



## Susceptibility for ischemic stroke in Sasang constitutional classification is associated with the interleukin-1 receptor antagonist polymorphism

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### SUMMARY

The Sasang constitutional medicine classifies the mankind into four constitutional types according to the individual psychological and physical traits. Differences in the Sasang constitutional medicine may be explained by the genetic factors. In order to determine the association of Sasang constitutional classification and interleukin-1 receptor antagonist (IL-1Ra) in genetic susceptibility to ischemic stroke, we classified the four constitutional types in ischemic stroke patients ( $n = 125$ ) and the healthy control subjects ( $n = 107$ ), and genotyped for IL-1Ra polymorphism by polymerase chain reaction (PCR) methods. The distribution of the IL1RN\*1/IL1RN\*2 genotype in the ischemic stroke patients was significantly different from the healthy controls ( $OR = 6.09$ ;  $P = 0.0134$ ). And the prevalence of IL1RN\*1/IL1RN\*2 genotype was increased in Taeum-in ischemic stroke patients, as compared to Taeum-in healthy controls ( $OR = 14.71$ ;  $P = 0.0144$ ). These results suggest that IL1RN\*1/IL1RN\*2 genotype in Taeum-in might be associated with the increasing risk for ischemic stroke. Furthermore, this relationship could provide the basis for a new approach in the investigation of the etiology of ischemic stroke.

**Key words:** Ischemic stroke; Sasang constitution; Interleukin-1 receptor antagonist; Polymorphism

### INTRODUCTION

The Sasang constitutional medicine, a unique medical theory of Korea traditional medicine, classifies the people into four constitutional types of Taeyang-in, Taeum-in, Soyang-in and Soum-in according to the individual psychological and physical traits (Lee, 1996; Chae *et al.*, 2003). It presumes that individual differences of disease susceptibility and drug response are depending on the constitution, and therefore the different treatment should be

applied to the each constitution (Um *et al.*, 2003). And these physiological, pathological and therapeutic differences may be explained by the genetic factors, and further may be linked to pharmacogenomics. Recent studies have shown the evidences that the Sasang constitution is associated with the psychological and physical traits, disease susceptibility of individuals (Chae *et al.*, 2003) and the genetic factor (Um *et al.*, 2004).

Recently, ischemic stroke has been recognized to be an acute local inflammatory disease (Hopkins *et al.*, 1995). Increasing production of several proinflammatory cytokines, including interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF) results in upregulation of adhesion molecules on endothelial cells, recruitment and activation of leukocytes, promotion of leukocyte-endothelium interaction,

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proliferation of the smooth muscle cell and thickening of the arterial wall, and conversion of the local endothelium to a prothrombotic state (Geng *et al.*, 1996; Grau *et al.*, 2001). As a consequence, cytokine is associated with the clinical progression and severity of ischemic stroke (Vila *et al.*, 2000).

Interleukin-1 receptor antagonist (IL-1Ra), an anti-inflammatory cytokine, can inhibit IL-1 activity by competing for receptor binding (Hannum *et al.*, 1990; Giovine *et al.*, 1992) and reduce the damage caused by experimental ischemic stroke (Garcia *et al.*, 1995). The IL-1Ra gene has been founded to have the variable numbers of an 86 base pair (bp) tandem repeat (VNTR) polymorphism in intron 2 (Tarlow *et al.*, 1993), which is associated with the various inflammatory diseases, obesity, diabetes, hypertension and ischemic stroke (Shu *et al.*, 2000; Cvetkovic *et al.*, 2002; Lee *et al.*, 2004; Um *et al.*, 2004).

In Sasang constitutional medicine, Taeum-in, one of four constitutional types, is characterized as relatively large body frame and heavy feeder (Lee, 1996), and it tends to be susceptible to the obesity, diabetes, hypertension and vascular disease such as ischemic stroke (Chae *et al.*, 2003). Therefore, we hypothesis that IL-1Ra polymorphism is associated with the susceptibility for ischemic stroke in Taeum-in.

And we classified ischemic stroke patients and the healthy control subjects into the four constitutional types, and genotyped for IL-1Ra polymorphism by polymerase chain reaction (PCR) methods.

## MATERIALS AND METHODS

### Subjects

The patient population consisted of all 125 ischemic stroke patients diagnosed between March 2002 and December 2003 in the Kyung Hee University Medical Center. Control subjects were comprised of 107 healthy blood donors attending the center for regular health check-ups during the same periods (Table 1).

