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원 저

Serotonin Transporter Gene Polymorphism in Korean Stroke Patients - 277 Case Control Study

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Abstract

한국인 중풍 환자의 Serotonin Transporter 유전자다형성 - 환자 대조군 연구 277례

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목적 : 본 연구는 serotonin transporter (5-HTT) 유전자다형성이 중풍의 발병과 관련이 있는지 알아보기 위해 수행하였다.

대상 : 경희의료원 한방병원에 입원한 중풍환자 139명과 각 병원의 중풍 기왕력이 없는 건강인 138명을 대상으로 하였다.

방법 : 각 그룹에서 개개인마다 DNA를 분리 정제한 후 Taq polymerase로 증폭하여 한천 겔에서 전기영동을 하여 잘려진 DNA fragment의 양상을 관찰하였다.

결과 : L/L, L/S, S/S의 세가지 유전자형이 검출되었으며 중풍군과 대조군 사이에 유의성 있는 차이는 발견되지 않았다. 개별 allele 빈도에 있어 중풍군과 건강인 사이에는 통계적인 유의성이 나타나지 않았다.

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결론 : 이상의 결과를 통하여 5-HTT 유전자다형성은 중풍의 발병과는 직접적인 관련이 없는 것으로 사려되며 더 많은 환자를 대상으로 다른 환경요인 또는 유전자와의 연관성에 대한 심도깊은 연구가 필요하다고 하겠다.

Key words : Stroke, Serotonin Transporter, Gene, Polymorphism

I. Introduction

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[1].

Serotonin (5-HT) is released following platelet aggregation. Serotonin induces vasoconstriction in the presence of atherosclerotic lesions, whereas, under normal conditions, serotonin causes vasodilatation or has no effect[2,3]. Platelets acquire serotonin from the extracellular space by serotonin transporter (5-HTT) on neurons, glial cells and blood platelets.

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 and control. There is a functional polymorphism in the 5-HTT gene promoter associated with transcriptional efficacy[4]. The genome organization of the 5-HTT gene has been determined and the promoter region of the gene characterized[5,6]. The recent identification of polymorphisms on the 5'-flanking region on the human 5-HTT gene has provided useful markers for investigation of the genetic contribution of 5-HTT gene[7,8]. Recently it was revealed that the 5-HTT promoter polymorphism influences platelet activation[9]. The polymorphism may have a potential role in gene regulation. To date, genetic polymorphism in the 5'-flanking region of the 5-HTT gene has not been described in stroke. We hypothesized that the 5-HTT and platelet are important candidates in the development of stroke. In this study, we investigated polymorphism in the 5-HTT promoter region in Korean stroke patients.

II. Subjects and Methods

1. Study Population

The control group consisted of 138 app-

arently healthy Korean. Controls were selected from healthy subjects who visited several hospitals. The stroke patient group consisted of 137 Korean stroke patients. At first 153 stroke subjects were selected from November 1, 2000, until November 30, 2001, who were admitted to the stroke service of the department of acupuncture & moxibustion, college of Oriental Medicine, Kyung-Hee University, Seoul city. Of these patients, 16 subjects were excluded from this study (1 was dead on arrival, 6 were transported to other hospitals, and 9 declined to give consent). Ultimately, 277 patients were enrolled in the current analysis.

2. Definition and Classification of Stroke

We included 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 (subdural) hematoma, brain tumors, and accidental or iatrogenic stroke. Final diagnosis of stroke subtypes 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. Intracerebral hemorrhage (ICH) was diagnosed when rapid evolution of focal neurological signs, quick progression into coma, signs of meningeal irritation, headache, and high-density areas in CT findings were observed. Subarachnoid hemorrhage (SAH) was diagnosed when such clinical observations as the sudden onset of severe headaches with a relatively momentary disturbance in consciousness, signs of meningeal irritation, absence of focal neurological signs, and presence of blood in the cerebrospinal fluid or the subarachnoidal space was indicated by high-density regions on CT images.

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, 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), according to the method of Lesch et al[4], with minor modifications. Oligonucleotide primers flanking the serotonin transporter promoter gene region (5-HTTLPR) and corresponding to the nucleotide positions -1416 to -1397 (5'-GGCGTTGCCG CTCTGAATGC) and -910 to -888 (5'-GAG GGACTGAGCTGGACAACCAC) of the 5-HTT

gene 5'-flanking regulatory region were used to generate 484- or 528-base pair fragments. PCR amplification was conducted in a final volume of 20 $\mu\ell$ consisting of 50 ng of genomic DNA, 2.5 mM deoxyribonucleotides, 20 pmol of forward and reverse primers, 10 mM tris/HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, and 1 U of Taq DNA polymerase. Annealing was conducted at 61℃ for 30 seconds, extension at 72℃ for 1 minute, and denaturation at 95℃ for 30 seconds for 35 cycles. PCR products were visualized by 2% agarose gel electrophoresis followed by ethidium bromide staining.

