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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 ($p = 0.001$; $\eta p^2 = 0.097$) and impulsivity ($p = 0.027$; $\eta p^2 = 0.044$) but not inattention ($p = 0.061$; $\eta p^2 = 0.032$), controlling for ASD severity, than children homozygous for the L_A allele. Conversely, mothers' ratings indicated that children with L_A/L_A genotype had more severe ASD social deficits than S or L_G allele carriers ($p = 0.003$; $\eta p^2 = 0.081$), 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.

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 and 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

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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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 and Praschak-Rieder, 2010).

Dysregulation of serotonergic processes has long been implicated in the pathogenesis of autism spectrum disorders (ASDs) (Lam et al., 2006), based initially on reports of platelet hyperserotonemia in a subset of individuals with ASD (Abramson et al., 1989; Schain and Freedman, 1961) and more recently on the role of serotonin in brain development (Whitaker-Azmitia, 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; Kistner-Griffin et al., 2011; Klauck et al., 1997) 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 and 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., 2008b; 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 to 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 non-ASD 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 dysregualtion 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., 2008c), (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 et al. (2006) and was carried out in a total volume of 20 μl with forward (5′-TCCTCCGCTTTGGCGCCTCTTCC-3′) and reverse primers for the 5-HTTLPR (5′-TGGGGGTTGCAGGGGAGATCCTG-3′). Each amplification contained 20 ng of genomic DNA, 1× multiplex master mix (Qiagen, Valencia, CA) and 0.20 μM of the 5-HTTLPR primers. Reaction conditions began with an initial denaturation at 95 °C for 15 min followed by 35 cycles of 94 °C for 30 s, 68.1 °C for 90 s, and 72 °C for 60 s, with a final extension step of 10 min at 72 °C. Next, 7 μl of PCR product were digested by HpaII (5U; New England Biolabs, Ipswich, MA) in a 20 μl reaction containing 1× NEBuffer 1 and 1× BSA at 37 °C for 3 h. A second set of mock digested samples were run in a parallel 20 μl 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.