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# Genetic, Behavioral, and Physiological Predictors of Phenotypic Variability in Typically Developing and High Functioning Children with Autism

Kim E. Ono

*University of Miami*, [k.ono@umiami.edu](mailto:k.ono@umiami.edu)

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UNIVERSITY OF MIAMI

GENETIC, BEHAVIORAL, AND PHYSIOLOGICAL PREDICTORS OF  
PHENOTYPIC VARIABILITY IN TYPICALLY DEVELOPING AND HIGH  
FUNCTIONING CHILDREN WITH AUTISM

By

Kim E. Ono

A THESIS

Submitted to the Faculty  
of the University of Miami  
in partial fulfillment of the requirements for  
the degree of Master of Science

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Kim E. Ono

Approved:

---

Heather A. Henderson, Ph.D.  
Associate Professor of Psychology

---

Terri A. Scandura, Ph.D.  
Dean of the Graduate School

---

Philip M. McCabe, Ph.D.  
Professor of Psychology

---

Michael L. Cuccaro, Ph.D.  
Associate Professor, Dr. John T.  
Macdonald Foundation Department  
of Human Genetics

ONO, KIM E.  
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of Phenotypic Variability in Typically Developing  
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There is extensive research focused on identifying predictors of autism, including biomarkers such as genes and neurophysiology. Because of inconsistent data, I explored these biomarkers as predictors of variability in behavioral outcomes (i.e., internalizing and externalizing symptoms), rather than indicators of the disorder per se. In a sample of children (ages 8-16) diagnosed with High Functioning Autism (HFA) and an age- and IQ- matched typically developing comparison group, individual differences in behavioral outcomes were assessed in relation to common genetic polymorphisms, 5-HTTLPR and DRD4, and neurophysiological (ERN) and behavioral (rate of self-correction) measures of response monitoring. Although the diagnostic groups did not differ on allele frequency for 5-HTTLPR, carriers of the L variant displayed attenuated ERN amplitudes at frontal-central sites, lower rates of self-correction following errors, and higher levels of parent-reported Somatization and Hyperactivity. With respect to DRD4, an overrepresentation of the 7-repeat allele was found in the HFA sample. Regardless of diagnostic group, 7-repeat allele carriers were rated as having more attention problems. These results suggest that genetics and neural correlates of response monitoring may

explain interindividual variations in social emotional functioning of both HFA and typically developing children alike. However, contrary to hypothesis, response monitoring did not mediate the association between 5-HTTLPR or DRD4 and outcome measures. Future directions of this research may look at how genes and measures of response monitoring affect etiology, course, and treatment of autism and other related disorders.

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## Chapter 1: Introduction

Autism is a severe condition in a group of neurodevelopmental disorders known as Autism Spectrum Disorder (ASD). Recent prevalence estimates are as high as 1 out of every 110 children (0.9%) (Autism and Developmental Disabilities Monitoring, 2009). Autism is four times more likely to be diagnosed in males than females (in the US, 1 in 70 boys are diagnosed with autism) and diagnoses appear across all racial and ethnic groups (Autism and Developmental Disabilities Monitoring, 2009).

ASD is commonly defined by a triad of conditions including problems with social communication, inflexible language and behavior, and repetitive, stereotyped sensory-motor movements (DSM-IV; American Psychiatric Association, 2000). The term “Autism Spectrum” in ASD describes the continuum on which this disorder and related behaviors present themselves. With respect to individuals with High Functioning Autism (HFA), clinical presentation appears to be particularly variable (Prior et al., 1998). Individuals with HFA often present with comorbid psychopathology most notably Attention Deficit Hyperactivity Disorder (ADHD), Anxiety, and Depression, as well as a variety of behavior problems including sleep abnormalities, self-injurious behavior, and aggression (Dominick, Davis, Lainhart, Tager-Flusberg, & Folstein, 2007). The high prevalence of co-occurring disorders and behavior problems with HFA makes differential diagnosis a challenge. Additionally, heterogeneous presentation patterns complicate studies related to etiology. One approach to better understanding individual differences is by examining non-syndrome specific biomarkers that may influence social and emotional presentation (Mundy et al., 2007). In the current study, I examined genetic markers (5-HTTLPR and DRD4) and physiological and behavioral indices of response

monitoring (error-related negativity and rate of self-correction) in relation to individual differences in symptom severity and social-emotional comorbidities in a sample of higher functioning children with autism (HFA) and an age- and IQ- matched typically developing sample.

### *Part I: Biological Pathways to Phenotypic Expression*

#### *Heritability of Autism Spectrum Disorder:*

Twin studies provide strong evidence for genetic risk factors in autism. Reports suggest a concordance rate for monozygotic (MZ) twins of 69 to 95% (Bailey et al., 1995; Folstein & Rutter, 1988; Ritvo, Freeman, Mason-Brothers, Mo, & Ritvo, 1985) while dizygotic (DZ) concordance rates range from 0-24%. It is also noted that 4.4 to 20.4% of siblings who do not meet criteria for ASD show subclinical traits related to autism, including difficulties with social interaction and isolation (Bolton et al., 1994; Piven et al., 1990). While the evidence for a genetic predisposition towards an autism diagnosis and the broader phenotype are strong, several genome-wide linkage studies have failed to reveal consistent associations between candidate genes and a diagnosis (see Table 1). Thus it is important to look at genetics, not only as risk factors for diagnosis, but also as predictors of variability in symptom profiles and severity (Devlin et al., 2005). In the current study, two polymorphisms (5-HTTLPR and DRD4) were examined in relation to measures of social emotional functioning (internalizing, externalizing, and attention problems) and symptom severity.

*Serotonin and Dopamine:*

Research has implicated two neurotransmitters in the pathophysiology of the broader autism phenotype, (1) the monoamine neuromodulator, serotonin (5-hydroxytryptamine, or 5-HT) and (2) the catecholamine neurotransmitter, dopamine (DA). 5-HT is vital to early brain development, promoting the binding of neurons and modulating sensory input and arousal in mood, sleep, aggression, impulsivity, and affiliation (Lucki, 1998). Serotonergic neurons innervate the amygdala and corticolimbic circuitry, which are involved in the expression of emotions and social behavior (Azmitia & Gannon, 1986; Brown & Hariri, 2006; Hariri et al., 2005; Hariri, Drabant, & Weinberger, 2006). DA is produced mainly in the substantia nigra, and the ventral tegmental area and is thought to affect a wide range of behaviors broadly associated with externalizing-type behavior problems including reward processing mechanisms, motor function, cognition, neuroendocrine regulation, and attention (Calne, Chase & Barbeau, 1975; Costa & Gessa, 1977; Roberts, Woodruff, & Iversen, 1978).

*Serotonin in Relation to ASD:*

Several lines of evidence suggest that 5-HT may be related to variations in behaviors characteristic of autism. Studies have documented elevated levels of platelet serotonin, also known as hyperserotonemia, in children with autism (Rolf, Haarmann, Grotemeyer, & Kehrer, 1993). Furthermore, it has been suggested that hyperactivity and compulsive and stereotyped behaviors in autism can be alleviated with selective serotonin reuptake inhibitors (SSRIs; Hollander, Phillips, & Yeh, 2003). In addition, Risperidone, a drug that blocks serotonin and dopamine post-synaptically, has demonstrated positive

results in alleviating aggression and self-injury in individuals with autism (Masi, Cosenza, Mucci, & Brovedani, 2003; McCracken et al., 2002). SSRIs and Risperidone have both been proven effective to treat specific symptoms in autism by inhibiting serotonin from leaving the system. This may seem contradictory to documented increased levels of serotonin in ASD. However, the effectiveness of the drugs may be specific to a subset of ASD individuals who display elevations in externalizing behaviors, such as hyperactivity, aggression, and self-injurious behavior (Bolton, Pickles, Murphy, & Rutter, 1998; Hollander et al., 2003). This evidence further supports the hypothesis that neurotransmitters, and the genes related to their transcription may have interindividual effects on phenotypic expression, as opposed to autism per se.

Biologically, serotonin (5-HT) is removed from the synaptic cleft through the activity of the serotonin transporter protein (5-HTT). The serotonin transporter promoter region (5-HTTLPR) regulates the transcription of the serotonin transporter gene and influences the amount of transporters produced (Mizuno et al., 2006). A common polymorphism (SLC6A4) located on the chromosome 17q11.1-q12 was identified in the human serotonin transporter gene. SLC6A4 is located in the promoter region and contains a polymorphism with “short” and “long” repeats, resulting in three allelic variants: a homozygous short (S/S) variant, a heterozygous short/long (S/L) variant, and a homozygous long (L/L) variant. Possession of one or two copies of the S allele accounts for nearly a 50% reduction in 5-HT availability, presumably resulting in a higher concentration of serotonin (Collier et al., 1996; Heils et al., 1996; Lesch et al., 1996). Because the S/S and S/L variant similarly affect 5-HT in the system, many studies combine these two variants into a single group of S variant and compare those to

homozygous L carriers (L variant). Within a sample of healthy individuals of northern European decent, the homozygous (S/S) and heterozygous (S/L) short form occurred with a frequency of 40% and the homozygous (L/L) long form occurred with a frequency of approximately 60%, but cultural variability in rates of the polymorphisms are noted (Mizuno et al., 2006; Tordjman et al., 2001; Hu et al., 2006; Roy et al., 2007; Hu et al., 2007). However, to date, published data are not available concerning the frequency distributions of alleles in Hispanic subjects.

In addition to the common allelic description of 5-HTTLPR (i.e., S variant versus L variant), recent studies have identified another polymorphism that may influence the expression of 5-HT. A single polymorphism within the L allele leads to an A or G substitution. The G variant of the L allele is identified as functioning similarly to the S variant (Hu et al., 2005), such that S, S/L and L<sub>G</sub> alleles are associated with less serotonin production relative to the L<sub>A</sub> allele (Hu et al., 2005; Praschak-Rieder et al., 2007; Reimold et al., 2007). As such, the traditional S and L variant, and the more recent L<sub>G</sub> and L<sub>A</sub> classification approaches were used in the current study.

#### *5-HTTLPR and Phenotypic Expression:*

Outside of the autism literature, 5-HTTLPR allele transmission has been associated with social and emotional responses characteristic of the broader phenotype, including social withdrawal, aggression, anxiety, depression, impulsivity, and harm avoidance (Bolton, Pickles, Murphy, & Rutter, 1998; Hollander et al., 2003). Specifically, externalizing-type behaviors including ADHD, impulsivity, and aggression are associated with the L variant, while more internalizing-type symptoms such as



anxiety, neuroticism, harm avoidance, and depression are more common among children in the S variant group (Bolton, Pickles, Murphy, & Rutter, 1998; Hariri et al., 2005; McDougle, Epperson, Price, & Gelernter, 1998; Nobile et al., 2004; Nobile et al., 2007; Lesch et al., 1996). Both externalizing and internalizing behaviors are comorbid with ASD and it is important to take into account these traits when understanding clinical heterogeneity (Hariri et al., 2005; Lesch et al., 1996; McDougle, Epperson, Price, & Gelernter, 1998; Nobile et al., 2004; Nobile et al., 2007).

