

# Association Between 5-HTTLPR Polymorphism and Tics after Treatment with Methylphenidate in Korean Children with Attention-Deficit/Hyperactivity Disorder

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## Abstract

**Objectives:** The purpose of this study is to examine the relationship between 5-HTTLPR polymorphism (44-bp insertion/deletion polymorphism of serotonin transporter gene) and methylphenidate (MPH) treatment response, as well as the association between the adverse events of MPH treatment and 5-HTTLPR polymorphism in children with attention-deficit/hyperactivity disorder (ADHD).

**Methods:** A total of 114 children with ADHD (mean age  $9.08 \pm 1.94$  years) were recruited from the child psychiatric clinic in a hospital in South Korea. We have extracted the genomic DNA of the subjects from their blood lymphocytes and analyzed 5-HTTLPR polymorphism of the SLC6A4 gene. All children were treated with MPH for 8 weeks, with clinicians monitoring both the improvement of ADHD symptoms and the side effects. We compared the response to MPH treatment and adverse events among those with the genotype of 5-HRRLPR polymorphism.

**Results:** There was no significant association between the 5-HTTLPR genotype and the response to MPH treatment in children with ADHD. Subjects with the S/L+L/L genotype tended to have tics and nail biting (respectively,  $p < 0.001$ ,  $p = 0.017$ ).

**Conclusions:** The results of this study do not support the association between the 5-HTTLPR polymorphism and treatment response with MPH in ADHD. However, our findings suggest the association between 5-HTTLPR polymorphism and the occurrence of tics and nail-biting as an adverse event of methylphenidate. This may aid in our understanding of the genetic contribution and genetic susceptibility of a particular allele in those ADHD patients with tics or nail biting.

## Introduction

ATTENTION-DEFICIT/HYPERACTIVITY DISORDER (ADHD) is the most common psychiatric disorder in children and adolescents (American Psychiatric Association 2013). Its worldwide prevalence is 5.29% (Polanczyk et al. 2007a). ADHD is known as a highly polygenic disorder. Its heritability is as high as 0.76 (Biederman and Faraone 2005). Previous genetic studies have investigated specific genes related to the etiology of ADHD. The complex interplay of genes related to dopamine, norepinephrine, and serotonin regulation in the brain is known to be the genetic etiology of ADHD (Cross-Disorder Group of the Psychiatric Genomics Consortium 2013). In particular, the involvement of genes related to the regulation of dopamine system such as the dopamine transporter (DAT) and D4 dopamine receptor (DRD4) genes in the pathogenesis of ADHD were supported by many previous studies and

confirmed by several meta-analysis studies (Purper-Ouakil et al. 2005; Gizer et al. 2009). However, these findings only explained a small portion of ADHD heritability, because their effect sizes were small. In addition, each susceptibility gene is likely to have low penetrance (Gizer et al. 2009; Akutagava-Martins et al. 2013).

Methylphenidate (MPH) has been reported to improve core symptoms of ADHD. However, ~30% of patients did not show satisfactory clinical response to MPH treatment (Swanson et al. 2002). Interindividual variability of clinical response to the same pharmacological agent has been reported in previous investigations (Husain et al. 2007). Identifying specific genetic variants underlying the clinical response to treatment is likely to explain the genetic contributor that partially affects the interindividual variability (Mazzelli et al. 2002). Further, the effort to investigate the genetic mechanism of response to medications might help us understand the underlying pathological process of ADHD. So far, pharmacogenetic

