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원 저

한국인 중풍 환자의 Catalase 유전자 다형성 - 환자 대조군 연구 251 례

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Abstract

Catalase Gene Polymorphism in Korean Stroke Patients - 251 Case Control Study

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목적 : 본 연구는 catalase 유전자 다형성이 중풍의 발병과 관련이 있는지 알아보기 위해 수행되었다.

대상 : 경산대학교부속구미 한방병원에 입원한 중풍환자 86 명과 종합건진센터에 내원한 중풍 기왕력이 없는 건강인 165 명을 대상으로 하였다.

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

결과 : T/T, T/A, A/A의 세가지 유전자형이 검출되었으며 중풍군과 대조군 사이에 유의성 있는 차이가 발견되지 않았다. 개별 allele 빈도에 있어서도 중풍군과 건강인 사이에 통계적인 유의성이 나타나지 않았다.

결론 : 이상의 결과를 통하여 catalase 유전자 다형성은 중풍의 발병과는 관련성이 없는 것으로 사려되며 더 많은 환자를 대상으로 다른 환경요인 또는 유전자와의 연관성에 대한 심도있는 연구가 필요하다고 하겠다.

Key words : Stroke, Catalase, Gene, Polymorphism

I. Introduction

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. The antioxidative enzyme system consists of several proteins scavenging oxygen radicals produced in various conditions[1]. Catalase is one of well-known antioxidative enzymes. This enzyme is found in all aerobic cells, and can therefore protect cells from the toxic effects of hydrogen peroxide (H_2O_2)[2),3)]. We report here a case-control study for the catalase gene in stroke patients. The catalase gene was selected as a candidate gene of antioxidation.

Catalase is a homotetrameric, heme-containing, peroxisomal enzyme that catalyses the conversion of hydrogen peroxide to water and oxygen, thereby preventing cell damage from highly reactive oxygen-derived free radicals[4]. In acatalasemia, the absence of catalase enzyme activity in blood and tissues predisposes patients to oral infections by peroxide-generating bacteria such as streptococci and pneumococci, but most forms of hypocatalasemia and acatalasemia are asymptomatic[4].

Many allelic variants of catalase have been reported, and the first form of acatalasemia described was found in a Japanese population[5]. The catalase gene consists of 13 exons spanning 34kb of genomic DNA[6], with the complete cDNA sequence revealing a coding region 1581 bp in length[7].

To date, genetic polymorphism in catalase gene has not been described in stroke, in this study, we investigated HinfI polymorphism in the catalase gene in Korean stroke patients.

II. Subjects and Methods

1. Study Population

The control group consisted of 165 apparently healthy Korean and the mean age of these individuals was 45.7 year (between 20 to 75). The stroke patient group consisted of 86

Korean stroke patients and the mean age of patients was 60.5 year (between 19 to 82). Controls were selected from January 1, 2002, until June 30, 2002, who visited to the health examination center, College of Oriental Medicine, Kyung-San University, Gumi, Korea. Stroke subjects were selected from the stroke service of the above hospital, at the same periods. And following baseline characteristics were recorded; age, gender. Of these patients, 11 subjects were excluded from this study (5 were transported to other hospitals, and 6 declined to give consent). Ultimately, 86 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. 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 haemorrhage (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 headache 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

Venous blood samples from controls and stroke patients were obtained without regard to the time of the last meal. This study was approved by the ethics review committee of the Medical Research Institute, Kyung-San 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, Indianapolis, IN, USA). The following polymorphisms were determined using polymerase chain reaction (PCR) amplification that specifically amplifies DNA region forward primer 5'-AATCAGAAGGCAGTCCTCCC-3' and reverse primer 5'-TCGGGGAGCACAGAGTGTAC-3'. And it was followed by HinfI restriction enzyme digestion: an A to T point mutation in the promoter region of the catalase gene[8], Reaction was amplified using a Perkin Elmer GeneAmp PCR system 9600 (Roche Diagnostic systems). The reaction profiles were as follows; denaturation at 94℃ for 30 seconds, annealing at 64℃ for 30 seconds, extension at 72℃ for 30 seconds, for 40 cycles. The cycling was preceded by a single 5 minutes denaturation at 94℃, and followed by a single cycle of

extension at 72℃ for 10 minutes. All PCR products were confirmed by loading 3% agarose gel electrophoresis, ten microliter of reaction mixture from each sample were digested with HinfI restriction enzyme and under recommended conditions (Boehringer Mannheim, Indianapolis, IN, USA). These products were loaded onto a 3% agarose gels in 0.5x TBE running buffer. The gels were stained with ethidium bromide and visualized by ultraviolet light.

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 SAS statistical package (release 6.12, SAS Institute Inc.) was used.

