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Multi-Method Investigation of Gene-Environment Interplay and ADHD

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James Janford Li

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ABSTRACT OF THE DISSERTATION

Multi-Method Investigation of Gene-Environment Interplay and ADHD

by

James Janford Li

Doctor of Philosophy in Psychology

University of California, Los Angeles, 2013

Professor Steve S. Lee, Chair

Attention-deficit/hyperactivity disorder (ADHD) is a childhood-onset disorder characterized by developmentally-aberrant and impairing levels of inattention and/or hyperactivity-impulsivity (Diagnostic and Statistical Manual of Mental Disorders, 4th edition - Text Revision; American Psychiatric Association, 2000). ADHD affects approximately 8-12% of school-aged children worldwide (Froehlich et al., 2007), and prospectively predicts a wide range of negative outcomes, including academic failure, criminality, substance abuse, and neuropsychological impairment (Barkley & Fischer, 2010; Langley et al., 2010; Molina et al., 2009).

Although there is consensus that ADHD is influenced by biological (e.g., genetics) and environmental factors (e.g., parenting) (Nigg, Nikolas, & Burt, 2010; Plomin, Owen, & McGuffin, 1994), relatively little research has directly assessed how biological and environmental factors interact in the development of ADHD and other forms of psychopathology (Moffitt, Caspi, & Rutter, 2006). Genetic influences on psychopathology are likely to depend on exposure to or the experience of varying environmental conditions such that some children exposed to environmental adversity (e.g., abuse, maladaptive parenting) develop psychopathology whereas others do not (i.e., gene x environment interaction; G×E) (Waldman, 2007). The primary aim of this study is to investigate the interactive effects of several functional polymorphisms and their interaction with environmental factors that are known to influence ADHD and related phenotypes. My dissertation will interrogate these influences using multiple research designs (i.e., cross-sectional, longitudinal) and methods (i.e., rating scales, experimental manipulations), including a large prospective longitudinal study of unselected youth and an intensively characterized sample of school-age children with and without ADHD being followed prospectively.

The dissertation of James Janford Li is approved.

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Li, J. J., & Lee, S. S. (2012a). Association of positive and negative parenting behavior with childhood ADHD: Interactions with offspring monoamine oxidase A (MAO-A) genotype. *Journal of Abnormal Child Psychology, 40*, 165-175.

Li, J. J. & Lee, S. S. (2012b). Interaction of dopamine transporter (DAT1) genotype and maltreatment for ADHD: A latent class analysis. *Journal of Child Psychology and Psychiatry, 53,* 997-1005.

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Li, J. J., Cutting, L. E., Ryan, M., Zilioli, M., Denckla, M. B. & Mahone, E. M. (2009). Response variability in rapid automatized naming predicts reading comprehension. *Journal of Clinical and Experimental Neuropsychology, 31,* 877-888.

SELECTED PRESENTATIONS

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Chapter I. Attention-Deficit/Hyperactivity Disorder (ADHD)

According to the *Diagnostic and Statistical Manual of Mental Disorder, 4th Edition-Text Revision* (DSM-IV-TR; American Psychiatric Association [APA], 2000), ADHD consists of two dimensions: inattention (e.g., difficulty sustaining attention, disorganization, avoidance of tasks that require sustained mental effort) and hyperactivity-impulsivity (e.g., difficulty sitting still, fidgeting or squirming, inability to think before acting, difficulty waiting for one's turn) There are three subtypes of ADHD based on these two dimensions: (1) Predominantly Inattentive type (Pre-I), which is characterized by at least six symptoms from the inattention cluster and fewer than six symptoms of hyperactivity and (2) Predominantly Hyperactive-Impulsive type (Pre-HI), which is characterized by at least six symptoms from the hyperactivity and impulsivity cluster and fewer than six symptoms of inattention. The Combined type (C) includes at least six symptoms from both clusters. Diagnostic criteria require that ADHD symptoms must persist for at least six months, be present in multiple settings (e.g., at home and school), and some symptoms must have been present prior to age of seven. In addition, there must be significant impairment in social, academic or occupational functioning and symptoms must be independent of a pervasive developmental disorder or other Axis I disorder (e.g., anxiety disorder).

Prevalence

ADHD affects approximately 8% of school-aged children in the United States and 8-12% of children worldwide (Froehlich et al., 2007; Faraone et al., 2003). ADHD is more prevalent in boys than girls, with ratios ranging from 2:1 to 9:1 depending on the subtype and setting (i.e., clinic referred vs. school) (APA, 2000). In a national interview survey of parents of 10,367 children (age 4 to 17) in the United States, 4.2% of males and 1.8% of females had clinically significant ADHD symptoms according to the Strengths and Difficulties Questionnaire (Cuffe,

Moore, & McKeown, 2005). However, these estimates varied by age (prevalence highest for boys 9 to 13 years old), race-ethnicity (highest for male African-Americans), and family income (boys in families reporting less than $20,000 income) (Cuffe et al., 2005). Comparable prevalence rates were reported in a nationally representative epidemiological study in the United States (aged 8 to 15, *n* = 3,082) (Froehlich et al., 2007) where approximately 8.7% of youth were diagnosed with ADHD according to a structured clinical interview. Consistent with Cuffe et al. (2005), impoverished children were more likely to be diagnosed with ADHD than wealthier children (adjusted odds ratio = 2.3). Overall, epidemiological studies suggest that ADHD is highly prevalent with variability as a function of the child's age, sex, race-ethnicity, and family income.

Comorbidity

ADHD is frequently comorbid with externalizing disorders [i.e., oppositional defiant disorder (ODD), conduct disorder (CD)], and to a lesser extent, internalizing disorders (e.g., anxiety, depression) (Angold, 1999; Jensen, Martin, & Cantwell, 1997). Based on a pooled population-based sample, Cohen et al. (1989) and Velez et al. (1989) interviewed 776 children (aged 9-18), and their parents using the Diagnostic Interview Schedule for Children (DISC) and found that among children with ADHD (n = 93), 56% had comorbid ODD, 57% had comorbid anxiety, and 13% had comorbid depression. In a large clinical sample of children with ADHD (Elia, Ambrosini, & Berrettini, 2008), 41% of youth had comorbid ODD, 21% had Major Depression, and 15% had Generalized Anxiety Disorder (GAD). ODD was significantly more common among the Hyperactive and Combined Type youth compared to the Inattentive Type (Elia et al., 2008), and only one-third of ADHD probands had "pure" ADHD (Elia et al., 2008).

Collectively, the literature strongly suggests that ADHD is highly comorbid and that studies of ADHD must adequately consider the influence of comorbid behavior and mood problems.

Impairment

ADHD is reliably associated with many adverse social, academic, behavioral, and emotional outcomes, independent of its comorbidity with externalizing and internalizing problems (Langley et al., 2010; Molina et al., 2009). In a four-year prospective study of biological siblings of children (age 6-17) with and without ADHD (n = 174 and 129, respectively), psychiatric, neuropsychological, academic, and psychosocial outcomes were significantly worse for siblings of ADHD youth than controls (Faraone et al., 1996). Siblings of ADHD probands showed significantly increased rates of psychiatric disorders and impairment compared to controls at the four-year follow-up, including ADHD, CD, ODD, major depression, and drug abuse, as well as substandard academic achievement (Faraone et al., 1996). Recent meta-analytic evidence persuasively suggests that children with ADHD are significantly more likely to develop substance abuse and dependence across multiple substances (i.e., alcohol, nicotine, cocaine, marijuana) (Lee, Humphreys, Flory, Liu, & Glass, 2011) and similar developmentally-sensitive studies further suggest that adult outcomes of children with ADHD are characterized by elevated rates of criminality, substance abuse, and occupational instability (Barkley & Fischer, 2010). Families of children with ADHD also reported higher levels of negative parent-child relationships, parenting stress, parental psychopathology and reduced parent efficacy (Harpin, 2005; Johnston & Mash, 2001). In addition to its clinical significance across multiple domains of child and family development, ADHD negatively affects society through its substantial economic burden on mental health and educational services (Pelham, Foster & Robb, 2007).

Chapter II. The Proposed Study

There is replicated evidence that environmental adversity, including prenatal exposure to maternal smoking (Motlagh et al., 2010; Froehlich et al., 2009), negative parenting behavior (Chronis-Tuscano et al., 2008), and maltreatment (Ford et al., 2000), are positively associated with the developmental course of ADHD. However, these associations do not necessarily reflect causal processes given that such experiences are not sufficient for developing ADHD (Caspi & Moffitt, 2006). Moreover, many environmental risk factors are not specific to ADHD. For example, childhood maltreatment (i.e., neglect, physical and sexual abuse) is not only a risk factor for ADHD, but is also a significant risk factor for cognitive deficits, ODD and CD, depression, and other anxiety disorders (i.e., multifinality) (Glaser, 2000; Ford et al., 2000; Kaufman & Charney, 2001). In addition, many children exposed to maltreatment still function reasonably well, perhaps due to resilience-promoting factors (Cicchetti et al., 2009; Heller et al., 1999). Given that bivariate associations betray the likely complex array of factors underlying the development of ADHD and its prognosis, conceptual models of ADHD must consist of an "interactive, multidimensional, multidirectional" framework involving environment, personality, and biological factors (Feiring & Lewis, 1987). Indeed, developmental psychopathology has long emphasized that psychopathology reflects dynamic developmental processes that interact or co-occur (Hinshaw, 2002). For example, parenting practices influence child development (through diverse processes including common genetic factors, socialization, modeling), but "child effects" are also salient (Lytton, 1990), given that child characteristics (e.g., behavior, temperament) influence parenting as well as other contextual influences, again reflecting transactional processes, especially over time (Hinshaw, 2002).

Given the importance of interactive models of environmental and biological influence in psychopathology, studies of gene-environment interaction (G×E) have become increasingly important. G×E research tests whether the association of genetic variation and outcome varies systematically as a function of environmental experience (Moffitt, Caspi, & Rutter, 2006). In the first measured G×E study for psychopathology, Caspi et al. (2002) found that the association of a common polymorphism in the monoamine-oxidase A (MAO-A) gene and violence and antisocial behavior (ASB) was moderated by childhood maltreatment among men in a large New Zealand birth cohort. That is, men who were maltreated as children were more likely to develop antisocial problems than non-maltreated men, but only among individuals with the low-expressing MAO-A genotype. Given the plausibility of genotype moderation of children's sensitivity to environmental adversity and subsequent replications (Foley et al., 2004; Kim-Cohen et al., 2006), including through meta-analysis (see Nelson & Trainor, 2007), this finding and overall approach has advanced our understanding of synergistic relations between individual differences in genetic liability and environmental experience and the development of ADHD, schizophrenia, substance use disorders, and mood disorders (see reviews by Nigg, Nikolas, & Burt, 2010; Wermter et al., 2010; Thapar et al., 2007).

Despite these initial contributions, several limitations of G×E studies have yet to be fully addressed. First, despite high heritability estimates, ADHD is frequently comorbid with ODD and CD (Nigg, Nikolas, & Burt, 2010; Jensen et al., 1997). In addition, inattention and hyperactivity/impulsivity are developmentally and etiologically distinct (Hinshaw, 1987), as suggested by divergent patterns of heritability, developmental course, and psychosocial correlates (Hinshaw, 2002). Thus, G×E models of traditional diagnostic categories are complicated given that psychiatric phenotypes are complex, multidimensional, and reflect

multiple etiologies. Additional work must be conducted on improving the accuracy and resolution of the ADHD phenotype, including multilevel and multivariate approaches, particularly within the context of longitudinal and experimental designs (Hinshaw, 2002; Rutter & Sroufe, 2000). For example, advanced latent clustering models can elucidate causal pathways within G×E models across disparate levels of influence (Li & Lee, 2010).

Second, although there is emerging evidence that offspring genotype interacts with environmental factors in the development of ADHD (see Thapar et al., 2007), relatively little is known about the mechanisms that account for these associations (Nigg, Nikolas, & Burt, 2010). G×E effects may also reflect unmeasured variables that influence both genetic and environmental variables (Belsky & Pleuss, 2009). In particular, genes not only increase susceptibility to a disorder, but also elicit environmental experiences (e.g., gene-environment correlation; *rGE*), thereby confounding G×E interpretations (Jaffee, & Price, 2007). Thus, it is necessary to identify mediators of genetic or environmental effects, which may shed light on the causal mechanisms that produce outcome. Although intermediate constructs (i.e., endophenotypes) may mediate genetic and/or environmental effects on ADHD, given their proximity to the latent substrates of the explicit phenotype (Gottesman & Gould, 2003), relatively few studies have explicitly tested endophenotypes as mediators of genetic influences on psychopathology. The endophenotype approach in G×E is a promising avenue for investigation because it may help explain causal mechanisms underlying genetic and environmental associations with ADHD, but few studies have formally evaluated its intermediate role between genetic variation and outcome.

Finally, G×E studies have traditionally focused on environmental adversity (e.g., prenatal exposure to teratogens, maltreatment) given the ascendancy of diathesis-stress conceptualizations in psychopathology (Belsky & Pleuss, 2009). Emerging models and reinterpretations of previous

findings now suggest that the same genetically "vulnerable" children are simultaneously more responsive to the effects from environmental enrichment, a phenomenon called "differential susceptibility" (Belsky & Pleuss, 2009; Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2007). In other words, genotype may concurrently confer heightened sensitivity to environmental enrichment and adversity (Belsky & Pleuss, 2009). For example, 3-year-old children carrying the dopamine D4 receptor (DRD4) VNTR 7-repeat allele exhibited the highest rates of externalizing behaviors at baseline (compared to carriers of the 4-repeat allele), but also had the greatest decrease in externalizing behaviors one year later following an intervention to promote positive and sensitive parenting (Bakermans-Kranenburg et al., 2008). Similarly, in their replication of the Caspi et al. (2002) findings, Kim-Cohen et al. (2006) found that 7-year-old children with the low-expressing MAO-A genotype had the most mental health problems and ADHD symptoms if they were victims of abuse, but concurrently had the fewest overall mental health problems if they were not exposed to abuse, compared to high-activity MAO-A youth. These studies suggest that genes may influence responsiveness to environmental conditions overall, rather than to specific reactivity to environmental adversity. However, empirical tests of differential susceptibility within the context of G×E are in their infancy, given that few studies have explicitly tested environmental variation across the continuum (i.e., positive *and* negative aspects). These investigations may carry important implications because they not only help explain potential mechanisms of influence, but may also impact intervention designs that focus on environmental change and enrichment (Belsky et al., 2007).