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research of ADHD has been focused on genes related to the dopamine and noradrenalin (NA) system, the primary site of action of MPH. Representatively, genetic polymorphisms of DAT (Winsberg and Comings 1999; Cheon et al. 2005; Stein et al. 2005; Joobar et al. 2007), dopamine receptor DRD2 (Leddy et al. 2009), DRD4 (Hamarman et al. 2004; Cheon et al. 2007; Ji et al. 2013), and DRD5 (Tahir et al. 2000) have been reported to contribute to the response to MPH treatment. For Korean children with ADHD, subjects with 4/4 genotype at DRD4 (homozygosity of the four repeat allele) were reported to have better response to MPH compared with other genotypes (Cheon et al. 2007). However, it was also reported that there was no association between the four repeat allele of the DRD4 gene and the response to MPH treatment (Ji et al. 2013). Although genes in the noradrenergic pathway have been examined less frequently than dopamine genes, prior studies mainly involved the G allele at the  $\alpha$ -2a-adrenergic receptor (ADRA2A) gene polymorphism (Polanczyk et al. 2007b; da Silva et al. 2008; Cheon et al. 2009) and norepinephrine transporter (NET) polymorphism (Yang et al. 2004). In line with these findings, better response to MPH was shown by homozygosity for the ADRA2A G allele (G/G) in children with ADHD in Korea (Cheon et al. 2009). In order to have a comprehensive understanding of ADHD, it is important to acknowledge the interaction of serotonergic system related to emotion and impulsivity regulation as well as the dopamine and norepinephrine system related to cognitive and reward processing (Nigg and Casey 2005).

5-HTTLPR (44-bp insertion/deletion polymorphism of serotonin transporter gene), a candidate gene for ADHD susceptibility that regulates serotonergic neurotransmission, is located on the long arm of chromosome 17 (Gelernter et al. 1995). 5-HTTLPR involves two common variants: S (short) allele and L (long) allele. The L allele compared with the S allele has increased 5-HTT mRNA expression that leads to faster serotonin reuptake from the serotonin transporter, which in turn results in low serotonin concentration in the synaptic clefts (Lesch et al. 1994; Heils et al. 1996). It has been reported that the S allele may be related to internalizing symptoms such as depression and anxiety, whereas the L allele may be related to externalizing symptoms such as impulsivity and aggression. In addition, more L allele carriers were found in children with ADHD (Cadoret et al. 2003; Kent et al. 2002). Recently, it has been suggested that the S allele at 5-HTTLPR polymorphism modulates an individual's response to environmental stress, with the S allele representing a higher sensitivity to stress, indicating that the S allele can be considered a susceptibility gene in ADHD in response to a high-stress environment (Caspi et al. 2010). For example, individuals who were S allele carriers showed a heightened vulnerability to ADHD within a high-stress environment (Muller et al. 2008; Retz et al. 2008).

Many pharmacological and neurobiological studies were conducted on the close interaction between dopamine and serotonin. Seeger et al. (2001) have found that children with hyperkinetic disorder (International Classification of Diseases, 10th Revision [ICD-10]) with the DRD4 and L/L genotype of 5-HTTLPR show a better treatment response to MPH (Seeger et al. 2001). However, two subsequent studies reported that 5-HTTLPR was not associated with MPH treatment response (Zeni et al. 2007; Tharoor et al. 2008). Nevertheless, one recent study suggested that ADHD children with the L/L genotype showed a better response to MPH when behavioral problems of ADHD were examined (Thakur et al. 2010). 5-HTTLPR was recently found to be a candidate gene in Tourette disorder as well as ADHD. New onset or aggravation of tic symptoms reported in Tourette patients with the L allele could be because 5-HTTLPR polymorphism indirectly affected the dopaminergic system. Some studies reported that change in the

serotonin receptor affected by different 5-HTTLPR polymorphisms would increase the secretion of dopamine, affecting the onset of Tourette disorder (Cath et al. 2001; Moya et al. 2013). In order to provide explanation for these inconsistencies in previous research results, the present study aimed to examine the relationship between 5-HTTLPR polymorphism and MPH treatment response, as well as the association between the side effects of MPH treatment and 5-HTTLPR polymorphism in children with ADHD.