Within the autism literature specifically, studies have found 5-HTTLPR allele variability to be associated with phenotypic expression. Tordjman et al. (2001) found no group differences in transmission of HTT promoter alleles in 71 probands with cognitively impaired autism (mean verbal IQ ranged from 45-57, mean of 45.5, and standard deviation of 2.2 on the Weschler intelligence scales) relative to their unaffected siblings. Among the diagnosed siblings, however, severity of impairments in the social and communication domains as measured by a combination of the Autism Diagnostic Interview-Revised (ADI-R) and the Pre-Linguistic Autism Diagnostic Observation Schedule (PL-ADOS) was associated with allelic groups. Specifically, probands of the S variant had greater social and communication impairment, while probands of the L variant were more mildly or moderately impaired. This relation between 5-HTTLPR alleles and severity of autistic symptoms suggests that genetic variation may be an important predictor of phenotypic variability *within* a diagnostic group rather than diagnostic group per se.

Further evidence for a genetic relation to phenotypic expression in autism is provided by the results of a study by Brune et al. (2006), in which the relation between 5-

HTTLPR and autism symptom presentation was examined. Seventy-three children diagnosed with autism, between the ages of 3 to 19, participated in the study. Behavioral phenotype was measured using the ADI-R. Results suggested a greater “failure to use nonverbal communication to regulate social interaction” (abbreviated B1 on the ADI-R) in the S variant group, and increased severity on “stereotyped and repetitive mannerisms” (abbreviated D3 on the ADI-R) in the L variant group among these unaffected siblings of autistic individuals (Brune et al., 2006). Collectively, these studies provide evidence for 5-HTTLPR as a biomarker for phenotypic expression in both typically developing and ASD children.

#### *Dopamine in Relation to ASD:*

Dopamine has also received notoriety in ASD literature due to its relation with phenotypic expression. Dopamine blockers (i.e., antipsychotics) have been used to treat hyperactivity, stereotypies, aggression, and self-injury in autism (Anderson & Hoshino, 1997; Young, Kavanagh, Anderson, Shaywitz, & Cohen, 1982). In addition, dopamine metabolite homovanillic acid was reported to be elevated in the cerebrospinal fluid in individuals with ASD (Gillberg & Svennerholm, 1987), however, these findings have not been replicated consistently (Narayan et al., 1993). Previous genetic studies have documented a higher prevalence of A1 allele of the dopamine D2 receptor in individuals diagnosed with ASD (Coming et al., 1991). Additionally, the dopamine D1 receptor gene was reported to be associated with higher severity of core ASD symptoms in a sample of at risk, male siblings of children diagnosed with autism (Hettinger, 2008). Furthermore, the 9- and 10- repeat alleles of the dopamine transporter (DAT1) have been associated

with hyperactivity, impulsivity, social anxiety, and tic symptoms in children with ASD (Gadow et al., 2008). Neurologically, PET studies reveal a lower levels of medial prefrontal dopaminergic activity in children with autism (Ernst et al., 1998) and increased dopamine D2 receptor binding in the caudate and putamen (Fernall et al., 1997). Collectively, these findings suggest that the dopaminergic system may play a critical role in the symptom expression in ASD.

To study the effects of dopamine, in the current study I focused on the highly polymorphic dopamine D4 receptor (DRD4) gene, due to its documented relation with comorbid symptoms of hyperactivity and conduct problems within autism (Battaglia et al. 1996; Gadow et al., 2010). Hyperactivity and inattention are such common comorbid symptoms in autism that the Diagnostic Statistical Manual for Mental Disorders Fourth Edition, Text Revisions (DSM-IV-TR; APA, 2000) and the International Classification of Disease (ICD-10: WHO, 1993) place exclusionary criterion on double diagnosing ASD with ADHD. DRD4 is a G protein-coupled receptor that produces inhibitory effects on the adenylyl cyclase and adenosine triphosphate (ATP) production. The DRD4 gene is located on chromosome 11 and contains a common polymorphism 48 bp variable number of tandem repeats (VNTR) within exon III. This polymorphism varies between 2 and 11 copies, with the 4- and 7-repeat allele (4r and 7r) being the most frequent (Lichter et al. 1993; Vallone et al. 2000). The 7-repeat variant is purported to lessen D4 receptor responsiveness and thus reduce dopamine binding efficiency (Asghari et al., 1995; Cravchik and Goldman, 2000; Tol et al., 1992; Kebir et al., 2009; Smith, 2010).

*DRD4 and Phenotypic Expression:*

In non-ASD samples, the long allele (6-8 repeats) of the DRD4 gene has been associated with novelty seeking in young adults (Ebstein, Nemanov, Klotz, Gritsenko, & Belmaker, 1997; Noble et al., 1998; Ono et al., 1997, Benjamin et al. 1999). There is a highly replicated association between exon III VNTR of DRD4 and impulsivity, sensation seeking, and aggression (Battaglia et al. 1996), Oppositional Defiant Disorder (Kirley et al., 2004), and antisocial behaviors (DiLalla et al., 2009; Fresan et al., 2007; Holmes et al., 2002; Schmidt et al., 2002, 2007). Similarly, ADHD has been documented to be associated with the 7-repeat variant of DRD4 in numerous reports (Faraone et al. 2005; Faraone et al. 2001; Li et al. 2006; Maher et al. 2002), although there are some failures to replicate this finding in the literature (Grady et al., 2005; Yirmiya et al., 2001).

Impulsivity, aggression, and conduct problems are associated behavior problems found with autism. Thus, it is not surprising to find relations between DRD4 7-repeat allele carriers and externalizing problems. One study conducted by Gadow et al. (2010a) investigated DRD4 allele variants in 59 mother-child and 53 father-child dyads of children diagnosed with ASD. Results revealed increased severity of tics, separation anxiety, obsessive-compulsive behavior, and oppositional behavior in matching parent-child 7-repeat genotype carriers. In addition, Gadow et al. (2010b) found that adolescents with ASD who carried at least one copy of the 7-repeat allele were rated by their mothers as more oppositional defiant and as displaying more obsessive-compulsive behaviors relative to non-carriers. Similar patterns of association between DRD4 7-repeat and externalizing symptomatology emerge between typically developing and ASD children,

furthering the evidence for a genetic component in the expression of social emotional functioning.

In summary, it is vital to address clinical heterogeneity when studying genetic associations with autism. Therefore a focus of this study is to examine not only standard measures of autism symptoms but also domains of social-emotional functioning that are highly comorbid with ASD (internalizing behaviors and externalizing behaviors), to better understand how 5-HTTLPR and DRD4 allele variations may relate to phenotypic differences. In non-ASD populations, both 5-HTTLPR and DRD4 have demonstrated effects on social emotional functioning and may play a key role in understanding individual differences in a typically developing sample. Extending this research to children with autism may provide a means for identifying genetically differentiated subgroups within a HFA sample, which will have a significant impact on our understanding of the etiology, course, and treatment of autism.

*Part II: Measures of Response Monitoring:*

Understanding the relation between allelic variability and phenotypic expression in ASD and a typically developing sample may be important to form a general picture of individual differences, but it is the underlying mechanisms that may provide a more accurate depiction of the association and provide clues to target intervention. Specifically, I am proposing a model in which measures of response monitoring may mediate the associations between allele variations (within 5-HTTLPR and DRD4) and variations in symptom presentation and social-emotional functioning.

Theoretical models and empirical findings relate cognitive executive functions such as self-monitoring and self-awareness to autism (Dawson & McKissick, 1984; Frith & Frith, 1999; Russell, 1997). Self-monitoring is the ability to continually check and monitor one's own progress towards a specified goal. Impairments in response monitoring may underlie some of the social impairments in autism (Henderson et al., 2006; Mundy, 2003; Santesso et al., 2011). This hypothesis is based on research indicating that children with autism have difficulty recognizing and correcting errors (Russell & Jarrod, 1998, Ono et al., 2009). In addition, functional imaging studies have suggested that patterns of activation in the dorsal medial-frontal cortex (DMFC) and anterior cingulate (AC) regions, which are implicated in response monitoring, are associated with standardized measures of social symptoms in children with autism (Haznedar et al., 2000; Ohnishi et al., 2000). Variations in functioning of the DMFC, amygdala, and AC may contribute to variability in social-emotional functioning in children with autism (Dawson et al., 2002; Mundy, 2003). Specifically, core features of autism, such as joint attention, responses to emotional displays of others, face recognition, and social orienting have all been related to abnormalities in these regions. Increased amygdala size is associated with impairments in joint attention in children with autism (Howard et al., 2000). Positron emission tomography (PET) studies have found reduced dopaminergic activity in the DMFC in individuals with autism (Ernst, Zametkin, Matochik, Pascualvaca, & Cohen, 1997). These literatures corroborate the hypothesis for a neurological pathway by which individual differences in brain functioning may potentially bias symptom presentation in autism.

For this study, I will examine an event-related potential (ERP) index of response monitoring, the error-related negativity (ERN). ERPs are time-locked averages of EEG activity derived from a continuous recording, that measure how the brain responds following the presentation of specific stimuli or following the performance of a specific response. When people make an error during a reaction time task, there is a negative deflection in the EEG within 100 ms of the response (referred to as the error-related negativity or the ERN), which is followed by a positive deflection within the next 100 ms (referred to as the error positivity or the Pe) (e.g., Luu et al., 2000; Buch et al., 2000; van Veen & Carter, 2002). Neural generators of the ERN and Pe have been localized to the DMFC and the dorsal anterior cingulate (dACC). Recent theory has suggested that ERN reflects reward-related medencephalic dopamine activity, and that specific neurotransmitters (i.e., dopamine and serotonin) play a role in generating the ERN signal (Frank et al., 2005; Holroyd and Coles, 2002; Taylor, Stern, & Gehring, 2007). The amplitude of the ERN is associated with behavioral indices of self-monitoring, such as error corrections and post-error slowing, in typically developing individuals (Buch et al., 2000; Holroyd & Coles, 2002; Stuphorn et al., 2000; van Veen & Carter, 2002). That is, larger ERN amplitudes are related to more strategic and cautious responding immediately following an error.

