Original Article

Association of interleukin-1 gene cluster polymorphisms with ischemic stroke in a Chinese population

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Background and Aims: Chronic inflammatory process plays an important role in atherothrombosis. Interleukin-1 (IL-1) is one of the key modulators of the inflammatory response and its activity is critically regulated by its receptor antagonist (IL-1Ra). A variable number tandem repeat polymorphism in intron 2 of IL-1Ra gene and a C to T single base polymorphism in the promoter of IL-1β gene ($C^{-511}\rightarrow T$) have been reported to affect the levels of IL-1 as well as its antagonist, IL-1Ra. It is also reported in several studies that these polymorphisms are associated with the susceptibility to cardio-cerebral vascular disease. However, data are limited in China. In this article, we studied the relationships between these polymorphisms and the risk of ischemic stroke in China. **Materials and Methods:** One hundred and twelve patients committed ischemic stroke were compared with 95 demographically matched healthy volunteers. **Results:** The frequencies of the IL-1Ra 1/1 genotype and IL-1Ra allele 1 (Ra*1 allele) in stroke patients were significantly higher than those in healthy volunteers [93.7% vs. 82.1%, *P*=0.014; 0.964 vs. 0.905, *P*=0.007]. No significant differences were found in the IL-1β-511 genotype and the allele distribution between the two groups. **Conclusions:** Our results implicated that IL-1 gene polymorphism might be associated with the susceptibility to ischemic stroke.

Key words: Cerebrovascular accident, genetics, interleukins, polymorphism

Atherosclerosis is considered as an inflammatory disease.[1] Infiltration of leukocytes is associated with plaque ruptures, which lead to narrowing and occlusion of the vessel lumen.[2] The proinflammatory cytokine interleukin-1 (IL-1) has been demonstrated to play an important role in atherosclerosis.[1] In endothelial cells, IL-1 induces the expression of adherence molecules for circulating leukocytes[3] and the synthesis of transforming growth factor-β, IL-6, fibrinogen, C-reactive protein and other inflammatory components.[4-7] The fact that increased levels of IL-1β mRNA have been detected in human atherosclerotic plaques[8] suggests that IL-1 may enhance the local immunoreaction. The activity of IL-1 (mainly IL-1β) is modulated by IL-1 receptor antagonist (IL-1Ra). The latter is secreted by the same cells secreting IL-1 and acts as an antagonist of IL-1 by blocking the IL-1 receptor.[9]

Several genetic polymorphisms have been detected in the genes of the IL-1 cluster. Their relationships with atherosclerotic diseases have been reported with contrary results, particularly the variable number tandem repeat (VNTR) polymorphism in intron 2 of human IL-1Ra gene.[10-15] This polymorphism has been reported to be associated with blood levels of IL-1Ra and its release from human monocytes upon stimulation.[16,17] Meanwhile, it has been considered that the secretion of IL-1Ra may be coordinately modulated by both IL-1Ra and IL-1β genes.[17]

However, data are limited in China. In this study, we investigated the relationship between the IL-1Ra and IL-1β($C^{-511}\rightarrow T$) polymorphisms and the risk of ischemic stroke in China.

Materials and Methods

Subjects

Two study groups from the same geographic area of Northern China were investigated: ischemic stroke (IS) group and healthy volunteers. Informed consents were obtained from all subjects. The IS group consisted of 112 ischemic stroke patients (72 men and 40 women, mean age 56.9±13.1 years) diagnosed by computerized tomography scan and/or nuclear magnetic resonance imaging analysis, who were admitted consecutively into the Department of Neurology, Peking Union Medical College (PUMC) Hospital between November, 2003 and February, 2005.

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Another 14 stroke patients admitted during this period refused to participate by refusing to sign the informed consent form. According to TOAST (Trial of ORG 10172 in Acute Stroke Treatment) criteria, IS patients were divided into large-artery atherosclerosis , small-artery occlusion and cardioembolism.[18] Considering that cardioembolism might have a different etiology origin, these patients were excluded from this study. Patients with clinical evidence of autoimmune disease or tumor were also excluded. The controls consisted of 95 healthy volunteers (60 men and 35 women, mean age 57.4 ± 10.0 years) without any sibship with the patients. They had no evidence of cardio-cerebral vascular disease, autoimmune disease or tumor. No difference was found in sex distribution between the two groups (P=0.982). There were no relationships between these two groups. Both of them are of Chinese ancestry and come from the northern regions of China. The study protocol conformed to the declaration of Helsinki and was approved by the institutional ethics committee at PUMC.