The clinical diagnosis of ischemic stroke was defined as an acute focal or global neurological deficit lasting more than 24 h without apparent cause other than that of vascular origin, consecutively confirmed by brain computed tomography (CT) or magnetic resonance imaging (MRI) scan within 72 h from the onset. Patients with cerebral hemorrhage, cerebral venous thrombosis, brain tumor and other infectious or inflammatory diseases were excluded. Control subjects were free of ischemic stroke, ischemic heart disease, transient ischemic attack,

**Table 1.** Clinical characteristics of the study populations

	Control	Stroke	P
N	107	125	
Age (years)	65.92 ± 3.65	64.31 ± 10.68	NS
Male : female	63 : 44	66 : 59	NS
Constitutions			
Taeum-in	50 (46.7%)	58 (46.4%)	NS
Soyang-in	33 (30.8%)	45 (36.0%)	NS
Soum-in	23 (21.5%)	22 (17.6%)	NS
Taeyang-in	1 (0.9%)	0 (0%)	NS
BMI (kg/m <sup>2</sup> )	24.27 ± 2.83	24.17 ± 3.46	NS
NIDDM	2 (1.9%)	65 (52.0%)	< 0.0001
Hypertension	6 (5.6%)	56 (44.8%)	< 0.0001
Glucose (mg/dl)	98.50 ± 16.38	130.7 ± 62.64	< 0.0001
Total-Cholesterol (mg/dl)	197.80 ± 31.70	195.70 ± 40.26	NS
HDL-Cholesterol (mg/dl)	44.17 ± 10.58	38.36 ± 9.73	< 0.0001

N, number; NS, no significance; BMI, body mass index; NIDDM, non-insulin independent diabetes mellitus

prior stroke or disease concerning vascular risk factor.

This study was approved by the ethics review committee of the Medical Research Institute at the Kyung Hee University Medical Center.

#### Determination of Sasang constitution

All subjects were classified into the four constitutional types (Taeyang-in, Taeum-in, Soyang-in and Soum-in) using the questionnaire for the Sasang constitution classification II (QSCCII; Neomyth, Seoul, Korea) and the interview with the Sasang constitution specialist.

QSCC II is the questionnaire program for objective classification of the four constitutional types under computer. The questionnaire is divided into four sections: the first deals with general mental and behavioral characteristics; the second with personal business and social lives; the third with physical characteristics; and the last with some general questions on health. It has been proved for providing its accuracy and universal logical ground with its standardized diagrams according to diagnostic clinical data (Joseph *et al.*, 2001).

The interview with Sasang constitution specialist consists of checking the personality, appearance, general symptom and response to the previous taken herbal medicines or acupuncture. And the several items of QSCC also are randomly rechecked, because QSCC rarely reflects the desire of answering persons. The Sasang constitution specialist has been major in Sasang constitution over ten years, and has determined the constitutions of the approximately two thousand subjects per year.

#### IL-1Ra gene analysis

DNA was isolated from venous blood using a commercially available genomic DNA purification kit (Nucleospin). PCR-based genotyping of the IL-1Ra polymorphism was performed as described (Tarlow *et al.*, 1993). Briefly, the PCR consisted of a denaturing at 94°C for 2 min, then 30 cycles at 94°C for 30 sec, 60°C for 30 sec and 72°C for 45 sec, and a final extension at 72°C for 5 min. The following primers were used: 5'-CTCAGCAACACTCCTAT-

3' and 5'-CCTGGTCTGCAGGTAA-3'. The PCR products for the IL-1Ra gene was analyzed by electrophoresis on 2.0% agarose gel and stained with ethidium bromide for visualization under ultraviolet light. The accuracy of the polymorphisms of the IL-1Ra gene was confirmed by three times analysis in all samples and direct sequencing in several randomly selected samples. The polymorphism of the IL-1Ra gene were designed as IL1RN\*1 (four repeats, 410 bp), IL1RN\*2 (two repeats, 240 bp), IL1RN\*3 (five repeats, 500 bp), IL1RN\*4 (three repeats, 325 bp), and IL1RN\*5 (six repeats, 595 bp).