In addition, Henderson et al. (2006) reported that individual differences in the amplitude of the ERN in a sample of higher functioning children with autism were related to variations in social-emotional functioning. Twenty-four HFA and 17 age- and IQ-matched comparison children completed a modified Flanker task. Results suggested an interaction between Diagnostic group and Verbal IQ on ERN amplitude, in which the

more verbally capable HFA children displayed significantly larger amplitude ERN responses than the comparison children. Within the HFA sample, enhanced ERN amplitudes were associated with fewer symptoms of social interaction impairments, but higher self-reported social stress (Henderson et al., 2006). Based on literature documenting associations between ERP measures of response monitoring and phenotypic expression, it is hypothesized that variation in social-emotional functioning and symptom severity will be associated with individual differences in response monitoring in a HFA and typically developing sample alike (Boksem, Tops, Kostermans, & De Cremer, 2008; Boksem, Tops, Wester, Meijman, & Lorist, 2006; Henderson et al., 2006).

*Genetic Association with Response Monitoring:*

Given the critical role of neurotransmitters, including serotonin and dopamine, in the development and functioning of cortical regions underlying response monitoring, a primary goal of the current study was to examine whether genetic variability in 5-HTTLPR and DRD4 allele frequencies were related to behavioral and physiological indices of response monitoring. Serotonergic and dopaminergic neurons densely innervate the amygdala and AC regions (areas associated with socio-emotional development in autism), thus implicating a unique sensitivity to imbalances in serotonergic and dopaminergic transmission (Azmitia & Gannon, 1986; Brown & Hariri, 2006; Hariri et al., 2005; Hariri, Drabant, & Weinberger, 2006).

Two studies have demonstrated that carriers of the 5-HTTLPR S allele had larger ERN responses than individuals with homozygous L alleles (Althaus et al., 2009; Fallgatter et al., 2004). However, discrepant results were recently reported by Olvet,



Hatchwell, and Hajcak (2010), whom documented larger ERN amplitude for individuals carrying the homozygous L allele, using an Erikson Flanker task. These contradictory findings were presumed to be the outcome of the small sample size across all three studies. Additionally, Olvet, Hatchwell, and Hajcak (2010) emphasized the importance of accounting for the G variant of the L allele ( $L_G$ ), which both Althaus et al (2009) and Fallgatter et al. (2004) did not. Olvet, Hatchwell, and Hajcak (2010) reanalyzed their results, re-categorizing individuals carrying at least one  $L_G$  allele in the S variant group. With the new regrouping, results indicated null effects of allelic variation on ERN amplitude. These findings highlight the importance of identifying the A/G SNP when analyzing 5-HTTLPR.

Additionally, DRD4 is associated with response monitoring, both neurophysiologically, as well as behaviorally. Behavioral differences in response monitoring have been reported to be associated with DRD4 in a sample of 20 healthy young adults (mean age = 21 years) (Kramer et al., 2009). 7-repeat carriers showed a tendency for more accurate responding on a Go/Nogo task compared to 4-repeat carriers. In addition, 7-repeat carriers presented with an increased Nogo related theta band ERP response. Theta band over the frontocentral areas have been shown to increase during cognitively demanding tasks, suggesting that 7-repeat carriers exerted more effort than 4-repeat carriers (Gevins, Smith, & McEvoy, 1997; Hanslmayr et al., 2008; Onton, Delorme, & Makelg, 2005). However, Birkas et al. (2006) did not find associations between the 7-repeat allele and early ERP responses to sound stimuli in a sample of 57 typically developing 6-year old children. Relatively small sample sizes in both studies may have confounded results.

Nonetheless, given the above-mentioned findings of associations between neurophysiological and behavioral measures of response monitoring, allelic variability, and phenotypic expression, we are proposing a mediation model to explain the relations. Specifically, it is hypothesized that the associations between allelic variability (5-HTTLPR: S and L variants; DRD4: 7-repeat, non 7-repeat) and individual differences in symptom severity, internalizing, and externalizing behavior problems, may be partially mediated by behavioral and physiological measures of response monitoring for children in both the HFA and comparison samples.

*Specific Aims:*

*Aim 1. Diagnostic Group Differences in Allele Frequencies*

To examine diagnostic group differences in allele frequencies in 5-HTTLPR and DRD4. Based on a mixed literature, it is hypothesized that there will be no significant association between diagnostic group and allele frequencies for either polymorphism.

*Aim 2: Diagnostic Group and Genetic Differences in Measures of Response Monitoring*

*Aim 2a.* To examine diagnostic group differences in behavioral (i.e., rate of self-correction) and physiological (i.e., ERN) indicators of response monitoring. It is hypothesized that ERN amplitude will not vary by diagnostic group.

*Aim 2b.* To examine the associations between allelic variations (in 5-HTTLPR and DRD4) and neurophysiological and behavioral indices of response monitoring. It is hypothesized that 5-HTTLPR S variant will have larger ERN amplitudes compared to the L variant, based of results from Fallgatter et al.

(2004) and Althaus et al. (2009). This association is hypothesized to exist in both the HFA and comparison group.

*Aim 3: Diagnostic Group and Genetic Differences in Symptom Severity and Social Emotional Functioning*

*Aim 3a.* To examine group differences in symptom severity and social emotional functioning (Attention Problems, internalizing and externalizing behaviors). It is hypothesized that participants in the HFA sample will be rated as having more symptoms and more social emotional difficulties compared to participants in the comparison group.

*Aim 3b.* To explore the association between 5-HTTLPR and DRD4 allele variability and individual differences in symptom severity and social emotional functioning (Attention Problems, internalizing and externalizing behaviors). Specifically, it is hypothesized that children in the 5-HTTLPR S variant group may display higher levels of Anxiety, Depression, and Somatization, whereas children in the L variant group may display more symptoms of Hyperactivity, Aggression and Conduct Problems (Brune et al., 2006; Hariri et al., 2005; Hariri et al., 2006; McDougle et al., 1998; Nobile et al., 2007). For DRD4 analysis, it is hypothesized that carriers of at least one 7-repeat allele will display more externalizing behaviors, including Hyperactivity, Aggression, and Conduct Problems, as well as Attention Problems (Faraone et al. 2005; Faraone et al. 2001; Li et al. 2006; Maher et al. 2002; Gadow et al. 2010). The gene-trait expression hypothesis is not specific to diagnostic group, and thus it is hypothesized that there will be similar patterns in both the HFA and comparison sample.

*Aim 4. Mediation Model*

To test the hypothesis that associations between 5-HTTLPR and DRD4 allelic variability and social-emotional functioning (i.e., internalizing, and externalizing behavior domains) are mediated in part by ERP and behavioral indices of response monitoring.

## Chapter 2: Method

### *Participants:*

Participants were part of a larger ongoing study of social functioning in a sample of HFA children and adolescents (8-15 years) and an age-, IQ-, and gender-matched sample of typically developing children. For the purpose of this study, the term higher functioning simply refers to a verbal IQ greater than 70, and our sample therefore includes participants with either Asperger Disorder or high functioning autism. Ninety-four (52 HFA, 42 comparison) children were eligible for inclusion in the current study. There were 44 males in the HFA sample and 36 males in the comparison sample. The parents of four eligible participants (2 HFA, 2 comparison) did not consent to genetic sampling. 5HTTLPR analysis was successfully completed on all 90 remaining participants (50 HFA, 40 comparison). However, DRD4 analysis was unsuccessful for two HFA participants (1 male, 1 female), thus DRD4 analyses are based on 88 participants (48 HFA, 40 comparison). Diagnostic groups were matched on age, gender distribution, and verbal IQ (see Table 2). The ethnic distribution of the sample was 46.6% Caucasian, 46.6% Hispanic, 3.4% Asian, and 3.4% African American. Ethnicity did not differ between the diagnostic groups,  $\chi^2(3, N = 90) = 4.45, ns$ .

Children in the HFA group had a community diagnosis of autism and were recruited from the University of Miami Center for Autism and Related Disabilities (CARD) center. The comparison sample was recruited through the local Miami-Dade school district. Diagnostic status was confirmed in the laboratory based on: (1) parent report on the Social Communication Questionnaire (SCQ; Rutter, Bailey, & Lord, 2003),

(2) parent report on the High-Functioning Autism Spectrum Screening Questionnaire (ASSQ; Ehlers et al., 1999), and (3) direct observations using the Autism Diagnostic Schedule (ADOS; Lord et al., 2001). Cutoff scores of 12 on the SCQ Total score, 13 on the ASSQ Total score and 7 on the ADOS Communication and Social Interaction domain were used to confirm ASD diagnosis. All children in the HFA sample met the established cutoffs on at least 2 of the 3 diagnostic measures. Likewise, all children in the TD sample scored below the cutoffs on at least 2 of the 3 measures.

Of the original 90 participants in the sample, children were excluded from some or all analyses if (a) parents did not complete BASC-2 ( $n = 8$ , 4 HFA, 4 Comparison), (b) technical problems during session, resulting in failure to collect EEG data ( $n = 6$ , 2 HFA, 4 Comparison), (c) refusal of EEG capping ( $n = 17$ , 12 HFA, 5 Comparison), (d) EEG data that could not be analyzed due to insufficient usable EEG data (i.e., participant had fewer than 10 artifact-free error trials on the flanker task ( $n = 12$ , 3 HFA, 9 Comparison), (e) or technical issues during data analysis (i.e., significant artifact, resulting in insufficient usable data) ( $n = 14$ , 12 HFA, 2 Comparison).

Due to the large number of participants removed from some analyses, comparisons were conducted to examine whether the included sample differed in any meaningful way from the excluded sample in terms of demographic characteristics. Included and excluded participants did not differ on VCI,  $F(1,78) = 2.63$ , *ns*, Gender,  $\chi^2(1, N = 80) = .62$ , *ns*, Ethnicity,  $\chi^2(1, N = 79) = 1.93$ , *ns*, SCQ,  $F(1,77) = .16$ , *ns*, ASSQ,  $F(1,76) = .05$ , *ns*, and ADOS,  $F(1,75) = .10$ , *ns*. Additionally, the analyzed group did not differ from the excluded group on any of the primary dependent variables including Internalizing Problems,  $F(1,72) = .52$ , *ns*, Externalizing Problems,  $F(1,72) = .01$ , *ns*,

average ERN amplitude,  $F(1,33) = .01$ , *ns*, and rate of self-correction,  $F(1,46) = 1.33$ , *ns*. However, groups differed on chronological age, such that the analyzed group was significantly older than the excluded group,  $F(1,78) = 6.30$ ,  $p = .02$ .