Overall, despite emerging evidence that measured G×E underlying psychopathology is both plausible and important, existing G×E models are often limited by (1) the imprecision of characterizing the phenotype, (2) confounds from other unmeasured genetic/environmental

variables, and (3) limited (i.e., unidirectional) investigation of the environmental criterion. Using a multi-method and multivariate approach (i.e., cross-sectional and experimental designs), the proposed set of studies collectively share the aim of identifying how *measured* candidate genes and *measured* environmental factors influence ADHD. Study I will employ latent class analysis (LCA) to uncover empirically-derived subtypes of disruptive behavior problems from a large population-based sample. LCA detects empirically-distinct categories (rather than relying on *a priori* categorizations based on theory or methods that may not be indicated in the sample) of ADHD and comorbid externalizing problems. This is an important complement to existing studies of G×E, which are largely based on traditional diagnostic designations. In Study II and III, I will test mediators and moderators of genetic effects on ADHD by featuring a more stringent test of environmental and genetic variation. This will be done by using a combination of observational and rating scale methods to assess the environmental criterion (i.e., parenting behavior) as well as assessment of putative endophenotypes (i.e., neurocognitive functions) for ADHD symptoms (Waldman, 2005; Willcutt et al., 2005). Finally, Study IV will utilize an experimentally-controlled examination of G×E (and concurrently, the differential susceptibility theory) by examining child genetic influences on behavior and cognitive performance *in-vivo,* via experimental manipulation of the environment (i.e., parenting). This approach will be complemented by traditional measures of the independent variable that are also collected longitudinally. These studies collectively represent a rigorous approach to G×E by using multiple designs and methods to understand gene-environment interplay in ADHD.

Chapter III. Genetics of ADHD

Emerging research in the field of behavioral genetics has shed light on the genetic factors that may play a role in the etiology of ADHD. For example, twin studies, which compare concordance rates of ADHD between monozygotic twins to dizygotic twins, estimates how much phenotypic variability can be decomposed into unique environmental, shared environmental, and additive genetic components (Sherman, Iacono, & McGue, 1997). To provide an overview of the genetic influences that underlie ADHD, the following sections review the magnitude of familial and genetic influences on ADHD and its putative subtypes based on family, adoption, and twin study designs. Subsequently, this discussion will be followed by a review of the literature pertaining to the molecular genetics of ADHD.

Behavioral Genetics

Several studies have reported that the risk of ADHD is significantly elevated among parents and siblings of children with ADHD (a two- to eightfold increase in risk) (see review by Faraone, Biederman, & Monuteaux, 2000). However, environmental differences (or similarities) may also account for the increase in risk of ADHD within families. That is, family members not only share genes, but also a common environment, thus necessitating designs that are sensitive to shared environmental factors. In family studies, ADHD is significantly associated within family members even in case-controlled, double-blind studies that specifically controlled for important environmental variables such as gender, family structure, and socioeconomic status (Biederman et al., 1990, 1992; Faraone et al., 2000a). Similarly, other family-based studies have found that the relative risk of ADHD is higher among first degree relatives with persistent ADHD (i.e., stable across the lifetime) than those with childhood-limited ADHD (Faraone et al., 2005), strongly suggesting that liability for ADHD is associated with the degree of genetic relatedness.

However, family studies are limited because they are unable to disentangle genetic and environmental influences. Adoption studies compare the prevalence of a disorder in the biological family to those in the adoptive family, with the implication that the degree of relatedness should be greater between the affected individual (i.e., adoptee) and his/her biological family than adoptive family, given salient genetic influences (Smoller & Finn, 2003). Hence, adoption studies are useful because they disentangle gene-environment correlation (i.e., adopted offspring are exposed to different environments), although some environmental influences are unlikely to be purely environmental (i.e., prenatal environment, which is biologically influenced) (Willcutt et al., 2005). Although there are relatively few adoption studies of ADHD (Willcutt et al., 2005), they provide further evidence of genetic influence. Alberts-Corush, Firestone and Goodman (1986) compared 176 biological and adoptive parents of hyperactive and control children and found that biological parents of hyperactive children showed more attentional difficulties, slower reaction times on a maze task, and fewer correct recognition hits on an apprehension task than adoptive parents. Sprich et al. (2000) estimated that 18% of biological parents of nonadopted ADHD probands had ADHD, compared to just 6% for adoptive parents of ADHD probands. These studies suggest that the familial risk of ADHD is likely genetically influenced, rather than due exclusively to shared environmental factors. However, adoption studies are also limited in several respects. Adoptive parents differ from non-adoptive parents, as adoption agencies tend to place adopted children with higher-functioning parents with greater resources (Plomin et al., 2001; Willcutt et al., 2005). In addition, records of biological parents of adopted children are very difficult to procure, and also explain why there are relatively few adoption studies at large (Cadoret, Leve, & Devor, 1997; Willcutt et al., 2005).

Twin studies more directly disentangle genetic and environmental influences (Faraone et al., 2005; Willcutt et al., 2005) because monozyotic ("identical") twins, who share 100% of their genes on average, can be compared to dizygotic ("fraternal") twins, who share 50% of their genes on average. Although results from twin studies across multiple cultural contexts vary in terms of their precise heritability estimate for ADHD, they nevertheless provide overwhelming evidence that ADHD is significantly heritable and to a degree that approximates other major forms of psychopathology including bipolar disorder, autism, and schizophrenia (Levy et al., 1997; Willcutt, Pennington, & DeFries, 2000). ADHD concordance rates among monozygotic twin pairs (58% - 82%) are consistently and significantly higher than same-sex dizygotic twin pairs (31% -38%) (Willcutt et al., 2005). With respect to heritability, Larsson, Larsson and Lichtenstein (2004) conducted a 5-year longitudinal study (children were age 8-9 at baseline) of ADHD symptoms among 1,480 monozygotic and dizygotic twin pairs born in Sweden and found that genetic factors accounted for 62% and 55% of the variance for boys and girls, respectively. Moreover, the stability of ADHD symptoms across the 5-year span was primarily due to genetic factors (Larsson, Larrson, & Lichtenstein, 2004). Heritability estimates from other large population-based samples have provided similar estimates, ranging from .4 to .9 (Thapar, Holmes, Poulton & Harrington, 1999), with a recent meta-analysis estimating that 73% and 71% of the variance in hyperactivity and inattention, respectively, was due to additive genetic factors (Nikolas & Burt, 2010). Crucially, in this study, both dimensions of ADHD were heritable, although they differed based on the type of genetic influence (dominant and additive genetic effects were stronger for inattention and hyperactivity, respectively).

Behavioral genetic studies provide compelling evidence that ADHD is significantly influenced by genetics. ADHD clusters in families, shows divergent patterns of association

between biological and adoptive families, and is significantly heritable across population-based studies (see recent meta-analysis by Nikolas & Burt, 2010). However, behavior genetic studies are limited to *inferring* heritability rather than directly isolating genetic differences (e.g., genotype). That is, although there are reliable heritability estimates, genetic contributions from family, adoption and twin studies do not reveal which specific genes are relevant to ADHD. Molecular genetic studies (i.e., candidate genes) provide additional insight into ADHD by clarifying its potential pathophysiology based on theoretical grounds.

Molecular Genetics

Substantial evidence suggests that ADHD is at least partly explained by dysfunction in the frontosubcortical system, which is crucial in regulating locomotor activity, inhibitory control, and reward sensitivity (Faraone & Biederman, 1998). The frontosubcortical system is also rich in dopamine and other catecholamines (i.e., serotonin and norepinephrine), which are the primary mechanism of action in pharmacological treatments for ADHD (Spencer et al., 2007; Faraone & Biederman, 1998). Thus, molecular genetic studies of ADHD have largely focused on dopaminergic genes (Gizer, Ficks, & Waldman, 2009). Meta-analytic studies have confirmed that genes for ADHD likely involve this system, with the strongest effect sizes emerging for the dopamine transporter gene (DAT1) and the dopamine D4 receptor gene (DRD4) (Gizer, Ficks, & Waldman, 2009; Brookes et al., 2006). This section will (1) provide an overview on role of dopamine in ADHD by summarizing pharmacological, neuroimaging studies, and (2) review the current literature on DAT1 and DRD4 genes and their association with ADHD, respectively.

Dopamine Neurotransmission of ADHD

Dopamine (DA) plays a central role as a mechanism of action for stimulant medication for ADHD (Solanto, 1998). Methylphenidates and amphetamines, the primary stimulant drugs

used to treat ADHD, elevate extracellular DA and norepinephrine levels by increasing receptor activation and signal transmission in the prefrontal cortex, hippocampus, nucleus accumbens, and the medial septal area (Arnsten, 2006; Swanson et al., 2005). Amphetamines additionally inhibit reuptake of monoamines (DA, norepinephrine, and serotonin) and effectively increasing intracellular release of DA (Rothman & Baumann, 2003). Stimulant medications are well documented to effectively reduce activity and increase attention in children (see "review of reviews" by Swanson et al., 1993). Specifically, very low doses of amphetamines (~0.5 mg/kg) and methylphenidates (4-5 mg/kg) stimulate DA autoreceptors and significantly reduce locomotor activity and reward sensitivity in humans (Solanto, 1998).

Neuroimaging Studies of ADHD

The crucial role of DA in ADHD is also implicated by studies on brain regions (i.e., prefrontal cortex) that are rich in DA (see review by Durston, 2010). Adult patients with discrete lesions in the orbitofrontal, dorsolateral and dorsomedial regions of the brain performed significantly worse on tasks of executive function compared to controls, including working memory, planning, and attentional set-shifting (Manes et al., 2002). Rubia et al. (2010) used functional magnetic resonance imaging (fMRI) of a cognitive switching task to compare boys with ADHD to conduct disorder-only and healthy controls. Among children with "pure" ADHD, they found significant underactivation in the right and left inferior prefrontal cortex compared to the other two groups, which suggests ADHD-specific neuroanatomical abnormalities.

In addition to structural differences in brain morphology in ADHD, imaging genetics have also revealed some of the neural consequences of genetic variation (Durston, 2010). Brain imaging techniques allows researchers to quantitatively assess brain neurochemistry *in-vivo* (e.g., DA transporter availability) (Durston, 2010). These methods also provide a more direct link

between gene expression and corresponding changes to brain structure, neurochemistry, and function. For example, Shaw et al. (2007) found a significant association for the 7-repeat allele of the 48-base pair (bp) variable number of tandem repeat (VNTR) polymorphism of the DA D4 receptor gene (DRD4) and a thinning of the right orbitofrontal/inferior prefrontal cortex and posterior parietal cortex among children with ADHD, compared to age-matched healthy controls. This finding is noteworthy because these regions of the brain are also associated with attentional and inhibitory control (Shaw et al., 2007). Similarly, electroencephalogram (EEG) frequency bands were associated with cortical activation, but only among parents and children with ADHD with the DRD4 7-repeat allele, suggesting that EEG is familial and may constitute an endophenotype for ADHD (Loo et al., 2010). Finally, Spencer et al. (2007) measured DA transporter (DAT) binding using position emission tomography (PET) and found significantly increased DAT binding in the right caudate of adults with ADHD compared to control adults, which is consistent with prevailing theories of DA dysregulation in ADHD. Collectively, these studies have provided a stronger link between genetic influences and brain structure and neurochemistry overall and on the functional role of DA in ADHD specifically (Durston, 2010).

Dopamine Transporter (DAT1)

The DA transporter gene (DAT1, SLC6A3) is located on chromosome 5p15.3 (Giros et al., 1992) and is responsible for coding proteins that transport DA from the synaptic cleft back to the pre-synaptic neuron. These proteins are mostly expressed in the striatum and nucleus accumbens and are primarily involved in DA regulation in those brain regions (Gizer et al., 2009). DAT1 is of particular interest in ADHD because methylphenidates and amphetamines, which are the most widely prescribed medications for ADHD, work by inhibiting the DA transporter and subsequently increasing the amount of extracellular DA in the brain. Increasing

DA activity typically results in therapeutic effects (e.g., increased attentiveness, decreased hyperactivity) in children and adults (Spencer et al., 2007). In addition, DAT1 knock-out mice exhibit behavioral features similar to ADHD, including increased hyperactivity and deficits in inhibitory behavior when compared to wild-type mice with intact copies of the DAT1 gene (Giros et al., 1996; Jones et al., 1999). Deactivation of DAT1 results in decreased levels of DA in presynaptic terminals, significant down-regulation of D1 and D2 receptor expression, and a loss of tyrosine hydroxylase (Fuke et al., 2001), suggesting that DAT1 is important in the expression of cortical levels DA in the synapse. Moreover, treatment with methylphenidate effectively normalized levels of hyperactivity in DAT1 knock-out mice (Giros et al., 1996). The neurobiological mechanisms (i.e., down-regulation of DA receptors) and behavioral findings (i.e., increased hyperactivity and lack of inhibition) reported from animal studies strongly suggest that DAT1 may have a similar role in humans as well.

In humans, the most widely studied polymorphism in DAT1 is the variable number of tandem repeats (VNTR) sequence in the 3' untranslated region (UTR) that is 40 base pairs (bp) long (Gizer et al., 2009). Although alleles with 3 to 13 repeats have been discovered (Fuke et al., 2001; Cornish et al., 2005), the 9 (440-bp) and 10 (480-bp) repeat alleles are the most frequent in the population (Kang et al., 1999). The polymorphism is also believed to be functional (Gizer et al., 2009). Single photon emission computed tomography was used to demonstrate that variation in the VNTR was associated with DA transporter availability and binding potential (Heinz et al., 2000; Gizer et al., 2009). This polymorphism has also been identified as a risk factor for ADHD (see meta-analyses by Swanson et al., 2000 and Gizer et al., 2009). Cook et al. (1995) were the first to demonstrate a significant association of the 10-repeat allele and ADHD in a sample of 49 families (i.e., mothers, fathers, and affected offspring). Auerbach et al. (2010) reported an

association between boys with the 10/10 DAT1 genotype and high hyperactivity/impulsivity ratings among 96 four-year old boys at risk for ADHD. However, results for this association have not been entirely consistent. Franke et al. (2010) found that the 9/9 DAT1 (and not the 10/10 genotype) genotype was associated with persistent ADHD in a pooled sample of 1440 patients and 1769 controls whereas Bruggemann et al. (2007) did not find an association between DAT1 and ADHD. In light of the inconsistent findings, it appears the precise nature of DAT1 functionality is unknown. However, given the role of DA on attentional and locomotor abilities, the evidence provided suggests that DAT1 is an important marker for ADHD.

