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A Polymorphism (rs10920568, A102A) of Adenosine A1 Receptor (*ADORA1*) Gene is Associated with Schizophrenia in Korean Population

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ABSTRACT

Adenosine A1 receptor (ADORA1) has a neuromodulatory activity in early stage of brain development. Recent studies have been suggested that a deficit in adenosinergic function may be a key factor in the pathophysiology of schizophrenia. To determine the genetic association between *ADORA1* gene polymorphism and schizophrenia in Korean population, we genotyped single nucleotide polymorphism (SNP) (rs10920568, A102A, exon5) in the *ADORA1* gene by using the direct sequencing. Among SNPs in the coding region of *ADORA1*, only one synonymous SNP's heterozygosity (rs10920568) is more than 0.05. Three hundred three control and 284 schizophrenia subjects were recruited. For the analysis of genetic data, EM algorithm, SNPStats, SNPAnalyzer, and Helixtree programs were used. Multiple logistic regression analysis with the codominant, dominant, and recessive models was performed. The genotype frequencies of rs10920568 showed statistically significant difference between schizophrenic patients and healthy control subjects. The rs10920568 SNP of *ADORA1* was weakly associated with schizophrenia in the dominant model (p=0.04, odds ratio=0.70, 95% confidence interval =0.50~0.98). The result suggests that the *ADORA1* gene may be associated with schizophrenia.

Key words: adenosine A1 receptor, association, schizophrenia, single nucleotide polymorphism

INTRODUCTION

Adenosine is a nucleoside that exists in the whole body. Although adenosine does not act as a classical neurotransmitter, adenosine acts as a neuromodulator controlling neurotransmitter release and neuronal excitability in the central nervous system (CNS) (Cunha, 2001; Dunwiddie and Masino, 2001; Fredholm et al., 2005). Boison (2008) also reported that adenosine is a modulator of brain function uniquely positioned to integrate excitatory and inhibitory neurotransmissions. Adenosine receptors are a four-member subfamily of G protein-coupled receptors. There are designated as A_1, A_{2A}, A_{2B}, and A_3 (Ribeiro et al., 2002; Gao and Jacobson, 2007). Recent reviews have shown that adenosine receptors are related to the pathophysiology of neuropsychiatric disorders and neurodegenerative

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diseases (Fredholm et al., 2005; Jacobson and Gao, 2006). Lara et al. (2006) reported the involvement of adenosine in the neurobiology of schizophrenia. Adenosine A1 receptor (ADORA1)-mediated inhibition of dopamine release from rat striatal slices is modulated by D1 dopamine receptor (O'Neill et al., 2007). Cao et al. (2007) reported the enhancement of dopamine D1 receptor desensitization by ADORA1 activation. Torvinen et al. (2004) found the biochemical identification of the dopamine D2 receptor domains interacting with the adenosine A_{2A} receptor (ADORA2A).

Deckert et al. (1995) searched a systematic mutation scan of the coding region of the *ADORA1* gene. They first detected a variant in the *ADORA1* gene. Deckert et al. (1998) also reported that polymorphisms of the *ADORA1* promoter do not play a major role in the development of bipolar affective disorder. Polymorphisms in adenosine receptor genes were associated with infarct size in patients with ischemic cardiomyopathy (Tang et al., 2007). Wright et al. (2004) reported the role of variants of adenosine-related genes in essential hypertension. Hong et al. (2005) reported the association study of the *ADORA2A* (1976T>C) polymorphism in Parkinson's disease and schizophrenia. However, a genetic study of the *ADORA1* gene in schizophrenia has not been performed yet. In this study, we investigated whether a synonymous polymorphism (rs10920568, A102A, exon5) of the *ADORA1* gene was associated with schizophrenia in Korean population.

