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The impact of serotonin transporter genotype on default network connectivity in children and adolescents with autism spectrum disorders

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ABSTRACT

Compared to healthy controls, individuals with autism spectrum disorders (ASD) have weaker posterior–anterior connectivity that strengthens less with age within the default network, a set of brain structures connected in the absence of a task and likely involved in social function. The serotonin transporter-linked polymorphic region (5-HTTLPR) genotypes that result in lowered serotonin transporter expression are associated with social impairment in ASD. Additionally, in healthy controls, low expressing 5-HTTLPR genotypes are associated with weaker default network connectivity. However, in ASD, the effect of 5-HTTLPR on the default network is unknown. We hypothesized that 5-HTTLPR's influence on posterior–anterior default network connectivity strength as well as on age-related changes in connectivity differs in the ASD group versus controls. Youth with ASD and healthy controls, ages 8–19, underwent a resting fMRI acquisition. Connectivity was calculated by correlating the posterior hub of the default network with all voxels. Triallelic genotype was assessed via PCR and Sanger sequencing. A genotype-by-diagnosis interaction significantly predicted posterior–anterior connectivity, such that low expressing genotypes (S/S, S/L_G, L_G/L_G) were associated with stronger connectivity than high expressing genotypes (L_A/L_A, S/L_A, L_A/L_G) in the ASD group, but the converse was true for controls. Also, youth with ASD and low expressing genotypes had greater age-related increases in connectivity values compared to those with high expressing genotypes and controls in either genotype group. Our findings suggest that the cascade of events from genetic variation to brain function differs in ASD. Also, low expressing genotypes may represent a subtype within ASD.

1. Introduction

Autism spectrum disorders (ASD) are neurodevelopmental conditions characterized by social and communicative impairments and rigid repetitive behaviors. The prevalence of ASD has sharply increased in recent years and is currently 1 in 88 (CDC, 2012). Deciphering the complex etiology of ASD is thus a priority, and progress will likely involve examining the condition using multiple methodologies, including neuroimaging and molecular genetics.

As alterations in brain connectivity have been repeatedly implicated in ASD (Hughes, 2007), attention has been focused on identifying perturbations in fundamental, large-scale networks, such as the default network, that may contribute to ASD symptoms. In healthy adults, the default network (including the posterior cingulate, angular gyri, superior frontal gyri/Brodmann's area (BA) 10, and anterior cingulate/BA 10) is active and functionally connected in the absence of a demanding task (Raichle and Snyder, 2007). Functional connectivity reflects structural connectivity of the default network in healthy adults (Greicius et al., 2009). The default network contains posterior and anterior hubs (Buckner et al., 2008) that typically display strong long-range connectivity but are distinct from one another (Horovitz et al., 2009).

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The primary purpose of the default network is a subject of debate. The default network may relate to basic central nervous system functions such as maintaining the balance of excitatory and inhibitory inputs or interpreting information from the environment (Raichle and Snyder, 2007). Alternatively, the primary purpose of the default network may be related to social cognition, including self-referential processes (Gusnard et al., 2001) and mentally projecting oneself into hypothetical situations (Buckner and Carroll, 2007).

Studies on adults with ASD (Cherkassky et al., 2006; Kennedy and Courchesne, 2008; Monk et al., 2009) as well as adolescents (Weng et al., 2010; Anderson et al., 2011; Wiggins et al., 2011) found weaker connectivity between the posterior and anterior default network compared to controls. Moreover, the weaker the posterior–anterior default network connectivity, the worse the social impairment in individuals with ASD (Monk et al., 2009; Weng et al., 2010).

A few studies have investigated the development of the default network. For healthy individuals, posterior–anterior connectivity is weaker during childhood and adolescence than during adulthood both functionally (Fair et al., 2008; Stevens et al., 2009; Wiggins et al., 2011) and structurally (Supekar et al., 2010). These studies indicate that connectivity of this network increases in strength over childhood and adolescence in healthy individuals. In contrast, youth with ASD have attenuated increases in posterior–anterior connectivity with age compared to controls (Wiggins et al., 2011).

Identifying the genetic factors that influence the default network in ASD is important to further elucidate the complex etiology of ASD. The serotonin transporter-linked polymorphic region variant (5-HTTLPR; Lesch et al., 1996) in the promoter region of the serotonin transporter gene (*SLC6A4*) is relevant to the default network in ASD. The S and L_G alleles of 5-HTTLPR are associated with decreased serotonin transporter expression relative to the L_A allele (A to G SNP in the L allele, rs25531; Hu et al., 2006). The low expressing alleles of 5-HTTLPR have been associated with worse social symptoms in ASD (Tordjman et al., 2001; Brune et al., 2006). In healthy adolescents, 5-HTTLPR is known to influence the default network: those with low expressing genotypes exhibit weaker posterior–anterior connectivity than adolescents with high expressing genotypes (Wiggins et al., 2012). Moreover, in healthy children and adolescents, 5-HTTLPR also impacts the development of default network connectivity such that youth with high expressing genotypes have greater age-related increases in posterior–anterior connectivity than those with low expressing genotypes (Wiggins et al., 2012). A previous study found that serotonin transporter binding in the anterior default network is decreased in individuals with autism (Nakamura et al., 2010). However, no study has yet examined how 5-HTTLPR affects default network connectivity or its development in individuals with ASD.

