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Sex-different association of DAO with schizophrenia in Koreans

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ABSTRACT

The gene encoding D-amino acid oxidase (DAO), which acts as a receptor for the schizophrenia-associated neurotransmitter, *N*-methyl-D-aspartate (NMDA), is regarded as a potential candidate gene for schizophrenia. However, the potential association of the DAO gene with schizophrenia has been the subject of some debate. Here, we tested three single nucleotide polymorphisms (SNPs) of DAO in a group of Korean schizophrenia patients, and found no significant association in the overall study subjects. Interestingly, however, we found gender-specific differences in allele distributions, with SNP rs2070586 appearing to act as a risk allele in female schizophrenia patients, but as a protective allele in males. Our data support the hypothesis that DAO plays a role in schizophrenia, possibly in a gender-dependent manner.

1. Introduction

Genetic research on schizophrenia has often raised contradictory findings, partly due to the complex aetiology and diverse clinical manifestations of this disease. Schizophrenia has long been recognised as a heterogeneous disorder in terms of clinical and epidemiological characteristics, since different aetiologies and symptoms are clustered under identical diagnostic criteria. This leads to heterogeneity within the disease. One example of this may be seen in the prominent gender differences in the phenotype of schizophrenia, including sex-specific differences in age of onset, treatment response, premorbid adjustment, co-morbid mood symptomatology, course of illness, etc. (Salem and Kring, 1998). Among these gender-related differences, the most prominent is a later age of onset among females compared to males.

Hypofunction of *N*-methyl-D-aspartate (NMDA) receptors has long been recognised as being involved in the core features of schizophrenia, such as cognitive deficits and negative symptoms (Javitt and Zukin, 1991). D-amino acid oxidase (DAO) is believed to function in schizophrenia through its NMDA-receptor activity. In the presence of glutamate, D-serine serves as an indispensable cofactor at the glycine binding site of the NMDA-receptor, allowing the ion channel to open (Miller, 2004). The concentration of D-serine is regulated by DAO (Schell, 2004), and there is an inverse relationship between DAO and D-serine concentrations in the brain (Mothet et al., 2000), suggesting that DAO is likely to modulate NMDA activity through regulation of D-serine levels. In support of this, a number of animal studies have shown that DAO might play a functional role in regulating NMDA neurotransmission. For example, application of purified DAO decreased NMDA-induced currents in animal model, and this inhibitory effect was fully reversed by exogenously applied D-serine (Mothet et al., 2000). Furthermore, in DAO-deficient mice, NMDA-receptor-mediated synaptic transmissions were enhanced by increased D-serine levels due to the absence of DAO activity (Wake et al., 2001). These findings indicated that decreases in DAO might lead to excessive metabolism of D-serine, which, in turn, might contribute to development of schizophrenia through NMDA hypofunction. Along with these animal studies, a recent human study found significantly higher cerebellar DAO activities in post-mortem samples from schizophrenia patients versus normal individuals, providing additional evidence that DAO might be associated with schizophrenia (Kapoor et al., 2006).

In the context of genetic analysis, Chumakov et al. (2002) showed that the genes encoding DAO and G72, a DAO activator, were linked with schizophrenia. Since then, the association of G72 with schizophrenia has been replicated in studies on patients from different ethnic groups (Detera-Wadleigh and McMahon, 2006). However, the case is less clear for DAO. Although significant associations have been found in four studies (Chumakov et al., 2002; Liu et al., 2004; Schumacher et al., 2004; Wood et al., 2006), a number of other studies have failed to find an association between the DAO gene and schizophrenia (Addington et al., 2004; Fallin et al., 2005; Goldberg et al., 2006; Liu et al., 2006). The reports supporting the association indicate positive linkage of markers rs2111902 (MDAAO-4), rs3918346 (MDAAO-5) and rs3741775 (MDAAO-6) with schizophrenia in French-Canadian and German samples (Chumakov et al., 2002; Schumacher et al., 2004). More recently, DAO was found to be significantly associated with schizophrenia in US subjects (Wood et al., 2006). However,

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inconsistent results have been obtained from Chinese subjects; one study found significant association of only one single nucleotide polymorphism (SNP) in the DAO gene, rs3741775, with schizophrenia (Liu et al., 2004), while another family-based study failed to show positive association of any of the above-mentioned SNPs with schizophrenia (Liu et al., 2006). Furthermore, a study in Japanese subjects showed no significant association (Yamada et al., 2005).

