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Association analysis between the C-1291G polymorphism in the promoter region of the adrenergic α2A receptor gene and polydipsia in schizophrenia

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ABSTRACT

Several lines of studies have shown the existence of an important inhibitory mechanism for the control of water intake involving adrenergic α2A receptors (ADRA2A). A human study using patients with schizophrenia demonstrated an exacerbation of polydipsia by the administration of clonidine, an ADRA2A-agonist, and a relief of polydipsia by mianserin, an ADRA2A-antagonist, suggesting the involvement of the central adrenergic system in the drinking behavior of patients with schizophrenia. Based on these findings we examined a possible association between the C-1291G polymorphism in the promoter region of the *ADRA2A* gene and polydipsia in schizophrenia using a Japanese case-control sample. Our sample includes 348 patients with schizophrenia (DSM-IV) (84 with polydipsia and 264 without polydipsia). No significant association between the *ADRA2A* C-1291G polymorphism and polydipsia was found. Our result suggests that the *ADRA2A* C-1291G polymorphism may not confer susceptibility to polydipsia in schizophrenia in our sample. Further studies with larger samples are warranted.

1. Introduction

Polydipsia is defined as either chronic or intermittent ingestion of large volumes of water. This occurs frequently among chronic psychiatric patients. Polydipsia, not explained by medically-induced polyuria, may be present in more than 20% of chronic psychiatric inpatients (de Leon et al., 1994). Polydipsia is most frequently diagnosed in patients with schizophrenia (SCZ). Among psychiatric disorders, approximately 80% of patients with polydipsia and water intoxication suffer from SCZ (Ferrier, 1985). Polydipsia may lead to bladder dilation, enuresis, incontinence, hydronephrosis, renal failure, and congestive heart failure. In addition, it may also lead to hyponatremic symptoms, which may be neurological in nature, and are often referred to as water intoxication: nausea, vomiting, delirium, ataxia, seizures, and even death (de Leon et al., 1994). Up to 5% of chronic inpatients develop water intoxication, though mild cases may have been missed (de Leon et al., 1994).

Although the underlying pathophysiology of polydipsia is poorly understood, two previous studies indicate a possible genetic component to this disorder. In the late 1950s, genetically polydipsic mice, STR/N, were discovered (Silverstein et al., 1958). Furthermore, our group reported a significant familial concordance of polydipsia in a SCZ family sample (Shinkai et al., 2003).

Adrenergic α2A receptors (ADRA2A) are presynaptic autoinhibitory receptors of noradrenergic neurons in the central and peripheral sympathetic nervous systems, which act to dynamically regulate neurotransmitter release. Signaling through the G_i/G_o family of G-proteins, the receptor subserves numerous homeostatic and central

Abbreviations: ADH, antidiuretic hormone; ADRA2A, adrenergic α2A receptor; AP, antipsychotics; Asn, asparagine; C, cytosine; CI, confidence interval; df, degrees of freedom; DNA, deoxyribonucleic acid; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; G, guanine; G-protein, guanine nucleotide binding protein; HPD-eq, haloperidol equivalents; ID, identification; LPBN, lateral parabrachial nucleus; Lys, lysine; MAP, mitogen-activated protein; NaCl, sodium chloride; OR, odds ratio; PCR, polymerase chain reaction; SCZ, schizophrenia; SE, standard error; SIADH, syndrome of inappropriate antidiuretic hormone secretion; SNP, single nucleotide polymorphism; SSRI, selective serotonin reuptake inhibitor; UOEH, University of Occupational and Environmental Health.

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Table 1
Demographic Characteristics of Cases and Controls.

	SCZ with polydipsia ($N=84$)	SCZ without polydipsia ($N=264$)	p
Gender (male/female)	55/29	134/130	0.018**
Age	50.4 ± 11.4	53.0 ± 11.3	0.048*
Age of onset (years old)	23.8 ± 7.5	26.7 ± 7.9	0.002*
Current AP dose (HPD-eq; mg/day)	21.5 ± 23.5	21.9 ± 22.9	ns*
Smoking (yes/no)	62/22	41/33 (190 unknown)	0.015**

Values are given as mean ± SD or number.
HPD-eq: haloperidol equivalents.
p values calculated using Mann–Whitney U-test*, χ^2 test** (gender: $\chi^2=5.56$, $df=1$; smoking: $\chi^2=5.87$, $df=1$).

nervous system functions. ADRA2A has been suggested to be involved in water intake behavior. Injections of ADRA2A-agonist into the lateral parabrachial nucleus (LPBN), a region having a close neuronal connection with the drinking center (lateral hypothalamus), enhances NaCl and water intake in rats (Andrade et al., 2004). In patients with schizophrenia, the α2A-adrenergic system has also been linked with the drinking behavior (Hayashi et al., 1997). Hayashi et al. (1997) have demonstrated that clonidine, a selective α2-adrenoceptor agonist, deteriorates the polydipsia in patients with SCZ, whereas mianserin, an α2-adrenoceptor antagonist ameliorates it.