*Data Collection:*

Children were asked to participate in four different laboratory visits in which a series of electrophysiological, behavioral, and cognitive measures were completed. Genetic samples were collected primarily during the 4<sup>th</sup> visit, but earlier for some participants. In addition, parents completed several questionnaires about their child's behaviors and emotions during the study.

During the first laboratory visit, children and parents filled out questionnaires and the ADOS and WISC-IV were administered to the child.

During the second and third laboratory visit, children participated in an EEG collection. The child was asked to sit quietly for approximately seven minutes with either their eyes open or closed to collect baseline data. Following this, EEG was recorded continuously as they completed a modified Eriksen Flanker task (described below).

During the final visit, children participated in a peer interaction behavioral task (that is not included in the current analyses). Additional questionnaires were administered, and saliva was collected for genetic analysis.

*Diagnostic Measures:*

Autism Diagnostic Observation Schedule (ADOS; Lord et al, 2001) is a semi-structured observational assessment of Pervasive Developmental Disorders that measures social, communicative, cognitive, and self-regulatory behaviors. The ADOS consists of a series of standard play based activities designed to allow the examiner to observe social, communication, and repetitive behaviors. The ADOS provides multiple items rated on a qualitative scale of 0 (not abnormal) to 3 (most abnormal) to assess 5 main domains: Language and Communication; Reciprocal Social Interactions; Imagination; Restricted, Repetitive Behaviors and Interests Scale; and Other Abnormal Behavior. The ADOS has sound inter-rater reliability (coefficients ranging from .82 to .93 for the subscales), excellent sensitivity (1.0), and specificity (.79) in a sample of 54 children ranging in age from 15 months to 10 years (Lord et al., 2001). A cut-off score of 7 on the ADOS Communication and Social Interaction domain was used in order to verify community diagnoses.

Social Communication Questionnaire (SCQ; Rutter, Bailey, & Lord, 2003) is a 40-item, parent-report screening device that measures communication skills and social functioning in children diagnosed with autism. In a sample of 200 children and adolescents, diagnostic differentiation of the SCQ was significant in all ranges of IQ (30-49, 50-69, > 70) but was strongest in the highest IQ cluster. The SCQ has documented sensitivity of 0.88 and specificity of 0.72 for the discrimination between ASD and non-ASD cases and a sensitivity of 0.90 and specificity of 0.86 for the discrimination between autism and non-autism cases (Bolte, Holtmann, & Poustka, 2008). Analyses of differentiation by domain score suggest that all three domain scores contribute to



diagnostic differentiation, but that the total score followed by the social interaction domain score provide the strongest differential. A cut-off score of 12 on the SCQ total score was used to confirm diagnostic status and domain scores (Social Interaction Domain, Communication Domain, and Repetitive Behavior Domain) were used to capture variability in symptom severity among all children in the sample.

High-Functioning Autism Spectrum Screening Questionnaire (ASSQ; Ehlers et al., 1999) is a brief 27-item screening instrument used to identify symptoms associated with ASD in children and adolescents. The ASSQ is rated on a 3-point scale (0 indicating normality, 1 some abnormality, and 2 definite abnormality). Normed on a sample of 1,407 children, the ASSQ has sound test-retest reliability (Pearson  $r = .90, p = .001$ ), and inter-rater reliability ( $r = .79, p = .001$ ). Ehlers et al. (1999) suggest a cutoff score of 13 to correctly identify a HFA and TD sample. A cut-off score of 13 on the ASSQ total score was used to verify community diagnoses.

*Intelligence Measures:*

Wechsler Intelligence Scale for Children, Fourth Edition (WISC-IV; Wechsler, 2003) measures intellectual functioning in four separate cognitive domains: Verbal Comprehension (VCI), Perceptual Reasoning (PRI), Working Memory (WMI), and Processing Speed (PSI). The WISC-IV was normed on a sample of 2,200 children divided in to eleven groups. The WISC-IV has strong internal consistency (coefficients ranging from .88 to .97 for composite scores), high test-retest stability (effect sizes ranging from .08 to .60), and strong validity ( $r = .89$  with WISC-III). For the purposes of this study, an abbreviated version of the subtests: Similarities, Vocabulary, were used to

calculate index scores for VCI. These subtests were chosen for several reasons: they have the highest loadings on the VCI factors, strongest estimates of internal consistency, best test-retest reliabilities, and the narrowest standard errors of measurement among the WISC-IV scales (Williams et al., 2003). The WISC-IV was used to assess IQ and to verify higher functioning cognitive abilities in both samples ( $IQ > 70$ ).

*Emotional Functioning Questionnaires:*

The Behavioral Assessment System for Children – Second Edition (BASC-2; Reynolds & Kamphaus, 2004) parent-report versions was used to evaluate the behaviors, thoughts, and emotions of children (ages 6 to 11) and adolescents (ages 12- 21). The BASC-2 Parent Report Rating Scales were normed on a sample of 3,483 parents. The scales have well-established reliability and validity. The BASC-2 Parent Rating Scales include measures of Hyperactivity (10 items,  $\alpha = .83$ ) Aggression (13 items,  $\alpha = .89$ ), Attention Problems (7 items,  $\alpha = .87$ ), Anxiety (7 items,  $\alpha = .77$ ), Depression (12 items,  $\alpha = .86$ ), and Somatization (11 items,  $\alpha = .78$ ). Of interest for 5-HTTLPR analyses are the externalizing behavior dimensions (Hyperactivity, Aggression, and Conduct Problems), and the internalizing behavior dimensions (Anxiety, Depression, and Somatization). Of particular interest for the DRD4 analyses are the externalizing behavior dimensions (Hyperactivity, Aggression, Conduct problems) as well as Attention Problems.

*Modified Eriksen Flanker Task:*

EEG data were collected in a dimly lit, sound attenuated room. Participants were seated approximately 70 cm from the computer monitor. The ERN paradigm was a

modified version of the Eriksen Flanker task, in which participants were asked to respond to the direction of a central target arrow (either right or left) with a button press flanked by either compatible arrows (>>>> or <<<<<) or incompatible arrows (<<<<< or >>>>). Trials consisted of a 200 ms warning cue (an asterisk), followed by a 300 ms delay, then one of four target displays (described above) presented for 200 ms. The four different target stimuli were presented in a counterbalanced order across each block. Participants responded on a two-button keypad that corresponded to the direction of the central arrow. Participants were asked to respond as quickly and accurately as possible. Feedback about the accuracy and the speed of the response were presented after each trial. Prior to EEG recording, children participated in a set of 20 training trials to ensure correct understanding of the task. A second training session was used to calculate each participant's mean reaction time, which was used to adjust the presentation time-window in order to increase the error rate for faster subjects and to avoid frustration by slower subjects and to increase the likelihood that participants committed at least 10 errors, to ensure that a reliable grand average of the ERN amplitude could be computed. The median reaction time was used to adjust the response time parameters for responding for children who performed at or above 70% accuracy on the second training session. The 75<sup>th</sup> percentile reaction time was used to select the response window for children who performed below 70% accuracy.

The task was presented in three blocks of 96 stimulus trials equally divided by compatible and incompatible trials, as well as left and right responses. The two warm-up sessions took about 30 seconds each to complete. Each trial took about 7 minutes to

complete. Participants were allowed to take short breaks in between the blocks. The entire task took about 25 minutes to complete.

*Electrophysiological Recordings:*

EEG was collected using a Lycra stretch electrocap with electrodes placed according to the international 10/20 electrode system. EEG was recorded from 15 scalp sites (central: C3, Cz, C4), (frontal: F7, F3, Fz, FCz, F4, F8), (anterior temporal: T7, T8), (parietal: P3, Pz, P4), (occipital: O1, O2), and (mastoid: M1, M2), with a ground electrode at site AFz. Similar to previous EEG studies by Henderson et al. (2006) and Sutton et al. (2005), data was collected referenced to Cz. Electro-oculographic (EOG) eye movements were recorded at the supra- and sub-orbit of one eye, as well as the outer canthi of each eye. Artifact EEG was response-locked (correct/error) and averaged for each participant, for analysis. The low pass filter setting was 100 Hz and a high pass filter setting was 0.1 Hz, and EEG and EOG signals were amplified by factors of 5000 and 2500, respectively.

*Analysis of EEG data from Flanker Task:*

For each participant, the amplitude of the ERN was scored from the grand average of all error and all correct trials averaged across the three blocks of trials. Analyses were conducted using the ERP Analysis System (James Long Company). Data was re-referenced offline to an average mastoid reference and re-filtered with a low pass setting at 30 Hz. The data was corrected for eye blink artifacts using a regression technique. Data was also manually inspected to remove additional eye movement and gross motor

movement artifacts. The ERN was scored as the most negative peak occurring in the time window 20 ms prior to the response and lasting 150 ms after the response. Data were analyzed from the four mid-line recording sites (Fz, FCz, Cz, & Pz). Preliminary analyses were conducted to examine site and trial type differences in the amplitude of the ERN responses.

#### *Analysis of Behavioral Data from Flanker Task*

As an additional index of response monitoring, the rate of self-correction on the Flanker task was analyzed. Rate of self-correction was computed as the number of self-corrected errors, divided by the total number of errors committed multiplied by 100 [ $\# \text{self-correct} / (\# \text{self-correct} + \# \text{repeat errors}) \times 100$ ].

#### *Genotyping:*

Cells derived from saliva were collected using Oragene DNA collection kits (DNA Genotek) and analyzed at the John P. Hussman Institute for Human Genomics Biorepository of the University of Miami for DNA extraction. Oragene DNA kits provide a high yield of DNA (median: 110ug) and have been proven to be successful in genotyping 5-HTTLPR and DRD4, within the MIHG Laboratory.

The 5-HTTLPR polymorphism was analyzed by polymerase chain reaction (PCR) amplification, restriction digest and agarose gel electrophoresis using the Autopure LS DNA extractor (Qiagen, Inc.). This automated extractor has been used for several years with consistent DNA quality and yield. The primers used to amplify this polymorphism were 5'FAM TGGCGTTGCCGCTCTGAATGC 3' (forward) and 5'-

AGGGACTGAGCTGGACAACCA -3' (reverse). 40ng of DNA was used to set up 20ul PCR reactions with the following conditions: 4ul of dNTP mix [4ul 100mM dATP, 4ul 100mM dCTP, 4ul 100mM dTTP, 2ul 100mM dGTP, 40ul 5mM 7-deaza GTP, 346ul dH<sub>2</sub>O]; 0.03 units/ul of Platinum Taq DNA Polymerase (Invitrogen); 2ul of 10x PCR Buffer (Invitrogen); 1.5 mM mgCL<sub>2</sub>; 7.5% DMSO; 0.8ul of each primer at 100ng/ul. Thermocycling was done using the ABI Veriti™ 96-Well Fast Thermal Cycler. PCR amplification yielded 5-HTTLPR/rs25531 (S<sub>A</sub>, S<sub>G</sub>, L<sub>A</sub>, L<sub>G</sub>) genotypes. Following the methods of Olvet, Hatchwell, and Hajcak (2010), 5-HTTLPR results were analyzed in two ways: (1) using a bi-allelic designation (i.e., analyzing the S and L variant, regardless of the A/G SNP), and (2) using the tri-allelic designation (i.e., re-categorizing L<sub>G</sub> allele carriers in the S variant group).

