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The association of functional polymorphisms of IL-6 gene promoter with ischemic stroke: Analysis in two Chinese populations

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ABSTRACT

Polymorphisms of G-572C and G-174C in the interleukin-6 (IL-6) promoter can affect both the transcription and secretion of IL-6 and may be involved in inflammation related to and the pathogenesis of ischemic stroke (IS). However, whether IL-6 polymorphisms are indeed risk factors for IS remains controversial. We recruited 748 Chinese IS patients diagnosed by magnetic resonance imaging (MRI) within 24 h of symptom onset and 748 normal healthy controls from two ethnic populations and performed two case–control studies in order to assess the nature of the polymorphisms of IL-6 and any links with IS. Common polymorphic loci in the IL-6 gene promoter were determined by TaqMan SNP genotyping assays. Multivariate logistic regression analysis was used to examine the association between IL-6 genotypes and a diagnosis of IS. We found that the C allele frequency at the -174 promoter region of IL-6 was extremely low in both IS patients and controls in both ethnic groups. The G allele of the promoter single nucleotide polymorphism (SNP) G-572C was more common in IS subjects than controls ($P = 0.004$, corrected for multiple testing) in the Han population but not in the Uyghur population. GC carriage therefore increased the risk of IS in the Han ethnic group (OR 1.45, 95% CI 1.13–1.86). In addition, the differences in GG and GC frequency between the two ethnic populations were significant. The C allele frequency at the -174 promoter region of IL-6 was rare in Chinese IS patients and controls from either ethnic group. We conclude that IL-6-572GC may be an independent risk factor for IS in the Chinese Han population.

Introduction

Ischemic stroke (IS), a common neurological disease with a variety of etiologies, is a leading cause of severe disability and death in both developed and developing countries. It is by far the most common kind of stroke, accounting for 85 to 90% of all strokes [1]. It is well known that the risk of IS is affected by genetic profiles, with no single common genetic variant exerting a major risk on stroke. The full range of contributory genes is yet to be determined [2].

Cerebral ischemia and inflammation are closely interrelated: Ischemia is a robust stimulus for potentially damaging inflammation while infection and the associated inflammation are known risk factors for IS [3–5]. Inflammation also contributes to ischemic events through the promotion of atherosclerosis [6]. Functional polymorphisms of inflammatory genes may thereby influence the incidence and outcome of IS.

Interleukin-6 (IL-6) is one of the confirmed major pleiotropic pro-inflammatory cytokines associated with atherosclerosis [7–10] and cardiovascular diseases [11–13] including stroke, and elevated serum IL-6 has been found in patients with acute IS. IL-6 has been implicated in the inflammation that contributes to both the injury and repair processes of IS [14,15]. Functional polymorphisms in the promoter regions of the genes coding for IL-6 are associated with increased plasma levels of this cytokine. The change from G to C at position 174 of the IL-6 gene creates a potential binding site for the transcription factor NF-1, resulting in repressed gene expression. The G allele, on the other hand, is associated with higher circulating IL-6 levels [16]. The activity of the promoter is also affected by nearby polymorphic sites at -572, which seem to control the influence of the polymorphism at position -174 [17,18].

It is well established that IL-6 is associated with atherosclerotic disease and is also a key mediator in the inflammatory response to cerebral ischemia, with many studies suggesting IL-6 may play a

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central role in the inflammatory response to cerebral ischemia [19–24]. Although some IL-6 promoter polymorphisms have been associated with carotid artery atherosclerosis and coronary heart disease, their relationship with IS is not well understood [25].

This study was undertaken to define how strongly the commonest genetic variation within the IL-6 gene promoter contributes to IS. We found that the presence of the G allele at the common IL-6 polymorphic promoter -572 loci was associated with IS events in our Han and Uyghur ethnic samples.

