

Apolipoprotein E Genotype in Korean Schizophrenic Patients

The apolipoprotein E (*APOE*) ϵ 4 allele is a known risk factor for the development of Alzheimer's disease, however, an association of the *APOE* genotype with schizophrenia is controversial. We investigated the association in 60 Korean schizophrenic patients and 60 healthy controls. *APOE* genotypes were identified by reverse hybridization-based line probe assay. There were significant differences in the distribution of *APOE* genotypes between schizophrenic patients and controls. *APOE* ϵ 2 and ϵ 3 allele frequencies in schizophrenic patients were significantly different from those in controls. Our results suggest that *APOE* alleles seem to be operative in the pathogenesis of schizophrenic disorders.

Key Words : Apolipoproteins E; Genotype; Schizophrenia

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INTRODUCTION

Schizophrenia is a clinical syndrome of severe mental illness characterized by organized thinking processes, hallucinations, delusions, and functional deterioration (1). Schizophrenia remains a diagnosis of exclusion, since none of its clinical features are pathognomonic (2), and applicable biochemical, neuroradiologic, physiologic, and psychological tests lack the sensitivity and specificity required for a decisive diagnosis. While numerous neuroanatomical, neurophysiological and neurochemical abnormalities have been described for the brains of patients with schizophrenia, the pathognomonic neurobiological mechanisms of the disorder have not yet been determined. Family, twin, and adoption studies indicate that there is a major heritable component to schizophrenia (3). Recent progress in molecular biology, neuroimaging, animal modeling, and genetics have significantly enhanced efforts to understand schizophrenia. Genetic studies have implicated several minor susceptibility loci, however the clinical impact of these loci on the neurobiology of schizophrenia is still unclear. In addition, researchers have reported an association of the disease with a polymorphism in candidate genes, such as the dopamine D3 receptor gene (4) and an expansion of trinucleotide repeats (5).

Apolipoprotein E (apoE) is found in plasma, where it has an important role in the transport of cholesterol and the modulation of atherogenic lipoprotein metabolism (6). It is also produced by astrocytes and oligodendrocytes in the central nervous system, where its physiological role is less certain (7). ApoE is suggested, however, to be involved in the transport

of cholesterol in myelin and neuronal membranes, and also to play important roles in neuronal growth and central nervous system response to injury (8). The gene for human *APOE* is found on the long arm of chromosome 19 (19q13.2) (9) and exists in three common allelic forms (ϵ 2, ϵ 3, and ϵ 4). These three alleles encode the main isoforms of apoE (E2, E3, and E4), which differ by amino acid substitutions at one or both of two sites, imparting distinctive physical and biochemical properties to each isoform (10).

Recently, it has been established that the *APOE* ϵ 4 allele is a risk factor for the development of Alzheimer's disease (AD) (11), while the ϵ 2 allele has been implicated as a protective factor against the development of dementia in AD (12). These genetic associations have led to many investigations on the role of *APOE* genotypes in other neuropsychiatric disorders. The association between *APOE* genotype and schizophrenia was first reported in 1995 (13) and subsequent studies on *APOE* genotype distribution in schizophrenia have reported conflicting findings (14-21). We examined the *APOE* genotype frequencies in Korean schizophrenic patients in order to corroborate the potential association between *APOE* genotypes and schizophrenia reported in previous studies.

MATERIALS AND METHODS

Sixty inpatients with schizophrenia (39 males, 21 females, mean age \pm SD: 32.95 \pm 7.04 yr) at the Department of Psychiatry, Chung-Ang University Hospital were selected for

this study. All patients had a diagnosis of schizophrenia according to DSM-IV criteria. Sixty healthy controls (30 males, 30 females, mean age \pm SD: 35.04 ± 8.93 yr) had undergone diagnostic assessments and were devoid of psychiatric and neurologic illnesses. The patients and controls were free of diabetes, dyslipidemia, and history of cardiovascular disease.

APOE genotypes were examined by reverse hybridization-based line probe assay (LiPA; INNO-LiPA *APOE*, Innogenetics, Belgium) (22). Specific oligonucleotides were immobilized at known locations on a membrane strip and were hybridized under strictly controlled conditions with biotin-labelled polymerase chain reaction (PCR) product. The hybrids formed are subsequently detected colorimetrically. The LiPA was performed as described in the user manual supplied. DNA was extracted from blood by a simple method using proteinase K (23). Target DNA was amplified in a PCR using the biotinylated primers. The PCR consisted of 30 cycles of 95°C for 30 sec, 60°C for 20 sec, and 72°C for 20 sec, followed by a final cycle of 10 min at 72°C. After gel analysis, PCR products would be denatured and hybridized to membrane-bound capture probes, followed by a color detection step that involved the addition of a conjugate (streptavidin) to which the enzyme alkaline phosphatase was bound. Chromogenic substrates (5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium) were added and converted into an insoluble purple-blue product by the alkaline phosphatase. Interpretation of the banding pattern allowed differentiation of *APOE* genotypes. Statistical analysis of *APOE* genotype distributions and allele frequencies were performed by the chi-square analysis and Fisher's exact test using Epi Info 2000 (version 1.05, Center for Disease Control and Prevention). $p < 0.05$ was considered significant. The chi-square test calculations for deviation from Hardy-Weinberg equilibrium were performed at the web site <http://www.bu-sdyr.kvl.dk/btm-1/kl/genetik/2/kitest.htm> (Prof. Knud Christensen).

