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Association Between *Monoamine Oxidase* Gene Polymorphisms and Attention Deficit Hyperactivity Disorder in Korean Children

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Attention deficit hyperactivity disorder (ADHD) is a common disorder of the school-age population. ADHD is familial and genetic studies estimate heritability at 80–90%. The aim of the present study was to investigate the association between the genetic type and alleles for the monoamine oxidase (*MAO*) gene in Korean children with ADHD. The sample consisted of 180 ADHD children and 159 control children. We diagnosed ADHD according to DSM-IV. ADHD symptoms were evaluated with Conners' Parent Rating Scales and Dupaul Parent ADHD Rating Scales. Blood samples were taken from the 339 subjects, DNA was extracted from blood lymphocytes, and polymerase chain reaction was performed for *MAO* polymorphism. Allele and genotype frequencies were compared using the chi-square test. We compared the allele and genotype frequencies of *MAO* gene polymorphism in the ADHD and control groups. This study showed that there was a significant correlation among the frequencies of the rs5906883 (odds ratio [OR] = 1.47, 95% confidence interval [CI] = 1.08–2.00, $p = 0.014$) and the rs3027407 (OR = 1.41, 95% CI = 1.03–1.91, $p = 0.029$) alleles of *MAO*, but the final conclusions are not definite. Follow-up studies with larger patient or pure subgroups are expected. These results suggested that *MAO* might be related to ADHD symptoms.

Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is a common childhood neuropsychiatric disorder characterized by behavioral problems such as attention deficit, hyperactivity, and impulsivity (American Psychiatric Association Committee on Nomenclature and Statistics, 1994). It has a prevalence of 2–7.6% among children of school age in Korea (Cho and Shin, 1994; Kim *et al.*, 1999). Family studies reported that ADHD showed a heredity as high as 80–90% (Faraone and Doyle, 2001), and molecular genetic studies are actively carried out accordingly. Recent genetic studies on ADHD have usually been conducted on the dopamine receptors and related neurotransmitters.

Monoamine oxidase *(MAO)* is an enzyme that affects the metabolism of dopamine to noradrenaline and plays an important role in degrading monoamine neurotransmitters (Weyler *et al.*, 1990; Lachman *et al.*, 1996). The *MAO* family includes Type A (*MAOA*) and Type B (*MAOB*), both of which are located on the X chromosome (Xp11.3 and Xp11.23) (Lan *et al.*, 1989). It is known that the *MAOA* enzyme level influences human behavior and traits. Some studies showed that an abnormal emotional response to environmental and social cue was found in a genetic polymorphism having a low level of *MAO* activity (Brummett *et al.*, 2008; Buckholtz and Meyer-Lindenberg, 2008). In addition, a family study reported that the *MAO* enzyme activity was highly related to impulsiveness. It is known that the *MAOA* enzyme activity is related to the EcoRV polymorphism of the *MAO* gene. The EcoRV polymorphism of the *MAOA* gene is a T/C polymorphism found at position 1460. When the EcoRV polymorphism has allele T, there exists an *Eco*RV restriction enzyme activity site and a high *MAO* activity is shown. On the other hand, when the EcoRV polymorphism has allele C, there is no *Eco*RV restriction enzyme activity site and a low *MAO* activity is shown (Hotamisligil and Breakefield, 1991).

It was reported that a male having a point mutation in the *MAOA* gene showed impulsive aggression, arson, attempted rape, exhibitionism, and borderline mental retardation. ADHD, along with inattention, is known to demonstrate features of impulsivity and hyperactivity clinically (Brunner *et al.*, 1993). Hawi *et al.* (2013) conducted a large-scale study with 270 subjects having ADHD and 540 family members

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residing in Ireland and Australia. The result showed that *MAOA* rs3027407 single-nucleotide polymorphism (SNP) did not have a significant correlation with ADHD. Kim *et al.* (2014) conducted a study with 275 schizophrenia patients and 289 controls by performing rs6323 and rs3027407 SNP. The result showed that the three SNPs were not correlated with schizophrenia, but presumably correlated with affective problems of schizophrenia such as the restricted affect and blunted affect. Yoo *et al.* (2009) conducted a study with 151 autism trios and 193 controls assessing rs5906883, rs1137070, and rs3027407 SNP. The result showed that the three SNPs were significantly correlated with the patient group and the control group. In addition, Park *et al.* (2013) also reported that the interaction between the *MAOA*-TCG, including rs6323, rs1801291, and rs3027407 SNPs, and the FOXP2-TCGC showed a correlation with autism and verbal communication. In addition, Brookes *et al.* (2006) conducted a large-scale study with 674 ADHD subjects and 808 sibling controls recruited from eight European countries. The result showed that the rs3027407 SNP was significantly correlated with ADHD. Guan *et al.* (2009) conducted a large-scale SNP study with 182 ADHD youths and 184 healthy controls in China and reported that the rs3027407 SNP was significantly correlated with ADHD. In addition, *MAOA* polymorphisms were reported to be associated with the hyperactive/impulsive type of ADHD and the development of borderline personality disorder (Liu *et al.*, 2011).

