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Allele-Specific Associations of 5-HTTLPR/rs25531 with ADHD and Autism Spectrum Disorder

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Abstract

Background—The aims of the present study were to examine the association between a common serotonin transporter gene (*SLC6A4*) polymorphism 5-HTTLPR/rs25531 with severity of attention deficit hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) symptoms.

Methods—Mothers and teachers completed a validated DSM-IV-referenced rating scale for ADHD and ASD symptoms in 118 children with ASD.

Results—Analyses indicated that children with at least one copy of the S or L_G allele obtained significantly more severe maternal ratings of hyperactivity ($p=0.001$; $\eta^2=0.097$) and impulsivity ($p=0.027$; $\eta^2=0.044$) but not inattention ($p=0.061$; $\eta^2=0.032$), controlling for ASD severity, than children homozygous for the L_A allele. Conversely, mothers' ratings indicated that children

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with L_A/L_A genotype had more severe ASD social deficits than $S+$ or L_G allele carriers ($p=0.003$; $\eta^2=0.081$), controlling for ADHD symptom severity. Teachers' ratings though consistent with mothers' ratings of hyperactivity and social deficits were marginally significant ($p=0.07/p=0.09$). There was some evidence that the magnitude of parent-teacher agreement regarding symptom severity varied as a function of the child's genotype.

Conclusion—The 5-HTTLPR/rs25531 polymorphism or its correlates may modulate severity of ADHD and ASD symptoms in children with ASD, but in different ways. These tentative, hypothesis-generating findings require replication with larger independent samples.

Keywords

5-HTTLPR; attention-deficit hyperactivity disorder; autism spectrum disorder; serotonin; *SLC6A4* gene

1. Introduction

Much interest has been focused on the potential role of the serotonin (5-hydroxytryptamine, 5-HT) system, particularly serotonin transporter protein (5-HTT, SERT) gene (*SLC6A4*) polymorphisms, in a variety of neuropsychiatric syndromes (Serretti et al., 2006) and personality characteristics (Ebstein, 2006) owing in part to the facts that (a) 5-HTT controls the strength and duration of neural transmission and recycles 5-HT into the presynaptic terminal (Murphy et al., 2008); (b) pharmacologic compounds that alter the actions of 5-HTT have important therapeutic applications (Kronenberg et al., 2008), and (c) 5-HT acts as a morphogen during embryogenesis (MacKenzie & Quinn, 1999; Whitaker-Azmitia, 2001). One of the most extensively studied *SLC6A4* polymorphisms is a functional variable number tandem repeat (VNTR) 43-bp insertion/deletion in the promoter, commonly known as the 5-HTT-linked polymorphic region (5-HTTLPR) (Wendland et al., 2006). There are two common variants, a long (L) 16-repeat and short (S) 14-repeat, that alter promoter activity differentially. Relative to the L allele, the S allele has a less efficient 5-HTT promoter (reduced expression of 5-HTT mRNA) and therefore produces less protein, which in turn leads to reduced 5-HT uptake from the synaptic cleft (Heils et al., 1996; Lesch et al., 1994). Carriers of the S allele evidence less 5-HTT density in the brain (Praschak-Rieder et al., 2007) and greater amygdala reactivity (Hariri et al., 2005), an area of the brain involved in the regulation of social and affective behaviors. There is an A to G substitution (rs25531) within the L allele, and the L allele with the A variant (L_A) is associated with increased 5-HTT mRNA expression compared with the S allele and L allele with the G variant (L_G) thus creating a triallelic polymorphism (Hu et al., 2006; Wendland et al., 2006). Of particular significance are the findings of three PET studies indicating that individuals with the L_A/L_A genotype exhibit higher 5-HTT binding and therefore greater 5-HTT density in several brain regions (Willeit & Praschak-Rieder, 2010).