A number of possible explanations could account for these inconsistencies. First, the DAO alleles associated with schizophrenia might vary depending on the ethnic group being examined, suggesting the need to examine all SNPs in DAO before excluding its candidacy for schizophrenia in a given population. Second, DAO might play a role in a specific subgroup of patients rather than in all patients affected with schizophrenia. To date, there has been no reported association of DAO with any specific subgroup of schizophrenia. However, the DAO activator, G72, has been shown to be associated with cognitive impairment of schizophrenia (Goldberg et al., 2006), childhood onset (Addington et al., 2004) and male schizophrenia (Hong et al., 2006). Thus, additional studies may be warranted to examine the association of DAO SNPs with specific subgroups of schizophrenia.

Despite the inconsistency in the genetic findings, the gene encoding DAO remains a strong candidate gene for schizophrenia based on its modulation of NMDA activity. Since the current evidence for the association between the DAO gene and schizophrenia is insufficient, further studies in various ethnic groups will be required. Only one prior report has examined the association of DAO with schizophrenia in Koreans. This previous study focussed on homicidal behaviour in just 92 male schizophrenia patients (Chung et al., 2007), which failed to find a significant association. In an effort to improve a power to detect, we decided to test whether DAO is associated with schizophrenia in Koreans with larger sample size than the previous study in Korean. Here, we report our findings of an apparently gender-specific association of DAO with schizophrenia in Koreans, and propose allelic heterogeneities between males and females.

2. Methods

2.1. Subjects

In total, 448 patients with schizophrenia (mean age and standard deviation, 33 ± 10.2 years; 263 male, 187 female) and 337 normal healthy controls (mean age and standard deviation, 37 ± 14.0 years; 158 male, 186 female) were recruited for this study. All patients were ethnically Korean and met the Diagnostic and Statistical Manual of Mental Disorder-IV (DSM-IV) criteria for schizophrenia. All patients were enrolled from a tertiary university hospital, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea. Diagnoses were made by a consensus diagnostic meeting that included the psychiatrists who were in charge of the patients, along with two or more independent experienced psychiatrists. A patient was excluded if there was any disagreement regarding diagnosis during the consensus meeting. Patients who had a history of head trauma, epilepsy, alcohol-related problems or other evident pathologies of the central nervous system were excluded. The normal controls were volunteers with no history of psychiatric problems, epilepsy or alcohol-related problems. Each control subject was asked about a history of psychiatric illness and treatment with unstructured screening questions. We obtained written consent forms from all participants after explaining the aims and procedures of the study. This study was approved by the Ethical Committee of Asan Medical Center.

2.2. Genotyping

For genotyping, 10 ml of venous blood was sampled from each study participant. Genomic DNA was isolated from peripheral blood leucocytes according to standard procedures, using proteinase K-RNase digestion followed by phenol–chloroform extraction. Fourteen SNPs within the gene encoding DAO were selected from dbSNP: rs2070585, 3220845, 2070586, 2070587, 2070588, 2111902, 7980427, 2302882, 3918346, 3741775, 3825251, 3918347, 4262766 and 4644682. These SNPs were screened for identification of polymorphic markers in Koreans by using a pooled DNA sequencing method (Lee et al., 2005). Markers were considered to be monomorphic when the peak patterns in both directions were clear and showed no evidence of background signals from a minor allele. Of the 14 tested SNPs, five (rs2070585, 7980427, 2302882, 4262766 and 4644682) were found to be monomorphic in our tested Korean population. We genotyped the remaining nine polymorphic markers in a randomly selected subset of our patient and control populations followed by in the whole sample using DNA sequencing method.