Some association studies were also conducted in Korea to investigate the correlation of the *MAO* gene with various psychiatric disorders. However, no association has been reported about the correlation between ADHD in youths and the *MAO* gene. Few studies have been conducted in Korea to show the correlation between *MAO* gene polymorphism and ADHD. Most of the studies were conducted with a small number of subjects. In addition, it is difficult to conclude that *MAO* gene polymorphism is not correlated with ADHD on the basis of the studies conducted in other countries having the limitation of popular stratification due to the multiracial and multiethnic features of the subjects.

The aim of the present study was to investigate the association between the genetic type and alleles for the *MAO* gene in Korean children with ADHD.

Materials and Methods

Subjects

A questionnaire was conducted with about 16,000 elementary school students in a city whose population is about 500,000 from September 2008 to August 2010. An interview was performed randomly with the children whose Korean version of the Dupaul Attention Deficit Hyperactivity Disorder Rating Sales (K-ARS)(Kim *et al.*, 2002) score was 19 or higher, and 180 ADHD children who consented to the genetic study were selected. For the control group, 159 children in the same area were selected by matching the sex and age of the subjects in the patient group. For both the patient and control groups, a clinical evaluation and the DSM-IV diagnosis (American Psychiatric Association Committee on Nomenclature and Statistics, 1994) were performed by a child psychiatrist. The number of ADHD children was 180, including 132 boys (73.3%) and 48 girls (26.7%), and the mean age was 8.67 ± 0.84. The number of children in the control group was 159, including 100 boys (62.9%) and 59 girls (37.1%), and the mean age was 8.59 ± 0.79. There was no significant difference in the sex and age between the two groups (Table 1). Subjects were excluded from the study if there was any evidence of conduct disorder, mood disorder, anxiety disorder, Tourette's disorder, pervasive development disorder, mental retardation ($IQ < 70$), or neurological disorders, including epilepsy. None of the children who participated in the study had ever undergone drug treatment before the evaluation. Informed consent was obtained before study entry. The study was also approved by the Hospital Ethics Committee. None of the children was taking psychostimulants at the time of the study.

On the day of visiting the hospital, the child psychiatrist performed a clinical interview as well as Kovacs Children's Depression Inventory (CDI) (Kovacs, 1983), State Anxiety Inventory (SAIC), Trait Anxiety Inventory (TAIC) (Cho and Choi, 1989), K-ARS (Kim *et al.*, 2002), computerized ADS(ADHD Diagnostic System) (Shin *et al.*, 2000), as well as completing a questionnaire survey regarding the pregnancy, infancy, developmental history, and anamnesis of the children with their parents. Subjects were included from our sample if they had a score over two standard deviations from the norm on the tests for ADS(T-score > 70). ADHD had a lot of comorbid disorders, such as depressive disorder and anxiety disorder. So, we excluded children with a high score of depressive symptoms and anxiety symptoms. Subjects with high anxiety scores (a Spielberger trait/state anxiety scale score $> 47/49$) on the Korean version of Spielberger trait-state anxiety scale for children were excluded, and subjects with high depression scores (Kovacs depression inventory score > 29) on Kovacs depression inventory for children were also excluded. Cho and Lee (1990) presented the score over 22 as the mild depressed state, over 26 as the middle depressed state, and over 29 as the severe depressed state in the Korean form of the Kovacs' Childhood Depression Inventory. In addition, Cho and Choi (1989) evaluated the reliability of the Korean State Anxiety Inventory for Children and reported that the scores 39–42 indicate a little high trait anxiety, scores 43–46 indicate a considerably high trait anxiety, and scores 47 or higher indicate very high trait anxiety in TAIC scales. In addition, a professional clinical psychologist performed a comprehensive psychological test, including an intelligence test, on each subject.