Dysregulation of serotonergic processes has long been implicated in the pathogenesis of autism spectrum disorders (ASD) (Lam et al., 2006), based initially on reports of platelet hyperserotonemia in a subset of individuals with ASD (Abramson et al., 1989; Schain & Freedman, 1961) and more recently on the role of serotonin in brain development (Whitaker-Azmitia et al., 2001), animal models of ASD (Altamura et al., 2007; McNamara et al., 2008; Veenstra-VanderWeele et al., 2012; Whitaker-Azmitia, 2001), correlation of lower levels of brain 5-HTT binding with impaired social cognition in adults with autism (Nakamura et al., 2010), and association of 5-HTTLPR genotypes with cerebral gray matter volumes in male children with autism (Wassink et al., 2007). There is also evidence of preferential transmission of 5-HTTLPR variants in individuals with ASD (Cook et al., 1997; Klauck et al., 1997; Kistner-Griffin et al., 2011) and association with ASD severity (Brune

et al., 2006; Mulder et al., 2005; Tordjman et al., 2001); however, findings are mixed (Devlin et al., 2005; Huang & Santangelo, 2008). For the most part these studies did not examine the triallelic 5-HTTLPR or consider co-occurring psychiatric symptoms.

Approximately one half of children with ASD meet symptom criteria for attention-deficit hyperactivity disorder (ADHD) (Gadow et al., 2005), which shows considerable phenomenological similarities with ADHD in non-ASD samples to include the differentiation of inattention and hyperactivity/impulsivity symptom phenotypes (Gadow et al., 2006; Lecavalier et al., 2009a), likely shares pathogenic processes with ASD (Rommelse et al., 2011) but may nevertheless be unique (Sizoo et al., 2010; Tudor et al., 2012). Moreover, a few studies of children with ASD describe possible ADHD symptom modulation for common gene variants of interest in ADHD (Gadow et al., 2008; Guerini et al., 2011; Roohi et al., 2009), but none have reported on the 5-HTTLPR. Animal models of ADHD indicate that serotonin acts to inhibit ADHD behaviors, particularly hyperactivity, through regulation of dysfunctional dopamine and norepinephrine signaling (Fan et al., 2011). Although findings of meta-analyses of studies that examined an association of the 5-HTTLPR with ADHD are contradictory as to whether the risk variant is the S (Landaas et al., 2010) or L (Gizer et al., 2009) allele, the extant literature pertains primarily non-ASD youth and for the most part neither examines the triad of ADHD symptoms separately, controls for co-occurring psychopathology, nor considers the triallelic 5-HTTLPR.

Our primary objective was to examine the association between the 5-HTTLPR/rs25531 variant with ADHD symptom severity (inattention, hyperactivity, impulsivity) in a restricted age range of children with ASD. Although the present study is by necessity exploratory, if ADHD is etiologically similar in both ASD and nonASD populations, then according to Landaas et al.'s (2010) analyses children with at least one copy of the S or L_G allele would likely have more severe ADHD symptoms. Owing to a number of nosological, phenomenological, and etiological overlaps between ASD and ADHD (Rommelse et al., 2011), analyses controlled for severity of ASD. A secondary objective was to see whether 5-HTTLPR/rs25531 variants were associated with ASD symptoms, particularly social deficits, which are central to the phenomenology of ASD, show similarities with the social cognition deficits of ADHD (Rommelse et al., 2011), and are associated with brain serotonin dysregulation in adults with autism (Murphy et al., 2006; Nakamura et al., 2010). Because numerous studies show that parent and teacher ratings of ADHD and ASD symptom severity evidence modest convergence (Gadow et al., 2006; Lecavalier et al., 2009b), ratings from each informant were analyzed separately.

2. Participants and methods

2.1. Participants

Participants were recruited from referrals to a university hospital developmental disabilities specialty clinic located on Long Island, New York. Families with at least one child with a confirmed diagnosis of ASD were contacted for participation in a genetic study. To maximize homogeneity, the study sample ($N=118$) was limited to individuals who were children (4–14 years old) when the diagnostic and behavioral evaluations were conducted. Demographic characteristics were as follows: age ($M=7.5$; $SD=2.7$), gender (86% male), ethnicity (91% Caucasian), IQ 70 (71%), socioeconomic status (SES) assessed with Hollingshead's (1975) index of occupational and educational social status ($M=41.8$; $SD=11.1$), single-parent household (9%), and receiving psychotropic medication when assessments were conducted (25%). Both referred (Gadow et al., 2005) and epidemiologic (Simonoff et al., 2008) samples of children with ASD exhibit high levels of co-morbid psychopathology. In the present study, the percentage of youth with T scores >65 for parent/teacher ratings were as follows: ADHD (63%/46%), oppositional defiant disorder (22%/

29%), generalized anxiety disorder (21%/25%), and major depressive episode (36%/39%). This study was approved by a university Institutional Review Board, informed consent was obtained, and appropriate measures were taken to protect patient (and rater) confidentiality.