Genetic analyses

Venous blood was collected from an antecubital vein anticoagulated with sodium citrate. Genomic DNA was purified from peripheral blood by a *salting out* method. The IL-1β and IL-1Ra polymorphisms were identified by polymerase chain reaction (PCR) as previously described.[19,20]

Interleukin-1Ra. Intron 2 of the IL-1Ra gene contains a variable number of identical tandem repeats of an 86-base-pair length of DNA. Primers listed in Table 1 were used to amplify the polymorphic region by PCR. The PCR product was analyzed on a 1.5% agarose gel stained with ethidium bromide.

Interleukin-1β (Promoter Region). Position -511 in the promoter region of this gene has a single base polymorphism (cytosine to thymine substitution, the cytosine allele completing an Ava I site). PCR amplification followed by Ava I (MBI Fermentas) digestion allowed the alleles to be identified on a 1.5% agarose gel stained with ethidium bromide. Gels were visualized under ultraviolet light.

Statistical analysis

The software SPSS for Windows, version 11.5, was used for statistical analysis. Tests for the Hardy-Weinberg's equilibrium, the differences of allelic and genotypic frequencies and the distribution of gender and smoker were performed by using the χ^2 test or Fisher's exact test where appropriate. Differences in age and blood lipid levels were tested by using the t test (two groups). Type I error rate of 5% was chosen for the analyses. Odds ratios (ORs) with 95% confidence intervals (CI) were also calculated. Conditional logistic regression analysis was used to assess the independent contribution of variables significantly associated in univariate analysis with the risk of stroke.

Results

The main characteristics of the IS patients and controls are listed in Table 2. The IS patients had a higher proportion of smokers, hypertension and diabetics and more unfavorable profile of plasma lipids.

The genotype distribution and allele frequencies of the IL-1Ra and IL-1β^{-511} polymorphisms in both groups are shown in Table 3. Only three of the six IL-1Ra alleles appeared in our study. The frequency of the minor IL-1Ra genotype was 0.9% (IL-1Ra 1/4). For each study group, no difference was found with respect to the Hardy-Weinberg's equilibrium for one locus (Controls: χ^2<0.001, P=1.0 of IL-Ra; χ^2=0.006, P=0.997 of IL-1β-511; Patients: χ^2=1.348, P=0.510 of IL-Ra; χ^2=0.005, P=0.998 of IL-1β-511). Interleukin-1Ra 1/1 genotype and IL-1Ra allele 1(Ra*1) were significantly higher in the IS group compared with healthy volunteers (93.7% vs. 82.1%; P=0.014 and 96.4% vs. 90.5%; P=0.007 OR =3.229, 95% CI 1.319 to 7.909). No significant differences were found in the IL-1β-511 genotype and allele distribution between the two groups.

Logistic regression analysis showed no correlation between genotype and clinical performance for the three models of inheritance [Table 4]. Adjustment for age, sex, blood pressure, diabetes mellitus, blood lipid levels and smoking habit did not change these results (data not shown).

Discussion

This study evaluated the potential role on the risk of ischemic

Table 1: Main characteristics of IL-1 gene polymorphisms and techniques used for screening

	IL-1β-511	IL-1Ra
Polymorphism type	Single base C/T	86-bp VNTR
Polymorphism site	-511	Intron 2
PCR primers		
Upstream	5'-TGGCATTGATCTGGTTCATC-3'	5'-CTCAGCAACACTCCTAT-3'
Downstream	5'-GTTTAGGAATCTTCCCACTT-3'	5'-TCCTGGTCTGCAGGTAA-3'
PCR conditions		
Denaturation	95°C 1 min	94°C 1 min
Annealing	55°C 1 min	55°C 1 min
Extension	74°C 1 min	72°C 1 min
Cycles (n)	35	35
Digestion	Ava I	
Allele size (bp)	C: 190+114	1:410, 2: 240, 3: 500
	T: 304	4:325, 5: 595, 6: 154

IL: interleukin, Ra: receptor antagonist, PCR: polymerase chain reaction, VNTR: variable number tandem repeat

stroke of the IL-1 gene cluster polymorphisms on a Chinese sample population, especially related to IL-1Ra gene.