Materials and methods

Study subjects. Subjects are either Han or Uyghur, who have the different religions and life customs. The study was carried out with prior approval from the local Ethics Committee. We enrolled inpatients attending the stroke units of five large general hospitals in Shenzhen, Heinongjiang and Xinjiang from September 2003 to February 2009. All subjects gave their written informed consent. The diagnosis of IS was established using the World Health Organization, International Classification [26] and stroke subtypes were defined using the Oxfordshire classification [27,28]. Screened by head CT or magnetic resonance imaging (MRI), a total of 748 subjects presenting within 24 h of symptom onset were enrolled in two case–control studies. Additionally, 748 age-, gender- and ethnicity-matched normal healthy controls were randomly selected from healthy volunteers from four local community-based populations. Subjects with a history of stroke, Alzheimer's disease, brain aneurysm, dementia, dystonia, Parkinson's disease or inflammatory disorders were excluded from the control group. A structured questionnaire was used to record general information, clinical history of IS and associated clinical parameters, and epidemiological data.

Sample processing. After obtaining informed consent from both groups, 5 ml blood samples were taken in ethylene diamine tetra acetic acid (EDTA) and plain vials. Genomic DNA was extracted from the peripheral blood leucocytes pellet using a DNA extraction kit (AXYGEN, California, USA). DNA samples were stored at −80 °C before use.

Genotyping of the IL-6 promoter genomic variants. The polymorphisms of IL-6-174G/C and -572G/C were determined using a TaqMan SNP genotyping technique. The IL-6-174G/C primers were forward, 5′-AGCCTCAATGACGACCTAAG-3′ and reverse, 5′-GGGGCTGATTG GAAACCTTA-3′. The TaqMan minor groove binding (MGB) probes for detection of G/C polymorphism (rs1800795) were FAM -AGTTGTGTCTTGCGATGC-MGB and HEX-AGTTGTGTCTTGCCATGC-MGB. The primers of IL-6-572G/C were forward, 5′-GCACGAAATTTGA GGATGGC-3′ and reverse, 5′-TCTGAGTTCTTCTGTGTTCTGG-3′. The TaqMan minor groove binding(MGB) probes for detection of G/C polymorphism (rs1800796) were FAM-TCTACAACAGCCCCTC-MGB and HEX-TCTACAACAGCCGCTCA-MGB. The primers and probes were commercially supplied by Shanghai GeneCore Biotechnologies. Thermal cyclings were performed on, and allele frequencies were determined by, Stratagene Mx4000 systems (Stratagene, La Jolla, CA, USA).

To improve the genotyping quality and validate our results, a random selection of 10% of the samples were re-genotyped by laboratory personnel not otherwise involved in the study, and the results were found to be reproducible with no discrepancies noted.

Statistical analysis. Allele frequencies, genotype frequencies and carriage rates of the alleles in all the groups were compared by the Fisher exact test using SPSS software (version 12.0, SPSS Inc., Chicago, Illinois, USA). Data on quantitative characteristics were expressed as means ± SD. Comparisons between groups were made with the χ^2 test (nominal data) or Student's *t*-test (interval data). Allele frequency was calculated as the number of occurrences of the test allele in the population divided by the total number of alleles. The carriage rate was calculated as the number of individuals carrying at least one copy of the test allele divided by the total number of individuals. All *P* values were two sided, and differences were considered statistically significant if $P < 0.05$. Hardy–Weinberg equilibrium was tested by the χ^2 method. Odds ratios (OR) at the 95% confidence interval (CI) were determined to describe the strength of any associations between IS and gene polymorphism using multivariate logistic regression modeling.

Results

Table 1 includes the demographic and clinical characteristics of the patients and controls from the Han and Uyghur populations. All cases are clinically diagnosed as the lacunar infarction. The ratio of males to females was 379:269 in the Han subjects and 52:48 in the Uyghur subjects. There were no significant differences in age between cases and controls in the two populations ($P = 0.102$ for Han, $P = 0.498$ for Uyghur). There was a significant difference in waist-to-hip ratio (WHR) between the patients and controls in the Han population ($P = 0.000$). There were significant differences in body mass index (BMI) ($P = 0.010$) and WHR ($P = 0.000$) between the patients and controls in the Uyghur population. The results suggest that hypertension and WHR increased the risk of IS in both the Han and Uyghur ethnic groups. As shown in Table 1, the Han IS group had a higher prevalence of conventional risk factors for vascular diseases, including a history of hypertension, diabetes, smoking and increased TG and/or LDL-C, compared to the Han control group. There was a trend for BMI, WHR and a history of hypertension to be risk factors for IS in the Uyghur population.

