Association Between *Dopamine Beta-Hydroxylase* Gene Polymorphisms and Attention-Deficit Hyperactivity Disorder in Korean Children

Ho Jang Kwon¹,² and Myung Ho Lim¹,³

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 dopamine beta-hydroxylase (*DBH*) gene in Korean children with ADHD. The sample consisted of 142 ADHD children and 139 control children. We diagnosed ADHD according to DSM-IV. ADHD symptoms were evaluated with the Conners’ Parent Rating Scales and Dupaul Parent ADHD Rating Scales. Blood samples were taken from the 281 subjects; DNA was extracted from blood lymphocytes, and polymerase chain reaction was performed for the *DBH* polymorphism. The alleles and genotype frequencies were compared using the chi-square test. We compared the allele and genotype frequencies of the *DBH* gene polymorphism in the ADHD and control groups. This study showed that there was a significant correlation among the frequencies of rs1611115 (odds ratio = 0.64, 95% confidence interval = 0.42–0.97, \( p = 0.034 \)) of the alleles of *DBH*, but the final conclusions are not definite. Follow-up studies with larger patient or pure subgroups are expected. These results suggested that DBH 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.

Dopamine beta-hydroxylase (DBH) is an enzyme that affects the metabolism of dopamine to noradrenaline, and plays an important role in catecholamine biosynthesis and the control of noradrenergic and adrenergic neurotransmission (O’Connor et al., 1994). DBH is mainly distributed in the sympathetic terminals, adrenal glands, and the prefrontal cortex, and especially a high concentration of DBH was identified in the human prefrontal cortex (Gaspar et al., 1989). The human DHBF-encoding gene is located on chromosome 9q34dp, and it is composed of 12 exons and consists of about a 23-kb sequence (Craig et al., 1988; Kobayashi et al., 1989).

In the animal study using DBH knockout mice, it was reported that the *DBH* gene is related to behavior and cognition, and that deactivation of the *DBH* gene alters social memory and reduces aggression (Marino et al., 2005). Thomas and Palmiter (1997) reported that lack of motor function as well as learning and memory was observed in the study of DBH knockout mice. In addition, there were studies on the relation between the *DBH* gene and human psychopathology. It was reported that decreased plasma DBH activity is related to bipolar disorder (Puzynski et al., 1983; Sofuoglu et al., 1995) and unipolar psychotic depression (Meyers et al., 1999; Cubells et al., 2002). However, some studies reported that there is no relation between the plasma DBH activity and unipolar or bipolar disorder. On the other hand, it was reported that a low plasma DBH activity is related with conduct disorder and behavior problem for boys (Rogeness et al., 1982; Gabel et al., 1995), and also reported that the plasma DBH activity is related with impulsive-hostile behaviors (Hess et al., 2009).

*DBH*-rs1611115 is located 1,021 bp upstream of the transcription site of 50-flanking region in the *DBH* gene, and the Taq1A, rs2519152, which is a nonsynonymous single-nucleotide polymorphism (SNP), is located at intron 5 (Thomas and Palmiter, 1997). *DBH*-rs1611115 explains 35%–52% of total variation for plasma DBH activity (Sofuoglu et al., 1995; Meyers et al., 1999; Cubells et al., 2002). This correlation shows a higher
Table 1. Epidemiological Characteristics Between the ADHD Group and the Control Group

<table>
<thead>
<tr>
<th>Rating</th>
<th>ADHD group (n = 142)</th>
<th>Control group (n = 139)</th>
<th>F or χ²</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>8.67 ± 0.84</td>
<td>8.65 ± 0.81</td>
<td>0.05</td>
<td>0.827</td>
</tr>
<tr>
<td>Sex(N,%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>41 (28.9%)</td>
<td>46 (33.1%)</td>
<td>0.52</td>
<td>0.262</td>
</tr>
<tr>
<td>Male</td>
<td>101 (71.1%)</td>
<td>93 (66.9%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These data represent mean ± S.D., by independent t test, or N (%), by chi-square test, significant p-value < 0.05.
ADHD, attention-deficit hyperactivity disorder.

The aim of the present study was to investigate the association between the genetic type and alleles for the DBH 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 and August 2010. An interview was performed randomly with the children whose Korean version of the Du-paul Attention Deficit Hyperactivity Disorder Rating Sales (K-ARS) (Kim et al., 2002) score was 19 or higher, and 142 ADHD children who consented to the genetic study were selected. For the control group, 139 children in the same area were selected by matching the sex and age of the subjects in the patient group. For both of 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 142, including 101 boys (71.1%) and 41 girls (28.9%), and the mean age was 8.67 ± 0.84. The number of the children in the control group was 139, including 93 boys (66.9%) and 46 girls (33.1%), and the mean age was 8.65 ± 0.81. 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 disorder, pervasive development disorder, mental retardation (IQ<70), and neurological disorders, including epilepsy. None of the children who participated in the study has 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 Kovac’s Children’s Depression Inventory (CDI) (Kovacs, 1983), State Anxiety Inventory (SAIC), Trait Anxiety Inventory (TAIC) (Cho and Choi, 1989), and Dupaul Attention Deficit Hyperactivity Disorder Rating Sales (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, as depressive disorder and anxiety disorder. Therefore, we excluded children with the high score of depressive symptoms and anxiety symptoms. Subjects with