Dopamine D4 receptor (DRD4)

The DA D4 receptor gene (DRD4) is another widely studied candidate gene in studies of ADHD (Brookes et al., 2006; Gizer et al., 2009). In humans, DRD4 is located on chromosome 11p15.5 (Gelernter et al., 1992) and codes DA receptors that are predominantly located in frontal lobe regions of the brain, including the orbitofrontal cortex and anterior cingulate (Gizer et al., 2009). Specifically, D4 receptors inhibit neural firing in the prefrontal cortex (Rubenstein et al., 1997), which suggests that the gene may be involved in executive control, inhibition, and other processes that regulate attention (Swanson et al., 1998). Moreover, DRD4 is consistently associated with novelty-seeking, which is also associated with impulsivity and hyperactivity in ADHD (Faraone et al., 1999). Animal studies have previously demonstrated that DRD4 knockout mice display reduced novelty-seeking. For example, Dulawa et al. (1999) found that DRD4 knockout mice exhibited significantly less responsiveness too novel situations, compared to wild-type mice. Rubinstein et al. (1997) found that compared to wild-type mice, DRD4 knockout mice exhibited hypo-motor locomotion, reduced novelty-seeking, and better performance on complex motor tasks. Taken together, the results suggest that DRD4 modulates

neural DA firing and acts as an inhibitory receptor that influences several cognitive and behavioral phenotypes related to ADHD.

Most studies of ADHD have focused on exon 3 of the 48-bp VNTR in DRD4 (Gizer et al., 2009). There is substantial variation in the VNTR, which includes alleles that are repeated between 2 and 11 times (Wang et al., 2004). The most frequent alleles in the population are the 2, 4 and 7-repeat alleles, which represent greater than 90% of the observed population diversity (Wang et al., 2004). The function of these alleles is not entirely clear. Some evidence suggests that the 7-repeat variant exhibits blunted cyclic-AMP levels compared to the 2- and 4-repeat variants (Asghari et al., 1995). However, the functional differences between these variants are minimal, and importantly, may or may not be of clinical relevance (Asghari et al., 1995; Gizer et al., 2009). Nonetheless, studies that have investigated this VNTR have repeatedly found significant associations with ADHD. Swanson et al. (1998) found over-transmission of the 7-repeat allele among 52 families with an ADHD proband. This association was also replicated by Gornick et al. (2007), who found significant over-transmission of the 7-repeat allele among children with ADHD (23%) versus healthy controls (17%). Moreover, results from meta-analyses have demonstrated an association of the 7-repeat allele and ADHD, albeit with small effect sizes (see Li et al., 2006 and Gizer et al., 2009).

However, conflicting results have been reported as well, with some studies reporting associations for non-7-repeat variants. Shaw et al. (2007) conducted a 6-year longitudinal study on the association between the 48-bp VNTR polymorphism in DRD4 and prefrontal brain development using structural MRI in children (mean age = 10) with and without ADHD. Although the 7-repeat allele was associated with cortical thinning in the prefrontal and parietal cortex, children carrying this allele with ADHD also had better clinical outcomes (i.e., fewer

symptoms of ADHD) at the 6-year follow up than children with ADHD who did not carry the 7-repeat allele. Johnson et al. (2008) investigated the association between the same VNTR in DRD4 and neurocognitive performance in 128 children with ADHD. They found that the group *without* the 7-repeat variant made significantly more errors of commission and exhibited more response variability on the Sustained Attention Task than children carrying at least one copy of the 7-repeat allele. Swanson et al. (2000) suggested that the 7-present ADHD subgroup may be characterized by a "partial ADHD syndrome," which includes behavioral difficulties but with intact cognition, versus the "full ADHD syndrome" of the 7-absent ADHD subgroup, which exhibit cognitive deficits in addition to behavioral difficulties. However, this hypothesis has yet to be empirically tested.

Summary

ADHD is associated with dysfunction in the frontosubcortical system of the brain, which is rich in DA and other catecholamines (i.e., norepinephrine, and serotonin) (Faraone & Biederman, 1998). Given that children and adults with ADHD show therapeutic responses to methylphenidates and amphetamines, both of which block the re-uptake of DA and lead to a subsequent increase in synaptic levels of DA, most candidate gene studies of ADHD have focused on the 40-bp VNTR 3'UTR polymorphism in DAT1 and the 48-bp VNTR polymorphism in the exon 3 terminal of DRD4 (Brookes et al., 2006). These genes are principally involved transcribing proteins that regulate DA levels in the brain. Indeed, DAT1 and DRD4 are currently the strongest candidate genes for ADHD based on meta-analytic reviews (see Brookes et al., 2006). Overall, DAT1 and DRD4 are strongly implicated in the etiology of ADHD, and are promising candidate genes for the present study.

Chapter IV. Gene-Environment Interaction

Environmental influences on ADHD are evident by virtue of the imperfect concordance between monozygotic twins and because environmental conditions influence gene expression (Moffitt, 2005). Furthermore, twin studies have indicated a significant contribution of shared and unique environmental influences on ADHD (Thapar et al., 2007; Burt , McGue, Krueger, & Iacono, 2007). However, environmental influences are likely underestimated given that they are typically subsumed into heritability estimates, suggesting the likelihood of gene-environment interactions (G×E) (Thapar et al., 2007). The following section will summarize the role of two well-established factors that influence ADHD and related phenotypes: maltreatment and parenting behaviors. The extant literature on G×E for maltreatment and parenting behaviors in ADHD are also discussed.

Maltreatment

Childhood maltreatment (e.g., child abuse, sexual abuse, neglect) is a robust risk factor for externalizing and internalizing disorders and cognitive deficits (see reviews by Glaser, 2000 and Kaufman & Charney, 2001). Despite its negative impact on child development, there are relatively few studies on the association between maltreatment and ADHD (Briscoe-Smith & Hinshaw, 2006). In a population-based sample of adolescents (n = 14,322), Ouyang et al. (2008) found that youth exposed to supervision neglect, physical abuse, and sexual abuse as children were more likely to report greater inattention and hyperactivity symptoms of ADHD. Similarly, girls (aged 6 to 12) who were exposed to child abuse were more likely to have ADHD, have higher rates of externalizing problems, and more likely to be rejected by peers than girls who were not exposed to child abuse (Briscoe-Smith & Hinshaw, 2006). These formative studies still

require replication, but given their negative impact across similar phenotypes (e.g., externalizing disorders), maltreatment is a likely and plausible risk factor for ADHD.

Child maltreatment also impacts the development of subcortical structures in the brain, making it a strong candidate in G×E studies (Moffitt et al., 2006). Maltreatment leads to disruptions to the hypothalamic-pituitary-adrenal (HPA) axis and attenuated development of the hippocampus, amygdala, and left neocortex (Teicher et al., 2003), which are associated with negative behavioral and cognitive outcomes. For example, early stress caused by maltreatment may cause abnormal development of the cerebellar vermis, which is known to play a critical role in attention, language, cognition and affect (Teicher et al., 2003). In addition, animal models suggest that early adversity may influence transcription (Champagne & Curley, 2005). For example, variability in licking/grooming (LG) behaviors leads to differentiated expression of glucocorticoid receptors (GR) in the hippocampus in rats (Weaver et al., 2004). These receptors are believed to regulate the attachment of methyl groups to DNA, which block transcription factors from gaining access to the gene, leading to gene silencing (Champagne & Curley, 2005). Weaver et al. (2004) found that adult offspring exposed to low LG had decreased GR expression and elevated DNA methylation compared to adult rats exposed to high LG. Moreover, these methylation patterns persisted throughout the lifespan, suggesting that early social experiences (e.g., neglect) influence developmental aspects of gene expression. Overall, the evidence presented implicate that early adversity in the form of maltreatment may interact with genotype to influence phenotypic expression.

In addition to the significant G×E involving child MAO-A genotype, child abuse and ADHD reported by Kim-Cohen et al. (2006), Kiive, Kurrikoff, Maestu, and Harro (2010) examined the interaction of the α2A-adrenergic receptor gene (ADRA2A), which indirectly

regulates dopamine and serotonin release, and maltreatment/poor family relationships on ADHD in a sample of 429 15-year-old Estonian youth. A significant interaction was observed, such that maltreated boys with the CC genotype had significantly more hyperactivity and inattention symptoms than non-maltreated boys (Kiive et al., 2010). Moreover, maltreated girls with the CC genotype had significantly higher aggression and inattention symptoms compared to maltreated girls with the GG genotype. In another study, a significant interaction was observed between a functional polymorphism in the serotonin transporter gene (5-HTTLPR) and childhood adversity (e.g., child abuse, poor financial status, frequent separation from family), such that boys with at least one-copy of the short allele scored higher on self-reported ADHD (Retz et al., 2008). In sum, emerging evidence suggests that maltreatment interacts with several dopamingeric genes, including MAO-A, ADRA2A, and 5-HTTLPR, to effect ADHD and other related phenotypes. However, no studies to date have investigated the role maltreatment and its interaction with DAT1 and DRD4, two of the most widely-replicated genes for ADHD (Brookes et al., 2006).

Parenting

Negative parenting (i.e., harsh punishment, inconsistent discipline) is an important environmental risk factor for ADHD because it is a well-established correlate of ADHD and it is biologically plausible in the context of G×E (Moffitt et al., 2006). Compared to parents of typically-developing children, parents of children with ADHD are more permissive, utilize harsher punishments, and less involved and more inconsistent in their discipline (Wells et al., 2000; Chronis-Tuscano et al., 2008). In a study of 33 predominantly hyperactive and 34 control boys (aged 47 to 62 months), parental laxness (i.e., inconsistent discipline) predicted child hyperactivity, even after controlling for conduct problems (Keown & Woodward, 2002). Furthermore, maternal and paternal inconsistent discipline significantly predicted dimensional

and categorical definitions of ADHD, independent of ODD and CD in a sample of 181 children (aged 6-12) (Ellis & Nigg, 2009). Meta-analytic findings confirm the prospective contribution of negative parenting on ADHD and disruptive behavior disorders (see Johnston & Jassy, 2007).

Despite the vast amount of research on the effects of negative parenting, there is substantially less literature on positive parenting (i.e., involvement, warmth, and positive reinforcement) and ADHD (Johnston & Mash, 2001). Negative *and* positive parenting may be crucial in the context of G×E, given that genotype may confer "differential susceptibility" to positive versus negative aspects of the environment (Belsky & Pleuss, 2009). Previous studies have demonstrated that positive parenting is unique from negative parenting, and is predictive of different outcomes in children. For example, Pettit, Bates and Dodge (1997) found that harsh/negative parenting at kindergarten better predicted grade 6 outcomes than positive parenting and each parenting domain was virtually independent from the other. Chronis et al. (2007) followed 108 preschool children with ADHD and observed positive parenting, but not negative parenting, inversely predicted CD 8 years later. These results suggest that negative and positive parenting dimensions are distinct, rather than opposite ends of the same continuum. Furthermore, they indicate the possibility that both dimensions of parenting may differentially interact with biological processes, paving the way for investigations of differential susceptibility.

Parenting behavior and maternal care more broadly are known to influence biological mechanisms in offspring. Environmental conditions can alter DNA methylation patterns, previously believed to be stable following embryogenesis (Szyf, McGowan, & Meaney, 2008), which can enhance or silence gene expression. Rats reared by genetically unrelated mothers who were frequently licked/groomed (LG) showed increased hippocampal expression, decreased hypothalamic corticotrophin releasing factor expression, and reduced hypothalamic-pituitary-

axis stress reactivity over the first 10 days of life, compared to rats that were raised by foster mothers with infrequent LG (Liu et al., 1997). Adult pups that were exposed to frequent LG were also more likely to demonstrate such behaviors to their own pups, suggesting that high quality maternal care can "program" adaptive biological responses to stimuli in offspring (Liu et al., 1997). In contrast, rats deprived of maternal care early in development exhibited significant down regulation of DA transporter and DA receptors D1, D2, and D3 relative to control rats, leading to deficits in spatial learning and memory (Zhu et al., 2010). These studies suggest that exposure to negative and positive nurturing behavior, particularly early in development, may change neurobiological systems in ways that may be partly influenced by genetic variation. However, G×E studies have largely focused on narrow definitions of negative parenting (most often maltreatment), despite evidence that positive parenting influences offspring biology and that positive parenting shows meaningfully different patterns of association with outcome relative to negative parenting (Bakermans-Kranenburg et al., 2008).

Although studies of parenting and its interaction with child genotype for ADHD are still in their infancy, G×E effects have been reported for other environmental conditions, including prenatal exposure to teratogens (Kahn et al., 2003) and maltreatment (Kim-Cohen et al., 2006; Retz et al., 2008; Kiive, Kurrikoff, Maestu, & Harro, 2010). Emerging evidence of interactive influences between parenting behavior and child genotype for externalizing behaviors may also be relevant to new G×E studies of ADHD given replicated evidence that the covariation between ADHD and externalizing behaviors reflects common genetic factors (Burt, McGue, Krueger, & Iacono, 2007; Dick et al., 2005; Nadder, Rutter, Silberg, Maes & Eaves, 2002). In a 7-8 year longitudinal study of 148 boys, the high activity MAO-A genotype was prospectively associated with conduct disorder, substance use disorder and ADHD, but only among youth exposed to less

positive parenting (Vanyukov et al., 2007). In a 5-8 year prospective longitudinal study of preschool children with and without ADHD (n = 162), the 40-bp VNTR polymorphism of the dopamine transporter (DAT1) gene interacted with observed maternal negative parenting to prospectively predict parent- and youth-reported adolescent CD such that negative parenting positively predicted CD more robustly in youth homozygous for the 9-repeat allele (Lahey et al., 2011). Finally, in a prospective longitudinal study of 250 Caucasian youth, parental physical discipline predicted delinquency and externalizing behavior, but only among individuals carrying the low-activity MAO-A allele (Edwards et al., 2010). Overall, these studies are noteworthy because MAO-A, DRD4 and DAT1 each regulate the availability of monoamines. Thus, DA genes may increase sensitivity to negative and positive parenting.