The present study addresses these two gaps in the literature on ASD: the role of 5-HTTLPR in default network connectivity and in the development of default network connectivity. This is accomplished by directly examining the influence of 5-HTTLPR variants on posterior–anterior default network connectivity as well on as age-related changes in connectivity in a sample of children and adolescents with ASD and controls. We hypothesized that the relationship between 5-HTTLPR genotype and posterior–anterior default network connectivity strength differs in the ASD group versus controls. Additionally, we hypothesized that the relationship between 5-HTTLPR and changes in connectivity across childhood and adolescence differs in the ASD group compared to controls.

2. Material and methods

2.1. Participants

Fifty-four children and adolescents with ASD and 66 healthy controls, aged 8.3 to 19.6 years, were included in this study (see Table 1 for participant characteristics). From a total of 105 participants with ASD and 82 controls recruited, 51 participants with ASD and 16 controls were excluded because of head movement exceeding 2.5 mm translation or 2.5° rotation, declining to complete the MRI scan due to discomfort, failure to return a saliva sample for genotyping, or technical problems with the MRI.

Controls were recruited through flyers posted at community organizations in the Ann Arbor, Michigan area. The University of Michigan Autism and Communication Disorders Center (UMACC) referred potential participants to our study and diagnosed participants with an ASD (autistic disorder, Asperger's syndrome, or pervasive developmental disorder — not otherwise specified) using the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2000), the Autism Diagnostic Interview—Revised (ADI-R; Lord et al., 1994), and clinical consensus (Lord et al., 2006). The University of Michigan Institutional Review Board approved the procedures. Participants over age 18 gave written informed consent; participants under age 18 gave written assent and their parents gave written informed consent. Cognitive functioning was evaluated for controls with the Peabody Picture Vocabulary Test (PPVT; Dunn and Dunn, 1997) and the Ravens Progressive Matrices (Raven, 1960); participants with ASD were given these measures or the Differential Ability Scales II — School Age (Elliott, 2005), the Stanford–Binet Intelligence Scales (Roid, 2003), the Wechsler Intelligence Scale for Children IV (Wechsler, 2003), or the Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999). Participants with orthodontic braces, medical conditions contraindicated for MRI, or history of seizures or neurological disorders were excluded. Control participants were screened for psychological disorders with the Child Behavior Checklist (Achenbach and Edelbrock, 1981), Social Responsiveness Scale (Constantino et al., 2003), Social Communication Questionnaire (Rutter et al., 2003), Obsessive Compulsive Inventory — Revised (Foa et al., 2010), Child Depression Inventory (Kovacs, 1992), and Multidimensional Anxiety Scale for Children (March et al., 1997). All control participants scored below clinical cutoffs for affected status. Individuals with the low and high expressing genotypes did not differ in any of the symptom measures or cognitive functioning in either the ASD or control group (Inline Supplementary Table S1). Prior studies utilized portions of this dataset (Weng et al., 2010, 2011; Wiggins et al., 2011, 2012).

Inline Supplementary Table S1 can be found online at http://dx.doi.org/10.1016/j.nicl.2012.10.008.

2.2. Genetic analyses

5-HTTLPR genotype was ascertained using previously published procedures (Wiggins et al., 2012). Participants donated saliva samples using the Oragene DNA kit (DNA Genotek; Kanata, Canada). PCR and agarose genotyping were used to determine S versus L allele. Sanger sequencing was utilized to determine the A to G single nucleotide polymorphism (SNP) in the L allele (rs25531; Hu et al., 2006) and to confirm PCR genotyping.

In autism, individuals with the low expressing genotype (S/S) have been shown to differ in neurochemical metabolism compared to L allele carriers in the anterior portion of the default network (Endo et al., 2010). As such, participants were put into two genotype groups: low expressing genotypes (S/S, S/L_G, L_G/L_G) versus medium and high expressing genotypes (L_A/L_A, S/L_A, L_A/L_G, hereafter referred to as "high expressing" genotypes). (The L_G allele is equivalent to the S allele in serotonin transporter expression level (Hu et al., 2006), so for the purposes of the analyses, the two alleles were grouped together.) This genotype grouping is consistent with a number of non-ASD studies that found recessive effects of the low expressing 5-HTTLPR alleles, often in adolescent populations (e.g., Cicchetti et al., 2007; Surguladze et al., 2008; Benjet et al., 2010). Nevertheless, we conducted additional analyses to examine whether our results still stood when the alleles were grouped differently (see Section 3.1.4 Alternative genotype groupings).