A single nucleotide polymorphism (SNP) of this receptor, which results in an asparagine to lysine substitution at amino acid 251 (Asn251Lys) of the third intracellular loop, was identified in the human population (Small et al., 2000). The Lys251 variant confers significantly increased agonist-promoted binding to Gi, leading to greater inhibition of adenylyl cyclase, activation of MAP kinase signaling, and stimulation of inositol phosphate accumulation (Small et al., 2000). However, the allele frequency of this variant, Lys251, is as small as 0.05 and 0.004 in African Americans and Caucasians, respectively, which would not be informative for association study. Another polymorphism identified by Lario et al. (1997) is a C to G polymorphism of the gene at position −1291 bp, which may be informative for the association study. Although the nature of this C-1291G polymorphism is unknown, studies have suggested that this SNP may be associated with some neurobehavioral developmental disorders such as attention-deficit hyperactivity disorder (Comings et al., 1999; Schmitz et al., 2006). Furthermore, Belfer et al. (2005) reported that a single haplotype block spanned *ADRA2A* gene; this haplotype block is composed by 9 different SNPs that distribute from the 5′ end to the 3′ end of *ADRA2A* locus, including the C-1291G and the functional polymorphism Asn251Lys.

In view of these findings, in the present study we examined the association between the *ADRA2A* C-1291G polymorphism and polydipsia in patients with schizophrenia using a Japanese case-control sample.

2. Subjects and methods

2.1. Subjects

A total of 84 unrelated in-patients (46 males, 25 females, age 50.4 ± 11.4 yr, mean ± S.D.) who met the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria for SCZ with polydipsia were recruited for participation in this study. Two hundred sixty four unrelated in-patients (134 males, 130 females, age 53.0 ± 11.3 yr, mean ± S.D.) who met the DSM-IV criteria for SCZ without polydipsia were also recruited as controls. None of the subjects had significant neurological comorbidity, epilepsy, mental retardation, or history of substance abuse. All subjects in the present study were Japanese and were recruited from psychiatric hospitals within a 70 km radius of the University of Occupational and Environmental Health (UOEH) in Kitakyushu City, Japan. Several epidemiological studies in psychiatric patients have shown polydipsia to be significantly associated with the chronicity of psychiatric disorders (de Leon et al., 1994). Therefore, in order to increase the confidence in the validity of the diagnosis of polydipsia, we limited the cases and controls to those who had been hospitalized for more than 3 years and whose serum sodium levels in the regular routine laboratory screening were able to be followed over more than 3 years. Most of patients used in this study were chronic schizophrenia inpatients (mean duration of illness, 25.8 ± 11.5 years). As well, we excluded those patients who were on any atypical antipsychotics from the study sample. These factors may be associated with the high current antipsychotic (AP) dose in the present sample (approximately 21 mg of haloperidol equivalents).

The diagnosis of polydipsia was made as previously described (Shinkai et al., 2003). Briefly, a diagnosis of polydipsia was made when the patient consistently drank excessive quantities of fluid and had a serum sodium level less than 135 meq/L at least one time on routine laboratory screening. Conventionally, the consumption of more than 3 L/day of fluid is defined as excessive (de Leon et al., 1994). Care-workers estimated the fluid intake of patients by close observation. Patients' symptoms were assessed by a research psychiatrist to confirm that their hyponatremia was associated with polydipsia and not attributable to a medical disorder such as hypothyroidism or cardiac, renal, or hepatic failure or to medications such as diuretics or carbamazepine; it has been reported that carbamazepine could induce hyponatremia via the syndrome of inappropriate antidiuretic hormone secretion (SIADH) (Van Amelsvoort et al., 1994). We also excluded those patients who were on lithium or selective serotonin reuptake inhibitors (SSRIs), which may be associated with the complaint of thirst, or associated with drug-induced hyponatremia and SIADH. Informed consent was obtained from all subjects, and this study was approved by the Ethics Committee of UOEH.

3. Genetic analysis

Two 7 ml EDTA tubes of blood were drawn from patients and their parents, and genomic DNA was extracted using standard procedures. Genotypes of the *ADRA2A* C-1291G polymorphism (SNP ID: rs1800544) were assessed by the TaqMan allele specific assay method (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocols in the Bio-information Research Center, UOEH. Two dual labeled probes centered on the SNP and differing in sequence by the 1-bp polymorphism of the SNP site itself were designed by Applied Biosystems Inc. The probes were labeled with 5′ reporter fluors VIC (corresponding to −1291C) or 6-FAM (corresponding to −1291G) and a 3′ quencher. Polymerase chain reaction (PCR) amplifications were performed on an ABI PRISM® 7000 Sequence Detection System (Applied Biosystems) with the reaction mixture in a total volume of 25 µl, consisting of 40 ng of genomic DNA, 2× TaqMan Universal Master Mix (Applied Biosystems), 20× TaqMan SNP Genotyping Assay Mix (Applied Biosystems), and deionized H_2O. After denaturing at 95 °C for 10 min, 45 cycles of PCR were performed under the following conditions: 92 °C for 15 s and 60 °C for 1 min. All genotypes were reported with the allelic discrimination program using the ABI

Table 2
Genotype and allele frequencies for the C-1291G polymorphism of the *ADRA2A* gene in patients with polydipsic vs. non-polydipsic schizophrenia.

	Genotype			Allele frequency	
	G/G	G/C	C/C	G	C
SCZ with polydipsia ($n=84$)	48 (57.1%)	33 (39.3%)	3 (3.6%)	129 (76.8%)	39 (23.2%)
SCZ without polydipsia ($n=264$)	135 (51.1%)	100 (37.9%)	29 (11.0%)	370 (70.1%)	158 (29.9%)

No significant difference in both genotype distribution ($\chi^2=4.28$, $df=2$, $p=0.12$) and allele frequency ($\chi^2=2.28$, $df=2$, $p=0.09$) between cases and controls.