Summary

The development of ADHD is likely explained by synergistic influences between environmental experience and genetic variation. Maltreatment and negative parenting are both well-established correlates of ADHD and externalizing behaviors. In addition, these factors are known to influence brain development and transcription, suggesting plausibility for G×E (Moffitt, 2005). However, few studies have examined the influence of positive/enriching environments on child genotype and ADHD. Emerging evidence suggest that genotype may concurrently confer heightened sensitivity to environmental enrichment and adversity in a "for better or for worse" manner (Belsky & Pleuss, 2009). To improve the specificity of interactive influences between parenting behavior and offspring genetic variation in the development of ADHD, I will test the independent and interact effects of the DAT1 and DRD4 child genotype and positive and negative parenting behavior on dimensions of ADHD.

Chapter V. Neurocognitive Functions as Endophenotypes of ADHD

Although genetic factors are known to influence psychopathology, researchers have yet to identify genes that *unequivocally* contribute its susceptibility, especially given the complexity of characterizing phenotypes with no known biochemical or anatomical markers (Bearden & Freimer, 2006). Even for diseases for which there are known biological markers with which to base the diagnosis (e.g., heart disease, diabetes, hypertension), genetic association studies have yielded similarly inconsistent results (see meta-analysis by Hirschhorn, Lohmueller, Byrne, & Hirschhorn, 2002). One promising approach to improve the consistency between candidate genes and complex disorders involves identifying intermediate markers between the phenotype in question and susceptibility loci (Waldman, 2005). These mediational or intervening constructs, known as endophenotypes, have provided some leverage on studies of ADHD because they are thought to be closer to the immediate products of the genes (Waldman, 2005) and to be genetically simpler than the explicit phenotype. Thus, endophenotypes are promising constructs to utilize in molecular genetic research because they have the potential to facilitate dissection of complex psychiatric disorders (Gottesman & Gould, 2003).

The search for potential ADHD endophenotypes is challenging because they, too, are multifactorial etiologically. Consequently, criteria for putative endophenotypes in molecular genetic studies have been proposed (Almasy & Blangero, 2001; Gottesman & Gould, 2003; Waldman, 2005). Doyle et al. (2005) integrated common elements of previous guidelines, stating that endophenotypes should: (1) co-occur with disease; (2) show evidence of heritability; and (3) show familial/genetic overlap with disease. In addition to these primary criteria, endophenotypes should also demonstrate "biologically plausibility" (i.e., association with neural systems that are

implicated with the disorder) (Gottesman & Gould, 2003). The criteria outlined above will be used to evaluate the suitability of the ADHD endophenotypes proposed herein.

Association with ADHD

Neurocognitive functions (NF) are defined as a set of maintenance tools used to regulate and control higher-order actions and goal-oriented behaviors, including response inhibition, working memory, cognitive flexibility, and self-regulation (Barkley 1997, Barkley et al., 2001; Willcutt et al., 2005). NF measures are highly sensitive at differentiating clinical groups from normal controls, but not all components of NF reliably distinguish clinical groups from each other (e.g., low specificity) (Sergeant, Geurts, & Oosterlaan, 2002). However, two NF components reliably differentiate ADHD from controls and other clinical groups (e.g., sensitivity *and* specificity): response inhibition (Barkley, 1997) and working memory (Doyle et al., 2005; Waldman, 2005; Castellanos & Tannock, 2002).

Among the most reliable correlates of ADHD is response inhibition, defined as the "suppression of an attentional or behavioral response to salient but irrelevant events" (Casey et al., 1997). On tasks that require quick and accurate inhibition to motor responses (e.g., Stop Signal Task), children and adults with ADHD exhibit slower response times and poorer inhibition than typically-developing controls (Oosterlaan, Logan, & Sergeant, 1998; DeVito et al., 2009). Individuals with ADHD not only exhibit response inhibition deficits compared to controls, but also greater *intra-variability* in their reaction times, a finding that has been replicated (Leth-Steensen et al., 2000; Castellanos et al., 2006; Vaurio, Simmonds, & Mostofsky, 2009). High intra-individual variability may confound the interpretation of reaction time, in absolute terms, thus prioritizing the appropriate measurement of response variability (Castellanos et al., 2006). It remains unclear, however, whether intra-individual variability is a stable deficit

in ADHD, independent of motivational factors (Andreou et al., 2007; Wodka et al., 2007). To date, most research examining response inhibition have not directly assessed item-level, intra-individual variability, despite evidence that response variability more strongly correlates with symptoms of ADHD compared to other measures of accuracy or total response times.

Working memory is also a promising endophenotype for ADHD (Barkley, 1997; Rapport et al., 2001; Martinussen et al., 2005). Working memory consists of a verbal and visuospatial storage system, as well as a "central executive system" that regulates the two storage systems (Baddeley, 1986). The verbal component stores verbal information for a short period of time before it decays whereas the visuospatial component stores spatial information. Few studies have separately examined the verbal and visuospatial components of working memory in ADHD (Martinussen et al., 2005; Martinussen & Tannock, 2006), although each component is activated by different hemispheric regions of the brain (Baddeley, 1998) and may be influenced by distinct neurobiological processes. For example, there is growing evidence from meta-analytic and empirical studies for a visuospatial working memory deficit in ADHD (Martinussen & Tannock, 2006; McInnes, Humphries, Hogg-Johnson, & Tannock, 2003). However, the literature is equivocal regarding whether children with ADHD also exhibit impaired verbal working memory deficits (Willcutt et al., 2005; Martinussen et al., 2005). Sowerby, Seal and Tripp (in press) reported that children with ADHD scored consistently lower on tests of visuospatial working memory compared to age- and gender-matched control children, but verbal working memory deficits were only observed in younger children with ADHD (age 6-7) and not for the older children (age 8-12). Similar results were reported in an earlier study (Seigel & Ryan, 1989), where verbal working deficits were only observed among younger children (age 7-8) with ADHD and not in older children (ages 9-10). These results help explain the discrepant findings

in the literature on working memory impairments in ADHD and underscore the importance of measuring both forms of working memory (verbal and visuospatial) and incorporating a development perspective (Sowerby et al., in press).

Heritability

Although studies of heritability for NF and its components have rarely been explored, these studies are emerging. Kuntsi and colleagues (2006) assessed performance on a go/no-go task and a continuous performance task in 400 7- to 9-year-old twin pairs, and reported a moderate degree of genetic influence for overall reaction times (50%) and inhibition (60%). Recently, Schachar and colleagues (2010) examined the genetic influences on several indices of response inhibition, including reaction time, response latency, and variability (from the Stop Signal Task) in a sample of 139 8-year-old twin pairs. Genetic factors accounted for 50% of the variance in reaction times, 40% of the variance in response latency and 23% of the variance in variability (Schachar et al., 2010). Although response variability was not significantly heritable, this may be due to the method variance in measuring variability across studies, including the use of standard deviations of reactions times and estimating parameters from ex-Gaussian distributions (Vaurio, Simmonds, & Mostofsky, 2009). Overall, their findings suggest that response inhibition and latency of the Stop Signal Task are significantly heritable and promising endophenotypes in genetic research.

Verbal and visuospatial working memory processes have also demonstrated significant heritability. Ando, Ono and Wright (2001) estimated that genetic factors accounted for 43% and 49% of the variance in verbal and spatial working memory tasks, respectively, from a sample of 143 monozygotic twins and 93 dizygotic twins from Japan, aged 16 -29. Similarly, genetic factors explained 43% and 56% of the variance on the Arithmetic and Digit Span subtests (i.e.,

verbal working memory) of the Wechsler Intelligence Scale of Children (WISC), respectively, in a sample 409 12-year-old twins and their siblings (Polderman et al., 2006). Although more studies are needed to replicate these findings, circumstantial evidence is also consistent with substantial heritability for working memory, as evidenced by high intra-class correlations for monozygotic twins versus dizygotic twin pairs (Doyle et al., 2005).

Overall, heritability estimates for response inhibition and working memory are *less* than for ADHD, which has been estimated at approximately .7 (Nikolas & Burt, 2009). Despite being less heritable, NF are useful endophenotypes because fewer genes are believed to contribute to NF than for ADHD; that is, the magnitude of effect for any single gene will be greater for NF (than for ADHD) because there are fewer genes implicated (Doyle et al., 2005).

Familial/Genetic Overlap with the ADHD

Several lines of behavioral genetic research support the notion that NF exhibit significant familial and genetic overlap with ADHD. Adoption studies indicated that biological parents of ADHD probands performed more poorly on measures of NF (including visual attention, reaction time, visuospatial working memory) than adoptive parents of ADHD children (Alberts-Corush, Firestone, & Goodman, 1986; Nigg, Swanson & Hinshaw, 1997). In family studies, Nigg and colleagues (2004) measured trail-making performance and reaction times/response variability on tasks of continuous performance (i.e., Stop Task) among 386 relatives of children with ADHD and controls. Mothers of ADHD probands had greater reaction time variability on the Stop Task and had more deficits on trail-making than mothers of controls (Nigg et al., 2004). Similarly, siblings of children with ADHD performed more poorly on NF tasks, including verbal learning and working memory, than siblings of unaffected children (Seidman et al., 2000). Finally, the twin studies of NF (Kuntsi et al., 2006; Schachar et al., 2011; Ando, Ono & Wright, 2001)

consistently concluded significant heritability for NF measures within ADHD populations. In sum, behavioral genetic studies have provided strong evidence that NF deficits are expressed within families of ADHD probands and, on the basis of twin studies, are likely to share genetic influences with ADHD.

Biological Plausibility

Dopamine is widely suggested to play a critical role in NF and ADHD, given its role in regulating motor and reward activity, attention, and problem-solving (Waldman et al., 1998). Components of NF are modulated by dopaminergic and noradrenergic systems in frontostriatal regions (Goldman-Rakic, 1996). For example, rats with poor baseline working memory significantly improved their working memory after administration of a dopamine D4 agonist (expressed primarily in the prefrontal cortex) (Pothuizen et al., 2004). In humans, noradrenaline and serotonin reuptake inhibitors effectively improved response inhibition in a group of healthy, male participants (Chamberlain et al., 2006). Furthermore, the therapeutic effects of stimulants in ADHD, which increase intracellular dopamine levels in the brain, also generalize to improved performance on neurocognitive tasks. Rats administered therapeutic doses of methylphenidate significantly improved their performance on a spatial alternation task in T maze, which requires spatial working memory, response inhibition and sustained attention, compared to rats administered high or low doses of methylphenidate (Arnsten & Dudley, 2005). In humans, Bedard, Martinussen, Ickowicz and Tannock (2004) found that methylphenidate treatment significantly improved visuospatial working memory performance (but not planning ability or recognition memory) in a clinic-referred sample of school-age children with ADHD from baseline levels. Specifically, methylphenidate was associated with fewer errors on self-ordered, computerized tasks of visual-spatial working memory, and improved capacity to store visual-

spatial information (Bedard et al., 2004). Thus, NF deficits associated with ADHD may be secondary to dopaminergic dysregulation and disrupted frontostriatal circuitry (Martinussen et al., 2005; Levy & Swanson, 2001).

Given the crucial role of dopamine in modulating NF processes, genes that regulate dopamine are logical candidates for NF as well for ADHD. Loo et al. (2003) found that ADHD children homozygous for the DAT1 10-repeat allele exhibited greater response variability and made more commission errors on a continuous performance task (CPT) than children with other genotypes. Cornish et al. (2005) reported a significant association between 10-repeat allele homozygotes and poor response inhibition among children with elevated levels of hyperactivity and/or inattentiveness compared to children with one or fewer copies of the 10-repeat allele. These preliminary findings suggest that the genetic variants associated increased transcriptional efficiency (leading to decreased intracellular dopamine levels in the brain), such as the DAT1 10-repeat allele, may be associated with poorer neurocognitive functioning and ADHD.

Summary

Endophenotypes are quantitative traits that are believed to be intermediate between a complex phenotype and its biological underpinnings (Bearden & Friemer, 2006). They are particularly valuable for molecular genetic studies because they can assist in the search for susceptibility loci for complex psychiatric disorders. Moreover, the endophenotype approach can afford greater statistical power to detect genetic effects of small size (since the gene is believed to be more proximal to the endophenotype than to ADHD) (Munafo, 2006). Neurocognitive functions, such as verbal and visuospatial working memory and response inhibition are promising endophenotypes for the study of ADHD because deficits in both domains are highly associated with ADHD, show emerging evidence of heritability, demonstrate familial and

genetic overlap with ADHD, and are "biologically plausible." Despite calls to test the potential meditational (or moderational) effects of endophenotypes in genetic association studies (Munafo, 2006), no known studies have utilized this approach.

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Study I: Uncovering Latent Class Subtypes of ADHD and Assessing Gene-Environment Interplay

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Abstract

Background: A challenge in understanding the etiology of attention-deficit/hyperactivity disorder (ADHD) is its heterogeneous clinical presentation. That is, variability *within* ADHD, attributable to differences in diagnostic and developmental subtypes complicate studies of etiology. Empirically-anonymous approaches (e.g., latent class analysis; LCA) may identify groups based on severity and clinical presentation that are not captured by traditional methods, such as dimensional or diagnostic categories. We explored the interaction between DAT1, DRD4 and maltreatment with ADHD symptoms defined dimensionally and using LCA. **Method:** We tested the association of the 40 base-pair variable number of tandem repeats (VNTR) polymorphism in DAT1, the 48-bp VNTR in DRD4, maltreatment, and their interactions in 2,488 boys and girls from the National Longitudinal Study of Adolescent Health**. Results:** In boys, ADHD symptoms were optimally defined by four classes (Combined, Hyperactive/Impulsive, Inattentive, and Normal), whereas in girls, ADHD symptoms were defined by three classes (Combined, Combined-Mild, Normal). A significant DAT1 x maltreatment interaction revealed that maltreated girls homozygous for the 10-repeat allele had more symptoms of ADHD, and were also 2.5 times more likely to be classified in the Combined ADHD group than in the Normal Group. **Conclusions:** The underlying structure of ADHD symptoms differed between boys and girls and DAT1 interacted with maltreatment to predict ADHD symptoms and ADHD status derived from LCA. Interactive exchanges between maltreatment and DAT1 for ADHD symptoms, and their implications for intervention, are discussed.

