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Original Article

A Study on SNP of IL10 in Cerebral Infarction Patients

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Abstract

Objectives : In this study, we investigated the SNP (single-nucleotide polymorphism) of IL10 in patients with stroke. The present study was undertaken to see if specific genotypic and allelic variations are associated with stroke in the Korean population.

Methods : Blood samples from all subjects were obtained for DNA extraction and collected in EDTA tube. Genomic DNA was extracted using DNA isolation kit for Mammalian Blood (Boehringer Mannheim, IN, USA). The extracted DNA was amplified by polymerase chain reaction (PCR). Pyrosequencing was performed according to manufacturer's standard protocol.

Results : There was no statistically significant genotypic distribution difference between control and stroke group. The frequencies of A/A homozygotes and A/C heterozygotes among control subjects were 91 (87.5%) and 13 (12.5%). The frequencies of A/A and A/C among the stroke patients were 85 (89.5%) and 10 (10.5%). There was not statistically significant allelic frequency difference between control and stroke group. The allelic frequency of A and C was 195 (93.8%) and 13 (6.2%) among the control subjects and 180 (94.7%) and 10 (5.3%) in stroke patients, respectively.

Conclusion : The cytokine IL10 may not be pathogenetic factors in stroke. But further studies including different cytokine gene can be a useful for predicting stroke. Establishment of more systemic approach and high quality of prospective cohorts will be necessary for the good prediction of genetic markers.

Key words : Stroke, Interleukin 10 (IL-10), Gene, Polymorphism

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I. Introduction

Stroke is the second most fatal disease following cancer in Korea. Stroke is a clinical concept of neurological disorder characterized by an acute faint, unconsciousness, excessive phlegm, hemiparalysis, dysphasia, facial palsy and motor disorder, etc.. Stroke develops several complications, among which sequela of stroke like motor disorder affects the family as well as the patient with great psychological and financial stress.

Gene expression can be regulated by a number of genetic elements located in the 5-upstream region of the gene. Variances in this upstream sequence can result in different level of gene expression.

For many cytokines and their receptors, genetic variants have been described[1-4]. Gene expression can be regulated by a number of genetic elements located in the 5-upstream region of the gene. Variances in this upstream sequence can result in different level of gene expression. IL10 is one of the immunomodulatory cytokines and it has anti-inflammatory capabilities[5]. To date, genetic polymorphism in the 5′-flanking region of the IL10 gene has not been described in stroke. We hypothesized that the IL10 gene is important candidate in the development of stroke and specific genotypic and allelic variations should be associated with stroke in the Korean population. In this study, we assessed the SNP (single-nucleotide polymorphism) of IL10 in patients with stroke.

II. Subjects and Methods

1. Study Population

The control group consisted of 95 apparently healthy Korean. Controls were selected from healthy subjects who visited for the health examinations at Jehan medical center in Daegu from March 2002 to April 2005. The patient group consisted of 104 Korean stroke patients. At first 116 stroke subjects were selected, who were admitted to the stroke service of the department of acupuncture & moxibustion, college of Oriental Medicine, Daegu Haany University. Of these patients, 12 subjects were excluded from this study (8 were transported to other hospitals, and 4 declined to give consent). Ultimately, 203 Koreans were enrolled in the current analysis.

2. Definition of Stroke

We included cerebral infarction patients with neurological symptoms lasting >24 hours accompanied by corresponding focal density changes detected by brain CT or MRI, and excluded patients suffering from epidural (or subdural) hematoma, brain tumors, and accidental or iatrogenic stroke. Intracerebral hemorrhage (ICH) and subarachnoid hemorrhage (SAH) was also excluded.

Final diagnosis of stroke was confirmed by serial CT or MRI findings. In case of CT, cerebral infarction was identified by gradual or sometimes rapid development of focal neurological symptoms and signs, such as hemiparesis, sensory impairment, and a low-density area in the CT image.

3. Blood Sample Collection

Blood samples were obtained from the antecubital vein without regarding to the time of the last meal. This study was approved by the ethics review committee of the medical research institute, Jehan medical center. Informed consent was obtained from all subjects. If patients were incommunicative, it was obtained from close relatives.