2.2. Procedure

Prior to the child's initial intake evaluation, parents and teachers were asked to complete a battery of measures. Parent/teacher ratings of psychiatric symptoms were available for 113/104 of the children, respectively. In most cases, parents' ratings were completed by the child's mother. Genotype status was determined using DNA isolated from either peripheral blood cells or buccal swabs. Diagnoses of ASD were confirmed by an expert diagnostician and based on five sources of information about ASD symptoms to verify DSM-IV criteria: (a) comprehensive developmental history, (b) clinician interview with child and caregiver(s), (c) direct observations of the child, (d) review of validated ASD rating scales including the Child Symptom Inventory-4 (CSI-4) (Gadow et al., 2008), (e) prior evaluations and, additionally ($n=98$), with (f) the Autism Diagnostic Observation Schedule (Lord et al., 2000) and/or Autism Diagnostic Interview-Revised (Rutter et al., 2003).

2.3. Genotyping

The PCR protocol was adapted from Wendland and colleagues (2006) and was carried out in a total volume of 20 μ l with forward (5'-TCCTCCGCTTTGGCGCCTCTTCC-3') and reverse primers for the 5-HTTLPR (5'-TGGGGGTTGCAGGGGAGATCCTG-3'). Each amplification contained 20 ng of genomic DNA, 1 $\times$ multiplex master mix (Qiagen, Valencia, CA) and 0.20 μ M of the 5-HTTLPR primers. Reaction conditions began with an initial denaturation at 95° C for 15 minutes followed by 35 cycles of 94° C for 30 seconds, 68.1° C for 90 seconds, and 72° C for 60 seconds, with a final extension step of 10 minutes at 72° C. Next, 7 μ l of PCR product were digested by HpaII (5U; New England Biolabs, Ipswich, MA) in a 20 μ l reaction containing 1X NEBuffer 1 and 1X BSA at 37° C for 3 h. A second set of mock digested samples were run in a parallel 20 μ l reaction in which water replaced HPAII. Finally, products were analyzed on a QIAxcel System (Qiagen, Valencia, CA) and genotype analysis was conducted by two investigators (D.O., V.S.) who were blind to the behavioral characteristics of the study sample.

2.4. Measures

The CSI-4 (Gadow & Sprafkin, 1986, 2002) is a behavior rating scale that assesses the behavioral symptoms of a broad range of psychiatric syndromes and has both parent and teacher versions. Individual items bear one-to-one correspondence with DSM-IV symptoms (i.e., high content validity). To assess symptom severity, items are scored (never=0, sometimes=1, often=2, and very often=3) and summed separately for each symptom dimension. Research indicates the CSI-4 has relatively high sensitivity and specificity for identifying children with autistic disorder and ASD (DeVincent & Gadow, 2009; Gadow et al., 2008), and scores of studies show the ASD and ADHD subscales demonstrate adequate internal consistency, reliability, and validity in community-based normative, clinic-referred non-ASD, and ASD samples (Gadow & Sprafkin, 2011). Moreover, confirmatory factor analysis of the CSI-4 with large a ($N=730$) sample of children with diagnosed ASD supports the construct validity of DSM-IV syndromes in general (Lecavalier et al., 2009a), as well as the triad of symptom dimensions that comprise the ASD and ADHD clinical phenotypes (Lecavalier et al., 2009a, b). As with all rating scales, mother and teacher ratings evidence modest convergence, and this is also true for ADHD and ASD and symptoms (Gadow et al., 2006; Lecavalier et al., 2009b). CSI-4 global scores show little relation to age, IQ, or SES.

2.5. Statistical Analyses

Chi-square tests (categorical variables), correlations (continuous variables), and ANOVAs (combined categorical and continuous variables) were used to test associations of demographic characteristics with genotype groups as well as dependent variables to identify potential covariates for subsequent analyses. ANCOVAs were conducted to examine main effects of genotype group covarying for global ASD symptom severity in the case of ADHD symptoms and ADHD for analyses of ASD symptoms. (Age and gender were considered as potential covariates, but none were linearly related to the dependent variables.) We report partial eta-squared (η^2) to gauge the magnitude of group differences. A rule of thumb for determining the magnitude of η^2 suggests the following: 0.01–0.06=small, 0.06–0.14=moderate, and >0.14 =large (Cohen, 1988).