Keywords: ADHD, latent class analysis, gene-environment interaction, DAT1, maltreatment.

A significant challenge to understanding the etiology of ADHD is its heterogeneous presentation, including frequent comorbidity with ODD/CD and inconsistent symptom profiles (Jensen, Martin, & Cantwell, 1997). Despite this fact, most genetic studies of ADHD have relied on using discrete classifications of ADHD using DSM-IV subtypes, despite its high rate of co-occurrence with aggression/antisocial behaviors (Jensen, Martin, & Cantwell, 1997; Acosta et al., 2008) and questionable validity (Valo & Tannock, 2010). One problem with this approach is the assumption of homogeneity within a group, despite the fact that diagnostics thresholds can be arbitrary (Blanton & Jaccard, 2006). For example, children who fell just short of diagnostic criteria for disruptive behavior disorders were often equally impaired as children who meet full criteria (Cho et al., 2009; Scahill et al., 1999). In addition, single-disorder designations (e.g., ADHD vs. controls) may exaggerate effect sizes because of high comorbidity and they may also artificially constrain groups based on theory and clinical observation. Empirically-driven approaches, such as latent class analysis (LCA), have identified groups based on ADHD severity and clinical presentation that are not reflected in current disorder-based approaches (Lee et al., 2007; de Nijs et al., 2007; Reinke et al., 2008). LCA is a person-centered approach that identifies groups based on similar patterns of responses (McCutcheon, 1987). Individuals within latent classes are homogenous and distinctive from other classes, provided the indicator variables are locally independent (Magidson & Vermunt, 2004). LCA is also a relatively anonymous approach where the number of classes is determined through an iterative process where the best fitting model determines the number of independent classes (Magidson & Vermunt, 2004). This is particularly important in the absence of strong theories and empirical precedent.

Studies of LCA for ADHD are currently underdeveloped, but recent findings are promising. Acosta et al. (2008) utilized LCA on symptoms for ADHD, ODD and CD among

1,010 children with and without ADHD, between the ages 7 and 18. For ADHD symptoms alone, they uncovered seven unique latent classes, corresponding to varying severity levels of inattentiveness, hyperactivity, and combined symptoms. However, when the LCA was conducted on ADHD, ODD and CD symptoms together, their findings revealed the presence of *13* unique symptom clusters. Their results suggest that the inclusion of comorbid externalizing symptoms with ADHD vastly changed the group composition and possibly presents a more valid presentation of the phenotype at-hand (Jensen, Martin, & Cantwell, 1997). More importantly, empirically-derived subtypes may represent differences in etiology, including genetic factors. For example, in our study from the National Longitudinal Study of Adolescent Health (Li & Lee, 2010), we utilized LCA on adolescent and young adult antisocial behavior (ASB) and found that the interaction of the 44-bp polymorphism of the serotonin transporter gene (5-HTTLPR) and maltreatment significantly predicted LCA-derived ASB phenotypes in girls, but not in boys, suggesting ASB subtype- and gender-specific G×E in the development of ASB.

Despite the promise and appeal of LCA, studies have yet to use this approach in testing G×E, particularly for ADHD. Utilizing the LCA approach would be particularly advantageous in G×E because they allow for the inclusion of "mild phenotypes" (i.e., phenotypes that fall below the clinical threshold) and comorbidities that would otherwise be omitted in genetic studies that employ traditional diagnostic categories (i.e., cases vs. controls). Thus, this study will employ LCA using a large, nationally-representative population using participants from National Longitudinal Study on Adolescent Health (Add Health). The primary aims of this study are to uncover the underlying structure of ADHD using LCA and to examine the association between a well-established risk factor for externalizing behaviors (i.e., childhood maltreatment) and its interaction with putative susceptibility genes in predicting latent class profiles of ADHD. In

addition, we examined predictive validity of the ADHD latent classes by testing their association with antisocial behaviors during adolescence and early adulthood.

Primary Study Goals

(1) To improve traction on the underlying structure of ADHD using latent class analysis (LCA) in a large, unselected sample.

(2) To examine the association of two candidate genes of ADHD (DAT1 and DRD4) and maltreatment in predicting subtypes of ADHD derived from LCA.

(3) To examine the interaction of genotype and maltreatment in predicting ADHD LCA-derived subtypes.

Secondary Study Goal

(1) To examine the predictive validity of ADHD latent classes on externalizing behaviors [i.e., antisocial behaviors (ASB)] in adolescence and early adulthood (ages 12-20).

Methods

Participants

We used data from the National Longitudinal Study of Adolescent Health (Add Health; Harris et al., 2008), which ascertained a stratified random sample of youth across 132 U.S. middle and high schools in 80 different communities, including urban, suburban, and rural, across the United States. Schools were stratified by region, ethnicity, size, and school type (i.e., parochial, private, public). In-school questionnaires were administered to 90,000 students, which ascertained data on information on social, emotional and health functioning. Additional information was obtained from a subsample of these adolescents through in-home interviews. A total of 20,745 youth and their caregivers participated in the comprehensive in-home interviews

at Wave I. Respondents were queried on issues pertaining to family support, engagement in risky behaviors (e.g., sexual behavior, rule-breaking, substance use), and their present health, mood and emotional functioning. At Wave I (1994 -1996), youth were between the ages of 12-20 (mean age = 15.22, SD = 1.65).

Wave II interviews were conducted one to one-and-half years after Wave I (1995 – 1996) (71% response rate) with the same adolescents (N = 14,738, mean age = 16.21, SD = 1.65). The attrition rate from Wave I to II was heavily influenced by graduation (high school graduates were not followed for the second interview) (Swallen, Reigher, Haas, & Meier, 2005; Harris et al., 2006). Most of the interview questions were retained from Wave I given that the participants were still adolescents. Wave III included 15,197 young adults that were interviewed 6-7 years after Wave I between 2001 and 2002 (73% response rate). Interview questions were changed in order to include more age-appropriate questions (e.g., marriage, children, employment history). Most of the respondents at Wave III were young adults (M age = 21.96, SD = 1.78). The rate of attrition over the seven year period was comparable to attrition rates in other longitudinal studies among young adults (approximately 32% attrition; Young, Powers & Bell, 2006).

Genetic information was obtained from a subsample of youth at Wave III, consisting of monozygotic and dizygotic twins, full siblings, half siblings and unrelated siblings raised in the same household. These individuals were identified through the in-school questionnaire administered at Wave I. The purpose of the collecting DNA from this subsample was to allow behavioral genetic studies "to move beyond variance decomposition…to testing specific hypotheses about the influence of individual genes and their expression in the context of environmental circumstances" (Harris et al., 2006). In total, the genetic subsample was comprised of 2,558 individuals (48% male). The genetic subsample was slightly older than the

non-genetic subsample (15.5 years vs. 15.2 years) (t = -8.97, df = 14736, p < .001). Furthermore, significant differences in racial-ethnic composition emerged as a function of whether or not adolescents had genetic or not (X^2 = 33.55, df = 3, p < .001), although they did not differ significantly with respect to sex (X^2 = 2.76, df = 1, p = .43). Finally, although the genetic subsample was ethnically diverse (57.5% Caucasian, 14.3% Hispanic, 18.1% African-American, 7.4% Asian, 1.7% Native American, and 0.9% "Other"), we emphasize that it is not a nationally representative sample.

Measures

ADHD. ADHD symptoms were assessed retrospectively during an in-home interview conducted at Wave III. Respondents were asked to rate their ADHD symptoms between 5 and 12 years of age on a four-point scale ("never or rarely," "sometimes," "often" and "very often"). 17 ADHD items were ascertained, including: "you failed to pay close attention to details or made careless mistakes in your work," "you had difficulty sustaining attention in tasks or fun activities" and "you had difficulty doing things quietly." For all analyses, we dichotomized the data where all responses of "often" and "very often" were coded positively. Cronbach's alpha for the 17 items of ADHD was adequate (.85).

Antisocial Behaviors (ASB). ASB items were ascertained during in-home interviews conducted at Waves I, II and III. These data were be analyzed separately given the instability of ASB across development, especially during adolescence and early adulthood. Respondents reported the frequency or presence of the following seven items "in the past 12 months," including: property destruction, stealing something worth more than $50, stealing something worth less than $50, breaking into a house or building, using a deadly weapon to threaten someone, selling drugs/marijuana, and participating in a fight in which someone was injured.

The same items were queried at all three Waves. Because the scaling of items was inconsistent (e.g., frequency counts, categorical), all items were converted to dichotomous data. Cronbach's alpha was adequate at each Wave (Wave I: .69, Wave II: .68, and Wave III: .62).

Maltreatment. Maltreatment was also assessed retrospectively at Wave III. Respondents reported the frequency of the following events prior to age 12: (1) parents or adult-caregivers did not take care of the respondent's basic needs (e.g., hygiene, food/clothing), (2) had been slapped, hit or kicked by parents or adult care-givers, and (3) had been touched in a sexual way, forced to touch someone else in a sexual way, or forced to have sexual relations with a parent or adult caregiver. If an event occurred at least once, it was scored as positive. 64.8% of youth reported no maltreatment history and 35.2% reported at least one episode. 11.7% of youth self-endorsed having experienced neglect, 29.5% endorsed having experienced physical abuse, and 4.8 % endorsed having experienced sexual abuse, all prior to age 12[1]. These estimates approximate rates in general population-based studies of maltreatment. Individuals with genotype data did not differ in maltreatment from individuals without those data ($F(1,14033) = .04, p = .84$).

Genotyping.

Saliva samples were collected from full siblings or twins to genotype for several candidate polymorphisms. Genomic DNA was isolated from buccal cells using standard methods. Dopamine transporter (DAT, SLC6A3) contains a 40-bp VNTR polymorphism in the 3' untranslated region. The 9-repeat (440 bp) and 10-repeat (480 bp) polymorphisms are the two most common alleles in the population. The primer sequences were: forward, 5'TGTGGTGTAGGGAACGGCCTGAG-3' (fluorescently labeled), and reverse:

[1] There were no sex differences in physical abuse [χ^2 (1) = 2.06, p = .15] or sexual abuse [χ^2 (1) = 2.73, p = .10], although neglect was more prevalent in boys than in girls [χ^2 (1) = 24.65, p < .001]. There was no main effect of neglect on ADHD symptoms, and the sex difference in neglect did not change the G×E results in any of the models (results available upon request).

5’CTTCCTGGAGGTCACGGCTCAAGG-3’. Given that 10-repeat homozygosity has been associated with increased mRNA expression (Mill, Ascherson, Browes, D’Souza, & Craig, 2002) as well as decreased DA transporter binding compared to non-10-repeat homozygotes (Heinz et al., 2000), the DAT1 analyses compared individuals homozygous for the 10-repeat allele (i.e., 10/10 repeat) versus individuals with at least a one copy of the 9-repeat allele (i.e., 9/9 + 9/10 repeat groups).

Dopamine D4 Receptor (DRD4) contains a 48-bp VNTR in the third exon. The most common polymorphisms in this VNTR are the 4 and 7 repeats. The primer sequences were: forward, 5’AGGACCCTCATGGCCTTG-3' (fluorescently labeled), and reverse, 5'-GCGACTACGTGGTCTACTCG-3'. Following recommendations, we compared children carrying at least one copy of the 7-repeat allele (i.e., “risk” group) to those who do not have the 7-repeat allele (Loo et al., 2010).

Statistical Analyses

To test the independent and interactive effects of DAT1, DRD4 and maltreatment with ADHD, we controlled for age and race-ethnicity and fit a zero-inflated negative binomial regression given that 31% (n = 4,549) of youth did not endorse any ADHD symptoms. We then conducted LCA on the 17 ADHD items using MPlus 4.0 (Muthen & Muthen, 2006) by fitting a two-class solution followed by successive models with an additional class until the best fitting model was indicated. We examined model fit using the Bayesian Information Criterion (BIC), adjusted BIC, as well as the Lo-Mendell-Rubin likelihood ratio test (LMR-LRT), which computes a p-value comparing the fit of a given model to a model with one less class. We then used multinomial logistic regression to examine the association of maltreatment, DRD4, and DAT1, and their respective interactions on membership in the LCA-derived subtypes based on

self-reported ADHD. To examine our secondary goal, antisocial behaviors (ASB) at each Wave were regressed on dimensional and latent-class designations of ADHD using zero-inflated negative binomial regression and multinomial logistic regression, respectively, controlling for age and race-ethnicity.

Results

Population Stratification and Gene-Environment Correlation (rGE)

Population stratification may confound the interpretation of potential G×E effects. DAT1 and DRD4 genotypes were related to race-ethnicity (X^2 = 46.58, df = 3, p < .01 and X^2 = 99.88, df = 3, p < .001, respectively), but not sex (X^2 = .65, df = 1, p = .42 and X^2 = 4.15, df = 1, p = .13, respectively). Thus, we controlled for race-ethnicity in all models. Exposure to maltreatment was unrelated to DAT1 (X^2 = .77, df = 1, p = .38) and DRD4 (X^2 = .31, df = 1, p = .58), thereby minimizing the possibility that rGE confounded our tests/interpretation of G×E.

Predicting Counts of ADHD Symptoms

DAT1. We first analyzed the association of DAT1, maltreatment, and their interaction with the total number of ADHD symptoms using zero-inflated negative binomial regression. The interaction of DAT1 and maltreatment was not significant for the overall sample (β = .03, SE = .09, p = .74), although there was a significant three-way interaction between sex, DAT1 and maltreatment (β = .57, SE = .18, p < .001). Among boys, significant main effects emerged for DAT1 genotype and maltreatment (β = .28, SE = .11, p < .05 and β = .38, SE = .15, p < .05, respectively) but their interaction was marginally significant (β = -.22, SE = .12, p = .07). In girls, there were no significant effects for DAT1 or maltreatment (β = -.23, SE = .13, p = .09 and β = -.17, SE = .14, p = .22, respectively), but their interaction was significant (β = .33, SE = .13,

$p < .01$) such that maltreatment positively predicted ADHD symptoms for girls with the 10/10 genotype ($\beta = .23$, $SE = .08$, $p < .001$), but not the 9/10 and 9/9 genotypes ($\beta = -.13$, $SE = .10$, $p = .21$).