RESULTS

The age ($p=0.159$) and sex ($p=0.140$) distribution of the schizophrenic patients were similar to those of control population. The *APOE* genotypes and allele frequencies in the schizophrenic patients and control population are given in Table 1. In both groups the genotype distributions were in Hardy-Weinberg equilibrium. The *APOE* genotypes in the schizophrenic patients were distributed as follows: $\epsilon 2/\epsilon 3$ ($n=3$, 5.0%), $\epsilon 3/\epsilon 3$ ($n=53$, 88.3%), $\epsilon 3/\epsilon 4$ ($n=4$, 6.7%). There were significant differences in the distribution of *APOE* genotypes between the two groups ($p=0.044$). *APOE* allele frequencies of $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ were 0.025, 0.942, and 0.033 in schizophrenic patients and were 0.108, 0.808, and 0.083 in normal controls, respectively. The frequency of *APOE* $\epsilon 3$ in schizophrenic patients was significantly higher than that in controls ($p=0.0017$; $\chi^2=9.75$; Odds ratio 3.83, 95% CI

Table 1. Comparison of apolipoprotein E genotypes and allele frequencies between schizophrenic patients ($n=60$) and control subjects ($n=60$)

	Schizophrenic patients	Control subjects
Genotype:		
$\epsilon 2/\epsilon 3$	3 (5.0%)	11 (18.3%)
$\epsilon 2/\epsilon 4$	0 (0%)	2 (3.3%)
$\epsilon 3/\epsilon 3$	53 (88.3%)	40 (66.7%)
$\epsilon 3/\epsilon 4$	4 (6.7%)	6 (10.0%)
$\epsilon 4/\epsilon 4$	0 (0%)	1 (1.7%)
Allele:		
$\epsilon 2$	3 (2.5%)	13 (10.8%)
$\epsilon 3$	113 (94.2%)	97 (80.8%)
$\epsilon 4$	4 (3.3%)	10 (8.3%)

1.48-10.3). In contrast, the frequencies of *APOE* $\epsilon 2$ ($p=0.0096$; $\chi^2=6.7$; Odds ratio 0.21, 95% CI 0.05-0.82) and $\epsilon 4$ ($p=0.098$; $\chi^2=2.73$; Odds ratio 0.38, 95% CI 0.1-1.37) in schizophrenic patients were lower than those in controls.

DISCUSSION

In this study, we found that the frequencies of two *APOE* alleles were significantly different between the schizophrenic patients and the controls. *APOE* $\epsilon 3$ showed a significant association with the schizophrenic patients, as compared with the controls. In contrast, the frequency of *APOE* $\epsilon 2$ in the schizophrenic patients was significantly lower than that in the controls. These findings are in agreement with only a few reports on the *APOE* genotypes in patients with schizophrenia (20, 21) and stand in contrast to most other reports (13-19). Although the basis for this discrepancy is unclear, there are several possible explanations for this discrepancy. First, the *APOE* allele frequency is dependent on the ethnic and genetic background of the population being examined (24). Thus, variations in the frequencies of *APOE* alleles in schizophrenic patients compared with controls may be attributable to this factor. Second, the differences may have been due to the clinical status of the patients. Our patients were inpatients in closed wards and therefore might have had more severe psychopathology than the average. Third, early methods for the detection of *APOE* isoforms were based on protein isoelectrofocusing (25). Later, *APOE* genotyping based on PCR and *HhaI* digestion were introduced (26). However, PCR-based assays are difficult to interpret because the *HhaI* enzyme yields several small fragments, not all of which are specific for the *APOE* genotypes. While most prior reports examined *APOE* genotype by PCR and *HhaI* digestion, our *APOE* genotyping was determined by reverse hybridization-based line probe assay. Different *APOE* genotyping methods might have affected discrepant reports on the frequencies of *APOE* genotypes and alleles. Finally, it is possible that random effects from the smaller sample size of our

study would account for the difference.

The frequency of the *APOE* $\epsilon 4$ was lower in patients with schizophrenia, but this was not statistically significant ($p=0.098$) and thus does not provide evidence of an association between the *APOE* $\epsilon 4$ allele and schizophrenia. This finding is similar to those of other reports (14-18).

In conclusion, our results suggest that two *APOE* alleles, $\epsilon 2$ and $\epsilon 3$, seem to figure in the pathogenesis of schizophrenic disorders. This correlation certainly needs further study with a large number of patients.

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