3. Results

3.1. Genotypes

The distribution of 5-HTTLPR/rs25531 genotypes (frequencies/percentages) was L_A/L_A (26/22%), L_A/L_G (12/10%), L_G/L_G (0%), S_A/L_A (46/39%), S_A/L_G (5/4%), and S_A/S_A (29/25%). Only one child had an S_G allele (S_A/S_G), and the overall distribution was generally consistent with Wendland et al.'s (2006) data. Genotype groups did not deviate from Hardy-Weinberg equilibrium, $X^2 = 5.97$, $df=6$, $p=0.46$ (www.husdyr.kvl.dk). For data analyses, children were sorted into one of three genotype groups: high expressing L_A/L_A , low expressing S/S (S_A/S_A , S_A/S_G , S_A/L_G) or heterozygous (L_A/L_G , S_A/L_A). Comparisons between genotype groups did not evidence statistically significant differences in age, gender, IQ, ethnicity, single-parent household, SES, current special education, or medication status at time of assessment.

3.2. ADHD Symptoms

The main effect of the three genotype group comparison was significant for maternal ratings of hyperactivity and marginally significant for impulsivity (see values in parentheses in Table 1). Post hoc analyses (Tukey HSD tests) indicated that both S/S ($p=0.013$) and heterozygous ($p=0.001$) groups had more severe hyperactivity symptoms than the L_A/L_A group. When children with at least one low expressing allele (i.e., S/S and heterozygous groups) were compared with youth homozygous for the high expressing L_A allele, analyses indicated that the former obtained significantly more severe maternal ratings of hyperactivity and impulsivity than the L_A/L_A group (Table 1), and findings for inattention were marginally significant ($p=0.061$).

Teacher ratings were not significant for the three genotype group comparison, but the main effect for hyperactivity was marginally significant ($p=0.067$) (Table 1). For the two genotype group comparison (i.e., L_A/L_A genotype vs. all other genotypes), hyperactivity was also marginally significant ($p=0.072$).

3.3. ASD Symptoms

There was one significant main effect for mothers' ratings of social deficits in the three genotype group comparison (see values in parentheses in Table 2), and post hoc analyses indicated that the L_A/L_A genotype exhibited more severe symptoms ASD social deficits than either the heterozygous ($p=0.003$) or the S/S ($p=0.019$) group. As expected, comparison of children homozygous for the L_A allele versus all other genotypes indicated that the former had more severe social deficits. Teacher ratings were not significant, but consistent with mothers' ratings the main effect in the two-genotype group comparison was marginally significant ($p=0.089$) for social skills deficits, with the L_A/L_A group having the most severe ratings (Table 2).

4. Discussion

Our findings provide preliminary evidence for an association between 5-HTTLPR/rs25531 genotype and severity of mother-rated ADHD symptoms in children with ASD, which lends additional support to the notion that the same gene variants implicated in the modulation of psychiatric symptoms in non-ASD populations warrant examination in children with ASD (Gadow et al., 2008; Roohi et al., 2009). Specifically, S or L_G allele carriers were at differentially greater risk for more severe ADHD symptoms, particularly hyperactivity, compared with children who were homozygous for the L_A allele. The effect size for hyperactivity symptoms was in the moderate range ($\eta^2=0.097$) and substantially larger than when not controlling for severity of ASD symptoms ($\eta^2=0.061$). Evidence of an association was less compelling for inattention symptoms, which was not unexpected owing to a voluminous literature supporting the differential validity of inattention versus hyperactivity-impulsivity, even in children with ASD (Gadow et al., 2006). As previously noted, a number of studies have examined the 5-HTTLPR as a potential risk factor for ADHD in non-ASD samples, but few deconstructed the ADHD diagnostic phenotype into component symptom phenotypes, controlled for co-occurring ASD symptom severity, or examined the triallelic 5-HTTLPR.

A secondary objective was to explore 5-HTTLPR/rs25531 variants as possible modulators of ASD severity, and analyses indicated a moderate-strength association ($\eta^2=0.083$) with social deficits where children with the L_AL_A genotype had the more severe symptoms than all other genotypes the S/S group. Failure to control for ADHD symptom severity resulted in a smaller effect size ($\eta^2=0.029$). Co-occurring ADHD is a risk factor for a wide range of psychiatric symptoms in children with ASD (Gadow et al., 2006), much as it is with other neurobehavioral syndromes such as chronic tic disorder (Gadow et al., 2002; Shapiro et al., 1988), and it is an important variable in characterizing associations between ASD symptom severity and a variety of other symptom phenotypes in children with ASD (e.g., Gadow et al., 2008; Gadow et al., 2012; Guttman-Steinmetz et al., 2010).