DRD4. We used parallel analytic strategies to test the association of DRD4, maltreatment, and their interaction with total ADHD symptoms, controlling for age and race-ethnicity. The three-way interaction between DRD4, maltreatment, and sex was not significant ($\beta = .03$, $SE = .26$, $p = .89$). Among boys, there were no effects for DRD4, maltreatment ($\beta = .16$, $SE = .12$, $p = .19$ and $\beta = .18$, $SE = .11$, $p = .12$, respectively) or their interaction ($\beta = -.15$, $SE = .19$, $p = .43$). In girls, there were similarly no effects for DRD4, maltreatment (($\beta = -.01$, $SE = .13$, $p = .91$ and $\beta = .12$, $SE = .11$, $p = .24$, respectively) or their interaction ($\beta = .09$, $SE = .18$, $p = .63$) in predicting total ADHD symptoms.

Latent Class Models

LCA fit indices are summarized in Table 1. Across the entire sample, the five-class solution was the best fit ($BIC = 174717.64$, $p < .001$). However, based on the significant three-way interaction between sex, maltreatment and DAT1 in our dimensional model, we also examined sex differences in the LCA. The probability of class membership differed significantly by sex ($X^2 = 3.50$, $df = 4$, $p < .001$), suggesting that the underlying structure of ADHD differed between boys and girls. For boys, a four-class solution was optimal ($BIC = 92990.04$, $p = .03$). When a five-class solution was modeled, the BIC dropped to 92698.55, but the LMR-LRT was no longer significant ($p = .64$), indicating that the four-class solution was optimal. For girls, a three-class solution was the best fit to the data ($BIC = 80908.48$, $p < .001$), given that LMR-LRT for the four-class solution was not significant ($BIC = 80395.19$, $p = .13$).

Figure 1 shows the prevalence of group membership for boys. Class 1 (10% of boys) was characterized by high probabilities for endorsing all ADHD symptoms (Combined Type), including symptoms of inattention ["easily distracted" (.93), "careless" (.78), and "can't pay attention" (.74)] and hyperactivity/impulsivity ["fidgety" (.80), "restless" (.79) and "runs on motor" (.72)]. Next, 20.2% of boys fell into Class 2 (Inattentive), characterized by distinctively high probabilities for endorsing inattention ["easily distracted" (0.48) and "forgetful" (0.35)] but low probabilities for hyperactivity/impulsivity ["can't stay seated" (.11), "not quiet" (.18), and "blurts out" (.13)], with the exception of "fidgety" (.53). Class 3 (Hyperactive/Impulsive) consisted of 15.2% of boys, and was characterized by relatively high probabilities for endorsing hyperactivity symptoms ["talkative" (.70), "blurts out" (.65), "fidgety" (.62) and "runs on motor" (.55)], but not inattention ["can't pay attention" (.18) and "disorganized" (.16)]. Finally, Class 4 constituted 53.7% of boys, as this group was characterized by having minor ADHD symptoms (Normal Group) (i.e., probabilities of endorsing ADHD symptoms were all below 20%).

Figure 2 shows the prevalence of class membership for girls. Nearly 64% of girls fell into Class 1, characterized by few ADHD symptoms (Normal Group). These individuals had low probabilities of endorsing all symptoms (i.e., < .20 for all items). Class 2 (Mild Combined) consisted of 29% of girls with higher probabilities for "fidgety" (.55), "runs on motor" (.32), "talkative" (.63), "blurts out" (.31), and "easily distracted" (.37), compared to the Normal Group. However, this group was also low on "avoids tasks" (.09), "does not finish things" (.09), and "can't stay seated" (.08). 7% of girls fell into Class 3 (Combined), which had high probabilities of endorsing most ADHD symptoms (i.e., > .50 for most items), although some symptoms were quite high [e.g., "fidgety" (.82), "talkative" (.82), and "easily distracted" (.95)].

Predicting Latent Class Membership

DAT1. We then separately modeled the probability of class membership using multinomial logistic regression for boy and girls (Tables 2 and 3). The odd ratios represent the likelihood of membership in each class *relative* to the Normal Group. First, we compared the probability of membership in the Combined Group versus Normal Group in boys. We detected a significant main effect for maltreatment, but not for DAT1 (β = 1.69, SE = .77, p = .03 and β = .43, SE = .30, p = .16, respectively). Although maltreated boys were 5.44 times more likely to be in the Combined Group than in the Normal Group, the interaction term was not significant (β = -.69, SE = .46, p = .13). We then compared the Inattentive Group versus the Normal Group for boys. No significant effects were detected for DAT1, maltreatment (β = .13, SE = .23, p = .58 and β = .31, SE = .66, p = .64, respectively), or their interaction (β < .01, SE = .39 p = .99). Finally, we compared the Hyperactive/Impulsive Group versus the Normal Group for boys. No significant effects for DAT1, maltreatment (β = .08, SE = .25, p = .73 and β = .71, SE = .68, p = .30, respectively), or their interaction (β = -.26, SE = .41 p = .53) were observed for this comparison.

For girls, we compared the Combined-Mild Group versus the Normal Group. There were no main effects for DAT1, maltreatment (β = -.20, SE = .18, p = .26 and β = .43, SE = .51, p = .40, respectively), or their interaction (β = .05, SE = .30, p = .87). Similarly, for the Combined Type Group versus the Normal Group, no main effects were detected for DAT1 or maltreatment (β = -.25, SE = .35, p = .48 and β = -2.97, SE = 1.57, p = .06, respectively), but their interaction was significant (β = 1.94, SE = .84, p = .02). *Post-hoc* analyses indicated that maltreated girls with the 10/10 genotype were 2.55 more likely to be in the Combined Group than in the Normal Group (β = .93, SE = .34, p < .01). Conversely, there was no association of maltreatment and latent class membership among girls with the 9/9 or 9/10 genotype (β = -1.14, SE = .77, p = .14).

DRD4. First, we compared the probability of membership in the Combined Group versus Normal Group in boys. There were no effects for DRD4, maltreatment (β = .23, SE = .44, p = .52 and β = .57, SE = .39, p = .15, respectively), or their interaction (β = -.05, SE = .70, p = .94). We then compared the Inattentive Group versus the Normal Group for boys. Similarly, no significant effects emerged for DRD4, maltreatment (β = -.33, SE = .38, p = .39 and β = .18, SE = .34, p = .39, respectively), or their interaction (β = .28, SE = .60, p = .63). Finally, we compared the Hyperactive/Impulsive Group versus the Normal Group for boys. We did not detect any significant effects for DRD4 (β = -.01, SE = .38, p = .97), maltreatment (β = .001, SE = .37, p = .99) or their interaction (β = .46, SE = .63, p = .47).

We adopted similar analytic strategies to analyze the interaction between DRD4, maltreatment and their interaction with ADHD latent classes in girls. Comparing the Combined-Mild Group versus the Normal Group, there were no main effects for DRD4 or maltreatment (β = -.34, SE = .54, p = .53 and β = .33, SE = .48, p = .50, respectively). There was no interaction between DRD4 and maltreatment in predicting membership into the Combined-Mild Group (β = .12, SE = .80, p = .88). Finally, we compared the Combined Group vs. the Normal Group. Maltreatment was positively predictive of group membership into the Combined Group (β = .73, SE = .28 p < .01). Maltreated girls were 2.08 times more likely to belong to the Combined group relative to the Normal group (95% CI: [1.21, 3.59]). There was no effect of DRD4 or the interaction between DRD4 and maltreatment, however (β = -.10, SE = .28, p = .73 and β = -.05, SE = .45, p = .91, respectively).

Predicting Antisocial Behaviors using Total Symptoms and Latent Classes

First, we employed a zero-inflated negative binomial regression to predict ASB at each Wave on total ADHD symptoms, controlling for age and race-ethnicity. For boys, ADHD

symptoms positively predicted ASB at Wave I (β = .03, SE = .007, p < .01), Wave II (β = .04, SE = .007, p < .01), and Wave III (β = .06, SE = .01, p < .01). Similarly, for girls ADHD symptoms predicted later ASB at all Waves (Wave I: β = .05, SE = .009, p < .01; Wave II: β = .04, SE = .01, p < .01; Wave III: β = .09 SE = .01, p < .01).

Next, we tested whether ADHD latent classes predicted ASB at each Wave, controlling for age and race-ethnicity. For the following analyses, the reference class was the Normal Group. For boys, membership to the Inattentive, Hyperactive, and Combined Groups prospectively predicted ASB at Wave 1 (β = .32, SE = .07, p < .01, β = .28, SE = .07, p < .01, and β = .26, SE = .09, p < .01, respectively). Membership to Inattentive, Hyperactive, and Combined Groups also predicted ASB at Wave 2 (β = .23, SE = .08, p < .01, β = .19, SE = .08, p < .05, and β = .41, SE = .09, p < .01, respectively). Finally, class membership to the Inattentive, Hyperactive, and Combined Groups predicted Wave 3 ASB (β = .40, SE = .10, p < .01, β = .30, SE = .09, p < .01, and β = .61, SE = .13, p < .01, respectively). For girls, class membership to the Combined Mild and Combined Groups prospectively predicted ASB at Wave 1 (β = .45, SE = .10, p < .01 and β = .33, SE = .07, p < .01, respectively), Wave 2 (β = .45, SE = .12, p < .01 and β = .26, SE = .07, p < .01, respectively), and Wave 3 (β = 1.03, SE = .17, p < .01 and β = .55, SE = .11, p < .01, respectively).

Discussion

Despite their independent associations with ADHD, there is relatively little knowledge about the potential interactive effects of DAT1, DRD4 and maltreatment. To address the phenotypic complexity of ADHD, we conducted LCA using a large population-based sample of adolescents and young adults and found four classes of ADHD in boys (Combined, Inattentive,

Hyperactive/Impulsive, and Normal) and three classes in girls (Combined, Combined-Mild, and Normal). Based on ADHD symptoms, maltreated girls homozygous for the DAT1 10-repeat allele had more ADHD symptoms than girls with the 9/9 or 9/10 genotypes. This pattern was replicated using LCA where maltreated girls with the 10/10 genotype had higher odds of being in the Combined ADHD group than maltreated girls with the 9/9 or 9/10 genotype, suggesting a potential G×E. No significant G×E was observed in boys using ADHD symptoms or LCA. Furthermore, we did not observe any effects between DRD4 and maltreatment in either our dimensional or latent-class multinomial models.

The latent structure of ADHD in boys approximated DSM-IV subtypes. However, they differed in that LCA classes included individuals who were unlikely to meet symptom criteria for ADHD. Interestingly, the latent structure for girls deviated from DSM-IV subtypes given that classes were organized around differences in severity rather than the nominal differences. This finding in girls diverges somewhat from a previous LCA study of adolescent female twins where different classes of severity corresponded well to DSM-IV subtypes (Hudziak et al., 1998). Indeed, sex differences in the underlying structure for ADHD symptoms were not entirely surprising given sex differences in the prevalence of ADHD as in neurocognitive and academic correlates (Gershon, 2002). It is also possible that including internalizing symptoms into the LCA may have changed the nature of the classes identified in girls (Neuman et al., 2001). Importantly, the ADHD latent classes prospectively predicted antisocial behaviors (i.e., externalizing problems) in adolescence and into young adulthood, independent from sex, confirming previous findings that childhood ADHD predicts the emergence of these behaviors later in life, regardless of severity or subtype (Thapar et al., 2006).

Among girls with the DAT1 10/10 DAT1 genotype, maltreatment positively predicted ADHD symptoms, but not in girls with the 9/9 and 9/10 genotype. LCA improved the specificity of our findings given that maltreated girls with the 10/10 genotype were nearly three times more likely to be in the Combined Group versus the Normal Group. However, this effect was *only* detected in the most severe class of ADHD for girls. One explanation may be that girls with the 10/10 genotype are the most sensitive to environmental stress (Caspi et al., 2010), which resonates with sex differences in sensitivity to stressful events in human and non-human studies (Oldehinkel & Bouma, 2010). For example, acute stress enhanced learning and memory in male rats, but impaired these functions in female rats (Shors, 2004). Similarly, adolescent girls experienced significantly more interpersonal stress (e.g., peers, parents) than boys, as well as higher levels of depression and anxiety as function of these stressors. Thus, girls may be more vulnerable to interpersonal stressors than boys, including potentially maltreatment (Rudolph & Hammen, 1999). Furthermore, the 10/10 genotype may confer additional sensitivity to positive and negative environmental factors through disrupted DA neurotransmission (Strathearn, 2011). Adolescents homozygous for the 10-repeat allele had the most inattention under high psychosocial adversity, but concurrently had the lowest inattention under low psychosocial adversity (Laucht et al., 2007). The rate of DA binding and subcortical reactivity may be contingent upon the salience of the event (i.e., highly positive or negative stimuli) such that DA neurons exhibit the highest firing rate prior to a salient reward, but the firing rate falls below baseline in anticipation of a harsh punishment (Mirenowicz & Schultz, 1996).

Our study detected specificity of the G×E involving DAT1, and not for DRD4. We found similar results to another pooled population-based study that compared the effects DAT1 and DRD4 on LCA-derived subtypes of ADHD (Todd et al., 2005), which found robust effects for

DAT1 but weak effects for the DRD4 in association with ADHD subtypes (although their study did not incorporate environmental factors for a G×E analysis). Despite being among the most studied susceptibility genes for ADHD, association studies between DRD4 and ADHD have produced mixed results (Shaw et al., 2007; Johnson et al., 2008). One reason for the contrastive findings may be due to the substantial genetic variability within the VNTR sequences in DRD4. A recent study found that children with ADHD carried more rare variants (beyond the more common 4-repeat and 7-repeat alleles) in the VNTR than healthy controls. Furthermore, assessing for rare variants uncovered a high prevalence of novel haplotypes in association with ADHD (29%) (Tovo-Rodrigues et al., 2011), suggesting that examining DRD4 VNTR rare variants (rather than repeat length variability) may provide more traction in ADHD genetic susceptibility.