Table 2. Single-Nucleotide Polymorphisms Considered in This Study

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>Chromosome</th>
<th>Position (Coordinate)</th>
<th>Distance</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1108580</td>
<td>9</td>
<td>coding</td>
<td>135494934</td>
<td>A/G</td>
</tr>
<tr>
<td>rs1611115</td>
<td>9</td>
<td>flanking_5</td>
<td>135490335</td>
<td>C/T</td>
</tr>
<tr>
<td>rs2519152</td>
<td>9</td>
<td>intron</td>
<td>135499454</td>
<td>A/G</td>
</tr>
</tbody>
</table>

NCBI gene ID (Accession) is 1621(NM000787.2).
DBH, dopamine beta-hydroxylase gene.
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. 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 DBH SNP was genotyped by polymerase chain reaction (PCR) according to the protocol described by studies (Cubells et al., 2000; Zabetian et al., 2001; Tang et al., 2006). DBH rs1108580, rs1611115, and rs2519152 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 (Park et al., 2005).

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 the statistical analysis, and the significance level was set to the p-value being < 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 281 subjects, and the power was 97.41%. This indicates that the association of the DBH 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 281 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 281 children. The children in both of the ADHD group and the control group had never taken any psychostimulant in advance. There was no difference in the age (F = 0.05, p = 0.827) and sex (F = 0.52, p = 0.262) between the control group and the ADHD children group (Table 2).

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

The DBH-rs1611115 genotypes of the 139 subjects in the control group and the 142 subjects in the ADHD group were C/C (55.8%: 68.6%), C/T (39.9%: 29.3%), and T/T (4.4%: 12.1%).
2.1%), and there was a significant difference in the frequency between the two groups ($\chi^2 = 2.18, df = 2, p = 0.029$) (Table 3).

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

For the $DBH$-rs1611115 genotype, the odds ratio was significant at 0.58 (confidence interval: 0.35–0.95, $p = 0.029$), and for the allele, the odds ratio was not significant at 0.64 (confidence interval: 0.42–0.97, $p = 0.034$) (Table 3).

**Discussion**

This study is a case-controlled study in which the frequency of the genotypes and alleles of $DBH$ were compared between the ADHD children and the control group in Korea. The correlation between the genotypes and alleles of three candidate $DBH$ SNPs was investigated. This study showed that there was a significant correlation between the frequencies of the $DBH$-rs1611115, and this result is reported for the first time in Korea. The association of $DBH$-rs1611115 in this study was matched up the result of Zhang et al.’s (2004, 2005) study, but were not matched up the result of Comings et al.’s (1996), Brookes et al.’s (2006), Bhaduri and Mukhopadhyay’s (2006), Kopecková et al.’s (2008), and Guan et al.’s (2009) study.

The lack of association of $DBH$-rs2519152 in this study was not matched by the positive result of Comings et al. (1996), Daly et al. (1999), Kirby et al. (2002), Wigg et al. (2002), Roman et al. (2003), Smith et al. (2003), Inskter et al. (2004), Bhaduri and Mukhopadhyay (2006), Tang et al. (2006), and Kopecková et al. (2008). In the only previous study of Korean children, Park et al. (2005) reported the association between the rs2519152 genetic polymorphism of the $DBH$ gene and ADHD. However, in this study, the correlation between ADHD and the $DBH$-rs2519152 genetic polymorphism was not found, but the correlation between ADHD and the $DBH$-rs1611115 genetic polymorphism was found in this study for the first time.

The lack of association of $DBH$-rs1108580 in this study was matched by the result of Hawi et al. (2003), Brookes et al.’s (2006), Bhaduri and Mukhopadhyay’s (2006), Kopecková et al.’s (2008), and Guan et al.’s (2009) study. However, one study found the association of $DBH$-rs1108580 and ADHD with A allele designated as the high-risk allele (Hawi et al., 2003).

Combining the results about the correlation between the $DBH$-rs1611115 risky alleles and ADHD, it can be understood that failure of $DBH$ regulation may cause changes in dopamine 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 with the symptoms found in the children with ADHD.

This study also suggests that the failure to regulate the $DBH$ expression causes changes in the dopamine expression and the structural development of the brain regions related with nerve activity, attention, and impulsivity. In this study, we assumed that the variation of the $DBH$ gene affects these SNPs that are the cause of ADHD. The analysis in the study of Guan et al., (2009) of 23 SNP genes showed that they were related to dopamine neurotransmission, including $DBH$, and that there was not a significant correlation between the $DBH$ gene and ADHD. Hence, the correlation between the $DBH$ gene and ADHD should be carefully handled, and the result of our study should be verified in the 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 142 ADHD children and 139 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 men 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 a random contact. Thus, the subjects in this study may be more appropriate to the characteristics of 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 children psychiatrists, applying the inclusion and exclusion criteria strictly, and thus the patient group was composed of subjects with pure ADHD.

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 hyperactivity-dominant group and attention-deficiency-dominant group, and the drug–response group.

**Acknowledgments**

The present research was conducted by the research fund of Dankook University in 2012.

**Author Disclosure Statement**

No competing financial interests exist.

**References**


Address correspondence to:
Myung Ho Lim, MD
Department of Psychiatry
College of Medicine
Dankook University
Manghyang Rho 359
Cheonan 330-715
South Korea
E-mail: paperose@dku.edu