There is a long-standing controversy in the ASD literature as to whether co-occurring symptoms such as ADHD are epiphenomena of ASD, traditional psychiatric disorders (“co-morbidity”), or possibly unique syndromes within the ASD clinical phenotype. In the present study, different alleles of the same gene were specifically associated with either hyperactivity or social deficits within the same clinical phenotype, thus (a) supporting to some extent the differential validity of ADHD within the ASD clinical phenotype, (b) providing one potential explanation for a growing literature indicating both phenomenological and etiological overlaps between the two spectra (Rommelse et al., 2011), and (c) suggesting a possible mechanism for “co-morbidity” within ASD. Alternatively, for another polymorphism, SNAP-25 rs363043, the same variant was found to be associated with both hyperactivity and ASD severity (Guerini et al., 2011). Along similar lines, other investigators have found different alleles of the same gene (Crespi & Thiselton, 2011) or duplications/deletions at the same locus (Crespi et al., 2010) confer specific risk for different disorders (autism or schizophrenia); nevertheless, the symptoms of these disorders appear to overlap in children with ASD (Gadow & DeVincent, 2012) as well as childhood-onset schizophrenia (Sporn et al., 2004). Collectively, the research literature supports multiple models of symptom occurrence and co-occurrence.

4.1 Informant

Findings for teachers’ ratings were marginally significant for both the exact same parent-rated symptom dimensions and genotypes, which was somewhat unexpected as the two informants often disagree about symptom severity. In this regard, correlations between parent and teacher ratings of hyperactivity ($r=0.51$, $p<.01$) and social deficits ($r=0.27$, $p<.01$)

were moderate to low. Effect sizes were lower for teachers' than parents' ratings, but with larger samples may nevertheless be significant. There is growing evidence that S allele confers differentially greater sensitivity to certain contextual features than the L allele (Belsky et al., 2009; Fox et al., 2011; Kuepper et al., 2012). This suggests one possible mechanism for behavioral plasticity (Komers, 1997; Piersma & Drent, 2003; Wilson, 1998), which *may* explain informant differences in perceptions of symptom severity. In other words, if the school (or home) is differentially more stressful/nurturing for a child, informants may actually observe different types of child behaviors. This could have special significance for ADHD, a disorder that appears to be particularly sensitive to contextual features (Gadow & Drabick, 2012), but this possibility remains largely unstudied. Interestingly, in the present study, parent-teacher agreement varied as a function of the child's 5-HTTLPR/ rs25531 genotype, and the discrepancy was particularly large in the case of ratings of hyperactivity and social skills deficits (Table 3). For hyperactivity, the correlation for the L_A/L_A group was significantly lower than for heterozygotes ($p=0.02$) but not significant for the L_A/L_A versus S/S comparison ($p=0.13$). Whether or not these data support a plasticity interpretation of informant discrepancy is open for discussion, but this phenomenon appears to warrant further study.

4.2. Strengths, Limitations, and Future Directions

The present study used well-validated measures, dimensional approach to symptom-specific phenotypes, and a restricted age range of children with ASD, and controlled for co-occurring symptoms. Moreover, classification of participants who were carriers of the L_G allele as members of the low-expression group (S+) generally resulted in more clearly defined gene-phenotype relations (particularly ASD social deficits) than the more traditional biallelic model (data not shown). Although findings also appear generally consistent with reports of some (but not all) comparable studies of children with ADHD or ASD, they are subject to several qualifications. We examined specific symptom dimensions (e.g., hyperactivity, social deficits) of each spectrum (ADHD, ASD) and not categorical diagnoses. Dimensional approaches to examining interrelations between disorders may be a useful strategy in future research, not only in terms of generating better models of pathogenesis but furthering our understanding of nosology. Owing to the predominance of males with ASD, we were unable to examine possible gender dimorphism in gene-behavior relations (Brummett et al., 2008). Because children with more and less severe symptoms were recruited and genotyped in identical fashion and at the same time, it is less likely that genotyping error confounded obtained results. There were few non-Caucasian participants, and not unexpectedly significant findings remained the same when data analyses (both two and three genotype group comparisons) were limited to White/Caucasian youth. The study sample was small according to current standards, and this has implications for both Type 1 (false positives) and Type 2 (false negatives) error. For these and other reasons, our results are best interpreted as hypothesis generating and recommend continued study with larger samples and a greater range of relevant variables.