#### Statistical analysis

Quantitative variables are presented as mean  $\pm$  standard deviation and qualitative variables as percentages. The differences of anthropometric and biochemical parameters between ischemic stroke patients and controls, and among Sasang constitution types were examined by Student's *t* test or Mann-Whitney rank test and the one-way analysis of variance (ANOVA). Genotype distributions between ischemic stroke patients and controls, among each constitution were compared by chi-square ( $\chi^2$ ) test or Fisher's exact test. Clinical characteristics and genotype were analyzed univariately in ischemic stroke and control groups. Multiple regression analyses adjusted for sex, age and risk factor were then performed to assess the independent contribution to ischemic stroke of IL1RN\*1/IL1RN\*2 genotype. Variables with a *P* value of  $< 0.10$  on univariate analysis were entered in the logistic model. Two-sided *P* values of  $< 0.05$  were considered significant. Odds ratio (OR) and 95% confidence intervals (CI) were calculated as estimates of the relative risks. Statistical analysis was performed with SPSS for Windows, version 10.0 (SPSS Inc).

## RESULTS

The characteristics of all study populations are presented in Table 1. The differences of ischemic stroke and healthy control group were detected in

the respect to hypertension, NIDDM, serum glucose and high density (HDL)-cholesterol level (Table 1). And the distribution of Sasang four constitutions in ischemic stroke patients was Taeyang-in (0%), Taeum-in (46.4%), Soyang-in (36.0%) and Soum-in (17.6%). When compared with healthy controls whose constitutional distribution was Taeyang-in (0.9%), Taeum-in (46.7%), Soyang-in (30.8%) and Soum-in (21.5%), there were no significant differences in the constitutional distributions between patients and healthy controls (Table 1). However, in the clinical characteristics according to the Sasang constitution, it shown that Taeum-in had the higher BMI, and that Soyang-in had the higher proportion of male than other constitutions (Table 2).

To determine whether the IL-1Ra polymorphism is associated with ischemic stroke, a total of 232 participants (107 controls, 125 ischemic stroke patients) were genotyped for the IL-1Ra polymorphism. The distribution of the IL1RN\*1/IL1RN\*2 genotype

(10.4%) in the patients with ischemic stroke were significantly different from the distributions seen in the healthy controls (1.9%) (OR = 6.09; 95% CI, 1.34 - 27.66;  $P = 0.0134$ ) (Table 3).

In the logistic regression analyses between the IL-1Ra polymorphism and clinical characteristics, the IL1RN\*1/IL1RN\*2 genotype was associated with diabetes (OR = 4.70; 95% CI, 1.04 - 21.37;  $P = 0.045$ ; data not shown), but not with the other clinical parameters, including the age, gender, BMI, hypertension, glucose, total cholesterol, HDL and LDL-cholesterol.

To determine the association between IL-1Ra genotypes and Sasang constitution, genotypes distribution according to the each constitution were assessed in the patient and healthy controls. While the significant differences of genotype distributions among constitutions were not observed in ischemic stroke patients and healthy controls, the IL1RN\*1/IL1RN\*2 genotype prevalence of

**Table 2.** Clinical characteristics according to the Sasang constitutions

	Taeumin	Soyangin	Soumin	<i>P</i>
N	108	78	45	
Age (years)	65.81 ± 7.81	63.92 ± 8.45	65.27 ± 8.94	NS
Male : female	52 : 56	54 : 24	22 : 23	0.0105
BMI (kg/m <sup>2</sup> )	25.81 ± 3.03	22.82 ± 2.17	22.48 ± 2.64	< 0.0001
NIDDM	29	24	14	NS
Hypertension	27	24	11	NS
Glucose (mg/dl)	120.0 ± 44.56	117.7 ± 48.09	123.0 ± 63.88	NS
Total-Cholesterol (mg/dl)	198.9 ± 37.90	193.7 ± 35.40	196.4 ± 33.79	NS
HDL-Cholesterol (mg/dl)	39.76 ± 9.63	41.90 ± 10.79	43.46 ± 10.31	NS
LDL-Cholesterol (mg/dl)	130.6 ± 39.15	119.5 ± 35.09	129.4 ± 33.55	NS

N, number; NS, no significance; BMI, body mass index; NIDDM, non-insulin independent diabetes mellitus

**Table 3.** The genotypes distribution of interleukin-1 receptor antagonist polymorphism in study populations

	Control (n = 107)	Stroke (n = 125)	OR (95% CI)	<i>P</i>
IL1RN*1/IL1RN*1	99 (92.5%)	109 (87.2%)	0.55 (0.23 - 1.34)	0.1844
IL1RN*1/IL1RN*2	2 (1.9%)	13 (10.4%)	6.09 (1.34 - 27.66)	0.0134
IL1RN*1/IL1RN*3	4 (3.7%)	3 (2.4%)	0.63 (0.14 - 2.89)	0.7064
IL1RN*2/IL1RN*2	2 (1.9%)	0 (0%)	0.17 (0.01 - 3.54)	0.2116
IL1RN*1/IL1RN*4	0 (1%)	0 (%)	-	-