4. DNA Preparation and Genotyping

Blood samples from all subjects were obtained for DNA extraction and collected in EDTA tube. Genomic DNA was extracted using DNA isolation kit for Mammalian Blood (Boehringer Mannheim,

IN, USA). The extracted DNA was amplified by polymerase chain reaction (PCR). The IL10 gene (113-bp) was amplified using 25 ng of DNA, 5 pmol of each primer. IL10 forward was 5′-GGGTAAAGGAGCCTGGAACAC-3′ and IL10 reverse was 5′-GGGTGGGCTAAATATCCTCAAAGT-3′. The polymerase chain reaction (PCR) amplification was performed by using 0.5 unit Taq polymerase (HT Biotechnology Ltd., Cambridge, United Kingdom). The 30 ul of PCR reaction mixtures were 10 mM Tris-HCl, pH 9.0, 1.5 mM magnesium chloride, 50 mM potassium chloride, 0.1% Triton-X 100, 0.01 % [v/v] stabilizer, 0.25 mM of each deoxynucleotide triphosphate (dNTP), 0.1 M of each oligonucleotide primer. The PCR steps were denaturation of 5 minute at 95℃, 30 cycles of 30 seconds at 95℃, 30 seconds at 60℃, and 30 seconds at 72℃ with a Gene-Amp PCR System 9600 (Perkin-Elmer, Foster City, CA, USA). The reverse primer was biotinylated to allow the preparation of single-stranded DNA. The quality of PCR products was controlled by 1.5% of agarose gel electrophoresis.

DNA Preparation for pyrosequencing was performed according to manufacturer's standard protocol (Pyrosequencing AB, Uppsala, Sweden)[6]. The streptavidin sepharose beads (Streptavidin Sepharose HP, Amersham Pharmacia Biotech, Uppsala, Sweden) were immobilized to PCR products. The sequencing primer of IL10 was 5′-CTGGCTTCCTACAG-3′ and it was designed so that the terminal residue hybridized to the base immediately adjacent to the A/G mutation from Pyrosequencing AB(http://www.pyrosequencing.com) [6]. By incubation at room temperature for 10 minutes, 20 ul of biotinylated PCR products were immobilized onto streptavidin-coated sepharose beads, the immobilized PCR products were transferred to a Millipore 96-well filter plate (Millipore, Bedford, MA, USA). Vacuum was used to eliminate the different solutions and reagents to obtain pure, single-stranded DNA while the beads remained in the wells[7]. In 55 ul of 4 M acetic acid containing 0.35 uM of IL10 sequencing primer the beads with the immobilized template were resuspended. Then the 45 ul of suspension was transferred to a PSQ 96 plate (Pyrosequencing AB, Uppsala, Sweden)[8]. By using PSQ 96 Sample Prep Thermoplate (Pyrosequencing AB, Uppsala, Sweden) the PSQ 96 plate containing the samples was heated at 90℃ for 5 minutes for sequencing primer annealing, and moved to room temperature for 10 minutes. Then the PSQ 96 Plate was placed into the process chamber of the PSQ 96 instrument (Pyrosequencing AB, Uppsala, Sweden)[9]. The enzymes, substrates, and nucleotides were dispensed from a reagent cassette into the wells by using the PSQ 96 SNP Reagent Kit (Pyrosequencing AB, Uppsala, Sweden), The light that was generated when a nucleotide is incorporated into a growing DNA strand[10]. From this process the polymorphism of the IL10 was genotyped automatically.

5. Statistical Analysis

To compare age of stroke patients and controls Student's t-test was used. To compare sex, the distribution of the genotypes and the frequency of alleles between Korean stroke patients and controls x^2 tests was used. The odds ratios (OR) and 95% confidence int ervals (CI) were used to quantify the association with stroke. As statistical package SAS program (release 8.2, SAS Institute Inc., Cary, NC, USA) was used.

Ⅲ. Results

1. Characteristics of the subjects

The characteristics of the patients and controls are shown in Table 1. There was significant difference between the patients and controls as for age and sex ($p<0.001$). Mean age of the controls and patients was 44.5 ± 12.8 and 61.6 ± 10.4 years. The number of male / female of the controls and patients was 23 / 81 and 53 / 42.

Table 1. Clinical Characteristics of Cerebral-infarction Patients and Controls

	Controls	Patients	P value
Age	44.5 ± 12.8	61.6 ± 10.4	p<0.001
No. of Male	23	53	
No. of Female	81	42	p<0.001

Student's t-test was used to compare age of Cerebral-infarction patients and controls. x2 test was used to compare the sex of Cerebral-infarction patients and controls.