5. Statistical Analysis

To compare the distribution of the genotypes and the frequency of alleles between Korean stroke patients and controls χ^2 tests was used. The odds ratios (OR) and 95% confidence intervals (CI) were used to quantify the association with stroke. AS statistical package SAS (release 6.12) was used.

Ⅲ. Results

Clinical characteristics of stroke patients and controls are shown in Table 1. The stroke subjects were significantly older (mean age= 62.4 years, SD=7.8) than the comparison subjects (mean age=50.3, SD=5.4) but they did not differ from the comparison group in percentage of male subjects (46.2% versus 43.3%).

Table 1. Clinical Characteristics of Stroke Patients and Controls

	Controls	Patients
Age	50.3±5.4	62.4±7.8
Maximum	76	86
Minimum	25	18
Male	43.3	46.2
Female	56.7	53.8

Our data provide no evidence for in vivo functional regulation of 5-HTT availability by 5-HTTLPR in between stroke subjects and control participants. There was not statistically significant genotypic distribution difference between control and stroke group. The frequencies of S/S (short allele) homozygotes, L/S heterozygotes, and L/L (large allele) homozygotes among stroke patients were 88 (63.3%), 49 (35.3%) and 2 (1.4%). The frequencies of S/S, L/S and L/L among the control subjects were 83 (60.4%), 54 (38.8%) and 1 (0.7%). And there was not statistically significant allelic frequency difference between control and stroke group. The allelic frequency of S and L was 80.9% and 19.1% among the stroke patients and 79.9% and 20.1% in control subjects, respectively (Table 2).

Table 2. Comparison of Genotype Distribution and Allele Frequencies Between Stroke and Control Participants

Genotype & allele	No. of Controls	No. of patients	P value
L/L genotype	1(0.01)	2(0.01)	NS
L/S genotype	54(0.39)	49(0.35)	NS
S/S genotype	83(0.60)	88(0.63)	NS
S allele	220(0.79)	225(0.81)	NS
L allele	56(0.20)	53(0.19)	NS

χ^2 test was used to compare values of stroke patients and controls for all parameters. NS means no significance

Ⅳ. Discussion and Conclusion

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.

Recently in stroke many polymorphism were investigated and some polymorphism such as α1-antichymotrypsin gene was associated[10] but some polymorphism such as promoter of lipopolysaccharide receptor CD14 was not related[11]. This is the first report to have shown the association of 5-HTT gene polymorphisms with stroke including subtypes by use of CT or MRI findings.

5-HT modulates diverse brain functions through interactions with 14 different 5-HT receptor subtypes[12]. However, recent evidence has shown that the complex 5-HT neuronal system is under bottleneck control by a single protein, 5-HTT[13]. By controlling reuptake of 5-HT from the extracellular space, 5-HTT regulates the duration and strength of the interactions between 5-HT and its receptors. There is a polymorphism in the promoter region of the 5-HTT gene[14,15]. In humans, the majority of alleles are composed of either 14 (S) or 16 (L) repetitive elements. The activity of the human 5-HTT gene promoter is regulated by these polymorphic repetitive elements, resulting in differences in the efficacy of 5-HTT reuptake among the allelic variants[13]. For example 5-HTTLPR short allele (S) has been associated with reduced 5-HTT expression when compared to cells carrying the 5-HTTLPR long allele (L)[6]. A polymorphism in the 5-HTTLPR has been shown to influence the quantity of serotonin transporter expressed in human cell lines[4] and the S allele has been suggested to be functionally dominant[5].

The present study was undertaken to see if specific allelic variations are associated with stroke in the Korean population. Our data failed to show any difference between stroke and control Korean. Moreover, the overall analysis revealed no significant interactions between genotype. The frequencies of S/S homozygotes, L/S heterozygotes, and L/L homozygotes among stroke patients were 88 (63.3%), 49 (35.3%) and 2 (1.4%). The frequencies of S/S, L/S and L/L among the control subjects were 83 (60.4%), 54 (38.8%) and 1 (0.7%). And the allelic frequency of S and L was 80.9% and 19.1% among the stroke patients and 79.9% and 20.1% in control subjects, respectively. The distribution of genotype in our control participants was, in general, consistent with previous reports with Japanese participants[13]. The frequencies of L allele shown in the present and previous studies[16] were all <20% in the Japanese

population, whereas a nearly 3-time higher frequency of the L allele has been found in Whites[17]. Our results suggest that the investigated 5-HTTLPR polymorphisms are not major susceptibility factors in the etiology of ischemia.

Recent study has characterized new variants within the human 5-HTTLPR[18], and regulatory gene sequences other than 5-HTTLPR have been described in the human 5-HTT gene region[19]. 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 5-HTT or other genes and lifestyles with regard to stroke risk is required.

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Footnotes

The first two authors contributed equally to this work.

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