Dopamine Transporter Genotype Influences the Attention Deficit in Korean Boys with ADHD

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Attention appears to be inheritable, stable and influenced by genetic factors. The use of the Continuous Performance Test (CPT), as an endophenotypic measure, is valuable for genetic studies because it may show increased sensitivity to specific dimensions in attention deficit hyperactivity disorder. However, few studies have been designed to examine the influence of the genotype on attention level measured by CPT in ADHD patients. This study examinee the difference between 10/10 and 10/* genotype in the attention deficits measured by the CPT in ADHD patients. Forty-four unrelated ADHD patients were recruited from the psychiatric outpatients' clinic at Kangbuk Samsung Hospital. Two child psychiatrists made the diagnoses of ADHD using the DSM-IV diagnostic criteria. The genomic DNA was extracted from the blood, and analyzed to the genotype. A 40-base pair variable number of tandem repeats (VNTR) polymorphism in the 3' untranslated region was amplified. The attention deficits were measured by the test of variables of attention (T.O.V.A.). Between the 10/10 genotype and 10/* genotype, standard scores of the T.O.V.A. were compared using a Mann-Whitney test. A comparison with the 10/10 genotype and 10/* genotype showed that those patients with the 10/10 genotype made less omission errors in the first quarter of the test (p<0.05, by Mann-Whitney test). No significant differences were observed in the errors of commission, response time, variety. This study found that the 10/10 genotype made less omission errors on the T.O.V.A. This suggests that the dopamine transporter genotype influences the attention deficits measured by T.O.V.A.

Key Words: ADHD, DAT1 genotype, attention deficits

INTRODUCTION

Patients with attention deficit hyperactivity disorder (ADHD) have symptoms of hyperactivity, inattention, and impulsivity. ADHD is believed to be an inheritable disorder, but the pattern of inheritance is unclear. 5-7 70-90% of ADHD symptoms are responsive to medications like, methylphenidate, dextroamphetamine, pemoline, and bupropion. The functional change in the dopamine transporter is related to the therapeutic effect of these drugs. Dopamine transporter gene (DAT1) knock out mice were reported to be several times more active than normal mice in novel situation. 10-13 These are the reasons why the dopamine transporter has been emphasized in understanding ADHD. The genetic locus encoding the DAT protein is SLC6A3, which is located in chromosome 5p15.3. 14 Since Vandenbergher reported the DAT1 polymorphism with a variable number of tandem repeats (VNTR), a 40-base pair (bp) VNTR in the 3' untranslated region has been the most studied for population variations. The 10 repeat allele was the most common allele, ranging from 52% of Greeks to 100% of South Americans. More than 90% of the Japanese, Mongolian and Korean population have the 10 repeat allele. 15-18

Findings concerning the association between the 10 repeat allele and ADHD is not conclusive. Some groups reported an association, 19-22 but others failed in replication. 23-25 Waldman reported that only hyperactivity - impulsivity symptoms were related to the number of 10 alleles, and inattention was not related to the number of 10 alleles. 26 It was postulated that only hyperactivity-impulsivity is associated with the 10/10 genotype. In contrast to Waldman's reports, the 10 repeat
allele was reported to be related to the symptoms of internalizing disorders (generalized anxiety disorder, major depression, panic disorder, separation anxiety disorder, social phobia, specific phobia) in both clinically referred children and controls. In addition, the 9 repeat allele was related to externalizing behavior problems in a community sample of children. Despite numerous efforts to elucidate the association between the 10 repeat allele of DAT1 and the behavioral outcomes, no specific behavioral outcomes are believed to be related to the 10 repeat alleles. Inconsistent findings regarding this issue increased interest in endophenotypic measurements. Well-defined endophenotypic measurements may increase the sensitivity to detect the differences between genotypes.

The attention function appears to be highly heritable, stable and influenced by genetic factors. Cornblatt tested normal subjects on a high-processing load Continuous Performance Test (CPT), and suggested that two types of attention (spatial and verbal) were independent and that each was heritable to some degree. The use of CPT as an endophenotypic measure is potentially advantageous for genetics studies since they may show increased sensitivity to specific dimensions related to complex psychiatric disorder. However, few studies have been designed to examine the influence of the genotype on attention measured by CPT in ADHD patients. This study examined that the difference between the 10/10 and 10/4 DAT1 genotypes in attention deficits measured by the CPT in ADHD patients.