The IL-1 gene cluster locates on chromosome 2, encoding three proteins: IL-1α,IL-1β and IL-1Ra. A penta-allelic VNTR polymorphism has been described in intron 2 of the IL-1Ra gene. This polymorphism contains a variable number of an 86-bp tandem repeat sequence and shows two major alleles: the most common allele 1 (four repeats) and the less common allele 2 (two repeats).[19] The IL-1β gene has a C/T single base variation at position -511 of the IL-1β promoter.[20] Interleukin-1Ra binds to IL-1 receptors, without activating them and therefore acts as an antagonist.[9] In monocytes, Ra*2 has been proved to be associated with an increased production of IL-1Ra and a decreased production of IL-1α protein.[21] So Ra*1 carriers are exposed to increased amounts of the IL-1 agonist and are less protected by the antagonist. On the other side, Ra*2 carriers showed to have higher blood levels or higher monocyte production rates of IL-1Ra and be more protected by the antagonist.

In this study, we found a significant difference in the frequency distribution of Ra*1 in ischemic stroke patients compared with healthy volunteers. This result is consistent with a former study in a Southern Italian population.[11] In our present study, the allele Ra*1 frequency is approximately 90%, while the reports of the frequency in the white population (70.8 to 77.3%)[10-15] and the North Indian (61.7%)[22] were relatively lower. Meanwhile, in contrast with the lower frequency in the white population (34 to 36%)[11,15] and higher rate in the population of North India (64.7%),[22] the allele IL-1β-511 T frequency in our present study is ~50%, which is consistent with a former study in our institution[23] [Table 5]. These findings indicated that there were differences in the distribution of allele frequencies in IL-1 genes among different ethnic groups. Interleukin-1 has been demonstrated to stimulate the thrombogenic response in endothelial cells and the production of endothelial-derived growth factor.[24] So, higher levels of IL-1 agonist as well as lower levels of IL-1 antagonist in atherosclerotic plaques would confer increased risk for plaque progression and atherothrombotic complications.[2,25] In addition, as with other clinical studies on genetic associations, the data presented here do not provide evidence for a causal role of the IL-1 cluster polymorphisms in the complex process of ischemic stroke. Some unknown genes may well be in linkage disequilibrium with the IL-1 gene cluster. In this case the IL-1 gene cluster polymorphisms would be serving as a marker for an unknown etiological gene.

There were some limitations in this study. This study was a relatively small sample study, the lack of power might potentially lead to spurious findings and large cohort studies would be advisable for evocative conclusions. The lack of measurements of blood levels or monocyte production of IL-1Ra or IL-1β is another limitation, because they might have provided some mechanistic insights into the association observed in this study. A third

Table 2: Characteristics of IS patients and healthy volunteers

	IS patients	Controls	*P* value
Age (±SD, years)	56.9 ± 13.1	57.4 ± 10.0	0.695
Male sex (%)	64.3	63.2	0.886
Hypertension (%)	65.5	0	ND
Diabetes (%)	31.9	0	ND
Total cholesterol (mg/dL)	186.6 ± 40.7	170.9 ± 44.5	0.009
LDL cholesterol (mg/dL)	124.7 ± 42.6	113.5 ± 38.1	0.048
Triglycerides (mg/dL)	151.8 ± 82.2	120.6 ± 78.4	0.006
HDL cholesterol (mg/dL)	44.2 ± 9.8	51.5 ± 10.2	<0.001
Smoker (%)	42.9	23.1	0.003

NS, not significant; ND, not done. Hypertension was defined as reported systolic blood pressure ≥ 140 mmHg, reported diastolic blood pressure ≥ 90 mmHg, patient's self-report of hypertension or use of antihypertensive drugs. Diabetes mellitus was defined as fasting blood glucose level ≥ 126 mg/dL, patient's self-report of diabetes or use of antidiabetic drugs. Smoking habit (current smoker, yes or no), IS - Ischemic stroke.

