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Regional brain perfusion before and after treatment with methylphenidate may be associated with the G1287A polymorphism of the norepinephrine transporter gene in children with attention-deficit/hyperactivity disorder

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ABSTRACT

The noradrenergic system modulates attention and arousal. Dysregulation of the noradrenergic system may be involved in the pathophysiology of attention-deficit/hyperactivity disorder (ADHD). This study intended to examine the differences in methylphenidate (MPH) treatment response and pre- and post-treatment cerebral perfusion associated with the G1287A and −3081(A/T) polymorphisms of the norepinephrine transporter (NET) gene in ADHD children. Thirty-seven drug-naïve ADHD children (8.9 ± 1.8 years old, M = 32, F = 5) were genotyped. Next, baseline single-photon emission computed tomography (SPECT) and clinical assessments were carried out for ADHD subjects. After 8 weeks of MPH treatment, SPECT and clinical assessment were repeated. There were no differences in baseline clinical assessments or cerebral perfusion based on genotype. However, after treatment, ADHD children with the G/G genotype at the G1287A polymorphism showed more improvement in symptoms than children without the G/G genotype as evaluated by the Clinical Global Impressions-Improvement scale ($p = 0.022$). Furthermore, ADHD children with the G/G genotype at the G1287A polymorphism showed hyperperfusion in the right inferior temporal gyrus ($p < 0.001$, uncorrected) and middle temporal gyrus ($p = 0.001$, uncorrected) compared to children without the G/G genotype. Although the results of this study should be interpreted cautiously, they suggest that polymorphisms of the NET gene may contribute to an intermediate phenotype. Further studies should clearly elucidate the relationship between treatment response and functional connectivity in the brain according to this genetic polymorphism.

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) is primarily characterized by inattention, hyperactivity and impulsiveness, and it affects about 3–10% of school-aged children [4]. The specific causes of ADHD are not known, but there is evidence that dysregulation of the noradrenergic system may be involved in the pathophysiology of ADHD [5]. Studies have indicated that the noradrenergic system modulates attention and arousal, and the activation of the noradrenergic system has a great amount of influence on the functions of the prefrontal cortex [2]. Further evidencing the close relation between the noradrenergic system and ADHD is the highly selective noradrenergic reuptake inhibitor atomoxetine, which is effectively used for treatment of ADHD [19,30].

Recently, many pharmacogenetic studies have been conducted to evaluate the efficacy of methylphenidate (MPH) treatment of ADHD according to genotype. Most of these studies have focused on dopaminergic genes because the dopamine transporter is the known site of action of MPH. However, it has been suggested that norepinephrine transporter (NET) is another site of action for MPH [24,31], and attention has been paid to the possibility that the NET is also connected with the response to MPH. NET not only reuptakes norepinephrine in the noradrenergic pathway but also reuptakes dopamine in the frontal cortex [8].

Several studies have investigated the association between genetic variants of the NET gene (*SLC6A2*), located at chromosome

Abbreviations: NET, norepinephrine transporter; MPH, methylphenidate; ADHD-RS, attention-deficit hyperactivity disorder rating scale.

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16q12.2, and ADHD, and the results have been heterogeneous [9,21]. Among the many polymorphisms of the NET gene, the G1287A polymorphism (rs5569), which is located at exon 9, and the −3081(A/T) polymorphism (rs28386840), in the promoter region, has received attention related to ADHD [3,17]. Several pharmacogenetic studies were also conducted with ADHD to examine MPH responses and the G1287A or −3081(A/T) polymorphism. Yang et al. [32] reported that symptom improvement after treatment with MPH is worse for ADHD with the A/A genotype of the G1287A polymorphism compared to those with other genotypes. Song et al. [27] reported that ADHD with the G/G genotype of the G1287A polymorphism have better responses to MPH treatment than those without the G/G genotype. Our previous study found that ADHD subjects who had a T-repeated allele at the −3081(A/T) polymorphism showed a better response to MPH treatment than those with the A/A genotype [11].

Recent studies have used a strategy of examining both genetic and functional neuroimaging data in a single study. Our recent genetic-imaging data suggested that ADHD subjects carrying the C allele at the MspI polymorphism show reduced perfusion in the bilateral orbitofrontal regions compared to those without the C allele [12]. Rohde et al. reported some significant findings when integrating MPH treatment response data with genetic and neuroimaging data [22].

However, to the best of our knowledge, no previous studies have integrated MPH treatment responses and pre- and post-treatment neuroimaging data associated with norepinephrine gene polymorphisms among ADHD children. Hence, this study sought to examine the differences in MPH responses and cerebral perfusion changes according to the G1287A and −3081(A/T) polymorphisms of the NET gene.