## Materials and Methods

### Subjects

The present study enrolled ADHD children (age 6–15 years) from child psychiatric clinics of two university hospitals in Korea. Inclusion criteria for the study were: 1) Being diagnosed with ADHD according to *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed. (DSM-IV) Diagnostic Criteria of Mental Disorders (American Psychiatric Association 1994), 2) their parents/guardians agreed to let them participate in the study with informed consent, and 3) had no history of exposure to psychostimulants such as MPH prior to the study participation. Exclusion criteria were: 1) A past or present history of brain damage or convulsive disorder; 2) mental retardation, autism, language difficulties, or developmental problems including learning disability; 3) not having agreement from parents/guardians for them to participate in the study. This study was approved by the Institutional Review Board for human subjects.

### Diagnostic and evaluation tools of clinical symptoms

The Korean version of the Kiddie-Schedule for Affective Disorders and Schizophrenia-Present and Lifetime Version (K-SADS-PL-K). K-SADS-PL was developed for the psychiatric diagnosis of children and adolescents between 6 and 17 years of age (Kaufman et al. 1997). This semistructured interview was used to assess the presence of current and past psychiatric disorders. This measure has been translated into Korean. Its reliability and validity on ADHD, tic disorder, and oppositional defiant disorder (ODD) for Korean children and adolescents were proven by Kim et al. (2004).

ADHD Rating Scale-IV (ARS). DuPaul et al. (1998) developed the ARS scale in order to measure the severity of ADHD symptoms according to the DSM-IV Diagnostic Criteria of Mental Disorders (DuPaul et al. 1998). This scale had 18 items that were divided into nine inattention and nine hyperactivity/impulsivity subscales. Each item was measured on a four point scale ranging from 0 to 3. The Korean version of ARS was developed and standardized by So et al. (2002).

Clinical Global Impressions – Improvement (CGI-I). CGI is composed of a symptom severity scale (CGI-S) and symptom improvement scale (CGI-I). CGI-I is a seven point scale requiring clinicians to assess how much the patient's illness has improved or worsened relative to a baseline state at the beginning of the intervention (Guy 1976). On the 7 point scale, 1 point was used for very much improved; 2 points for much improved; 3 for minimally improved; 4 for no change; 5 for minimally worse; 6 for much worse; and 7 for very much worse.

### MPH administration and treatment response

All subjects participating in this study were administered MPH for 8 weeks. MPH dosage was increased until sufficient therapeutic effect was observed in the subjects. The therapeutic effect was determined by the degree of improvement and side effects reported by the parents.

We adjusted the MPH doses at visit 1 (1st week), visit 2 (2nd week), visit 3 (4th week), and visit 4 (6th week) and performed posttreatment assessments at visit 5 (8th week). The posttreatment assessments were evaluated using the ARS score reported by parents and the CGI score rated by clinician. A clinical response to the treatment was determined as follows. A "good" response indicated an improvement of >50% from the baseline ARS score, and an improvement in CGI-I score of 1 or 2 points after 8 weeks of treatment. A "poor" response indicated an improvement in the ARS scores of <50% and a CGI-I score in the range of 3–7 points. All procedures were performed by clinicians who had no information about the results of 5-HTTLPR genotype analysis.

#### Assessment for adverse events induced by MPH

Clinicians identified the most common adverse effects such as sleep disturbance, loss of appetite, tics, and nail biting. Adverse effects were also reported by subjects and their parents at each visit. Those who showed a "marked" adverse event according to the clinician's judgment in the 8th week were considered as those who developed adverse effects related to the MPH treatment in this study. "Marked severity" was defined as a symptom severity that caused the impairment of functioning or social embarrassment to a degree that the benefits of medication must be considered to justify the risks of continuing medication (Pelham 1993). More specifically, the frequency of adverse events was more than three times per week, and the intensity of adverse events were severe enough to reduce conformity to medication, and the duration was reported through all 8 weeks of MPH treatment. However, "tics" as an adverse event was described as a categorical variable, dividing them into a group of "new onset or aggravation of tics" and a group of "no change of tics or no tics."