DNA extraction and genotyping

DNA was extracted from leukocytes using a commercial DNA extraction kit, the Wizard Genomic DNA purification kit (Promega, Madison, WI). The *MAO* SNP was genotyped by polymerase chain reaction (PCR) according to the protocol described by studies (Guan *et al.*, 2009; Yoo *et al.*, 2009; Park *et al.*, 2013; Kim *et al.*, 2014); *MAO* rs5906883 and rs3027407 were genotyped by Illumina, Inc. (San Diego, CA) through the use of their Integrated Bead Array System. We supplied Illumina with barcoded DNA microtiter plates containing the DNA quantified with Pico Green to be at 100 ng/mL and Illumina delivered genotypes with quality scores calculated by proprietary Illumina algorithms. Genotyping methods for the Korean samples were previously reported (Yoo *et al.*, 2009; Park *et al.*, 2013; Kim *et al.*, 2014).

Table 1. Epidemiological Characteristics Between the ADHD Group and the Control Group

Rating scale	*ADHD group (*n = *180) Mean ± SD*	*Control group (*n = *159) Mean ± SD*	F or χ^2	p-*Value*
Age[a]	8.67 ± 0.84	8.59 ± 0.79	0.06	0.813
Sex (*n*, %)[b]				
Female	48 (26.7%)	59 (37.1%)	3.79	0.052
Male	132 (73.3%)	100 (62.9%)		

These data represent mean ± SD, by independent *t*-test,[a] or *n* (%), by chi-square test,[b] significant *p*-value < 0.05.
ADHD, attention deficit hyperactivity disorder.

Statistical analysis

We performed independent *t* tests for age, chi-square tests for sex, and chi-square tests to compare the results of the control group and the ADHD group through the frequency of the genotypes and alleles. SPSS PC software (version 15.0) was used for statistical analysis and the significance level was set to the *p*-value being less than 0.05. The calculation revealed that a sample size of 210 subjects is required to obtain a power that is 95% or higher in the chi-square test between the control group and the patient group. Our study was conducted with 339 subjects and the power was 97.41%. This indicates that the association of the *MAO* gene polymorphism and ADHD can be sufficiently accounted for by the results in this study. However, we performed the power program analysis for the chi-square test with 339 subjects and the result showed that the effect size was 0.46 (moderate level).

Results

Demographic characteristics of the subjects

The subjects were a total of 339 children. The children in both the ADHD group and the control group had never taken any psychostimulant in advance. There was no difference in the age ($F = 0.06$, $p = 0.813$) and sex ($F = 3.79$, $p = 0.052$) between the control group and the ADHD children group (Table 2).

Comparison of the frequency of the genotypes and alleles with genetic polymorphism of MAO between the control group and the ADHD group

The *MAO* rs5906883 allele of the 159 subjects in the control group and the 180 subjects in the ADHD group were A alleles (63.3%: 53.9%) and C alleles (36.6%: 46.1%), and there was a significant difference in the frequency of allele between the two groups ($\chi^2 = 2.45$, df = 2, $p = 0.014$). The *MAO* rs3027407 allele of the 159 subjects in the control group and the 180 subjects in the ADHD group were A alleles (61.7%: 53.4%) and C alleles (38.3%: 46.6%), and there was a significant difference in the frequency of allele between the two groups ($\chi^2 = 2.18$, df = 2, $p = 0.029$ (Table 3).

Odds ratio of the genotypes and alleles with genetic polymorphism of MAO between the control group and the ADHD group

For the *MAO* rs5906883 allele, the odds ratio was significant at 1.47 (confidence interval: 1.08–2.00, $p = 0.014$). In addition, for the *MAO* 3027407 allele, the odds ratio was significant at 1.41 (confidence interval: 1.03–1.91, $p = 0.029$) (Table 3).

Discussion

This study is a case-controlled study, in which the frequency of the genotypes and alleles of *MAO* was compared between the ADHD children and the control group in Korea. The correlation between the genotypes and alleles of two candidate *MAO* SNPs was investigated. This study showed that there was a significant correlation between the frequencies of the *MAO* rs3027407. This result is reported for the first time in the study by Brookes *et al.* (2006). The association of *MAO* rs3027407 in this study was similar to the results of Brookes *et al*. (2006) and Guan *et al.* (2009), but was not similar to the result of Hawi *et al.* (2013).

This study showed that there was a significant correlation between the frequencies of the *MAO* rs5906883 and ADHD; this result is reported for the first time in our study. In the study of Korean children, which is the only previous study about the association between Autism and *MAO*, Yoo *et al.* (2009) were the first to report the association between rs5906883 genetic polymorphism of the *MAO* gene and autism in Korea. However, in this study, the correlation between ADHD and *MAO* rs5906883 genetic polymorphism was found for the first time.

Combining the results about the correlation between the *MAO* rs3027407 and rs5906883 alleles and ADHD, it can be understood that the failure of *MAO* regulation may cause changes in catecholamine and may be correlated with the vulnerability of various psychiatric diseases, including ADHD and movement disorder. These receptors can affect the dopamine-mediating action, which is related to the symptoms found in the children with ADHD.