There were several limitations to our study. ADHD was assessed via retrospective self-report, which may have been influenced by mood, psychopathology or inaccurate recall. However, adults with ADHD are better at self-reporting their own ADHD symptoms than their partners or their parents (Kooij et al., 2008), suggesting that patients may remember their externalizing behavior better than other forms of psychopathology. Although items related to other dimensions of psychopathology were ascertained, these data were only available for concurrent psychopathology (i.e., when participants were ages 12-20) rather than for retrospective psychopathology as it was for ADHD (ages 5-12). Moreover, because ADHD symptoms were not assessed using a formal diagnostic interview, the groups that were ascertained in the LCA should not be interpreted as clinical groups. Finally, latent transition analysis would have provided improved traction on the developmental trajectory of ADHD

symptoms. However, the present study was cross-sectional because ADHD data were retrospective and only available at Wave III.

This study characterized the latent structure of ADHD in a population-based sample with evidence that a biologically-plausible interaction between DAT1 and maltreatment predicted severe ADHD, but only among girls. Diagnoses of DSM-IV ADHD must be done cautiously as these categories may include phenotypically heterogeneous subgroups that inadvertently reduce statistical power, a longstanding concern in genetic association studies. We anticipate that integrated models of risk, incorporating genetic and environmental constructs with empirically-derived groups of youth, will facilitate the development and implementation of targeted interventions to reduce the considerable burden associated with significant ADHD.

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Appendix: Tables and Figures

Table 1: Fit indices for LCA models

	Number of Classes	BIC	Sample Size Adjusted BIC	*p*
Combined (n = 13,963)	2	181392.43	181274.84	<.01
	3	176495.42	176317.46	<.01
	4	175516.74	175278.40	<.01
	5	**174717.64**	**174418.92**	**<.01**
	6	174343.40	173984.30	.08
Boys (n = 6,611)	2	95825.82	95708.25	<.01
	3	93425.09	93247.14	<.01
	4	**92990.04**	**92751.71**	**.03**
	5	92698.55	92399.84	.64
	6	92526.43	92167.34	.11
Girls (n= 7,352)	2	83222.25	83104.68	<.01
	3	**80908.48**	**80730.53**	**<.01**
	4	80395.19	80156.86	.13
	5	80021.26	79722.55	.02
	6	79908.23	79549.14	.33

Table 1. Fit indices for LCA models

Note. Bold indicates best fitting model; BIC = Bayesian Information Criterion; *p* = Lo-Mendell-Rubin likelihood ratio test for *k* versus *k* - 1 classes.

Table 2: Multinomial logistic regression analyses: DAT1 x maltreatment in boys

Variable	B	SE	RRR	95% CI Lower	95% CI Upper	*Sig.*
Combined Group						
Age	-0.07	0.07	0.94	0.81	1.06	0.32
Race-ethnicity	-0.51	0.16	0.6	0.29	0.92	0
DAT1	0.43	0.3	1.53	0.94	2.12	0.16
Maltreatment	**1.69**	**0.77**	**5.44**	**3.93**	**6.94**	**0.03**
DAT1 x Maltreatment	-0.69	0.46	0.5	-0.4	1.4	0.13
Inattentive Group						
Age	-0.03	0.05	0.97	0.86	1.07	0.56
Race-ethnicity	-0.04	0.09	0.96	0.77	1.14	0.66
DAT1	0.13	0.23	1.14	0.68	1.6	0.58
Maltreatment	0.31	0.66	1.36	0.07	2.65	0.64
DAT1 x Maltreatment	0	0.39	1	0.25	1.76	0.99
Hyperactive Group						
Age	-0.11	0.06	0.9	0.79	1.01	0.07
Race-ethnicity	-0.35	0.13	0.71	0.46	0.95	0.01
DAT1	0.08	0.25	1.09	0.61	1.57	0.73
Maltreatment	0.71	0.68	2.03	0.7	3.36	0.3
DAT1 x Maltreatment	-0.26	0.41	0.77	-0.03	1.58	0.53

Table 3: Multinomial logistic regression: DAT1 x maltreatment in girls

				95% CI		
Variable	B	SE	RRR	Lower	Upper	*Sig.*
Combined Mild						
Age	-0.05	0.04	0.95	0.87	1.04	.25
Race-ethnicity	-0.10	0.08	0.90	0.74	1.06	.21
DAT1	-0.20	0.18	0.82	0.46	1.17	.26
Maltreatment	0.43	0.51	1.53	0.53	2.54	.40
DAT1 x Maltreatment	0.05	0.30	1.05	0.46	1.64	.87
Combined						
Age	-0.02	0.08	0.98	0.82	1.15	.85
Race-ethnicity	-0.23	0.17	0.79	0.45	1.14	.19
DAT1	-0.25	0.35	0.78	0.08	1.47	.48
Maltreatment	-2.97	1.57	0.05	-3.02	3.13	.06
DAT1 x Maltreatment	**1.94**	**0.84**	**6.97**	**5.33**	**8.61**	**.02**

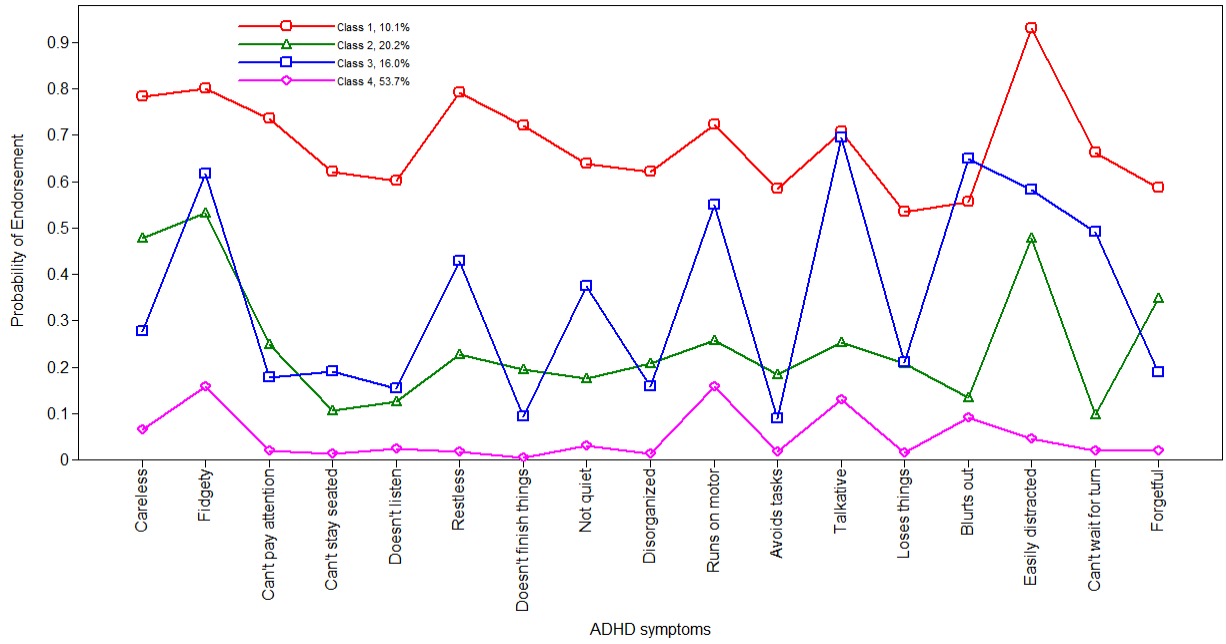
Table 4: Multinomial logistic regression analyses: DRD4 x maltreatment in girls

				95% CI		
	B	SE	RRR	Lower	Upper	Sig.
Inattentive Type						
Age	-0.09	0.07	0.91	0.79	1.05	0.21
Race-ethnicity	-0.10	0.20	0.90	0.61	1.33	0.60
DRD4	-0.01	0.38	0.99	0.47	2.08	0.97
Maltreatment	0.00	0.38	1.00	0.48	2.10	1.00
DRD4 x Maltreatment	0.46	0.63	1.58	0.46	5.41	0.47
Hyperactive Type						
Age	-0.02	0.08	0.98	0.84	1.15	0.82
Race-ethnicity	0.10	0.15	1.11	0.82	1.49	0.51
DRD4	-0.33	0.38	0.72	0.35	1.51	0.39
Maltreatment	0.18	0.34	1.19	0.61	2.33	0.61
DRD4 x Maltreatment	0.29	0.60	1.33	0.41	4.29	0.63
Combined Type						
Age	-0.15	0.11	0.86	0.69	1.07	0.17
Race-ethnicity	-0.45	0.20	0.64	0.43	0.95	0.03
DRD4	0.23	0.44	1.26	0.53	2.98	0.61
Maltreatment	0.57	0.39	1.76	0.82	3.81	0.15
DRD4 x Maltreatment	-0.05	0.70	0.95	0.24	3.75	0.94

Table 5: Multinomial logistic regression analyses: DRD4 x maltreatment in girls

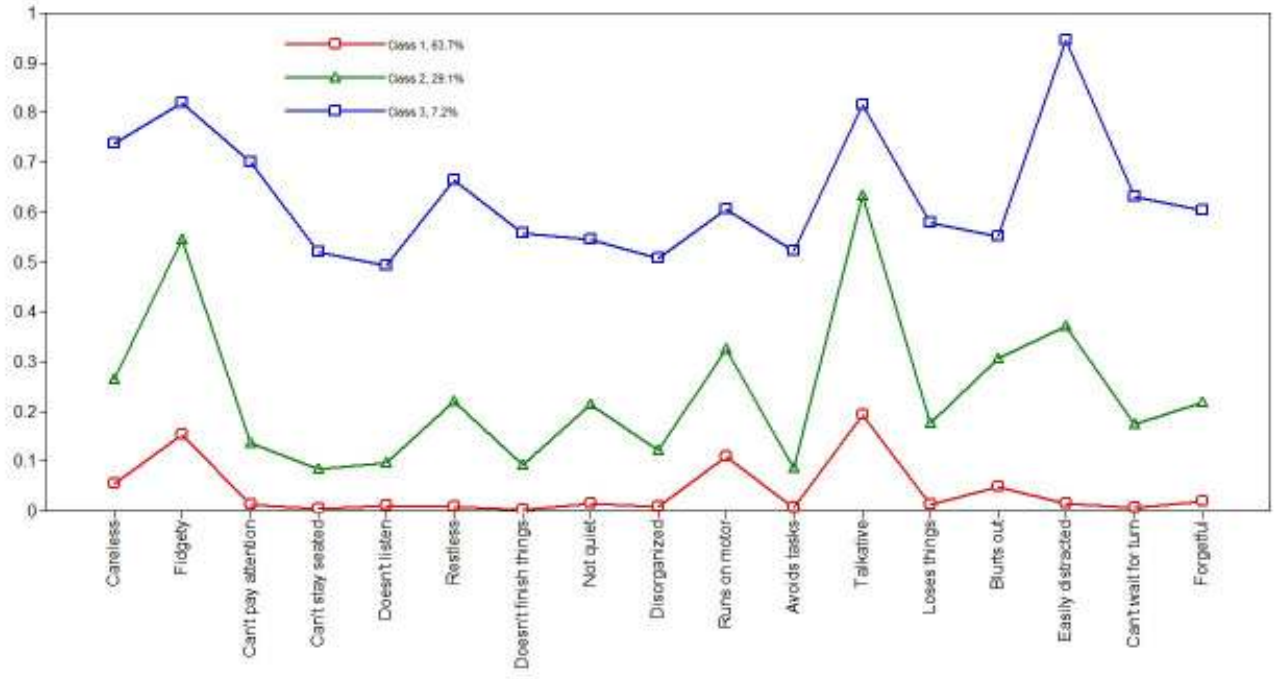
				95% CI		
	B	SE	RRR	Lower	Upper	Sig.
Combined-Mild						
Age	0.00	0.14	1.00	0.76	1.31	0.98
Race-ethnicity	-0.23	0.19	0.80	0.55	1.16	0.23
DRD4	-0.34	0.54	0.72	0.25	2.06	0.53
Maltreatment	0.33	0.48	1.39	0.54	3.58	0.50
DRD4 x Maltreatment	0.12	0.80	1.12	0.23	5.38	0.88
Combined Type						
Age	-0.08	0.06	0.93	0.82	1.05	0.23
Race-ethnicity	0.03	0.12	1.03	0.81	1.30	0.83
DRD4	-0.10	0.28	0.91	0.53	1.56	0.73
Maltreatment	0.73	0.28	2.08	1.21	3.59	0.01
DRD4 x Maltreatment	-0.05	0.45	0.95	0.40	2.28	0.91

Figure 1: Latent class membership for boys



Note. Class 1 = Combined Group; Class 2 = Inattentive Group; Class 3 = Hyperactive Group; Class 4 = Normal group (reference class).

Figure 2: Latent class membership for girls



Note. Class 1 = Normal group; Class 2 = Combined-Mild Group; Class 3 = Combined Group.

Study II: A Test of Differential Susceptibility in ADHD

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Abstract

Objective: Most studies of G×E have focused on environmental adversity, given the primacy of diathesis-stress conceptualizations in psychopathology. Far less is known about *high quality* environmental factors, such as warmth, low family conflict, and positive parent-child communication in the context of G×E. Positive family conditions may interact with genotype in ways that are consistent with "differential susceptibility" whereby the same genotype may simultaneously increase sensitivity to environmental enrichment and adversity. We examined the interface between child genotypes and a range of parenting behaviors with childhood ADHD. Method: We ascertained 150 six to nine year-old boys and girls with and without ADHD. Objective counts of parenting behavior (i.e., negativity and praise) were assessed from a validated parent-child interaction task as well as from a self-report questionnaire on parenting behavior. The 40 base-pair VNTR in DAT1 and the 48-bp VNTR in DRD4 were genotyped in children. ADHD data was gathered from a structured interview with parents. Results: Youth with 9/9 and 9/10 DAT1 genotypes were simultaneously *more likely* and *less likely* to have Inattentive Type (IA) and Hyperactive/Impulsive Type (HI) ADHD (relative to youth with the 10/10 genotype) if they were exposed to high levels and low levels of parental negativity, respectively. However, DAT1 and DRD4 genotypes did not interact with praise or positive parenting for ADHD. Conclusions: These preliminary results suggest that interactive exchanges between parenting behavior and child genotype potentially contribute to the development of ADHD. Clinical implications for interactions between parenting behavior and child genotype are discussed.

Keywords: Parenting, ADHD, gene-environment interaction, DAT1, DRD4.