#### Serotonin transporter gene (SLC6A4) genotyping

Genomic DNA was extracted from whole blood lymphocytes using Genomic DNA Extraction Kit (Bioneer, Korea). The 5-

HTTLPR polymorphism of the SLC6A4 gene was analyzed by polymerase chain reaction (PCR). Oligonucleotide primers (5'-GGC GTT GCC GCT CTGAAT GC-3' and 5'-GAG GGA CTG AGC TGG ACA ACC A-3') were used in PCR. Allele resolution was confirmed by agarose gel electrophoresis.

#### Statistical analysis

Descriptive statistics in numbers and percentages were used to analyze demographic and the clinical characteristics of the participants.  $\chi^2$  test or Fisher's exact test was used to examine the association between the genotype of 5-HTTLPR and MPH treatment response or between the genotype of 5-HTTLPR and the adverse events after MPH treatment response. A *p* value of 0.0125 (two tailed) was chosen as the Bonferroni-corrected significance threshold to adjust for multiple comparisons in four adverse events related to MPH treatment. SPSS 20.0 software (SPSS Inc., Chicago, IL) was used for statistical analysis.

## Results

### Demographic and clinical characteristics

This study initially included 131 children with ADHD, but 17 participants dropped out because of adverse events; in 15 (11.5%) these were related to MPH treatment (7 for loss of appetite, 5 for sleep disturbance, 3 for depressed mood/irritability). Another two were excluded from the final analysis because of blood storage problem. The mean age of the 114 ADHD subjects used in this study was  $9.08 \pm 1.94$  years. The 114 subjects included 95 (83.3%) boys and 19 (16.7%) girls. Of the 114 subjects with ADHD, 70 (60.9%) had the SLC6A4 S/S genotype, 40 (34.8%) had the S/L genotype, and 4 (3.5%) had the L/L genotype. There was no significant difference in the demographic or clinical characteristics between patients with S/S and those with S/L+L/L genotypes (Table 1). The genotypic distribution of 5-HTTLPR polymorphism

TABLE 1. COMPARISON OF DEMOGRAPHIC AND CLINICAL CHARACTERISTICS ACCORDING TO THE GENOTYPE OF 5-HTTLPR

Characteristics	Total (% of total) (n = 114)	S/S (n = 70)	S/L + L/L (n = 44)	p
Age (years)	9.08 ± 1.94	8.99 ± 1.99	9.23 ± 1.89	0.674 <sup>a</sup>
Male (%)	95(83.3%)	56(80.0%)	39(88.6%)	0.228 <sup>b</sup>
FSIQ	104.78 ± 16.20	103.13 ± 17.56	107.31 ± 14.91	0.575 <sup>a</sup>
ADHD subtype				
Combined (% of each genotype)	49(43.0%)	25(35.7%)	24(54.5%)	
Inattentive (% of each genotype)	53(46.5%)	37(52.9%)	16(36.4%)	0.139 <sup>b</sup>
Hyperactive/Impulsive (% of each genotype)	12(10.5%)	8(11.4%)	4(9.1%)	
Comorbidity				
Conduct disorder (% of each genotype)	2(1.8%)	1(1.4%)	1(2.3%)	1.000 <sup>b</sup>
ODD (% of each genotype)	5(4.4%)	1(1.4%)	3(6.8%)	0.297 <sup>b</sup>
Mood (% of each genotype)	20(17.5%)	13(18.6%)	7(15.9%)	0.716 <sup>b</sup>
Anxiety disorder (% of each genotype)	12(10.5%)	9(12.8%)	3(6.8%)	0.432 <sup>b</sup>
Tic disorder (% of each genotype)	11(9.6%)	6(8.6%)	5(11.4%)	0.747 <sup>b</sup>
ARS Baseline Scores				
Total	32.19 ± 8.01	32.99 ± 7.70	30.93 ± 8.42	0.184 <sup>a</sup>
Inattentive	16.67 ± 4.20	16.87 ± 4.36	16.34 ± 3.95	0.513 <sup>a</sup>
Hyperactive/impulsivity	15.53 ± 6.29	16.11 ± 6.25	14.59 ± 6.30	0.209 <sup>a</sup>
Mean dosage of MPH (mg/day)	29.47 ± 7.61	29.86 ± 7.51	28.84 ± 7.81	0.556 <sup>a</sup>

<sup>a</sup>Calculated by one way analysis of variance test.