Ⅲ. Results

Clinical characteristics of stroke patients and controls are shown in Table 1. The catalase-AA genotype and HinfI*2 (A) alleles were not more frequent in patients with stroke than in healthy controls. Genotypic distributions and allelic frequencies of HinfI polymorphism in normal Korean controls and related Korean stroke patients group are shown in Table 2. The HinfI restriction fragment length polymorphism (RFLP) was examined in 86 stroke patients. The heterozygous form of HinfI polymorphism (T/A) was 41.86% of stroke patients, while those homozygous forms for HinfI*1 form (T/T) and HinfI*2 form (A/A) were 44.19% and 13.95%, respectively. On the other hand in 165 Korean healthy controls, the heterozygous form of HinfI polymorphism (T/A) was noted in 41.21% of the normal population, while HinfI*1 homozygous form (T/T) and HinfI*2 homozygous form (A/A) were 45.45% and 13.33%, respectively. There was no significant genotypic distribution difference between control and stroke group (p=0.86). In stroke patients the frequency of HinfI*1 and HinfI*2 alleles were 65.12% and 34.88%, respectively. On the other hand in healthy controls the frequency of HinfI*1 and HinfI*2 alleles were 66.06% and 33.94%, respectively. There was no significant allelic frequency difference between control and stroke group (p=0.74).

Table 1. Clinical Characteristics of Stroke Patients an Controls.

	Controls	Stroke Patients
Age(mean±SD)	45.7±5.1	60.5±6.8
Maximum	75	82
Minimum	20	19
Male(n=123)	78	45
Female(n=128)	87	41

Table 2. Comparison of Genotype Distribution an llele re uencies et een Stroke an Control Participants.

Genotype & Allele	No. of Controls(%)	No. of Stroke Patients(%)	P value
T/T genotype	75(45.45)	38(44.19)	0.86
T/A genotype	68(41.21)	36(41.86)	
A/A genotype	22(13.33)	12(13.95)	
T allele	218(66.06)	112(65.12)	0.74
A allele	112(33.94)	60(34.88)	

χ^2 test was used to compare values of stroke patients and controls for all parameters

IV. Discussion

Apoptosis is a genetically controlled programmed cell death mechanism serving homeostatic functions. Apoptosis is important to determining development, plays a critical role in neurodevelopment[9]. It is involved in the pathogenesis and pathophysiology of several known human diseases, such as autoimmune dysfunction, cancer, stroke and neurodegenerative diseases. In neurodegenerative changes, specific neurons under apoptotic cell death characterized by DNA fragmentation increased levels of pro-apoptotic genes and apoptotic protein[10].

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 sequelae of stroke motor disorder affects the family as well as the patient with great psychological and financial stress. Stroke is the second most fatal disease following cancer in Korea.

Recently in stroke many polymorphism were investigated and some polymorphism such as α 1-antichymotrypsin gene was associated[11] but some polymorphism such as promoter of lipopolysaccharide receptor CD14 was not related[12]. This is the first report to have shown the significant association of catalase gene polymorphisms with Korean stroke patients by use of CT or MRI findings.

In the present study, we raised the question whether the polymorphisms of the antioxidative enzyme (catalase) genetically determined or not. The allele frequencies of catalase of these controls were similar to those reported previously for control and nondiabetic subjects[8),13),14)]. Previous studies have focused on the possible association of chronic heart disease or other atherosclerotic vascular complications with the genetically determined polymorphisms of the antioxidative enzymes[8),13),14),15)]. Our results didn't support for the hypothesis that the polymorphisms of the antioxidative enzyme (catalase) could be among the factors that explain the high incidence of stroke. The functional significances of catalase promoter region polymorphisms are yet unknown but theoretically they could be in linkage disequilibrium with a nearby locus that has a functional significance[15].

There are several possible mechanisms for the catalase gene polymorphism in stroke patients. One of the mechanism is the possible inhibition of catalase activity by elevated levels of H_2O_2 from various sources, as high levels of substrate can irreversibly inactivate the heme active site of catalase[16),17)].

There could also be, as yet unreported, polymorphisms that may significantly contribute to the development of stroke. 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 catalase or other genes and lifestyles with regard to stroke risk is required.

V. Conclusion

These studies have shown no clear relationship in subtypes of stroke. The findings of this study need to be confirmed in larger patient samples and further studies. Additional genetic examinations will be helpful in the prevention of stroke through the promotion of more suitable lifestyles. Overall, these results suggest that the catalase gene may not be a susceptibility gene in some stroke patients, and didn't support the oxidative stress model for stroke pathogenesis.

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VII. Footnotes

The first two authors contributed equally to this work.

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