The DRD4 polymorphism was extracted using a gel-based size standard and values were assigned to the bands based on the theoretical per product according to the reference data. Fragment analysis software was used, assigning values to the peaks based on the size standard of the reaction (ABI LIZ600). The primers used to amplify the rs1805186 STR were 5'FAMcgcgactacgtggtctactcg3' (forward) and 5'aggacctcatggccttg3' (reverse). 10ng of DNA was used to set up 15ul PCR reactions with the following conditions: 1.5ul of dNTP mix [20ul 100mM dATP, 20ul 100mM dCTP, 20ul 100mM dTTP, 20ul 100mM dGTP, 920ul dH<sub>2</sub>O]; 0.03 units/uL of Platinum Taq DNA Polymerase (Invitrogen); 1.5ul of 10x PCR Buffer (Invitrogen); 1.0 mM mgCL<sub>2</sub>; 2.0mM Betaine; 0.6ul of each primer at 100ng/ul. Thermocycling was done using the ABI Veriti™ 96-Well Fast Thermal Cycler. The qualities of the PCR products were confirmed using 2% agarose gel electrophoresis. From the DRD4 polymorphism:

fragments between 2 to 11 copies were extracted. Participants were classified based on whether a 7-repeat allele was present on either allele of the polymorphism. These individuals were identified as 7-repeat present. All other individuals were identified as non 7-repeat.

## Chapter 3: Results

### *Preliminary Medication Analysis*

Due to the potential influence of medication on physiological and behavioral measure of interest, it was important to examine the effects of medication on each of our independent and dependent variables. Within the HFA sample, 13 children were taking stimulant medication and 4 were taking selective serotonin reuptake inhibitors (SSRIs). One comparison child was taking stimulant medication. Chi-square analyses were conducted to explore the relation between medication use and allele frequency. Results indicated that there was not a significant association between general medication use and 5-HTTLPR allele frequency,  $\chi^2(1, N = 90) = .99, ns$ , or between stimulant specific medication and 5-HTTLPR bi-allelic frequency,  $\chi^2(1, N = 90) = 1.88, ns$ . Additionally, DRD4 allele frequency was not related to general medication use,  $\chi^2(1, N = 88) = .12, ns$ , and stimulant specific medication,  $\chi^2(1, N = 88) = .02, ns$ .

A series of multivariate analyses of variance (MANOVAs) were conducted to explore the effect of general medication use on dependent measures of symptom severity (SCQ Social Interaction, Communication, and Repetitive Behavior Domains) and social emotional functioning (BASC-2 Parent reported Hyperactivity, Aggression, Conduct Problems, Anxiety, Depression, Somatization, and Attention Problems). Medication use was significantly associated with symptom severity,  $F(3, 85) = 3.02, p = .03, \eta_p^2 = .10$ . Univariate post hoc analyses revealed an association between medication status and SCQ Repetitive Behavior Domain scores,  $F(1,87) = 8.87, p = .004, \eta_p^2 = .09$ . Participants taking medication were rated as having more severe Repetitive Behavior problems (see



Table 3). However, medication use was not associated with social emotional functioning,  $F(7, 74) = 1.20, p = .31, \eta_p^2 = .10$ . Although medication status did not have an overall multivariate effect on social emotional functioning, exploratory univariate analyses revealed significant associations with internalizing behaviors of Depression and externalizing behaviors of Attention Problems, as well as trend level significance with Hyperactivity (see Table 3). In summary, children taking medication were reported to exhibit higher levels of symptom severity, as well as more Depression, Attention Problems, and Hyperactivity.

Analyses were conducted to explore the effects of medication use on neural and behavioral indices of response monitoring (a) ERN amplitude at Fz, FCz, and Cz, and (b) rate of self-correction. Multivariate analyses indicated that the relation between medication status and ERN amplitude approached significance,  $F(3, 37) = 2.20, p = .10, \eta_p^2 = .15$ . Post hoc analysis revealed a trend level association between medication status and ERN amplitude at Cz,  $F(1, 39) = 3.93, p = .05, \eta_p^2 = .09$ . Individuals taking medication had significantly larger amplitude ERN responses at Cz (Medicated  $n = 7, M = -6.91, SD = 7.30$ ; Non Medicated  $n = 34, M = -1.34, SD = 6.69$ ). However, medication use was unrelated to the rate of self-correction,  $F(1, 48) = .16, p = .688, \eta_p^2 < .01$ .

Based on these preliminary analyses, medication use was used as a covariate in all analyses that included the dependent variables in which relations were revealed.

#### *Preliminary Response Monitoring Analysis*

Analysis was conducted between the two indices of response monitoring. A trend level negative correlation existed between mean ERN amplitude (average of ERN across

Fz, FCz, and Cz sites) and rates of self-correction,  $r = -.27$ ,  $p = .100$ , such that larger amplitude ERN responses were marginally associated with higher rates of self-correction. Because ERN amplitude and rates of self-correction did not significantly correlate together, these variables were analyzed separately in the following analyses.

*Aim 1. Diagnostic Group Differences in Allele Frequencies:*

Chi-square analyses were used to examine diagnostic group differences in allele frequencies for both 5-HTTLPR (bi-allelic grouping: S variant and L variant and tri-allelic grouping: S/L<sub>G</sub> variant and L<sub>A</sub> variant) and DRD4 (7-repeat or non-7repeat). There was not a significant association between diagnostic group and 5-HTTLPR allele frequency using either the bi-allelic grouping,  $\chi^2(1, N = 90) = .17$ , *ns*, or the tri-allelic grouping,  $\chi^2(1, N = 90) = .03$ , *ns*. However, there was a significant association between diagnostic group and DRD4 allele frequency,  $\chi^2(1, N = 88) = 6.93$ ,  $p = .02$ , with HFA individuals carrying the 7-repeat allele more frequently than comparison participants. Seventeen percent of the comparison participants ( $n = 7$ ) carried at least one 7-repeat allele, while 43.8% of the HFA sample ( $n = 21$ ) were carriers. Results for the comparison sample were consistent with previous findings of roughly a 25% 7-repeat allele frequency in typically developing samples (Wang et al., 2004; Sheese et al., 2007), and suggested an over-representation of the 7-repeat allele in the HFA sample.

*Aim 2a. Diagnostic Group Differences in ERN:*

A repeated measures ANOVA was conducted to examine the spatial distribution and amplitude difference of ERPs to correct versus error responses on the Flanker task.

A 2 (correct vs. error) x 2 (diagnostic group) x 4 (midline scalp sites: Fz, FCz, Cz, Pz) ANOVA was conducted. Analysis with Greenhouse-Geisser adjusted degrees of freedom revealed main effects of site,  $F(1.83, 71.51) = 33.72, p < .001, \eta_p^2 = .47$ , and response type,  $F(1, 39) = 6.77, p = .01, \eta_p^2 = .15$ , which were qualified by an interaction between site and response type,  $F(1.86, 72.56) = 11.18, p < .001, \eta_p^2 = .22$ . Across both diagnostic groups, ERN amplitude was larger for error versus correct trials at frontal-central sites, Fz,  $t(40) = -3.82, p < .001$ , FCz,  $t(40) = -3.46, p = .001$ , and Cz,  $t(40) = -2.13, p = .039$ , but did not differ at Pz (see Figure 1). Therefore, the three frontal-central sites (Fz, FCz, and Cz) were retained as focal areas for the following analyses.

Diagnostic group, independently or in interaction with response type or site, was not related to ERN amplitude. Thus across both diagnostic groups, there was a frontal-central distribution of the ERN which is comparable to prior studies of children with autism and typically developing children in this age range (e.g., Henderson et al., 2006).

*Aim 2b. Associations Between Allelic Variations and Response Monitoring:*

Two indices of response monitoring were analyzed: (1) neurophysiological: ERN amplitude and (2) behavioral: rates of self-correction. MANCOVAs were conducted to examine the effect of diagnostic group and allele frequency variants on the ERN amplitude at frontal-central sites (Fz, FCz, and Cz), with medication status serving as the covariate. Using a bi-allelic grouping, there were not main effects of diagnostic group or allele frequency on the ERN amplitude across all the sites. However, univariate analysis revealed between subject effects of 5-HTTLRP on ERN amplitude at FCz,  $F(1,37) = 4.18, p = .04, \eta_p^2 = .10$ , and Cz,  $F(1,37) = 4.93, p = .03, \eta_p^2 = .12$ , but not at Fz sites. At

both sites, regardless of diagnostic group, the L variant was associated with a smaller ERN amplitude. However, using the tri-allelic grouping, 5-HTTLPR was unrelated to ERN amplitude at all sites including FCz,  $F(1, 37) = 1.86, p = .18, \eta_p^2 = .05$ , and Cz,  $F(1, 37) = 3.32, p = .07, \eta_p^2 = .08$ .

A second index of response monitoring, rate of self-correction, was also analyzed. With bi-allelic grouping, an ANOVA revealed (a) a main effect of diagnostic group,  $F(1,29) = 12.56, p < .001, \eta_p^2 = .30$ , and (b) a main effect of 5-HTTLPR allelic frequency,  $F(1,29) = 10.08, p < .001, \eta_p^2 = .26$ , and (c) an significant interaction between diagnostic group and 5-HTTLPR allele frequency,  $F(1, 29) = 7.86, p = .01, \eta_p^2 = .21$ . Children in the comparison group self-corrected themselves significantly more than children in the HFA sample and across both diagnostic groups, the S variant self-corrected significantly more than the L variant. Post hoc analyses revealed that HFA carriers of the S variant did not self-correct more than S variant carriers in the comparison sample,  $t(35) = .43, ns$ , however HFA children carrying the L variant self-corrected less than comparison children carrying the L variant allele,  $t(11) = -2.49, p = .03$  (see Figure 2). Comparable results were found with the tri-allelic grouping, such that there remained a significant interaction between diagnostic group and allele frequency on rates of self correction,  $F(1, 29) = 11.62, p = .002, \eta_p^2 = .30$ . Tri-allelic post hoc analysis revealed similar patterns of association as the bi-allelic categorization.

