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Assessment of an Apo-1/Fas Promoter Polymorphism in Korean Schizophrenia Patients

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Apoptosis has been hypothesized to be involved in the pathogenesis in schizophrenia. A large number of genes are known to mediate the apoptotic process; Apo-1/Fas (CD95) is a well-known example of such genes. In the present study, *Mva*I restriction fragment length polymorphism, a polymorphic marker present within the Apo-1/Fas gene, was examined in a population consisting of 226 control subjects and 110 schizophrenia patients, all of them Korean in ethnicity. No statistically significant difference in the genotypic distribution and allelic frequencies was observed between the control and the schizophrenia patient group. To find out the precise effect of Apo-1/Fas gene polymorphisms on the susceptibility to schizophrenia, further studies are warranted to investigate possible involvement of other polymorphisms with a larger sample population.

Key Words: Schizophrenia, Apoptosis, CD95, Promoter regions, Restriction fragment length polymorphism

INTRODUCTION

Schizophrenia is generally considered to be a syndrome, which manifests itself through various disturbances in cognition, reality perception, mood, interpersonal relations, and social and occupational performances. Various reports have suggested that schizophrenia is associated with neurodevelopmental abnormalities, which could be explained by apoptosis. Consequently, investigators have hypothesized a possible involvement of apoptosis in the pathophysiology of schizophrenia (Harrison, 1997; Catts & Catts, 2000).

A large number of genes are known to mediate apoptosis. Apo-1/Fas (CD95) is one such gene; its up-regulation precedes apoptosis in many cell types (Leithauser et al, 1993). The genome organization and the promoter region of the Apo-1/Fas gene have been analyzed (Cheng et al, 1995; Rudert et al, 1995). Two sites of polymorphism on the 5' flanking region on the human Apo-1/Fas gene have been identified, which could serve as the potential markers to investigate the role of the gene (Huang et al, 1997). *Mva*I restriction length fragment polymorphism (RFLP), a polymorphic marker within the Apo-1/Fas gene, is located 670 nucleotides upstream in the enhancer region and results from a GA→GG substitution, which creates an *Mva*I restriction site. This polymorphic site is situated on a consensus sequence of the gamma-activated sequence, which is expected to play a potential role in gene regulation (Shuai, 1994). To date, this polymorphic site has not yet been studied with respect to schizophrenia. In the present study, we have investigated *Mva*I RFLP in the Apo-1/Fas promoter region as a possible lesion in Korean schizophrenia patients was investigated.

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METHODS

Human subjects

The control group consisted of 226 apparently healthy Koreans without personal or familial history of psychiatric or neurologic illness, and the mean age was 40.7 years (ranging between 19 to 75). The schizophrenia patient group is consisted of 110 unrelated Korean schizophrenia patients, diagnosed according to DSM-IV by the trained psyhiatrists; the mean age of the patients was 39.4 years (ranging between 19 to 65). All individuals participating in the study signed the written consent form.

Polymerase chain reaction

Blood samples from all subjects were obtained in EDTA tubes. Genomic DNA was extracted using a DNA Isolation Kit for Mammalian Blood (Boehringer Mannheim, Indianapolis, IN, USA). *Mva*I RFLP was studied by polymerase chain reaction (PCR) for amplification of genomic DNA followed by *Mva*I restriction enzyme digestion of the reaction products. The selected sequences of the PCR primers which was used to amplify the stretch of genomic DNA examined in the present study have been previously published [6] and are as follows: 5'-CTACCTAAGAGCTA-

ABBREVIATIONS: RFLP, restriction length fragment polymorphism; PCR, polymerase chain reaction; CI, confidence intervals.

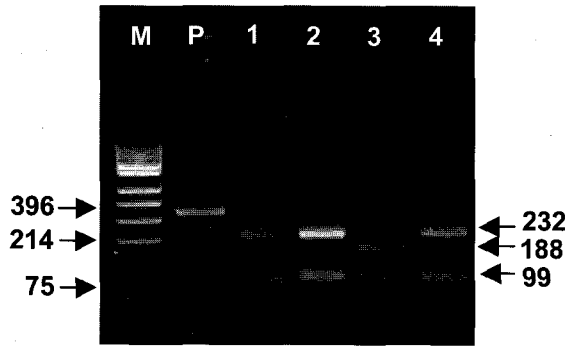

Fig. 1. Genotype analysis of *Mva*I polymorphism in Apo-1/Fas gene. The PCR products were digested with *Mva*I and subjected to electorophoresis in a 3% agarose gel, followed by ethidium bromide staining. Lane M, size marker; Lane P, undigested PCR products (331 bp); Lane 1, heterozygous *1/*2 genotype (232 bp, 188 bp, 99 bp, 44 bp); Lane 2 and 4, homozygous *1/*1 genotype (232 bp, 99 bp); Lane 3, homozygous *2/*2 genotype (188 bp, 99 bp, 44 bp). The 44 bp bands in Lane 1, 3 were not shown.