Table 2 shows the distributions of the genotypes and allelic frequencies of IL-6-174G/C and -572G/C polymorphisms. There were no statistically significant differences in the distribution of IL-6-174G/C polymorphism between the patients and controls in either ethnic group ($P > 0.05$). The C allele frequencies at the -174 promoter region of IL-6 were extremely low in both the Chinese Han and Uyghur populations. However, there were significant differences in the distribution of IL-6-572G/C polymorphism between the patients and controls in the Han group ($P < 0.01$) but not in the Uyghur group ($P = 0.38$). This suggests that IL-6-572GC may be a risk factor for IS in Han Chinese. With IL-6-572CC as the reference genotype, IL-6-572GC had an odds ratio for IS of 1.49 ($P < 0.01$) in Han and 0.75 in Uyghur ($P = 0.38$) people. The Han patient group had an increased frequency of the G allele compared to the Han control group and the odds ratio for the G allele, as opposed to the C allele, was 1.30 in Han Chinese ($P = 0.00$) and 0.67 in Uyghur Chinese ($P = 0.04$). The IL-6-572G/C gene polymorphism was Hardy–Weinberg equilibrium in both ethnic groups ($P > 0.05$).

Fig. 1 compares the IL-6-572G/C genotype frequency distribution between IS patients and controls in the two ethnic groups. There were significant differences in IL-6-572GC heterozygous and GG homozygous frequency between patients and controls in both ethnic groups. However, there were no significant differences in TNF-α -572CC wild-type homozygous frequency ($P = 0.053$).

Table 3 shows a multiple logistic regression model for evaluating the relative effects of IL-6 polymorphism on the risk of IS. The relationship between -572G/C polymorphism and IS was further assessed by multivariate logistic regression analysis, and variables were entered into a logistic regression model if they were statistically significant following univariate analysis. In the Han group, after adjustment for Hypertension, Diabetes, Smoking, Tea drinking, Alcohol drinking, BMI and WHR, the IL-6-572 G allele was associated with an increased risk of IS, with an overall OR of 1.433 and 1.446 when assuming either Recessive or Additive models of inheritance, respectively ($P < 0.001$). However, after adjustment for the same risk factors in the Uyghur population, the

Table 1
Demographic characteristics and distribution of traditional risk factors in two ethnic groups.

Characteristic	Cases (n = 648)	Han		Cases (n = 100)	Uyghur	
		Controls (n = 648)	P value[c]		Controls (n = 100)	P value[c]
Gender (M/F)	379/269	379/269		58/42	58/42	
Age (years)	61.12 ± 9.98	60.21 ± 9.89	0.102	64.12 ± 9.36	63.18 ± 10.19	0.498
BMI (kg/m^2)						
>25	255	225	0.084	50	32	0.010
<25	393	423		50	68	
WHR						
M > 0.9 or F > 0.8	541	402	0.000	65	25	0.000
M < 0.9 or F < 0.8	107	246		35	75	
History of hypertension[a]	366	176	0.000	59	11	0.000
History of diabetes	148	100	0.001	10	8	0.621
History of heart diseases	77	81	0.734	8	0	0.007
Current smokers[b]	163	119	0.003	17	19	0.713
Alcohol drinking	141	172	0.044	16	26	0.083
Tea drinking	175	332	0.000	98	100	0.155
Triglyceride (mmol/l)	1.86 ± 1.35	1.57 ± 0.97	0.000	1.69 ± 1.07	1.66 ± 0.92	0.849
Cholesterol (mmol/l)	5.25 ± 1.49	5.28 ± 1.01	0.691	4.53 ± 1.24	5.65 ± 1.23	0.000
HDL-C (mmol/l)	1.23 ± 0.51	1.34 ± 0.40	0.000	1.06 ± 0.37	1.39 ± 0.38	0.000
LDL-C (mmol/l)	3.46 ± 1.31	2.83 ± 0.78	0.000	2.70 ± 0.77	2.78 ± 0.53	0.767