Table 2. Comparison of IL10 Genotype Distribution Between Cerebral-infarction and Control Participants

Genotype	No. of Controls	No. of patients	OR(95% CI)	P value
A/A	91 (87.5)	85 (89.5)	0.82 (0.34-1.97)	0.663
A/C	13 (12.5)	10 (10.5)		

x^2 test was used to compare values of Cerebral-infarction patients and controls

Table 3. Comparison of Allele Frequencies of IL10 Between Cerebral-infarction and Control Participants

Allele	No. of Controls	No. of patients	OR(95% CI)	P value
A	195 (93.8)	180 (94.7)	0.83 (0.35-1.94)	0.673
C	13 (6.2)	10 (5.3)		

x^2 test was used to compare values of Cerebral-infarction patients and controls

2. IL10 genotype Distribution

There was no statistically significant genotypic distribution difference between control and stroke group (p=0.663, OR (95% CI) ; 0.82 (0.34-1.97)). The frequencies of A/A homozygotes and A/C heterozygotes among control subjects were 91 (87.5%) and 13 (12.5%). The frequencies of A/A and A/C among the stroke patients were 85 (89.5%) and 10 (10.5%). These results are shown in Table 2.

3. Allele Frequencies Distribution

There was not statistically significant allelic frequency difference between control and stroke group (p=0.673, OR (95% CI) ; 0.83 (0.35-1.94)). The allelic frequency of A and C was 195 (93.8%) and 13 (6.2%) among the control subjects and 180 (94.7%) and 10 (5.3%) in stroke patients, respectively (Table 3).

Ⅳ. Discussion

Stroke is a leading cause of morbidity and mortality in Korea. Physical and psychological impairment from stroke may negatively affect quality of life. Stroke is a multifactorial disease and various factors such as atherosclerosis, hypertension, diabetes, hyperlopidemia and smoking interact to increase the risk of developing stroke. Lou et al revealed platelet hyperaggregability was seen in young patients with completed stroke[11].

Cytokines take important part in the infectious and inflammatory processes. They serve to transduce antigen specific signals, and focus inflammatory response where it is needed. That is, as in other disease states, some of the cytokines play a role in stroke, but importance of this role has not been established delicately to date. Interleukines and TNF-a are the cytokines which are released by

certain cells (mononuclear phagocytes, endothelial cells, epithelial cells, T cells, natural killer cells, etc.), and act in the generation of immune response, inflammation, acute phase reaction, fever, etc[12].

IL10 is one of the immunomodulatory cytokines. IL10 is produced by a variety of cell types, including monocytes and B cells[13]. It is an up-regulator of B lymphocyte production and differentiation[14], but has anti-inflammatory abilities that can directly down-regulate TNF ■, IL-1, IL-8 and interferon-■ production[15]. Three of IL10 SNPs have been studied in some detail: -1082(G to A), -819(C to T) and -592(C to A)[16-18].

Genetic factors appear to contribute to virtually every human disease, conferring susceptibility or resistance, affecting the severity or progression of disease, and interacting with environmental influences. In trying to get the information about genetic variation is important for understanding how genes function or malfunction, and how genetic and functional variation are related.

Recently in stroke many polymorphism were investigated and some polymorphism such as α 1-antichymotrypsin gene was associated[19], but some polymorphism such as promoter of lipopolysaccharide receptor CD14 was not related[20]. This is the report to have shown the association of IL10 gene polymorphisms with stroke, especially cerebral infarction, by use of CT or MRI findings. In this study, we investigated the SNP (single-nucleotide polymorphism) of IL10 in patients with stroke. The present study was undertaken to see if specific genotypic and allelic variations are associated with stroke in the Korean population.

The overall analysis revealed no significant interactions between genotype. There was no statistically significant genotypic distribution difference between control and stroke group. The frequencies of A/A homozygotes and A/C heterozygotes among control subjects were 91 (87.5%) and 13 (12.5%). The frequencies of A/A and A/C among the stroke patients were 85 (89.5%) and 10 (10.5%). These results are shown in Table 2. There was not statistically significant allelic frequency difference between control and stroke group. The allelic frequency of A and C was 195 (93.8%) and 13 (6.2%) among the control subjects and 180 (94.7%) and 10 (5.3%) in stroke patients, respectively.

Genetic factors and environmental factors are both critical in the development of stroke. So far it is very difficult to apply the results from genetic studies to clinic patients. The cytokine IL10 may not be pathogenetic factors in stroke. But further studies including different cytokine gene can be a useful for predicting stroke. Establishment of more systemic approach and high quality of prospective cohorts will be necessary for the good prediction of genetic markers.

There are some limitations of this study. Firstly, the IL10 serum of the patients was not taken, which makes the information somewhat heterogenous. In the further studies, these limitations should be improved. The findings of this study need to be confirmed in larger patients samples and further studies. Additional epidemiologically based studies of the effects and relationship between IL10 or other genes and lifestyles with regard to stroke risk is required.

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VI. References

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