With respect to DRD4, there were not effects of allele frequency on ERN amplitude at any of the three scalp sites. Additionally, there were no significant effects of DRD4 allele frequency on the rates of self- correction.

*Aim 3a and 3b. Differences in Symptom Severity and Social Emotional Functioning:*

A series of 2 (Diagnostic group [HFA, Comparison]) x 2 (5-HTTLPR allele frequency [S variant, L variant]) MANCOVAs were conducted to examine associations with measures of social emotional functioning. Separate MANCOVAs were conducted for (a) internalizing behaviors (BASC-2 parent reported Anxiety, Depression, and Somatization), (b) externalizing behaviors (BASC-2 parent reported Hyperactivity, Aggression, and Conduct problems), and (c) symptom severity (SCQ Social Interaction Domain, Communication Domain, and Repetitive Behavior Domain). Medication use was controlled for in all following analyses. As expected there were main effects of diagnostic group on internalizing behaviors,  $F(3,75) = 8.23, p < .001, \eta_p^2 = .25$ , externalizing behaviors,  $F(3,75) = 3.80, p = .01, \eta_p^2 = .34$ , and symptom severity,  $F(3,82) = 40.30, p < .001, \eta_p^2 = .60$ . Additionally, with bi-allelic grouping, there was a trend level effect of 5-HTTLPR allele variability on internalizing problems,  $F(3,75) = 2.52, p = .06, \eta_p^2 = .09$ , as well as externalizing problems,  $F(3,75) = 3.00, p = .06, \eta_p^2 = .07$ . Post hoc ANCOVAs revealed a significant association between 5-HTTLPR and parent reported Somatization, Hyperactivity, and a trend with Conduct Problems (see Table 4). That is, across both diagnostic groups, carriers of the L variant were reported to be overly sensitive and complained about relatively minor physical problems and discomforts (Somatization). They were also described as over active, rushed through work or activities, and acted without thinking, as reported by their parents (Hyperactivity). Results were comparable using the tri-allelic grouping, with significant associations between 5-HTTLPR grouping and Hyperactivity,  $F(1,77) = 4.03, p = .05, \eta_p^2 = .05$  and Somatization,  $F(1,77) = 6.13, p = .016, \eta_p^2 = .07$ .

Separate 2 (Diagnostic group [HFA, Comparison]) x 2 (DRD4 allele frequency [7-repeat, non 7-repeat]) multivariate ANCOVAs were conducted with measures of (a) BASC-2 parent report Attention Problems, Conduct Problems, Aggression and Hyperactivity and (b) symptom severity: SCQ Social Interaction Domain, Communication Domain, and Repetitive Behavior Domain, as dependent variables were conducted, again controlling for medication. As reported above, there were main effects of diagnostic group on both social emotional functioning,  $F(4,73) = 7.37, p < .001, \eta_p^2 = .29$ , and symptom severity,  $F(3.80) = 41.03, p < .001, \eta_p^2 = .61$ . In addition, while DRD4 did not predict any measures of symptom severity, there was a significant association between DRD4 allele frequency and measures of social emotional functioning,  $F(4,73) = 2.69, p = .04, \eta_p^2 = .13$ . Post hoc ANCOVAs revealed a significant effect of DRD4 on Parent Reported Attention Problems  $F(1,76) = 6.74, p = .02, \eta_p^2 = .08$ . Across both diagnostic groups, Attention Problems were reported to be higher in carriers of at least one 7-repeat allele (non-7 repeat  $n = 54, M = 51.63, SD = 11.70$ ; 7-repeat  $n = 27, M = 61.22, SD = 10.27$ ).

*Aim 4. Mediation Model:*

Given the association between 5-HTTLPR and DRD4 allele frequency and measures of response monitoring (ERN amplitude at FCz and Cz and rates of self-correction) and social emotional functioning (BASC-2 parent reported measures of Somatization, Hyperactivity, and Attention Problems), analyses were conducted to determine whether response monitoring mediated the associations between genes and behavioral expression. Using Baron and Kenny's (1986) method, a series of multiple

regressions were conducted to examine the effect of ERN at FCz and Cz as a mediator of the relationship between allelic variability and our dependent variables (DV) of social-emotional functioning. Mediation analyses were restricted to the 39 children (20 HFA, 19 Comparison) with complete genetic, behavioral and neurophysiological measures of response monitoring, and behavioral data.

Step 1 in testing the mediation hypothesis was to confirm a significant relationship between the predictor (i.e., allelic variability in 5-HTTLPR) and the criterion variable (i.e., social emotional functioning: Somatization and Hyperactivity). Univariate ANCOVAs covarying medication, with diagnostic group and allelic frequency as fixed variables were employed. For the 5-HTTLPR analysis, results indicated that the overall model with this reduced sample did not account for a significant portion of the variance for either Somatization,  $F(1,31) = 2.30, p = .14$ , or Hyperactivity,  $F(1,31) = .23, p = .64$ . For the DRD4 analysis, results also indicated non-significant results for Attention Problems,  $F(1,31) = .30, p = .580, \eta_p^2 = .01$ .

Due to the fact that the first criterion for testing a mediation model was not met, we did not proceed with employing the second step of testing whether the independent variable (i.e., allelic variability in 5-HTTLPR [S variant, L variant]) significantly predicted the hypothesized mediator (i.e., ERN amplitude at FCz and Cz). Additionally, we did not employ the final step of testing whether the mediator significantly predicted social emotional functioning. Thus, conducting a Sobel test of significance of the indirect effect was not necessary for this analysis.

## Chapter 4: Discussion

In the current study, genetic polymorphisms (i.e., 5-HTTLPR and DRD4) and measures of response monitoring (i.e., ERN and rates of self-correction), were examined in relation to individual differences in general autism symptoms and social emotional functioning. Collectively, the findings suggest that the wide range of variability in the manifestation of ASD may be explained, in part, by genetic and neural factors related to attention and self-regulation. Importantly, in most cases, comparable effects were noted for typically developing children, suggesting that these factors are related to core non-syndrome specific processes that affect social and emotional development. However, contrary to hypotheses, electrophysiological and behavioral measures of response monitoring did not mediate the relation between gene expression and internalizing and externalizing symptoms. Nonetheless, results provide evidence for a connection between genetics, neural mechanisms, and phenotypic expression.

### *Diagnostic Group Differences in DRD4 Allele Frequency:*

An interesting finding, although unexpected, was that children with HFA were more likely than children in the comparison sample to be carriers of at least one 7-repeat allele. This finding is interpreted as an overrepresentation in the HFA sample (versus underrepresentation in the comparison sample), given that the comparison group had similar rates as those reported in prior studies (e.g., Wang et al., 2004; Sheese et al., 2007). The high rate of this variant in the HFA sample likely reflects the elevated rate of attention problems typically present in children with HFA. This finding, in conjunction



with previous literature (Faraone et al., 2005; Faraone et al., 2001; Li et al., 2006; Maher et al., 2002; Gadow et al., 2010) suggests that DRD4 may convey risk for ADHD type symptoms, as opposed to autism symptoms per se. In fact, at the time of the study, 14 HFA children were taking stimulant medication for ADHD related symptoms.

Additionally, Attention Problems was the *only* domain of social emotional functioning that was significantly elevated in children taking stimulant medication compared to children on SSRIs or no medication. These findings do not specifically suggest DRD4 as a biomarker for autism, but rather as a biomarker for global attention problems, a predominant comorbid symptom of autism.

#### *Response Monitoring Measures*

Response monitoring is defined as the ability to continually check one's progress toward a goal and has been hypothesized to underlie some of the social impairments in autism (Russell & Jarrold, 1998, Ono et al., 2009). Within this study, ERN and rates of self-correction were used to measure response monitoring in the HFA and comparison sample. Neural regions implicated in cognitive control, such as the DMFC and ACC, are densely innervated by 5-HT and DA neurotransmitters and are facilitated by the combined effects of 5-HT and DA (Daw, Kakade, & Dayan, 2002). Thus, functional genes, 5-HTTLPR and DRD4, were studied in relation to response monitoring.

#### *5-HTTLPR and Response Monitoring:*

Consistent with the results reported by Althaus et al. (2009) and Fallgatter et al. (2004), in the current study L/L carriers, regardless of diagnostic group, had significantly

smaller ERN amplitudes at frontal-central sites, indicating poorer response monitoring abilities. In contrast, the L variant was associated with significantly lower rates of self-correction, but only among HFA participants. This may indicate that the effects of the L variant are amplified among children with HFA. Consistent with findings of Olvert et al. (2010), however, the effects of 5-HTTLPR on ERN amplitude were reduced to non-significance when using the tri-allelic grouping. Nonetheless, rate of self-correction retained significance with the tri-allelic recategorization. Together these results demonstrate that 5-HTTLPR allele frequency may have a profound effect on neurophysiological as well as behavioral measures of response monitoring.

#### *DRD4 and Response Monitoring:*

DRD4 allele variations were not associated with response monitoring in the current study. However, this does not necessarily preclude the possibility that dopamine is involved in the genesis of ERN. In fact, DA has been repeatedly documented as playing a key role in performance monitoring, including medication challenges (de Bruijn et al., 2004, 2006; Zirnel et al., 2004). Studies report that administration of dopamine agonist amphetamine drugs led to an increase in ERN amplitude (de Bruijn et al., 2004, 2006). It is important to note that DRD4 is one of many polymorphisms that convey expression of DA. In future studies it will be important to investigate other functioning polymorphisms (e.g., monoamine oxidase A: MAO-A, DAT, DA D2 receptor: DRD2, and Catechol-O-methyltransferase: COMT), which may provide further evidence for the influence of dopaminergic biomarkers on response monitoring. For example, there is evidence that the Met allele of COMT is associated with greater working memory

abilities (Bruder et al., 2005; Egan et al., 2001; Mattay et al., 2003) and that the Val allele is associated with poorer attentional control (Blasi et al., 2005). The DAT gene has been documented to affect response monitoring (Yucubian et al., 2007) and DRD2 A1-allele carriers were reported to respond less to negative feedback and have less posterior medial frontal cortex activation than non-carriers (Klein et al., 2007). These studies provide evidence for relations between a variety of dopaminergic polymorphisms and related executive functions including response monitoring, attentional control, and working memory.

*5-HTTLPR and Social Emotional Functioning and Symptom Severity:*

The results of the current study provide support for the role of 5-HTTLPR in predicting variations in social emotional functioning in all children, regardless of diagnostic status. Results supported an association between the L variant and both internalizing (Somatization) and externalizing (Hyperactivity) behavior problems. Given the concurrent nature of the data in the present study, it is unclear whether 5-HTTLPR directly affects both externalizing and internalizing behaviors, or whether 5-HTTLPR influences behavior in one domain (e.g., externalizing behavior; hyperactivity), which subsequently lead to more (internalizing) complaints later in life, or vice versa. Clearly, longitudinal studies are necessary to tease apart these relations.