Table 3: Genotype and allele distribution of IL-1Ra and IL-1β-511 gene polymorphisms in IS patients and controls

	Patients (n=112)	Controls (n=95)	*P* (OR, 95% CI)
IL-1Ra genotype frequencies			
1/1	105 (93.7)	78 (82.1)	0.014
1/2	5 (4.5)	16 (16.8)	0.007 (3.229,
2/2	1(0.9)	1 (1.1)	1.319-7.909)
IL-1Ra allele frequencies			
1	216 (96.4)	172 (90.5)	0.007 (3.229,
2	7 (3.1)	18 (9.5)	1.319-7.909)
IL-1β-511 genotype frequencies			
C/C	25 (22.3)	30 (31.6)	0.202
C/T	55 (49.1)	46 (48.4)	0.071 (1.430,
T/T	32 (28.6)	19 (20.0)	0.970-2.109)
IL-1β^{-511} allele frequencies			
C	105 (46.9)	106 (55.8)	0.071 (1.430,
T	119 (53.1)	84 (44.2)	0.970-2.109)

IS, ischemic stroke; OR, odds ratio; CI, confidence interval; IL, interleukin; Ra, receptor antagonist. Data presented as number, with percentage in parentheses, for patients and controls. Because one person has two alleles, the total number of alleles is twice as much in the number of genotypes.

Table 4: Odds ratios of IL-1 polymorphisms for stroke

Model	OR	95% CI	*P*
IL-1β^{-511} C>T			
Additive	1.128	0.378-3.371	0.829
Dominant	1.115	0.401-3.098	0.835
Recessive	0.943	0.311-2.855	0.917
IL-1Ra VNTR			
Additive	0.000	0.000-1.72 × 10^{216}	0.966
Dominant	0.260	0.050-1.336	0.107
Recessive	0.000	0.000-2.69 × 10^{-92}	0.935

OR, odds ratio; CI, confidence interval; VNTR, variable number tandem repeat. IL-1β^{-511} C>T: additive, CC vs. CT vs. TT; dominant, CC vs. CT/TT; recessive, CC/CT vs. TT. IL-1Ra VNTR: additive, 11 vs. 12 vs. 22; dominant, 11 vs. 12/22; recessive, 11/12 vs. 22.

Table 5: Allele frequencies of IL-1Ra and IL-1β^{-511} polymorphism of different populations

	This study (n=190)	Seripa *et al*[11] (n=2606)	Bid *et al*[22] (n=300)
Ra*1	172 (90.5)	1866 (71.6)	185 (61.7)
Ra*2	18 (9.5)	665 (25.5)	97 (32.3)
C	106 (55.8)	1657 (63.6)	194 (64.7)
T	84 (44.2)	949 (36.4)	106 (35.3)

Ra, receptor antagonist, Data presented as number, with percentage in parentheses, for patients and controls.

limitation lies in the way the subjects were recruited. The stroke population considered by this research may constitute by itself a systematic bias in this study, because patients with more severe conditions (they might die before they could be sent to our hospital) might not be included. Further genetic studies on patients who did not survive after the ischemic event may answer the question whether Ra*1 individuals are also exposed to greater mortality. The significant difference of characteristic risk factors between the patients and the controls might influence the study results too. So when respected to the logistics regression analysis, IL-1Ra polymorphism was not proved to act as an important factor of ischemic stroke. Our findings require confirmation in more strictly designed case control studies.

The biological control of IL-1 is complex. Concentrations of IL-1 and IL-1Ra *in vivo* vary in parallel, suggesting a coordinated pattern of regulation.[26] Recently, the first randomized phase II study of IL-1Ra in acute stroke patients has been finished and showed benefit to acute stroke patients.[27] Different genotypes may result in different effects and safety to a drug. Our study may add some data to further research on IL-1Ra.

Conclusion

The results reported here suggest a significant association between IL-1 Ra*1 and the susceptibility to ischemic stroke in a Chinese population. Our results may be a useful clue for pharmacological intervention in IL-1 production, but requires replication/confirmation in other studies.

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