<sup>b</sup>Calculated by  $\chi^2$  test or Fisher exact test.

FSIQ: Full scale intelligence quotient; ADHD, attention-deficit/hyperactivity disorder; ODD, oppositional defiance disorder; ARS, ADHD Rating Scale; MPH, methylphenidate.

TABLE 2. ASSOCIATION BETWEEN 5-HTTLPR GENOTYPE AND RESPONSE TO MPH TREATMENT IN ADHD SUBJECTS

Response to MPH n (% of total within genotype)	5-HTTLPR genotype		Total (% of total)	p
	S/S (n = 70)	S/L + L/L (n = 44)		
Poor (ARS Change <50%)	26(37.1%)	20(45.5%)	46(40.4%)	0.379
Good (ARS Change ≥50%)	44(62.9%)	24(54.5%)	68(59.6%)	
Poor (CGI score:3 ~ 7)	24(34.3%)	19(43.2%)	43(37.7%)	0.340
Good (CGI score:1 or 2)	46(65.7%)	25(56.8%)	71(62.3%)	

$\chi^2$  test was used for association analysis; significant at  $p < 0.05$ .

MPH: methylphenidate; ADHD, attention-deficit/hyperactivity disorder; ARS, ADHD Rating Scale; CGI, Clinical Global Impressions.

was consistent with the expected values of those computed from Hardy–Weinberg Equilibrium ( $\chi^2 = 0.352$ ,  $df = 1$ ,  $p = 0.55$ ).

#### Correlation between the response to MPH treatment and the 5-HTTLPR polymorphism

The mean MPH doses for the S/S group and for the S/L+L/L group were not significantly different (Table 1). A total of 44 (62.9%) subjects with S/S genotype and 24 (54.5%) subjects with S/L+L/L genotype showed good response to MPH treatment as measured by the ARS. There were no significant group difference either in response to MPH treatment when assessed by ARS ( $\chi^2 = 0.775$ ,  $p = 0.379$ ) or by CGI-I ( $\chi^2 = 0.910$ ,  $p = 0.34$ , Table 2). In addition, there was no significant difference between the changes in ARS scores before and after MPH treatment, depending on the genotype of 5-HTTLPR ( $t = 0.716$ ,  $p = 0.461$ ).

#### Adverse events after the use of MPH

After receiving MPH treatment, 9 (7.9%) and 20 (17.5%) subjects reported sleep disturbances and loss of appetite, respectively (Table 3). There was no significant difference in adverse events between the two groups. Nail biting was more frequently reported in the S/L+L/L group (22.7%,  $n = 10$ ) than in the S/S group (7.1%,  $n = 5$ ). This difference was significant ( $\chi^2 = 5.743$ ,  $p = 0.023$ ). However, in a post-hoc analysis, nail biting did not survive Bonferroni-corrected level of significance (0.0125). In patients with the S/S genotype, nine (12.9%) reported a new occurrence or aggravation of tics. In contrast, 20 (45.5%) patients with the S/L+L/L genotype had new onset of tics or aggravated tics ( $\chi^2 = 15.136$ ,  $p < 0.001$ , Table 3).

#### Discussion

Our results revealed that there was no significant association between the 5-HTTLPR genotype and the clinical response to MPH

treatment in Korean children with ADHD. Although the association between ADHD and 5-HTTLPR has already been reported, there were only a few studies regarding the association between the 5-HTTLPR genotypes and the treatment response of MPH. However, those results were inconsistent.