Furthermore, 5-HTTLPR is one of many polymorphisms that convey 5-HT expression. Intron 2 12/12 genotype has been linked to greater impairment in the rigid-compulsive domain of the ADI-R. Reports suggest that the intron 2 is a transcriptional regulatory domain and functions as a differential enhancer of 5-HT (Fiskerstrand,

Lovejoy, & Quinn, 1999; MacKenzie & Quinn, 1999). Intron 2 has been associated with affective disorders, including bipolar disorder, depression, and anxiety (Collier et al., 1996; Kunugi et al., 1997; Rees et al., 1997; Bellivier et al., 1998; Furlong et al., 1998; Gutierrez et al., 1998a, Gutierrez et al., 1998b; Hoehe et al., 1998). These results demonstrate that other functional polymorphisms may also influence symptom severity in ASD children and social emotional functioning. Thus, it is important to keep in mind that these results only focus on a subset of the potential variables that may influence behavioral expression.

Nonetheless, these results may provide preliminary evidence for 5-HTTLPR as a modifier for neural and behavioral changes that affect response monitoring, and social emotional deficits in both typically developing children and children diagnosed with HFA. Specifically, carriers of the L variant of 5-HTTLPR may have deficits in their ability to self-regulate, thus producing social emotional difficulties, such as hyperactivity and increasing sensitivity to minor physical problems and discomforts.

#### *DRD4 and Social Emotional Functioning:*

While previous research has reported associations between the DRD4 7-repeat allele and conduct problems, obsessive-compulsive behaviors, and hyperactivity (Faraone et al. 2005; Faraone et al. 2001; Li et al. 2006; Maher et al. 2002; Gadow et al. 2010), the current study only found an association between Attention Problems and the 7-repeat variant. It is interesting to note that attention-related problems are a component of all the disorders above (whether it be overattending or underattending) (Casey et al., 2002). In fact, common neural pathways (i.e., mesolimbic branches in the cortico-striato-thalamo-

cortical circuitry) have been identified as a link between these disorders and may help to explain the similarities in presentation (Bradshaw & Sheppard 2000; Durston & Konrad, 2007; Miller & Cohen, 2001; Rauch et al., 2001; Sonuga-Barke, 2005). Thus, results from previous literature and the current study provide evidence for DRD4 7-repeat having a broad impact on the development of attention control and regulation. Within the present study, across both diagnostic groups, carriers of the 7-repeat allele were described by their parents as being easily distracted or having difficulty concentrating. This suggests that DRD4 7-repeat functions similarly in both HFA and typically developing children.

*Generalization Across Diagnostic Groups:*

5-HTTLPR and DRD4 are polymorphisms found in all individuals, and thus it is important to highlight that results reflect similar genetic effects in the HFA and comparison sample. While the HFA group displayed more syndrome specific behaviors (SCQ: Social Interaction Domain, Communication Domain, and Repetitive Behavior Domain) and non-syndrome specific behavior problems (BASC-2 Somatization, Hyperactivity, Attention Problems) compared to the typically developing sample, the L variant of 5-HTTLPR and the 7-repeat of DRD4 affected social emotional functioning similarly across both groups. However, there was no relation between either 5-HTTLPR or DRD4 allele frequency and symptom severity. Collectively, results implicate these polymorphisms as markers for individual variability, as opposed to risk for autism or psychopathology per se.

*Closer Look at Medication Analysis:*

While medication use was not a main focus of the study, results demonstrated interesting associations between medication use and several outcome measures. Children taking stimulant medication or SSRIs were reported by parents to be *more* symptomatic as well as to exhibit higher levels of Depression, Attention Problems, and Hyperactivity. While initially counter-intuitive, it is likely that parents who view their children as having more difficulties, both non-syndrome specific and syndrome specific, would be more likely to resort to medication to alleviate symptoms. However, children taking medication also had larger amplitude ERN responses, generally regarded as an indicator of enhanced response monitoring abilities. Studies have demonstrated that dopamine agonists are associated with an enhanced ERN response and dopamine antagonists are associated with attenuated ERN amplitude. However there is an absence of effects of selective serotonin reuptake inhibitors on ERN (de Bruijn et al., 2004; de Bruijn et al., 2006). Many stimulant drugs (e.g., Ritalin, Concerta, and MPH) are purported to increase levels of dopamine and norepinephrine (agonists) in the brain through reuptake inhibition of the monoamine transporters. Thus, our findings corroborate previously documented effects of dopamine agonist medication on enhanced ERN amplitude.

It appears, however, that while medication has a direct effect on neural measures of response monitoring, it did not have an effect on behavioral measures. Research has suggested that stimulant medication D-amphetamine (dopamine agonist) leads to an enhanced ERN amplitude, however does not affect reaction time (de Bruijn et al., 2004; de Bruijn et al., 2006). Results from the current study corroborated these findings, such that ERN amplitude was enhanced in children taking stimulant and SSRI medication,

however medication use was not associated with rate of self-correction. Collectively, these results may suggest that while medication may physiologically enhance response monitoring abilities, other mechanisms may be interacting with the neural circuitry, to deflect behavioral changes. Further research is needed to better understand the complexity of medication use on the neural circuitry and behavioral outcome. However, it is important to note that the current study did not look at the specific type of medication, and thus can only give a general account of the relations between medication use on response monitoring and behavioral expression. Temporal information (including length of time on medication as well as pre and post medication assessments), dosage, and specificity of medication are important to better understand the course and trajectory of medication treatment.

*Clinical Implications:*

The results of this study suggest that autism and the most common comorbid conditions, internalizing disorders, such as Anxiety and Depression, as well as externalizing disorders, such as ADHD, may be related at a biological level. It is important for clinicians to note this relation between common comorbid disorders and autism, for treatment and diagnostic purposes. The current DSM-IV TR and ICD-10 are structured such that it places exclusionary criteria on giving a comorbid diagnosis of ADHD when an individual is diagnosed with an Autism Spectrum Disorder (APA: DSM-IV-TR, 2000; ICD-10: WHO, 1993). However, findings from the current study suggest that ADHD-like symptoms may in fact be present (stemming from a genetic predisposition), in combination with autism symptoms, thus providing evidence for the

existence of a dual diagnosis. Diagnosing an individual with both ADHD and autism not only clarifies clinical presentation of symptoms, but may also be beneficial for treatment purposes. Many clinicians find it important to utilize a stimulant medication regimen as a first response to treat ADHD, while behavioral intervention is usually the first line of treatment for autism. By excluding ADHD from a diagnosis, a child may not receive proper treatment for their symptoms.

Additionally, the effects of functional polymorphisms may be particularly important in the HFA sample, not only for treatment, but for diagnoses as well. Anxiety, Depression, and ADHD often appear in conjunction with ASD and presentation of these symptoms may influence a diagnosis and developmental course. Many times clinicians will use standardized assessments, including the BASC-2, to measure these types of behavioral difficulties. BASC-2 specifies that T-scores between 60-69 are in the At-Risk range, and scores 70+ are in the clinically significant range. In the HFA sample, the L variant of 5-HTTLPR appeared to elevate T-scores on multiple domains in to the At-Risk range. Hyperactivity, Anxiety, Depression, and Somatization were all in the At-Risk range for HFA children in the L variant group. Whereas HFA children in the S variant group did not have any scores in the At-Risk range on all domains of social emotional functioning (see Table 4). Likewise, DRD4 7-repeat carriers in the HFA group were reported be At-Risk for Attention Problems, while non 7-repeat carriers in the HFA group were in the normal range for Attention Problems. Results highlight the importance of genetic factors influencing results on behavioral assessments, and the impact it may have on formulating a diagnosis.



*Limitations and Future Directions:*

While the current model of response monitoring mediating the relation between genes and social emotional functioning was not supported, this does not necessarily invalidate the model. First and foremost, the reduction in sample size due to missing ERN and outcome data may have affected the significance. Due to the nature of the study and the sensitivity of this population, EEG data collection was difficult to complete on many individuals. Regardless, the mediation model may also be explained by other variables, which have been previously reported to affect phenotypic expression, such as environmental influence (Greenberg et al., 2006; Pakenham et al., 2005). For example, DRD4 7-repeat may predispose a child to seek out stimulating environments, and subsequently these children are reported to have difficulty with attention and focus.

Another way of looking at individual differences is with interactions, as opposed to mediation analyses. Studying individual polymorphisms may not fully account for the complexity of behavioral expression and thus researchers in the past have looked at gene-gene, as well as gene-environment interactions. Environmental factors, including but not limited to, family cohesion, social economic status, therapy and intervention, and parental education may influence response monitoring and modify the expression of behavior (Greenberg et al. 2006; Pakenham et al. 2005). Thus, both gene-gene as well as gene-environment influences are important factors in understanding the neural circuitry and phenotypic expression in HFA and typically developing children.

Furthermore, these results are established on parent report measures of symptom severity and behavior problems and are susceptible to reporter bias. Parents of children diagnosed with ASD may be more likely to overestimate symptoms due to their

knowledge of the disorder. Future studies may include peer-, teacher-, and self-report measures of behavioral expression, as well as behavioral observations to better qualify differences in social emotional functioning. In addition, this sample was studied at a single time point and therefore we are only able to describe a snapshot of each child's life. Longitudinal studies are needed to investigate and track the trajectory of behavior and neural mechanisms across the developmental lifespan of a child.

While the current study used a typically developing control sample, it may be beneficial to look at the effects of 5-HTTLPR and DRD4 in psychiatric control samples as well. By extending the research to other psychiatric populations, such as ADHD, Anxiety, and Depression, researchers can gain a better understanding of whether the effects seen in this study can be generalized across disorders. This will strengthen the hypothesis that simple polymorphisms can have a trans-diagnostic effect.

*Summary:*

Mundy et al. (2007) used the modifier model to explain how non-syndrome specific processes refract or alter the expressions of autism symptomatology and contribute to the heterogeneous behavioral and psychological presentation found in this disorder. Within the current study, genetic variability and measures of response monitoring were examined as non-syndrome specific factors that serve as potential modifiers contributing to the phenotypic variability in typically developing and children with higher functioning autism. Results provide insight into the processes by which genes and neural mechanisms broadly related to attention and attention regulation seem

to modify behavioral expression and may have profound effects on the presentation and developmental course of disorders such as autism.

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Table 1.