TCTACCGTTC-3' (sense) and 5'-GGCTGTCCATGTTGTG-GCTGC-3' (antisense). PCR was carried out using a Perkin Elmer GeneAmp PCR system 9600 (Roche Diagnostics Corporation, Indianapolis, IN, USA). The conditions employed in the PCR cycles were as follows; 40 cycles, each consisting of denaturation at 94°C for 30 sec, annealing at 58°C for 1 min, and extension at 72°C for 1 min. The reaction cycles were preceded by a single 5 min period of denaturation at 94°C, and were followed by a 10-min period of extension at 72°C. Formation of proper PCR products was confirmed via electrophoresis of whole PCR products on 1.5% agarose gels.

Analysis of polymorphic restriction enzyme site

The PCR product of each sample was then digested with the *Mva*I restriction enzyme (Boehringer Mannheim, Indianapolis, IN, USA). The digestion products were electrophoresed on 3% agarose gels in a 0.5×TBE running buffer. Two polymorphic alleles, *1 (G: fragment of length 188 bp) and allele *2 (A: fragment of length 233 bp), were visualized, depending on the presence of A→G substitution at −670 bp (Fig. 1).

Statistical analysis

The distribution of the *Mva*I genotypes in the schizophrenia patient group was compared to that of the control group using the χ^2test (3×2 contingency table). To compare the allelic frequencies of *1 and *2 of the two groups, a 2×2 contingency (χ^2) test was used. Odds ratios and 95% confidence intervals (CI) were used to quantify any association between the polymorphism and schizophrenia. The Statistic Analysis System program was used for statistical analysis.

RESULTS

The genotypic distribution and allelic frequencies of *Mva*I RFLP in the control group and schizophrenia patient group are shown in Table 1. The frequency of heterozygosity (*1/*2) for the *Mva*I RFLP was 54.55% in the schizophrenia patient group, while the frequencies of homozygosity for the *1 and *2 alleles ((*1/*1) and (*2/*2), respectively) were similar, at 23.64% and 21.82%, respectively. The allelic frequencies of *1 and *2 were 50.91% and 49.09%, respectively. In the control group, the frequency of heterozygosity (*1/*2) for *Mva*I RFLP was 48.67%, while *1 homozygotes (*1/*1) and *2 homozygotes (*2/*2) were 31.42% and 19.91%, respectively, of the group's population. The allelic frequencies of *1 and *2 were 44.25% and 55.75%, respectively. There was no statistically significant difference in genotypic distribution and allelic frequencies between the control and the schizophrenia patient groups (p=0.183, 0.104).

Table 1. The *Mva*I polymorphism: genotypes and corresponding allele frequencies in Korean control group and Korean schizophrenia patient group

Samples	Genotypes (%)			Allele frequency (%)	
	*1/*2	*1/*1	*2/*2	*1	*2
Control (N=226)	110 (48.67%)	45 (19.91%)	71 (31.42%)	200 (44.25%)	252 (55.75%)
Schizophrenia (N=110)	60 (54.55%)	26 (23.64%)	24 (21.82%)	112 (50.91%)	108 (49.01%)
χ^2 value		3.401		2.640	
df		2		1	
P value		0.183		0.104	
Odd ratio (95%CI)				0.765 (0.554~1.057)	

DISCUSSION

Numerous reports are available on the occurrence of neurodevelopmental abnormalities such as altered cortical organization and cytoarchitecture in the brain, for example, in schizophrenia, apoptosis induced by tumor suppressor gene has been implicated (Harrison, 1997; Catts & Catts, 2000). In addition, it has been established by several epidemiological studies that the incidence of cancer is lower in patients with schizophrenia than in those who do not have the disorder (Mortensen, 1994; Mortensen, 1989). Because apoptosis suppresses neoplastic change by eliminating potential tumor cells, it could be rationalized that the basal rate of apoptosis might be increased as it appeared to be the case in schizophrenia patients whose tumor resistancy is expected, and that this is the case in schizophrenia patients observed by epidemiological studies.

Apo-1/Fas, also known as CD95, is a member of the tumor necrosis factor/nerve growth factor receptor superfamily, and it transduces death signals in susceptible target cells (Nagata, 1994). Since, the essential role the Apo-1/Fas gene is known to play in the regulation of immune system, *Mva*I RFLP of the human Apo-1/Fas gene has been applied to evaluate several immunologic diseases, such as multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus (Huang et al, 1999; Huang et al, 2000). However, these studies have failed to answer any clear and strong association between these diseases and the *Mva*I RFLP of the Apo-1/Fas gene. For several neuropsychiatric disorders, including Alzheimer's disease and Down syn-

drome, the possible involvement of Apo-1/Fas gene in apoptosis of neural and glial cells has been reported (DelaMonte et al, 1997; DelaMonte et al, 1998; Seidl et al, 1999).

In the present study, we have not observed any clear association between MvaI RFLP of the Apo-1/Fas gene and schizophrenia in a Korean population. Furthermore, between Caucasians and Koreans, there were no significant differences in allele frequency and genotype distribution of MvaI RFLP (data not shown; Huang et al, 1999; Huang et al, 2000). Additional investigation with more advanced designs and ideas should be conducted to integrate the interaction of genetic and various environmental factors.

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