Weizman et al. (1987) suggested that MPH treatment could reduce prolactin level. Shapira et al. (1992) showed that the increased serotonin level from the selective serotonin reuptake inhibitor (SSRI) injection caused the release of prolactin. In this context, Seeger et al. investigated the change in prolactin level by creating a group having both DRD4 and 5-HTTLPR polymorphisms from children with hyperkinetic disorder (ICD-10). Their findings showed that when MPH was administered in subjects with both the DRD4-7 allele and the 5HTTLPR/L genotype, the prolactin level was increased, but the degree of improvement in hyperactivity and impulsivity was decreased (Seeger et al. 2001). Although it was not a study solely on 5-HTTLPR, that study made it possible to predict that 5-HTTLPR might interact with the dopamine system and affect the treatment response in ADHD.

Subsequently, Zeni et al. (2007) and Tharoor et al. (2008) reported the absence of association between 5-HTTLPR and treatment response in ADHD (Zeni et al. 2007; Tharoor et al. 2008). However, these studies had some limitations, in that observations for clinical response were made only for a relatively short period of time (4 weeks), and assessments were retrospectively performed based on parents' subjective reports. In the most recent crossover study on 5-HTTLPR and the clinical response to MPH treatment (Thakur et al. 2010), ADHD children with S/S genotypes ( $L_G/L_G$ ,  $S/L_G$  and  $S/S$ ) were improved even in a placebo condition. In contrast, patients with the L/L genotype ( $L_A/L_A$ ) showed no improvement in a placebo condition. However, they had sufficient improvement after MPH treatment. In subjects with the S/L genotype ( $L_G/L_A$  and  $S/L_A$ ), some improvement was made in both the placebo condition and after MPH treatment, showing an intermediate profile between

TABLE 3. DIFFERENCE IN THE ADVERSE EVENTS AFTER THE USE OF MPH ACCORDING TO THE GENOTYPE OF THE 5-HTTLPR

Adverse events n (% of total within genotype)	5-HTTLPR Genotype		Total (% of total)	p
	S/S (n = 70)	S/L + L/L (n = 44)		
Difficulty falling asleep	6(8.6%)	3(6.8%)	9(7.9%)	0.517 <sup>a</sup>
Decreased appetite	13(18.6%)	7(15.9%)	20(17.5%)	0.716 <sup>a</sup>
Nail biting	5(7.1%)	10(22.7%)	15(13.2%)	0.023 <sup>a</sup>
Tics after the use of MPH				
New onset or tics aggravated	9(12.9%)	20(45.5%)	29(25.4%)	<0.001 <sup>b</sup>
No change or no tics	61(87.1%)	24(54.5%)	85(74.6%)	

<sup>a</sup>The  $\chi^2$  test was used of association, significant at  $p$  value  $< 0.05$ .

<sup>b</sup>At a Bonferroni-adjusted level of significance ( $p = 0.0125$ ), significant differences in tics after the use of MPH.

MPH, methylphenidate.

the S/S genotype and the L/L genotype. These results indicate that the clinical response to MPH treatment was better when 5-HT transmission of the L<sub>A</sub> allele became more efficient.