Table 2. Single-Nucleotide Polymorphisms Considered in This Study

SNP ID	*Chromosome*	*Location*	*Position (coordinate)*	*Distance*	*Alleles*
MAO					
rs5906883	X	Intron	43411886	−11281	A/C
rs3027407	X	3UTR	43489784	[1080/1227]	A/G

MAO, monoamine oxidase gene, NCBI gene ID(Accession) is 1621(NM000787.2).
SNP, single-nucleotide polymorphism.

Table 3. Multivariate Model for Genotype Distributions and Allele Frequencies in the ADHD Group and the Control Group

	Control		*ADHD*					
Characteristics	N	%	N	%	*OR*	*95% CI*	χ^2	p
MAO rs5906883 (A/C)								
Genotype					1.26	0.99–1.60	1.91	0.056
AA	87	55.41	82	46.07				
AC	25	15.92	28	15.73				
CC	45	28.66	68	38.20				
Allele					1.47	1.08–2.00	2.45	0.014
A	199	63.38	192	53.93				
C	115	36.62	164	46.07				
MAO rs3027407 (A/G)								
Genotype					1.23	0.97–1.56	1.68	0.093
AA	85	53.80	81	45.51				
AG	25	15.82	28	15.73				
GG	48	30.38	69	38.76				
Allele					1.41	1.03–1.91	2.18	0.029
A	195	61.71	190	53.37				
G	121	38.29	166	46.63				

These data represent *N* (%) by chi-square test, significant p-value < 0.05.

This study also suggests that the failure to regulate the *MAO* expression causes changes in the dopamine expression and the structural development of the brain regions related to nerve activity, attention, and impulsivity. When the EcoRV polymorphism has the major allele T, there exists an *Eco*RV restriction enzyme activity site and a high MAO activity is shown. On the other hand, when the EcoRV polymorphism has the minor allele C, there is no *Eco*RV restriction enzyme activity site and a low MAO activity is shown (Hotamisligil and Breakefield, 1991). In our study, we found that allele C in the ADHD group was more frequent than in the control group; this may be a risk allele for ADHD. We suspect that the differences of major allele A and minor allele C may lead to the change of amino acid in MAOA enzyme activity function. The analysis in a study of 12 SNP genes (Guan *et al.*, 2009) showed that they were related to dopamine neurotransmission, including *MAO*, and that there was not a significant correlation between the *MAO* gene and ADHD. Hence, the correlation between the *MAO* gene and ADHD should be carefully handled and the result of our study should be verified in a future study with a large number of independent samples.

The limitations of this study are as follows: first, the number of subject children was small. The subjects of this study were 180 ADHD children and 159 children in the control group. Second, the results of this study may not be generalized for the cases of other racial or ethnic groups since the frequency of alleles can vary due to local or racial differences. The distribution of the allele frequency in the ADHD patient, children, and parent group in this study was also different from that of other countries. Third, only a few SNPs were investigated in this study among the many genes related with the various ADHD phenotypes. Although it is clear that not just one genetic factor causes the increased ADHD vulnerability, we did not consider the interaction with other risk factors.

Despite the methodological limitations described before, this study has several advantages. First, the patient group and the control group were matched so that there was no difference in the frequency of sex and age. The prevalence of ADHD is higher among males and in adolescence; thus, the sex and age characteristics can have a great effect. Considering this, our study was evaluated by matching the age and sex of the patient group and the control group with each other. Second, this study used population-based samples. Previous studies in Korea were hardly considered to represent the general population because the subjects were usually ADHD children who visited hospitals for their clinical symptoms. In this study, the subjects in the risk group were selected by the questionnaire survey from the whole population in a region and the patient and control samples were obtained by random contact. Thus, the subjects in this study may be more appropriate to the characteristics of a general population than those of the study performed with the patients who visited hospitals. Third, this study might have compared relatively homogenous groups that had the characteristics of Koreans, different from the studies conducted in other countries with subjects from various ethnic groups and nations. Fourth, both the patient group and control group in this study underwent clinical evaluation and DSM-IV diagnosis by child psychiatrists, applying the inclusion and exclusion criteria strictly, and thus, the patient group was composed of pure ADHD-diagnosed subjects.

We expect that different allele distribution results may be produced from future studies on the quantitative correlation of the ADHD performance in the pure ADHD group from which coexisting diseases are excluded; the patient group composed of only boys or girls, the subtype groups such as the hyperactivity dominant group and attention deficiency dominant group, and the drug response group.

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Author Disclosure Statement

No competing financial interests exist.

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