Data are shown as mean ± standard deviation, unless otherwise indicated M, male; F, female; BMI, body mass index; WHR, waist-to-hip ratio; FH, family history.
[a] Blood pressure ⩾140/90 mm Hg for >1 year.
[b] More than 5 pack-years and smoking within last 12 months.
[c] Continuous data were tested using two-tailed Student's t-test, and categorical data were tested using a χ^2 test (with df = 1) or Fisher's exact test for difference between case (patient) and control (normal) groups.

Table 2
IL-6 promoter genotype and allele distribution between cases and controls of two ethnic populations.

	Cases (n = 648)	Han			Cases (n = 100)	Uyghur		
		Controls (n = 648)	P	OR (95% CI)		Controls (n = 100)	P	OR (95% CI)
IL-6 (-174)								
GG	648	645			99	98		
GC	0	3	0.25	1.00 (0.99–1.01)	1	2	1.00	2.02 (0.18–22.65)
IL-6 alleles								
G	1296	1293			199	198		
C	0	3	0.25	1.00 (1.00–1.01)	1	2	1.00	2.01 (0.18–22.35)
MAF	–	0.23%			0.50%	1.00%		
IL-6 (-572)								
CC	341	401			32	23		
GC	269	212	0.00	1.49 (1.19–1.88)	57	55	0.38	0.75 (0.39–1.43)
GG	38	35	0.32	1.29 (0.79–2.07)	11	22	0.02	0.36 (0.15–0.88)
GC + GG	307	247	0.00	1.46 (1.17–1.82)	68	77	0.15	0.64 (0.34–1.19)
IL-6 alleles								
G	345	282	0.00	1.30 (1.09–1.56)	79	99	0.04	0.67 (0.45–0.99)
C	951	1014			121	101		
MAF	26.62%	21.76%			39.50%	49.50%		

MAF, minor allele frequency.

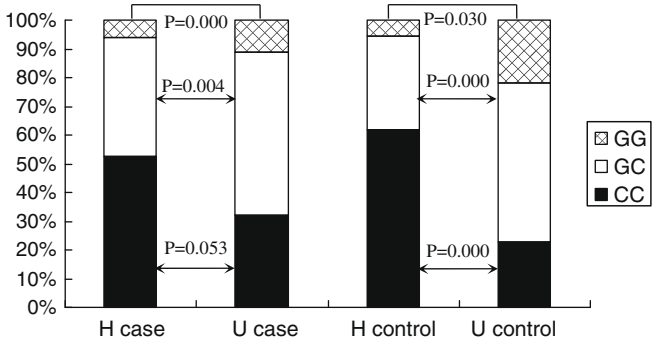

Fig. 1. the Comparisons of IL-6-572G/C genotypes frequency distribution between cases and controls, respectively, among two ethnic groups.

Table 3
Risk of ischemic stroke and IL-6 genepolymorphism in cases and controls of two ethinic populations.

	Han		Uyghur	
	Adjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value
IL-6 (-572G/C)				
Recessive	1.433 (1.121–1.833)	0.004	0.476 (0.212–1.069)	0.072
Additive	1.446 (1.125–1.859)	0.004	0.982 (0.477–2.021)	0.961
Dominant	0.971 (0.577–1.633)	0.911	2.721 (1.028–7.201)	0.044

Multivariate logistic regression analysis after adjustment for hypertension, diabetes, smoking, alcohol drinking, tea drinking, BMI, WHR.

IL-6-572 G allele was associated with an increased risk of IS only when assuming a Dominant model of transmission, with an overall OR of 2.721 (OR 95% CI 1.028–7.201, P = 0.044).