Some children were receiving psychotropic medication at the time their behavioral evaluations were being conducted. Although genotype groups did not differ in the percent receiving treatment, we nevertheless considered this variable as a potential covariate. In the case of hyperactivity and social deficits, medication status was not linearly related to the dependent variable. As for impulsivity, children receiving medication had significantly more severe symptoms, and when medication status was treated as a covariate, the effect of genotype group became nonsignificant ($p=0.077$) in the two group comparison. It would have been ideal to obtain all assessments off medication, however, withdrawal from treatment solely for the purpose of obtaining ratings raises a number of concerns. Equally

problematic is their exclusion, which could potentially bias the sample with regard to the very behaviors that were the object of study.

It is premature to offer specific hypotheses about 5-HTTLPR/ rs25531 genotypes, brain function, and child behavior. This having been said, research suggests that maternal, placental, and child serotonin may all interact with environmental variables to influence brain development (Bonin et al., 2011; Zhang et al., 2010). In the case of ASD, the replicated finding of seasonal fluctuations brain 5-HTT binding (Willeit & Praschak-Rieder, 2010) and autism births (Lee et al., 2008; Gadow & DeVincent, 2012), the effects of seasonal light cycles on serotonin signaling in the brain mediated by the circadian system (Ciarlegio et al., 2011), and possible involvement of circadian system regulatory genes in autism (Hu et al., 2009) all suggest multiple avenues of inquiry. Alternatively, our finding that the high expressing L_A/L_A genotype was associated with more severe ASD social deficits combined with research indicating (a) lower levels of brain 5-HTT binding in adults with ASD and correlation with social cognition deficits (Nakamura et al., 2010), (b) reduction in cortical serotonin 2A receptors in adults with Asperger syndrome and association with social communication deficits (Murphy et al., 2006), but (c) higher 5-HTT levels in non-ASD adults with the L_A/L_A genotype (Willeit & Praschak-Rieder, 2010) suggest possible disruption of neuronal homeostatic mechanisms (Ramocki & Zoghbi, 2008); nevertheless, divergent hypotheses based on results from neurodevelopmental, animal, and human cell line studies are also plausible.

5. Conclusion

Children with ASD who had at least one copy of the S or L_G allele had more severe symptoms of hyperactivity than children homozygous for the L_A allele. Conversely, children with the L_A/L_A genotype had more severe ASD social deficits than S or L_G allele carriers. These results suggest one potential model for co-morbidity within the ASD clinical phenotype. The magnitude of parent-teacher agreement about the severity of hyperactivity and social deficits varied as a function of child's genotype suggesting that these alleles *may* influence behavioral plasticity.

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Abbreviations

A	Adenine
ADHD	attention-deficit hyperactivity disorder
ANCOVA	analysis of covariance
ASD	autism spectrum disorder
CSI-4	Child Symptom Inventory-4
G	Guanine
L	Long allele
S	Short allele

SES	Socio-economic status
SSRI	Selective serotonin reuptake inhibitor
5-HTT	Serotonin transporter protein
VNTR	variable number tandem repeat
5-HTTLPR	Serotonin transporter-linked polymorphic region.

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Highlights

- 5-HTTLPR/rs25531 is associated with both ADHD and autism spectrum disorder.
- S+ or L_G+ genotypes had more severe hyperactivity than the L_A/L_A group.
- The L_A/L_A genotype had more severe ASD social deficits than the S/S group.
- Results suggest a potential model for co-morbidity within the ASD clinical phenotype.

Table 1

Mean (SD) Rating of ADHD Symptoms for Child 5-HTTLPR/rs25531 Genotypes Controlling for Severity of ASD