MATERIALS AND METHODS

Patients selection

The Ethics Committee of the KangbukSamsung Hospital approved this study. All subjects participated after giving informed consent. Forty-four unrelated ADHD patients were recruited from the psychiatric outpatients’ clinic at Kangbuk Samsung Hospital. Two child psychiatrists made the diagnoses of ADHD using the DSM-IV diagnostic criteria. All the patients were scored higher than 15 points with Conners Parent-Teacher Questionnaire by the parents and teachers. All patients with a comorbid diagnosis including patients with an IQ score lower than 75 by the Korean Educational Development Institute-Wechsler Intelligence Scale for Children (KEDI- WISC) were excluded.

DAT genotyping

The genomic DNA was extracted from the blood, and analyzed to determine the genotype. The chromosome location of DAT1 was 5p15.3. The DAT1 site was amplified by a polymerase chain reaction using the primers 5'-TGT GTA GGG AAC GGC CTG AG-3' and 5'-CTT CCT GGA GGT CAC GGC TCA AGG-3'. 25 μl of the mixture consisted of 12.5 μl of GC buffer (Takara, Japan), 1.5 U of Taq polymerase (Takara, Japan), 10 pmol/25 μl of each primers, 2.5 mM dNTP (each), and 200 ng of template DNA. The PCR conditions were 1 min. of an initial denaturation step at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 60°C and 30 s at 72°C. A final extension of 10 min at 72°C was done. 2% agarose gel with ethidium bromide was used for electrophoresis.

Measurement of inattention and impulsivity

The Test Of Variables Of Attention (T.O.V.A.) is a computerized continuous performance test. The patients were asked to push a button connected to a computer when they recognized the target on the monitor. The target means a small square appearing on the upper part of a rectangle. The non-target means a small square appearing on the bottom of the rectangle. A stimulus flashed on a screen every 2 seconds. The target is presented on 22.5% and 77.5% of the trials during the first and second halves, respectively. The data was obtained in the domains of the omission error, the commission error, the response time, and variability. All the variables are recorded for each 5-min quarter and 10-min halves, as well as the overall total scores for each variable. The scores are compared to the standardized norms, and the interpretation of data is reported in a printable form.
Statistical analysis

A comparison of the 10/10 genotype and 10/* genotype showed a difference in the standard scores of T.O.V.A were computed by Mann-Whitney test.

RESULTS

Forty-four ADHD patients were included in this study. The mean age of the children was 8.59 ± 1.9. The allele and genotype frequencies are shown in Table 1 and Table 2.

The 10 repeat allele was the most commonly observed and consisted 87.5% of the total alleles. The 10/10 genotype was found in 77.3%, 10/* genotype was found in 20.4%.

Patients with the 10/10 genotype made less omission errors compared to those with the 10/* genotype in the first quarter of tests (p < 0.05, by Mann-Whitney test, Table 3). In the errors of the commission, response time, variability, no significant differences were observed between the two groups.

DISCUSSION

A well-defined phenotype will promote the success of a genetic study. Defining the phenotype is a significant problem in psychiatry where the diagnosis is made by the symptoms reported by the patients or observers, rather than by the objective signs or laboratory findings. Despite the fact that a clinically acceptable definition is attained through a major improvement in the reliability of the diagnostic criteria, this definition, which is based on the reported symptoms, may not be valid for genetic studies. ADHD is also diagnosed by the reported symptoms. Researchers have been deliberating whether or not hyperactivity is a dimensional or categorical problem. To answer the question whether or not hyperactivity exists is not that simple. Even in the data from a population-based sample, a wide variation of hyperactivity is observed. A diagnosis based on the dichotomous concept of hyperactivity will not be valid for the phenotype. A quantitative analysis of hyperactivity will be more suitable for genetic studies. Since attention has been observed with wide variation in the general population, a similar notion was applied to attention. Attention is understood to be a dimensional problem. A quantitative measurement of attention is believed to be more reliable than a dichotomous classification in a genetic study. Endophenotypes are heritable quantitative traits, which are an index of an individual’s liability to develop diseases.