Discussion

IL-6, one of the most important mediators of the in vivo inflammatory reactions associated with atherosclerotic disease, is likewise a key mediator in the inflammatory response to cerebral ischemia [29]. Additionally, it has been suggested that the serum level of the cytokine IL-6 rises markedly in IS [20]. There is mounting evidence confirming that IL-6 is a key regulator of inflammatory mechanisms that play an important role in the pathophysiology and development of IS [30]. A part of the SNPs identified in the IL-6 gene, especially within the non-coding promoter sequence, has been shown to have a powerful influence on the expression of the gene [31–33]. Our study shows a significant association between certain promoter genotypes of IL-6 and IS.

Some previous studies have found a unique association between the CC genotype of the -174 G/C IL-6 polymorphism and stroke, however, the results are different in different populations [34–38]. In addition, it has been reported that IL-6 promoter polymorphisms are associated with other inflammation- and atherosclerosis-related diseases, such as periodontitis, coronary artery disease, otitis media, celiac disease and obstructive sleep apnea [39–43]. Recently, the -174G>C promoter polymorphism has been linked with increased risk of cervical cancer and developmental delay [44,45]. However, we failed to demonstrate an association between functional polymorphism of -174G/C in the IL-6 and the occurrence of IS in two ethnic groups of Chinese population. Interestingly, we found that the C allele frequency at the -174 promoter region of IL-6 was extremely low in the both Han and Uyghur IS patients and controls. The reason for the rarity of C allele in the Chinese population is unclear and needs further ethnicity-specific studies.

It has been shown that the other important promoter variant (G-572C) can influence IL-6 transcription through a complex interaction, and the G allele at these loci has been found to be associated with increased transcription efficiency of IL-6 [17]. Our results may confirm this finding; the frequency of the IL-6-572 G allele was significantly greater in IS patients than in controls in the Han group, which may indirectly explain the high serum level of the cytokine IL-6 previously reported in IS cases [20], whereas a statistically significant lower prevalence of the IL-6-572 G allele in patients compared to controls was observed in the Uyghur group. Furthermore, we analyzed the IL-6-572G/C genotypes frequency discrepancy between patients and controls in the two ethnic populations. There were significant differences in IL-6-572GC heterozygous frequency and GG homozygous frequency when IS patients and controls were compared, and this was true for both ethnic groups. However, there was no significant difference in IL-6-572CC wild-type homozygous frequency when patients from the two ethnic groups were compared ($P = 0.053$). All these interesting findings suggest to us that there may be genetic variations between Chinese Han and Uyghur populations that may affect the risk of IS.

Our study has certain strengths, including a relative large sample size in the Han population, rigorous methods used to diagnose IS including formal assessment of the reliability of interpretations of head CTs or MRI findings, and a biologically plausible a priori rationale for choosing the candidate genes. Our study also has some limitations. First, it is a case–control study and a selection bias could not be completely excluded for the group of patients with IS. Second, due to the small size of the Uyghur population in China and the harsh environment in which this ethnic group is clustered, the number of Uyghur people enrolled in the study was relatively small and might not be a representative sample of the population as a whole. In addition, although controls with diseases potentially related to ischemic disorders were debarred from entry to the study, there may have been enrollees with undiagnosed disease, so a selection bias cannot be categorically excluded. Third, it is possible that our findings might apply only to the Chinese population. Finally, only a small number of polymorphisms have been determined at this time. It is likely that with a more refined technology, such as genome-wide association studies, additional polymorphisms will be identified.

From the current study we conclude that the C allele frequency at the -174 promoter region of IL-6 is extremely low in the Chinese population. The IL-6 polymorphism at -174 is unlikely to contribute significantly to a susceptibility to, or affect the progression of, IS in either Han or Uyghur populations. Moreover, our findings lend support to the notion of an association between promoter variations in the IL-6 gene-572 and IS and suggest that IL-6-572GC may be an independent risk factor for IS in the Chinese Han population and IL-6-572CC in the Uyghur population. These findings may affect the diagnosis and treatment of IS.

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