The discrepancy between this present study and previous studies might be explained as follows. First, it may be because of polygenic contribution or phenotypic heterogeneity. In the polygenic mode of inheritance, each of multiple genes can contribute to a certain phenotype as a small percentage (or has a small effect on risk). Therefore, it might be difficult to reach statistical significance. Second, we divided our samples into S/S and S/L+L/L genotypes because of the limited number of subjects with L/L genotypes. As a result, three-group comparison using the traditional grouping of S/S, S/L, and L/L could not be conducted for our samples. In addition, the L allele consists of the subtypes of L<sub>A</sub> allele and L<sub>G</sub> allele. The L<sub>G</sub> allele equivalently expresses HTT mRNA with similar function as a short (S) variant. As a result, similar to the S allele, the L<sub>G</sub> allele has low serotonin transporter expression and serotonin reuptake activity. If there are many L<sub>G</sub> in S/L and L/L genotypes, the effect of 5-HTTLPR might have been underestimated (Hu et al. 2006). For more detailed comparison, consideration should be given to the fact that 5-HTTLPR is functionally triallelic. Thakur et al. (2010) have suggested that the L<sub>G</sub> allele is functionally equivalent to the S allele (Thakur et al. 2010). They investigated the association of clinical response to MPH treatment in children with ADHD by categorizing both L<sub>G</sub>/L<sub>G</sub> and S/L<sub>G</sub> genotypes into the S/S group, L<sub>G</sub>/L<sub>A</sub> and S/L<sub>A</sub> into the S/L group, and L<sub>A</sub>/L<sub>A</sub> into the L/L group. Third, recent studies suggest that the 5-HTTLPR polymorphism could moderate the effect of environmental stressors (Nikolas et al. 2010; Jonassaint et al. 2012; van der Meer et al. 2014). However, we could not investigate the effect of environmental factors with our study design.

Van der Meer et al. (2014) found that neither the genetic factor of 5-HTTLPR polymorphism nor the environmental factor of stress could independently affect symptoms in children or young adults with ADHD, but that they did affect symptoms by interacting with each other (van der Meer et al. 2014). In particular, a positive correlation between stress and ADHD severity was reported in S allele carriers. This is consistent with the existing theories suggesting that carrying the S allele associated with anxiety-related traits and higher reaction to stress (such as increased heart rate and increased blood flow in the limbic system) is linked to stress and the resultant negative results (Lesch et al. 1996; Muller et al. 2008; Caspi et al. 2010). We did not consider the possibility that the S allele could aggravate ADHD symptoms by the mediation of stress, therefore affecting the clinical response.

Although the association between 5-HTTLPR polymorphism and the clinical response to MPH treatment was not confirmed in our study, we did find that the more L variants there were, the more correlations there were between 5-HTTLPR polymorphism and tics and nail biting (possible side effect of MPH treatment). It is well known that ADHD has a high comorbidity with tic disorder (Taurines et al. 2010; El Malhany et al. 2015). Until recent years, whether the use of the stimulants causes or aggravates tic symptoms has been a debated issue. It is a common view that using stimulants in children with ADHD does not cause tic symptoms. However, there is a strong association between stimulant use and tic symptoms (Lowe et al. 1982; Kurlan 2003; Erenberg 2005). In addition, the high comorbidity rate of ADHD, tic disorder, and obsessive-compulsive disorder (OCD) suggests a genetic relationship among these disorders (Gillberg et al. 2004; Taurines et al. 2010). A family study suggests that OCD and tic disorder are considered to share genetic susceptibility factor, whereas the association between

ADHD and tic disorder is more complicated. Such association might be mediated in part by OCD (Mathews and Grados 2011; O'Rourke et al. 2011).

More recently, however, 5-HTTLPR, a candidate gene for ADHD and OCD, was found to be a candidate gene in Tourette disorder as well. Moya et al. (2013) revealed a relationship between the L<sub>A</sub> allele or L<sub>AC</sub> haplotype of 5-HTTLPR polymorphism (5-HTTLPR/rs25531/rs25532) and Tourette disorder. By increasing serotonin clearance, the L<sub>A</sub> allele and L<sub>AC</sub> haplotype caused low serotonin levels and upregulated postsynaptic 5-HT<sub>2A</sub> receptor (Moya et al. 2013). Such a change in the serotonin receptor is reported to increase the secretion of dopamine, affecting the onset of Tourette disorder (Cath et al. 2001).