Variable	L _A /L _A M(SD)	L _A /L _G M(SD)	S/L _A M(SD)	S/L _G M(SD)	S/S M(SD)	F ratio ^a	p= ^a	η ² _a
Mothers' ratings	n=25	n=11	n=45	n=4	n=28			
Hyperactivity	6.5 (3.9)	9.5 (4.4)	9.2 (4.1)	7.0 (3.7)	9.2 (4.1)	11.62 (6.00)	.001 (.003)	.097 (.101)
Impulsivity	3.8 (3.6)	6.3 (2.8)	5.2 (3.0)	4.5 (2.6)	4.8 (2.6)	5.02 (3.10)	.027 (.049)	.044 (.055)
Inattention	14.5 (6.7)	15.1 (6.5)	17.2 (5.4)	17.8 (4.9)	15.5 (6.3)	3.57 (2.34)	.061 (.101)	.032 (.042)
Teachers' ratings	n=22	n=11	n=44	n=5	n=22			
Hyperactivity	6.1 (4.8)	7.3 (5.7)	6.9 (5.3)	5.3 (2.3)	7.6 (5.0)	3.2 (2.78)	.072 (.067)	.032 (.053)
Impulsivity	3.8 (3.2)	4.5 (4.5)	4.0 (3.4)	4.8 (3.7)	4.6 (3.9)	0.45 (0.71)	.502 (.492)	.005 (.014)
Inattention	16.1 (7.4)	17.2 (7.8)	15.2 (7.5)	12.0 (6.3)	14.9 (7.6)	0.00 (.113)	.983 (.893)	.000 (.002)

NOTE: ADHD=attention-deficit/hyperactivity disorder; ASD=autism spectrum disorder; L=long allele; S=short allele.

^aValues for comparison of the high expressing L_A/L_A genotype versus all other (low expressing) genotypes. Values in parentheses are for comparison of L_A/L_A, S/S, and heterozygous (L_A/L_G, S/L_A) genotypes.

Table 2

Mean (SD) Rating of ASD Symptoms for Child 5-HTTLPR/rs25531 Genotypes Controlling for Severity of ADHD

Variable	L _A /L _A M (SD)	L _A /L _G M (SD)	S/L _A M (SD)	S/L _G M (SD)	S/S M (SD)	F ratio ^a	p= ^a	ηp ^{2a}
Mothers' ratings	n=25	n=11	n=44	n=4	n=27			
Social deficits	7.6 (3.0)	6.0 (3.8)	6.5 (3.5)	9.3 (2.8)	6.0 (3.1)	9.49 (4.83)	.003 (.010)	.081 (.083)
Communication deficits	6.7 (3.4)	5.4 (3.4)	6.0 (3.6)	7.0 (3.4)	6.7 (4.0)	1.87 (1.82)	.174 (.168)	.017 (.033)
Perseverative behaviors	5.1 (3.3)	6.7 4.0	5.4 3.4	7.8 (3.3)	5.9 (3.2)	.073 (0.57)	.788 (.568)	.001 (.011)
Teachers' ratings	n=22	n=11	n=43	n=5	n=22			
Social deficits	8.0 (3.6)	6.9 (3.7)	7.1 (4.1)	6.0 (1.0)	6.0 (3.4)	2.95 (2.46)	.089 (.090)	.023 (.047)
Communication deficits	6.3 (4.2)	5.6 (4.2)	5.9 (3.5)	3.5 (3.4)	5.9 (3.5)	0.89 (0.79)	.766 (.459)	.001 (.016)
Perseverative behaviors	5.7 (3.4)	6.3 (3.5)	5.5 (3.3)	4.0 (2.8)	4.4 (3.3)	0.87 (2.48)	.355 (.089)	.009 (.048)

NOTE: ADHD=attention-deficit/hyperactivity disorder; ASD=autism spectrum disorder; L=long allele; S=short allele.

^aValues for comparison of the high expressing L_A/L_A genotype versus all other (low expressing) genotypes. Values in parentheses are for comparison of L_A/L_A, S/S, and heterozygous (L_A/L_G, S/L_A) genotypes.

Table 3

Correlation (Pearson) Between Parent and Teacher Ratings of ADHD and ASD Symptoms for Specific Child 5-HTTLPR/rs25531 Genotypes

	L _A /L _A (n=21)	Heterozygotes (n=53) ^a	S/S (n=25)
ADHD Symptoms			
Inattention	0.18	0.32 *	-0.06
Hyperactivity	0.12	0.63 **	0.54 **
Impulsivity	0.52 *	0.30 *	0.36
ASD Symptoms			
Social deficits	0.43	0.29	0.01
Communication deficits	0.65 **	0.50 **	0.55 **
Perseverative behaviors	0.22	0.32 *	0.06

NOTE: ADHD=attention-deficit/hyperactivity disorder; ASD=autism spectrum disorder; L=long allele; S=short allele.

^aL_A/L_G and S_A/L_A, genotypes.

*
p<0.05

**
p<0.01