MATERIALS AND METHODS

Subjects

All subjects used in this study were obtained from Kyung Hee University Medical Center (IRB number, 20040915; genetic institute, no89). Three hundred three control subjects with no clinical evidence of any other disorders (150 men, 40.0±5.8 years (mean age±SD); 153 women, 33.4±6.3) and 284 schizophrenic subjects (165 men, 42.8±10.9; 115 women, 43.6±10.8) were recruited. Clinical diagnosis was conducted according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, 1994). Medical record of each patient was reviewed. The Brief Psychiatric Rating Scale (BPRS) (Flemenbaum and Zimmermann, 1973), the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1982), and the operational criteria (OPCRIT) checklist (McGuffin et al., 1991) were also applied to patients with schizophrenia. Patients with SCZ were also divided according to onset age (onset age ≤20 years, 60 patients: onset age >20 years, 192 patients). All studies were carried out according to the guidelines of the Declaration of Helsinki (Rickham, 1964). Control subjects were recruited as mentally healthy based on a general health checkup program. DNA was isolated from a peripheral blood using the Core One Blood Genomic DNA Isolation Kit (CoreBioSystem, Seoul, Korea). The study was approved by the ethics Review Committee of the Medical Research Institute, Kyung Hee University Medical Center, Seoul, Korea. Written informed consent was obtained from all subjects.

Genotyping

We initially selected SNPs within exon regions of the *FRK* gene using the following websites: (1) human SNP websites (http://www.ensembl.org; www.ncbi.nlm.nih.gov/SNP), (2) HapMap database (http://www.hapmap.org), (3) tag SNPs website (http://broad.mit.edu/mpg/tagger). When the SNPs with unknown heterozygosity and minor allele frequency (below 5%) were also excluded, we obtained only one SNP (rs10920568). Genotyping of rs10920568 was conducted by direct sequencing. Genomic DNA was amplified using the following primers for rs10920568 (sense, 5'- TGCTGGTGATCTGGGCGGTGAAGGTG-3'; antisense, 5'- CATCTGGCTTACTTGGGGTCTTATGC-3'). The samples were sequenced using an ABI Prism 377 automatic sequencer (PE Applied Biosystems, Foster City, CA, USA). Sequence data were analyzed using the SeqManII software (DNASTAR Inc., Madison, WI, USA).

Statistical Analysis

Chi-square test was used to compare the observed numbers of each genotype with the expected results for a population in Hardy-Weinberg equilibrium ($p > 0.05$). Multiple logistic regression models were used for the odds ratio (OR), 95% confidence interval (CI), and p value, controlling for age and gender as covariables. We also used SNPStats, SNPAnalyzer

(ISTECH Inc., Goyang, Korea), and Helixtree software (GoldenHelix, MT, USA). The significance level was set at 0.05.

RESULTS

In this study, we investigated whether an *ADORA1* gene polymorphism (rs10920568) was related to schizophrenia in Korean population. In the control group, genotype distribution of rs10920568 SNP was in Hardy–Weinberg equilibrium ($p > 0.05$). The genotype distributions of rs10920568 in schizophrenia and controls are summarized in Table 1. The rs10920568 showed statistically significant difference between schizophrenia and controls. In a dominant model, rs10920568 was weakly associated with schizophrenia (p=0.04, OR=0.70, 95% CI=0.50～0.98). TT, GT, and GG genotype frequencies in Korean control subjects were 0.653, 0.310, and 0.036, which are similar to those in Japanese or Chinese (http://www.ncbi.nlm.nih.gov/SNP). However, genotype distributions (TT, 0.570 GT, 0.373 GG, 0.056) of rs10920568 in Korean schizophrenia were different, compared to controls (Fig. 1, Table 1).

We also evaluated any association between the SNP (rs10920568) and onset age. Onset age was not related to any statistically significant difference in genotype and an allelefrequency analysis between the two groups (onset age≤20 years and onset age＞20 years; data not shown).

DISCUSSION

Adenosine activates adenosine receptors, which in turn regulate various physiologic functions including cardiac contractility, platelet function, lipolysis, renal function, smooth muscle tone, sedation, and release of neurotransmitters. Popoli et al. (1994) reported that modulation of striatal adenosine A1 and A2 receptors induces rotational behaviour in response to dopaminergic stimulation in intact rats. However, no genetic study concerning the association between *ADORA1* and schizophrenia has been reported.