2. Methods

2.1. Participants and procedures

A total of 39 children (8.9 ± 1.9 years old, M = 34, F = 5) who visited the Department of Child and Adolescent Psychiatry of the Seoul National University Hospital and were diagnosed for ADHD according to DSM-IV criteria were recruited to this study.

Children meeting the following criteria were excluded: (1) an IQ score below 70 based on the Korean version of Wechsler's Intelligence Scale for Children [20]; (2) past or current neurological disease; (3) any evidence of comorbid psychiatric conditions except oppositional defiant disorder or anxiety disorder if they were mild enough that they did not require medication.

All of the participants were drug-naïve at the time of recruitment. Before medication, clinical assessments and baseline brain perfusion single-photon emission computed tomography (SPECT) were conducted. Subjects received 0.4–1.5 mg/kg/day extended-release MPH once a day in the morning for 8 weeks. The treatment included medication only. Doses of MPH were titrated depending on symptoms and adverse effects at the 2nd and 4th weeks. On each visit, we checked with the parents verbally regarding compliance. If a subject skipped medication more than 3 times throughout the total treatment period, he/she was excluded from the study. After 8 weeks, subjects were evaluated with the same clinical assessments as at baseline, and follow-up SPECT was carried out. The study was approved by the Institutional Review Board (IRB) for human subjects at the Seoul National University Hospital.

2.2. Clinical assessments

The Kiddie-Schedule for Affective Disorders and Schizophrenia-Present and Lifetime Version (K-SADS-PL) was used for diagnoses of ADHD and comorbid psychiatric disorders. For the clinical evaluation of ADHD, the ADHD rating scale-IV (ADHD-RS) [26] by parents were administered. The severity of ADHD symptoms was estimated using the Clinical Global Impressions-Severity (CGI-S) scale [7], which ranges from 1 (normal) to 7 (the most extremely ill patients), and was administered by a child psychiatrist (K.J.W.). The improvement of ADHD symptoms was estimated using the Clinical Global Impressions-Improvement (CGI-I) [7], which ranges from 1 (very much improved) to 7 (very much worse); this was administered by the same child psychiatrist. The child psychiatrist was blind to the children's genotypes and imaging data. The Korean versions of all instruments used in this study are known to have good validity and reliability.

2.3. Genotyping

The *SLC6A2* polymorphisms were genotyped as previously described with slight modifications. Genomic DNA was extracted from whole blood according to standard protocols. The detection of a single nucleotide polymorphism was based upon analysis of primer extension products generated from previously amplified genomic DNA using a chip-based matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry platform (Bruker-Sequenom, CA, USA). The details of protocols are available on demand.

2.4. Image acquisition

SPECT images were acquired using a triple detector (Triad; Trionix Research Lab., Twinsburg, OH) with a low energy, high-resolution pinhole collimator. On the day of follow-up SPECT, patients were instructed to take MPH in the morning as usual, and SPECT was performed 4 h after the MPH dose. All subjects were lying in the supine position with their eyes closed in a quiet room with dimmed lights. Based on the body weights of subjects, 9–10 mCi of technetium-99m-hexamethylporphylenamine oxime (99mTc-HMPAO) was administered intravenously 2–5 min before SPECT acquisition. A total of 120 frames were acquired in step-and-shoot, circular mode with a 40 frame/1 detector. The energy window was 140 keV with a width of 20%. The scan time was 15–20 s/frame. Reconstruction was carried out with a Butterworth filter, and the images were corrected for attenuation using the methods of Chang [6].

2.5. Statistical analysis

All reconstructed images were converted to the analyze format and spatially normalized to the SPECT standard templates using Statistical Parametric Mapping 8 (SPM8) software (Wellcome Department of Imaging Neuroscience, Institute of Neurology, University College London, UK). We performed affine transformation to determine the optimal parameters to minimize the least squares distances between individual scans and templates. We performed nonlinear registration using the weighted sum of the predefined smooth basis functions, and discrete cosine transformation was used to remove global nonlinear differences between images. The spatially normalized images were smoothed by convolution using an isotropic Gaussian kernel with a 16-mm full-width at half-maximum to increase the signal to noise ratio. Global effects, such as arbitrary changes in global signals due to variations in the radioactivity delivered to each participant, were removed by normalizing the scan voxel counts by proportional scaling. SPM 8 was used for imaging analysis. Pre- or post-treatment SPECT images between ADHD children according to genotype were compared using a *t*-test with covariates for age, gender, and IQ. Statistically significant differences between sets of images were assessed at