In this study, the reason that the group with the L allele showed a new onset or aggravation of tic symptom could be because 5-HTTLPR polymorphism indirectly affected the dopaminergic system. It is also possible that the etiologies of ADHD and OCD, as well as those of ADHD and tic disorder, are genetically linked together. Meanwhile, nail biting known as body-focused repetitive behavior disorder has a tendency to be transmitted in the family of the patients with OCD, which is considered to be part of familial OCD spectrum (Bienvenu et al. 2000). Although there is no consensus yet, the L<sub>A</sub> allele of 5-HTTLPR is associated with OCD (Taylor 2013). In addition, the L<sub>A</sub> allele of 5-HTTLPR has a strong association with early onset OCD, with younger age having more power to explain the genetic etiology (Walitza et al. 2014). Because nail biting as an OCD spectrum disorder partly shares 5-HTTLPR polymorphism, it might be more sensitive to dopamine and/or serotonin turnover change when MPH is administered. In this study, there was no difference in baseline tic symptoms between the S/S and S/L+L/L groups, but the existence of a significant difference in tic symptoms between the two groups after MPH treatment suggests that there was significant association between 5-HTTLPR polymorphism and the occurrence of tics and nail biting as adverse events of MPH treatment in ADHD children. However, we cannot disentangle the causal relationship between the MPH treatment and the occurrence of tics with our current study design. The association between MPH treatment and tic symptom is complicated. We cannot exclude the possibility that multiple factors, such as genetic predisposition, including 5-HTTLPR L genotype, and stimulant treatment or stress, may trigger tic symptoms in ADHD children by interacting with each other.

### Limitations

Our study has the following limitations. First, a relatively small sample size made it impossible to obtain statistical significance. In complex neuropsychiatric disorders, including ADHD, sample sizes in at least the thousands or tens of thousands are necessary to expect a robust finding from a candidate gene study in ADHD, and, therefore, our findings need to be replicated in larger samples. In addition, the samples were biased in terms of gender, as male participants accounted for 80% of the whole sample. However, there was no difference in the ratio of gender between the two groups. Second, our study design is not a randomized placebo-controlled study, and, therefore, is not able to exclude the effect of placebo response. Third, our findings may be difficult to generalize to other races and ethnic groups. Based on a previous study that reported a 5-HTTLPR allelic frequency difference among different ethnicities — the lower L allele frequency in Asian populations compared with those of European or African descent (Goldman et al. 2010) — our results about the effectiveness and adverse events of MPH treatment may not be generalized across different

populations. Fourth, in our sample, ADHD's comorbidity with disruptive behavior disorders (for example, CD or ODD) was relatively low (at 6.2%), whereas that with internalizing disorders (for example, mood disorder or anxiety disorder) was higher (at 28%). Compared with the ADHD comorbidity in the previous study (Biederman 2005), the ratio of disruptive behavior disorder in this study was relatively lower. However, no significant difference was found between the two genotype groups. Fifth, when evaluating side effects in patients, we did not use an objective scale tool such as the Side Effect Rating Scale (SRS). However, well-trained psychiatrists have carefully examined their side effects through parents' reports and clinical interviews with the subjects.

## Conclusions

In conclusion, our results revealed that there was no significant association between 5-HTTLPR polymorphism and the response to MPH treatment in ADHD children. There has been report of inconsistent results for this issue in previous studies. Further studies are needed to clarify this issue. Given the fact that 5-HTTLPR is triallelic, the genotypes should be grouped in a more refined way. In addition, the effect of environmental factors should be considered. However, we did find that the S/L or L/L genotypes of 5-HTTLPR polymorphism were associated with tics after MPH treatment. Carrying L alleles could affect serotonin transporter gene expression by indirectly accelerating dopamine release through the serotonergic pathway. This may aid in our understanding of the genetic contribution and genetic susceptibility of this particular allele in those ADHD patients with tics.

## Clinical Significance

To our best knowledge, our result is the first one to show the relationship between the serotonin transporter gene and tics after treatment with MPH in ADHD children.

## Disclosures

No competing financial interests exist.

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