2.3. Statistical analysis

Differences in the genotype and allele frequencies between schizophrenic and controls were evaluated by χ^2 test or Fisher's exact test. The logistic regression analysis was used to adjust for age and sex to calculate odds ratio (OR) and 95% confidence interval (CI). When we divided the samples into two groups according to sex, Bonferroni correction was performed (significance level $P < 0.025$). Haplotype frequencies were estimated using the SNP Alyze program (Dynacom Co., Yokohama, Japan), which uses an expectation-maximisation (EM) algorithm to determine the maximum-likelihood frequencies of multi-locus haplotypes in diploid populations. We tested haplotypes for association, and all *P*-values were computed with 1000 permutations. When the disease association of a haplotype was statistically significant, the individual haplotype was tested for association by grouping all others together and applying a χ^2 test. All statistical tests were carried out by using SPSS for Windows K12.0.

3. Results

3.1. Single SNP analysis

Of the nine polymorphic SNPs tested in 96 controls and 96 cases, three SNPs (rs2070586, rs2070587 and rs3918347) showed statistically significant differences between cases and controls, and were selected for further analysis. Markers rs2070586 and rs2070587 are separated by 22 base pairs (bp), while rs2070587 and rs3918347 are separated by 15,578 bp. The allele frequencies and genotype distributions of rs2070586, rs2070587 and rs3918347 are shown in Table 1. All three were compatible with the Hardy–Weinberg equilibrium distribution (rs2070586 case $P = 0.75$, control $P = 0.99$; rs2070587 case $P = 0.88$, control $P = 0.54$; rs3918347 case $P = 0.39$, control $P = 0.92$), and the allele and genotype frequencies did not differ significantly between the schizophrenia patients and controls in our study population (Table 1).

However, although the tested schizophrenia patient population did not show a significant association between the disease and the SNPs in DAO, we observed significant associations when we analysed male and female subjects separately (Table 1). The three SNPs were concordant with Hardy–Weinberg equilibrium in each sex (in females, rs2070586 case $P = 0.88$, control $P = 0.77$, rs2070587 case $P = 0.63$, control $P = 0.32$, rs3918347 case $P = 0.80$, control $P = 0.99$; in males, rs2070586 case $P = 0.99$, control $P = 0.93$, rs2070587 case $P = 0.98$, control $P = 0.98$, rs3918347 case $P = 0.09$, control $P = 0.64$). In females, three SNPs showed statistically significant differences in the allele frequencies between cases and controls, and the results of two SNPs, rs2070586 and rs2070587, remained significant after the *P*-values were corrected for multiple comparisons with the alpha value set at 5% (Table 1). Differences in the genotypes of rs2070586 and rs2070587 between female cases and controls also reached a statistically significant level, whereas those of rs3918347 were marginally significant. Among the three SNPs, SNP rs2070587 showed a prominent association with schizophrenia in females and had the highest combined genotype (heterozygote and minor homozygote)-specific adjusted odds ratio (OR = 1.78; 95% confidence interval (CI) = 1.17–2.71; $P = 0.007$). The other two SNPs, rs2070586 and rs3918347, yielded combined genotype (heterozygote and minor homozygote)-specific adjusted odds ratios of 1.58 (95% CI = 1.04–2.39; $P = 0.031$) and 1.67 (95% CI = 1.06–2.64; $P = 0.028$), respectively.

In male subjects, only rs2070586 showed a significant difference in allele frequency between cases and controls before correction for multiple comparisons. Intriguingly, the minor allele (A) of rs2070586 trended in opposite directions in males and females. In females, the A allele was significantly more frequent in female cases than controls (34.1 vs. 24.6%, $P = 0.005$; adjusted OR (aOR) = 1.58 (1.04–2.39)), suggesting that it acts as a risk allele. By contrast, the frequency of the A allele in males was lower in cases than controls (22.8 vs. 29.7%, $P = 0.025$; aOR = 0.66 (0.44–0.99)), suggesting that the allele might