*Summary of Published Studies 5-HTTLPR Allele Transmission.*

<i>Study</i>	<i>Results</i>	<i>Sample Size</i>
Cook et al., 1997	S	86 trios
Conroy et al., 2004	S	84 trios
McCauley et al., 2004	S	123 multiplex
Devlin et al., 2005	S	103 trios
Tordjiman et al., 2001	L	69 trios
Klauck et al., 1997	L	65 trios
Yirmiya et al., 2001	L	34 trios
Kim et al., 2002		81 trios
Persico et al., 2000		86 trios 5 multiplex
Coutinho et al., 2004		182 trios
Maestrini et al., 1999		8 trios 82 multiplex
Betancur et al., 2002		43 trios 53 multiplex

*Table shows: the study, whether results indicated an overtransmission of at least one short allele (S) or overtransmission of the long allele (L), or no difference (blank), as well as the sample size.*

Table 2.

	<i>HFA (n=50)</i>		<i>Comp (n=40)</i>		<i>Analysis</i>	
	<i>Mean (SD)</i>	<i>Range</i>	<i>Mean (SD)</i>	<i>Range</i>	<i>F value</i>	<i>p value</i>
<i>Age (months)</i>	152.28 (30.39)	98-199	153.85 (26.76)	107-193	.07	.80
<i>Gender</i>	44 M, 6 F		36 M, 4 F		.69	.41
<i>VIQ</i>	103.72 (16.82)	75-140	108.88 (13.30)	89-155	2.51	.12
<i>SCQ</i>	18.44 (6.20)	3-33	5.08 (3.72)	0-20	141.29***	<.001
<i>ADOS</i>	11.44 (4.05)	0-21	2.89 (3.82)	0-15	97.70***	<.001
<i>ASSQ</i>	26.76 (8.09)	12-45	4.26 (4.40)	0-23	239.46***	<.001

*Note: SCQ and ASSQ are Total Scores. ADOS is the Communication and Social Interaction Domain*

\*\*\*  $p < .001$

Table 3.

	Medicated (n=18)		No Med (n=71)		Analysis F value	p value	Effect Size ( $\eta^2$ )
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)			
<i>Symptom Severity</i>							
<i>Social Interaction Domain</i>	5.11 (3.46)	3.86 (3.71)			1.74	.19	.26
<i>Communication Domain</i>	5.37 (2.54)	4.17 (2.57)			3.27	.07	.43
<i>Repetitive Behavior Domain</i>	4.74 (2.38)	2.67 (2.75)			8.87**	.004	.84
<i>Social Emotional Functioning</i>							
Externalizing Behaviors							
Hyperactivity	59.88 (15.88)	53.45 (11.15)			3.72	.06	.48
Aggression	51.71 (9.51)	49.75 (10.46)			.49	.49	.11
Conduct Problems	51.47 (11.60)	49.77 (8.73)			.44	.51	.11
Attention Problems	60.41 (15.02)	52.51 (12.10)			4.98*	.028	.60
Internalizing Behaviors							
Anxiety	54.94 (11.27)	51.57 (11.90)			1.11	.30	.18
Depression	60.41 (15.02)	52.51 (12.10)			5.19*	.025	.61
Somatization	53.41 (18.73)	48.86 (11.24)			1.63	.21	.24

Note: Symptom Severity measured by the Social Communication Questionnaire (SCQ).

Social Emotional Functioning

measured by the Behavioral Assessment System for Children- Second Edition (BASC-2).

Means for the BASC-2 are

listed as T-scores. Values between 60-69 are At-Risk and 70+ are Clinically Significant.

\*  $p < .05$

\*\*  $p < .01$

Table 4.

		S Variant		L Variant		Analysis		Effect Size ( $\eta_p^2$ )
		Mean (SD)	n	Mean (SD)	n	F value	p value	
<i>Social Emotional Functioning</i>								
<i>Externalizing Behaviors</i>								
<i>Hyperactivity</i>								
	HFA	53.07 (12.00)	59	59.17 (12.77)	23	4.53*	.04	.06
	Comparison	55.21 (12.36)	34	66.92 (12.72)	12			
<i>Aggression</i>								
	HFA	50.16 (11.07)	25	50.73 (5.52)	11	.71	.40	<.01
	Comparison	49.61 (11.02)	59	51.57 (7.97)	23			
<i>Conduct Problems</i>								
	HFA	51.12 (12.81)	34	54.17 (8.26)	12			
	Comparison	47.56 (7.78)	25	48.73 (6.90)	11			
<i>Internalizing Behaviors</i>								
<i>Anxiety</i>								
	HFA	48.88 (8.77)	59	53.30 (10.20)	23	3.61	.06	.51
	Comparison	49.12 (7.90)	34	56.00 (11.64)	12			
	Comparison	48.56 (10.00)	25	50.36 (7.87)	11			
<i>Depression</i>								
	HFA	51.63 (11.36)	59	53.91 (12.94)	23	1.32	.25	.02
	Comparison	55.79 (10.60)	34	60.92 (10.94)	12			
<i>Somatization</i>								
	HFA	45.00 (14.14)	59	46.27 (10.65)	11	.10	.75	<.01
	Comparison	54.00 (14.14)	59	54.52 (10.06)	23			
	Comparison	58.97 (14.59)	34	61.08 (8.47)	12			
	Comparison	47.24 (10.39)	25	47.36 (5.97)	11			
	HFA	47.54 (11.08)	59	55.61 (16.21)	23	7.54**	.007	.09
	Comparison	50.18 (12.77)	34	61.42 (19.37)	12			
	Comparison	43.96 (7.00)	25	49.27 (8.99)	11			

Note: ANCOVA results, covarying medication use. Social Emotional Functioning measured by the Behavioral Assessment System for Children- Second Edition (BASC-2). Means for the BASC-2 are listed as T-scores. Values between 60-69 are At-Risk and 70+ are Clinically Significant.

\*  $p < .05$

\*\*  $p < .01$

Figure 1.  
*ERP waveforms for error and correct trials at Fz, FCz, Cz, and Pz for entire sample (n = 40).*

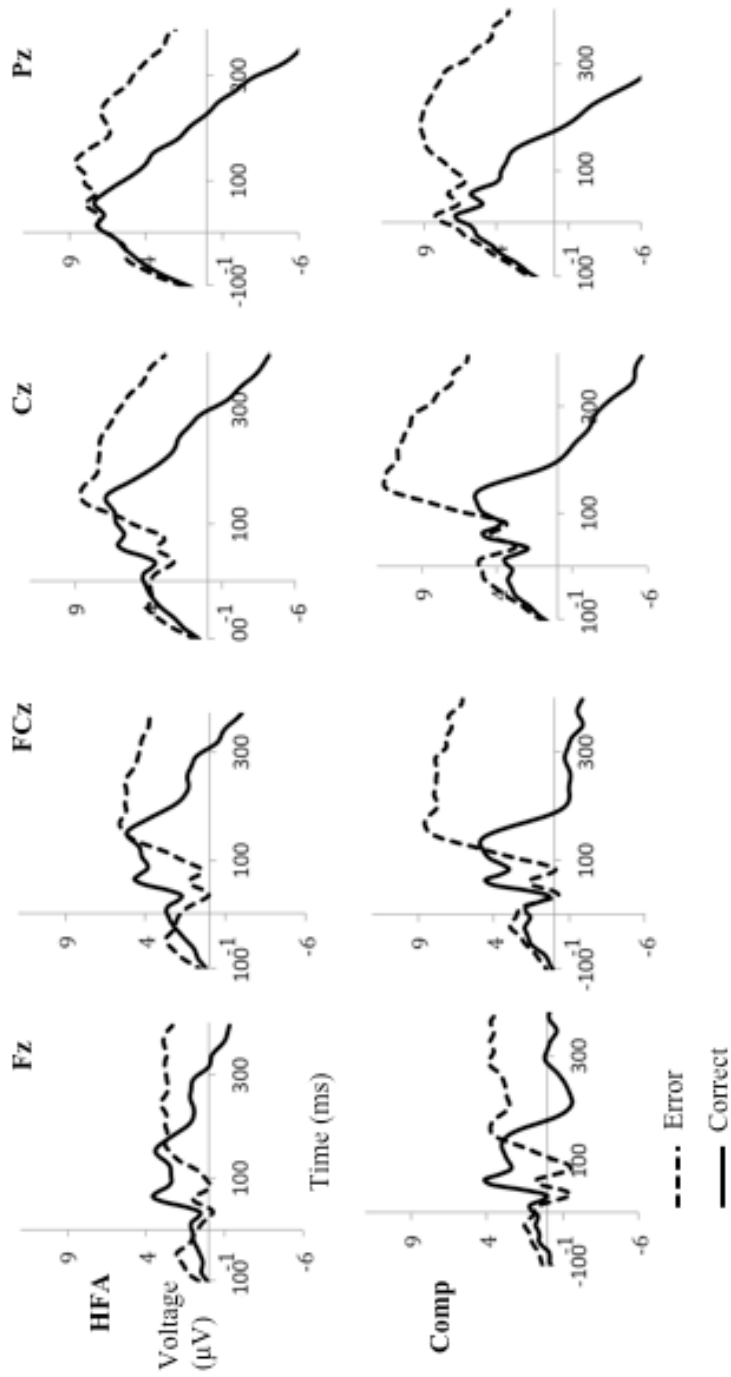
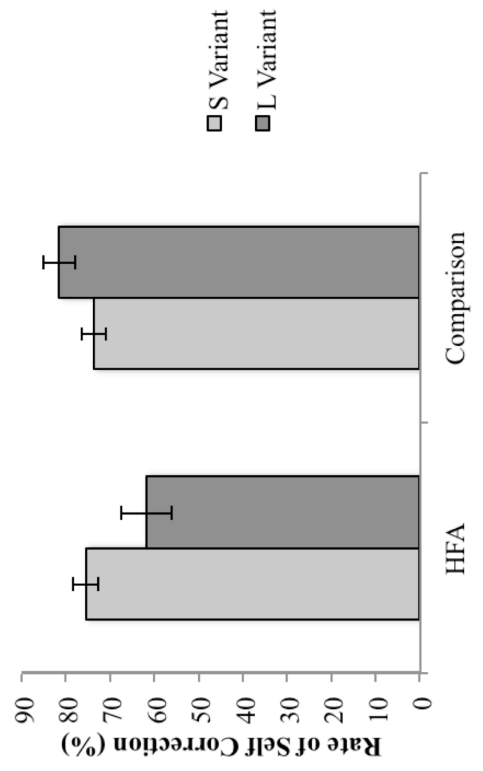


Figure 2.  
5-HTTLPR Bi-Allelic Categorization and Diagnostic Group Differences in Rate of Self Correction



Note: Rate of self-correction is reported in percentage (0-100).