The rs10920568 is located on exon 5, and is a synonymous SNP (A102A) with 0.318 heterozygosity (http://www.ncbi.nlm.nih.gov/SNP). ADORA1 protein (P30542) consists of 326 amino acids, and belongs to G-protein coupled receptor. Amino acids from 11 to 33 comprise potential 1st transmembrane domain, 47 to 69 potential 2nd transmembrane domain, 81 to 102 potential 3rd transmembrane domain, 124 to 146 potential 4th transmembrane

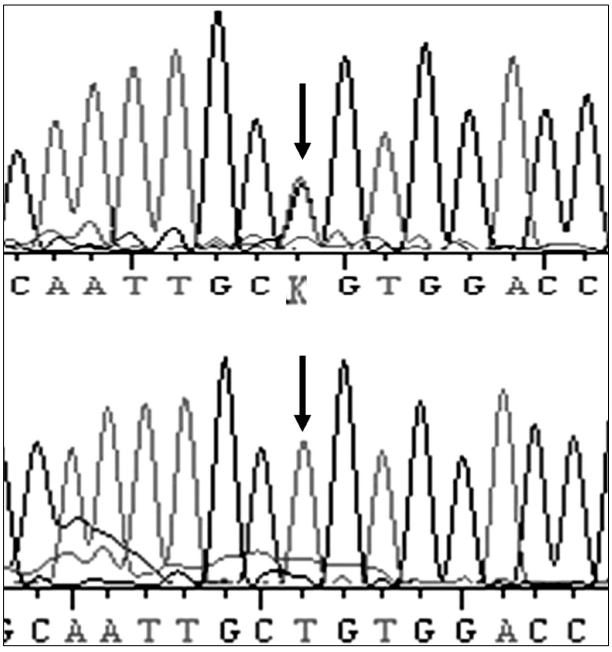

Fig. 1. Direct sequencing of PCR-amplified DNA including an SNP (rs10920568) of the adenosine A1 receptor (*ADORA1*) gene. Arrows indicate electropherograms of nucleotide showing the heterotype GT (top) and homotype TT (bottom). K means G and T nucleotides.

Table 1. Logistic regression analysis and genotype frequency of adenosine A1 receptor (*ADORA1*) polymorphism in schizophrenia and control subjects

Locus	Genotype	Schizophrenia	Controls	Model	OR (95% CI)	p
		Frequency (%)	Frequency (%)			
rs10920568 (A102A)	T/T	162 (57.0)	198 (65.3)	Codominant	0.56 (0.25～1.25)	0.09
(Exon5)	G/T	106 (37.3)	94 (31.0)	Dominant	0.70 (0.50～0.98)	**0.04**
	G/G	16 (5.6)	11 (3.6)	Recessive	0.63 (0.29～1.38)	0.25

OR: odds ratio, CI: confidence interval.

domain, 177 to 201 potential 5th transmembrane domain, 236 to 259 potential 6th transmembrane domain, and 268 to 292 potential 7th transmembrane domain (UniProt, http://beta.uniprot.org; SwissProt, http://www.expasy.org). The rs10920568 in potential 3rd transmembrane domain was associated with schizophrenia (Table 1). T and G allele frequencies are reported to be 0.681 and 0.319 in European, 0.789 and 0.211 in Chinese, 0.818 and 0.182 in Japanese, and 0.915 and 0.085 in Sub-Saharan African, respectively (http://www.ncbi.nlm.nih.gov/SNP). In Korean population, T and G allele frequencies were 0.809 and 0.191, which are similar to those in Japanese and Chinese.

However, our study had several limitations. Firstly, we analyzed one synonymous SNP of the coding region in the *ADORA1* gene. Therefore, future study will be needed to assess the association between additional SNPs of the *ADORA1* gene and schizophrenia. Secondly, another replication study on rs10920568 will be investigated.

In conclusion, we detected a significant association between the *ADORA1* gene and schizophrenia. The result suggests that the *ADORA1* gene may be related to the development of schizophrenia in Korean population.

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