The VNTR of the 3'-UTR of the DAT gene may have regulatory effect on expression and influence levels of DAT protein in the brain. Nine repeat alleles of the 3'-UTR segment of a human DAT allele showed a significantly higher levels of reporter gene expression than the 10 repeat allele. Increased levels of DAT1 expression were associated with the number of 10-repeat alleles.

The study results from the in vivo study with single photon emission computed tomography (SPECT) also suggests the differential effect of the VNTR of the DAT gene on protein function. Heinz reported that individuals with the 9/10

| Table 1. Allele Frequency of the Dopamine Transporter Gene in ADHD Patients |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                             | Allele 7 (360bp)            | Allele 9 (440bp)            | Allele 10 (480 bp)          | Total                       |
| Number (%)                  | 2 (2.3%)                    | 9 (10.2%)                  | 77 (87.5%)                  | 88 (100%)                   |

bp, base pairs.
PCR product size in this study.

<p>| Table 2. Genotype Frequency of the Dopamine Transporter Gene in ADHD |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                             | 7/10                        | 9/10                        | 10/10                       | 9/9                         | Total                       |
| Patients                    | 2 (4.5%)                    | 7 (15.9%)                   | 34 (77.3%)                  | 1 (2.3%)                    | 44 (100.0%)                 |</p>
<table>
<thead>
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<th></th>
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<td>77.7 (±22.7)</td>
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<tr>
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Numbers in parenthesis are standard deviation.
Q1, first quarter of total test time; Q2, second quarter of total test time; Q3, third quarter of total test time; Q4, fourth quarter of total test time.

\( p < 0.05 \) by Mann-Whitney test.

genotype showed a reduced DAT protein-binding capacity in the putamen compared to the 10/10 genotype individuals.\(^{36}\) In contrast, Jacobsen et al reported that individuals with the 9/10 genotype had an increased DAT protein binding capacity.\(^{37}\) Although these results support the assumption that VNTR polymorphism have a differential effect on the protein function, the mechanism is not clearly understood. Despite efforts to link the DAT genotype and the function of the DAT protein, the bottom-up approach appears to be less effective when trying to clarify this issue.

The top-down approach to examine the relationship between the behavioral outcome and DAT genotype did not generate consistent findings. Inconsistent findings from an association of VNTR and the behavioral outcomes raised the issue of endophenotypic measurements. It has been reported that attention is feasible for making an endophenotypic measurement.\(^{29,38,39}\) In the normal population, subjects homozygous for the rare 9-repeat allele showed a modestly lower score in executive attention than the pooled scores for the more common 10-repeat homozygotes and 9/10 heterozygotes.\(^{39}\) Sustained attention deficit, as measured by the continuous performance test, identical pairs version (CPT-IP) are heritable, developmentally stable, independent of clinical state in patients with schizophrenia.\(^{36,39}\) Rowe reported that mothers with the 10/10 genotype had higher levels of inattention symptoms.\(^{40}\) The main limitation of Rowe’s study was that the data were collected based on the parental report retrospective to their teen-age years. Little research has been conducted on the objective endophenotypic measurement of attention in ADHD patients. This study measured the attention quantitatively, and compared the attention between the patients with the 10/10 genotype and 10/* genotype. In this study, patients with the 10/10 genotype made less
omission errors during T.O.V.A. During T.O.V.A., the subjects were asked to push the button when a small square flashed on the upper part of a rectangle. The task insisted that subjects should recognize the location of the small square. Recognition of the shape is accompanied by the activation of the posterior parietal lobe (PPL). The appropriate activation of PPL is expected to perform the T.O.V.A successfully. Patients with the 10/10 genotype appear to have an advantage on the attention task accompanied with activation of PPL. Attention consists of several networks, which are alerting, orienting, and executive control. It was suggested that each network had a different genetic background. Attention measured by a test other than T.O.V.A. can have different results. Therefore, additional data based on the measurement of attention by T.O.V.A. will be compared with the data from this study.

The small sample size is the major limitation of this study. In this study, only the standard scores of the first quarter of the omission error were statistically significant. The standard scores from the second to the fourth quarter of the test would reach the significance in a larger sample. Further research with a larger sample is expected. Another limitation was that the normal control group was not included in this study. Further study including a normal control group is expected in the future.

This study found that the 10/10 genotype made less omission errors on T.O.V.A. The study suggests that the dopamine transporter genotype influences the attention deficits measured by T.O.V.A.

REFERENCES


