]. Further studies have greatly needed to confirm the association between *IL-10 -1082A/G* gene polymorphism and tobacco smoking in the development of ischemic stroke.

Two limitations should be considered in our study. First, the control subjects were selected from one hospital, which suggests that the selected subjects could not well represent the general population, and thus selection bias may not be avoided in this study. Second, other polymorphisms may influence the development of ischemic stroke except for *IL-10* gene polymorphism. Third, the sample size of our study is relatively small, which may limit the statistical power to find the difference between groups, and could explain our failure in finding an association with the *IL-10 -819T/C* polymorphisms.

In conclusion, our study suggests that *IL-10* gene polymorphism contribute to the development of ischemic stroke, especially in tobacco smokers. Further studies using larger sample sizes are greatly needed to confirm our finding.

Disclosure of conflict of interest

None.

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References

[1] Meschia JF, Bushnell C, Boden-Albala B, Braun LT, Bravata DM, Chaturvedi S, Creager MA, Eckel RH, Elkind MS, Fornage M, Goldstein LB, Greenberg SM, Horvath SE, Iadecola C, Jauch EC, Moore WS, Wilson JA; American Heart Association Stroke Council; Council on Cardiovascular and Stroke Nursing; Council on Clinical Cardiology; Council on Functional Genomics and Translational Biology; Council on Hypertension. Guidelines for the primary prevention of stroke: a statement for healthcare professionals from the American Heart Association/American Stroke Association. Stroke 2014; 45: 3754-832.

[2] Oliveira-Filho J, Ornellas AC, Zhang CR, Oliveira LM, Araújo-Santos T, Borges VM, Ventura LM, Reis FJ, Aras R, Fernandes AM, Rosand J, Greenberg SM, Furie KL, Rost NS. COX-2 rs20417 Polymorphism Is Associated with Stroke and White Matter Disease. J Stroke Cerebrovasc Dis 2015; 24: 1817-22.

[3] Shi JH, Niu LD, Chen XY, Hou JY, Yang P, Li GP. Investigation on the IL-18 -607A/C and -137C/G on the susceptibility of ischemic stroke. Pak J Med Sci 2015; 31: 198-202.

[4] Chen QY, Liu N, Ma J, Fang Y, Cao Y, Li H, Liu YC. Effect of a pre-microRNA-149 (miR-149) genetic variation on the risk of ischemic stroke in a Chinese Han population. Genet Mol Res 2015; 14: 2582-9.

[5] Kumar P, Yadav AK, Kumar A, Sagar R, Pandit AK, Prasad K. Association between Interleukin-6 (G174C and G572C) promoter gene polymorphisms and risk of ischaemic stroke: A meta-analysis. Ann Neurosci 2015; 22: 61-9.

[6] Yan HQ, Yuan Y, Zhang P, Huang Z, Chang L, Gui YK. CYP4F2 gene single nucleotide polymorphism is associated with ischemic stroke. Genet Mol Res 2015; 14: 659-64.

[7] Zhang X, Cao X, Xu X, Li A, Xu Y. Correlation between the -1562C/T polymorphism in the matrix metalloproteinase-9 gene and hemorrhagic transformation of ischemic stroke. Exp Ther Med 2015; 9: 1043-7.

[8] Prabhakar P, Majumdar V, Kulkarni GB, Christopher R. Genetic variants of vitamin D receptor and susceptibility to ischemic stroke. Biochem Biophys Res Commun 2015; 456: 631-6.
[9] Zhu XY, Hou RY, Pan XD, Wang YC, Zhang ZS, Guo RY. Association between the methylenetetrahydrofolate reductase (MTHFR) gene C677T polymorphism and ischemic stroke in the Chinese population: a meta-analysis. Int J Neurosci 2015; 125: 885-94.
[10] D'Andrea A, Aste-Amezaga M, Valiante NM, Ma X, Kubin M, Trinchieri G. Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. J Exp Med 1993; 178: 1041-8.
[11] Qi XF, Feng TJ, Yang P, Feng HY, Zhang P, Kong LY, Liang DL, Li PF, Na W, Li YW, Wang Y. Role of inflammatory parameters in the susceptibility of cerebral thrombosis. Genet Mol Res 2014; 13: 6350-5.
[12] Munshi A, Rajeshwar K, Kaul S, Al-Hazzani A, Alshatwi AA, Sai Babu M, Usha A, Jyothy A. Interleukin-10-1082 promoter polymorphism and ischemic stroke risk in a South Indian population. Cytokine 2010; 52: 221-4.
[13] Sultana S, Kolla VK, Jeedigunta Y, Penagaluru PK, Joshi S, Rani PU, Reddy PP. Tumour necrosis factor alpha and interleukin 10 gene polymorphisms and the risk of ischemic stroke in south Indian population. J Genet 2011; 90: 361-4.
[14] Xie G, Myint PK, Zaman MJ, Li Y, Zhao L, Shi P, Ren F, Wu Y. Relationship of serum interleukin-10 and its genetic variations with ischemic stroke in a Chinese general population. PLoS One 2013; 8: e74126.
[15] Park HK, Kim DH, Yun DH, Ban JY. Association between IL10, IL10RA, and IL10RB SNPs and ischemic stroke with hypertension in Korean population. Mol Biol Rep 2013; 40: 1785-90.
[16] Yu GI, Cho HC, Cho YK, Park HS, Yoon HJ, Kim HS, Nam CW, Kim YN, Kim KB, Ha E, Shin DH, Hur SH. Association of promoter region single nucleotide polymorphisms at positions -819C/T and -592C/A of interleukin 10 gene with ischemic heart disease. Inflamm Res 2012; 61: 899-905.
[17] Jin L, Ni P, Wu J, Fu Y, Ge H. The correlation between gene polymorphism of IL-10-819 C/T and-1082 G/A and cerebral infarction. Lab Med 2011; 26: 717-21.
[18] Yin GT, Ma YT, Zheng YY, Yang YN, Li XM, Fu ZY, Zhang JZ, Dai CF, Liu F, Chen BD, Gai MT, Xie X. Polymorphisms of interleukin-10 genes on the risk of ischemic stroke in a meta-analysis. Int J Clin Exp Med 2015; 8: 1888-95.
[19] Seifart C, Plagens A, Dempfle A, Clostermann U, Vogelmeier C, von Wichert P, Seifart U. TNF-alpha, TNF-beta, IL-6, and IL-10 polymorphisms in patients with lung cancer. Dis Markers 2005; 21: 157-65.
[20] Hsu HJ, Yang YH, Shieh TY, Chen CH, Kao YH, Yang CF, Ko EC. Role of cytokine gene (interferon-γ, transforming growth factor-β1, tumor necrosis factor-α, interleukin-6, and interleukin-10) polymorphisms in the risk of oral precancerous lesions in Taiwanese. Kaohsiung J Med Sci 2014; 30: 551-8.