OR, odds ratio; CI, confidence intervals

**Table 4.** The distribution of IL1RN\*1/IL1RN\*2 genotype of interleukin-1 receptor antagonist polymorphism according to the Sasang constitution in study populations

	Control	Stroke	OR (95%CI)	P
Taeum-in	0	7	14.71 (0.82 - 264.6)	0.0144
Soyang-in	0	5	9.10 (0.49 - 170.7)	0.0691
Soum-in	2	1	0.50 (0.04 - 5.95)	1.0000

OR, odds ratio; CI, confidence intervals

Taeum-in with ischemic stroke was significantly higher than that of Taeum-in healthy controls (OR = 14.71; 95% CI, 0.82 - 264.6;  $P = 0.0144$ ) (Table 4).

## DISCUSSION

In this study, we found that Taeum-in had the high BMI, and that Soyang-in had the high proportion of male. These anthropometric and biochemical differences among Sasang constitutions will be helpful to classify the constitutions, in addition of the psychological and physical traits. The distribution of Sasang constitution was approximately corresponded with the other Korean study (Kim *et al.*, 1996), but it different from that in Western study (Koh *et al.*, 1999). Generally, the distribution of four constitutions in Korean is that Taeum-in takes almost half, Soyang-in and Soum-in take the other half, and Taeyang-in is very rare (Kim *et al.*, 1996). On the other hand, in American, the proportion of Soyang-in and Soum-in increase to the almost three fourth, and that of Taeum-in decrease to the below one fourth (Koh *et al.*, 1999). These differences of constitution distribution between two countries could be influence to the incidence of diseases. For example, Asian populations have a lower incidence of coronary heart disease but a higher incidence of stroke, compared with Western populations (Song *et al.*, 2004). And Taeum-in who has a high susceptibility for stroke takes a larger proportion in Korean, which might be induced the high incidence of stroke in Korean, combined with the genetic and environmental factors.

In this study, the genotype distribution of the IL-1Ra polymorphism in control subjects was consistent

with those reported in Asian study (Shu *et al.*, 2000). The IL1RN\*1/IL1RN\*2 genotype was associated with the increased risk for ischemic stroke, and it played as a more accelerating factor in the condition of diabetes. These results are corresponded with previous reports that IL1RN\*1/IL1RN\*2 genotype was associated with the susceptibility of ischemic stroke and diabetes (Blakemore *et al.*, 1996; Lin *et al.*, 2002). And the prevalence of IL1RN\*1/IL1RN\*2 genotype in Taeum-in with ischemic stroke was higher than Taeum-in healthy controls. And considering the 6.09 relative risk in all constitutions and the 14.71 relative risk in only Taeum-in with ischemic stroke, it was suggested that the incidence of ischemic stroke in Taeum-in might be strong related with IL-1Ra polymorphism compared with other constitutions. Furthermore, it will be helpful to predict the incidence of ischemic stroke, in addition of the diabetes, hypertension and other related risk factors.

Sasang constitutional medicine, a major branch of Genuine Korea medicine, is established by Je-Ma Lee in 1894. Sasang constitutional medicine explains that each individual has the special psychological and physical traits in the respects of emotion, taste and appearances. And these individual's inherent characteristics cause the physiological weakness and strength of each internal organ and bowel, which ultimately result in the unique pathological disorders. Finally, Sasang constitutional medicine classifies the people into four constitutional types of Taeyang-in (greater yang), Taeum-in (greater yin), Soyang-in (smaller yang) and Soum-in (smaller yin) according to the individual psychological, physical, physiological and pathological

traits.

Furthermore, Sasang constitutional medicine explains that distribution of all constitutions can show difference according to the family, race, region and nation. It could be linked to the familial and racial diseases, and genetic disorders. In this study, we present the differences of constitution distribution between countries, and those of characteristics among Sasang constitutions, and finally the association between the genetic factor and Sasang constitution. These findings will be helpful to understand the differences of individuals, races and nations in physiological and pathological characters.

In conclusion, that IL-1Ra polymorphisms was associated with a risk of ischemic stroke in Taeumin. Our results suggest that Sasang constitution might be related with genetic factor, and it further could provide a new approach in the investigation of the etiology of ischemic stroke.

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