There is replicated evidence that environmental adversity, such as parental negativity (i.e., harsh comments, hostility, and low warmth), is associated with attention-deficit/hyperactivity disorder (ADHD): compared to parents of typically-developing children, parents of children with ADHD made more harsh and critical statements, and were less physically involved with their offspring (Wells et al., 2000; Chronis-Tuscano et al., 2008). In addition, mothers were significantly more critical and exhibited less positivity and warmth with ADHD probands than with their non-ADHD siblings (Cartwright et al., 2011). Meta-analytic findings further substantiate this notion given the prospective association of parental negativity (and negative parenting behavior more broadly) with ADHD (Johnston & Jassy, 2007). Beyond negative parenting per se, poor parent-child *relationships*, which reflect reciprocal and "child effects,'' also characterize families of children with ADHD (Lifford et al., 2009). Through cycles of negative parent-child interactions, negative parenting and child development must consider reciprocal and transactional effects (Lifford et al., 2009).

Dimensions of positive parenting (i.e., involvement, warmth, and praise) have shown unique patterns with ADHD relative to negative parenting, which is consistent with previous studies on the empirical independence of positive and negative parenting behavior with respect to child outcome more broadly (Pettit, Bates, & Dodge, 1997). However, relatively few studies of ADHD and behavior problems more generally have made this important distinction. Self-reported negative parenting (i.e., inconsistent discipline, low involvement) positively predicted ADHD symptoms, controlling for child ODD, CD, and parental ADHD, but positive parenting did not (Ellis & Nigg, 2009). Similarly, negative parenting (i.e., harsh discipline, inconsistent discipline), but not positive parenting (i.e., positive reinforcement, parental involvement) was independently associated with parent- and teacher-ratings of ADHD symptoms (Li & Lee, in

press). Finally, in a prospective study of preschool children with ADHD, observed positive parenting (but not negative parenting) inversely predicted conduct disorder (CD) symptoms eight years later (Chronis et al., 2007). Collectively, these results suggest that negative and positive parenting may be empirically distinct (rather than simply reflecting opposite ends of a single underlying continuum) and that studies must separately examine their association with outcome.

Study II employs a validated structured observational parent-child interaction task to test the association of parental negativity and praise, child genotype (DAT1 and DRD4), and their respective interactions with child ADHD from parents. Study II will provide an important complement to Study I by focusing on a more specific developmental period (6 to 9 years) that yields greater precision with respect to being developmentally-informative. More importantly, whereas previous G×E studies have largely focused on a restricted range of the environmental criterion, the present study examines *multiple* aspects of parenting (i.e., parenting styles and behaviors) across multiple dimensions, including both positive (e.g., involvement, praise) and negative (e.g., harsh discipline, inconsistent discipline) behaviors.

Study II also tests differential susceptibility (Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2007). Briefly, the steps for testing differential susceptibility are as follows: (1) test for statistical interaction; (2) test for the independence of the susceptibility factor and the predictor; (3) test the association between susceptibility factor and outcome (if non-zero, there is no support for differential susceptibility); (4) compare the interaction plot with prototypical models of interaction (for differential susceptibility, there must be a cross-over effect in which the slope of the susceptible subgroup is significantly different from zero and concurrently, significantly steeper than the slope for the non-susceptible subgroup); and (5) test specificity of

the model by replacing susceptibility factors and outcomes. All five criteria must be met to detect differential susceptibility.

Primary Study Goals

(1) To test whether children with the putative vulnerability genotype (7-repeat allele in DRD4, 10-repeat allele in DAT1) have fewer ADHD symptoms when exposed to high positive parenting, and simultaneously more ADHD symptoms when exposed to high negative parenting, compared to children without the vulnerability genotype.

Methods

Participants

We recruited 150 ethnically diverse (56% Caucasian, 8% African America, 8% Hispanic or Latino, and 28% Mixed or Other) 5 to 10 year-old children (mean age = 7.4, SD = 1.1, 71% male) with and without ADHD. Recruitment methods included mailings to local schools, flyers and advertisements, presentations to self-help groups, and referrals from local mental health service providers. To be eligible for the study, all participants were required to have a Full Scale IQ above 70, to live with one biological parent at least half the time, and to be fluent in English. Exclusionary criteria included current or previous diagnosis of mental retardation, seizure, autism, or pervasive developmental disorders. Diagnostic status for ADHD was assessed using the Diagnostic Interview Schedule for Children, 4th edition (DISC-IV; Shaffer et al., 2000), a fully structured interview keyed to all DSM-IV criteria (i.e., age of onset, symptom persistent, cross-situational presence). Youth were considered ADHD probands (vs. non-ADHD control group) if they met full diagnostic criteria on the DISC-IV. To avoid recruiting a control group that was unrealistically high functioning, children were placed in the control group if they met

diagnostic criteria for any disorder other than ADHD. This procedure has been used in similar studies of childhood ADHD (Lahey et al., 1998).

Procedures

Eligibility was determined after parents completed a telephone screening and eligible families who were interested in participating were mailed rating scales. Families were then invited to our laboratory for in-person assessments. Following parent consent and child assent, parents completed the DISC and other measures related to parenting, child behavior, family functioning, personality, and their own psychopathology. During that same time, children completed standardized test of cognitive ability and academic achievement. Whenever possible, children were assessed without medication. Similarly, parents and teachers were asked to complete rating scales based on the child's unmedicated behavior. All interviewers were blind to the child's diagnostic status. The Institutional Review Board approved all study procedures.

Genotyping

DNA was extracted from saliva using DNA Genotek Oragene^{TM} Self-Collection Kits (DNA Genotek, Inc., Ottawa, Canada). DA transporter (DAT, SLC6A3) contains a 40-bp VNTR polymorphism in the 3'UTR. The 9-repeat (440 bp) and 10-repeat (480 bp) polymorphisms are the two most common alleles in the population. The primer sequences were: forward, 5'TGTGGTGTAGGGAACGGCCTGAG-3' (fluorescently labeled), and reverse: 5'CTTCCTGGAGGTCACGGCTCAAGG-3'. The DAT1 analyses were conducted by comparing individuals homozygous for the 10-repeat allele (i.e., 10/10) (n = 65; 43.5%) versus individuals with at least a one copy of the 9-repeat allele (i.e., 9/9 + 9/10) (n = 85; 56.5%).

Dopamine D4 Receptor (DRD4) contains a 48-bp VNTR in the third exon. The most common polymorphisms in this VNTR are the 4 and 7 repeats. The primer sequences were:

forward, 5'AGGACCCTCATGGCCTTG-3' (fluorescently labeled), and reverse, 5'-GCGACTACGTGGTCTACTCG-3'. Following recommendations, we compared children carrying at least one copy of the 7-repeat allele (i.e., "risk" group) to those who do not have the 7-repeat allele (Loo et al., 2010).

Parenting Measures

Dyadic Parent Child Interaction Coding System (DPICS; Eyberg, Nelson, Duke, & Boggs, 2005). Parenting behavior was coded using the DPICS, a well-validated system of rating parent-child interaction in children with disruptive behavior disorders (Chronis-Tuscano et al., 2008). 88% of parents who participated in the study were mothers. Discrete parent and child behaviors were coded continuously. We created composite categories of parenting quality that appear in the literature (Chronis-Tuscano et al., 2008; Eyberg et al., 2001), including parental negativity, praise, and child noncompliance. Parental negativity was coded when parents utilized a harsh or brash tone of voice, made critical or hostile comments (e.g., "that's a terrible drawing," "you better try harder or else you'll be punished"), issued negative commands (e.g., "stop doing that right now!"), or made sarcastic and condescending remarks (e.g., "you think you're real good at building that don't you?"). Examples of praise included positive appraisals for a child's behavior or attribute, or a product that the child created (e.g., "you're a good builder," "that's a really pretty picture of a dog you drew"). Child noncompliance was coded when the child refused parental commands and/or ignored questions. We tallied the total counts of parent and child behaviors across each condition (e.g., number of times the parent praised the child) and divided this by the total minutes that were coded. Interactions were recorded using a digital recorder.

Six undergraduate research assistants were intensively trained in the DPICS coding procedures outlined in the manual until at least 70% agreement was attained. Coders participated in a full day of training followed by two months of practice where each coding category was discussed and reviews/quizzes were completed. We held weekly coding meetings to ensure reliability and to resolve disagreements. 20% of the videos were randomly selected and coded by two separate raters to estimate reliability. DPICS composite categories have shown moderate to substantial inter-rater and test-retest reliability (Chronis-Tuscano et al., 2008). The intra-class correlations for our composite categories ranged from acceptable to excellent: negativity (ICC = .75), praise (ICC = .88), and child noncompliance (ICC = .78).

*Alabama Parenting Questionnaire (*APQ; Frick, 1991). Parents self-reported 42 items related to child-rearing practices, each rated on a 5-point scale [1 ("Never") to 5 ("Always")] Previous factor analyses supported several distinct factors, including positive (i.e., involvement; 10 items), positive reinforcement (6 items) and negative dimensions (i.e., inconsistent discipline; 6 items), poor monitoring (10 items) and corporal punishment (3 items) (Shelton, Frick & Wootton, 1996). We calculated and standardized separate positive and negative parenting composite score, which has been utilized in previous studies of disruptive behavior disorders (Frick & Dantagnan, 2005). Negative and positive parenting dimensions demonstrated adequate internal consistency in this sample (ICC = .67 and .80, respectively), comparable to previous studies (Dadds, Maujean, & Fraser, 2003).

ADHD

Diagnostic Interview Schedule for Children, Version IV (DISC-IV; Shaffer et al., 2000). The DISC-IV is a computer-assisted, fully structured diagnostic interview with the parent used to assess child psychopathology based on the Diagnostic and Statistical Manual, 4th Edition

(American Psychiatric Association [APA], 1994). The ADHD module of the DISC-IV has good psychometric properties, including high test-retest reliability (r = .79 after one year) and internal consistency (ICC = .84 for symptoms counts, ICC = .77 for criterion counts) among the parents from a large community sample (see Shaffer et al., 2000). We analyzed ADHD categorically (e.g., ADHD subtypes) and dimensionally using full DSM-IV diagnostic criteria and counts of the total number of DSM-IV ADHD symptoms, respectively.

Statistical Analysis

Based on recommendations for molecular genetic studies of ADHD (Thapar et al., 2006), we characterized ADHD using categorical and dimensional approaches. This strategy allows for comparisons with previous genetic studies and improves model estimation by increasing power to detect main effects (Royston, Altman, & Sauerbrei, 2006). We controlled for child age, race-ethnicity, sex, household income, and parental depression from the Beck Depression Inventory, given its association with negative parenting and childhood ADHD (Chronis et al., 2007). To account for potential "child effects," we controlled for child noncompliance during the interaction so that parenting behavior was not exclusively a reflection of negative child behavior.

For categorical models, parent ratings of ADHD were analyzed based on whether the child met full DSM-IV criteria for each subtype of ADHD (i.e., inattentive type, hyperactive/impulsive type, and combined type). Using multinomial logistic regression, we separately regressed parent-rated ADHD subtype status (the non-ADHD group served as the reference group in each comparison) on (1) parental negativity, (2) child genotype (DAT1 and DRD4 were entered into separate models) and (3) their interaction. We then conducted parallel analyses for parental praise in which parent-rated ADHD subtype status was regressed on (1) parental praise, (2) child genotype, and (3) their interaction. In the dimensional analyses, we used

negative binomial regression to account for the over-dispersed ADHD count data and separately regressed the total number of parent-rated ADHD symptoms using the same hierarchical structure: (1) parental negativity and praise in separate models, (2) child DAT1 genotype, and (3) separate models that included interaction terms for parental negativity×DAT1 and praise×DAT1.

Results

Population Stratification and Gene-Environment Correlation

Population stratification can produce spurious effects and confound the interpretation of potential G×E. Passive and evocative gene-environment correlations (rGE) may also confound tests of G×E (Jaffee & Price, 2007). To address these concerns, we first tested the association of *parent* DAT1 and DRD4 genotypes with parenting behaviors. Parent DAT1 and DRD4 genotypes were unrelated to parental negativity (B = .20, SE = .12, p = .09 and B = .08, SE = .06, p = .18, respectively) and praise (B = 0.02, SE = .09, p = .77 and B = .03, SE = .08, p = .66, respectively). Thus, our conceptualization of parenting as the "environment" in the context of G×E was not complicated by the influence of parental genotype. To address evocative rGE, where the child's genotype predicted exposure to the environment, negativity and praise did not differ significantly by offspring DAT1 genotype [F(1, 149) = 0.04, p = .59 and F(1,149) = 0.11, p = .48, respectively] or DRD4 genotype [F(1, 149) = 0.002, p = .96 and F(1,149) = 0.09, p = .77, respectively]. Furthermore, child DAT1 genotype was unrelated to their age (X^2 = 4.44, df = 5, p = .49), sex (X^2 = .001, df = 1, p = .98) and race-ethnicity (X^2 = 11.37, df = 7, p = .12). Similarly, child DRD4 genotypes were unrelated to their age (X^2 = 4.04, df = 5, p = .54), sex (X^2 = .38, df = 1, p = .54) and race-ethnicity (X^2 = 6.48, df = 6, p = .37). Thus, our findings are robust to rGE.

Observed Parenting Behaviors: Categorical ADHD

We analyzed observed parental negativity from the DPICS, child genotypes, and their interactions on parent-rated ADHD (i.e., subtypes) using multinomial logistic regression, controlling for age, sex, race-ethnicity, parental depression symptoms, family income, and child noncompliance during the DPICS. We then reproduced this model but with praise, instead of negativity. In all cases, the non-ADHD group served as the reference group with the relative risk ratio (RRR) representing the risk of being in each class [i.e., ADHD inattentive type (IA), ADHD hyperactive/impulsive type (HI), and ADHD combined type (CT)] *relative* to the non-ADHD group. First, we compared IA to non-ADHD youth. The negativity×DAT1 interaction (RRR = 0.21, $p < .05$; 95% CI = [0.06, 0.74]) suggested that a one standard deviation (SD) increase in negativity increased the risk of IA by 3.87 ($p < .05$; 95% CI = [1.11, 13.42]), but only among youth with the 9/9 or 9/10 genotype. No association was observed between negativity and IA among youth with the 10/10 genotype (RRR = 0.99, $p = .98$; 95% CI = [0.45, 2.18]). Similarly, no significant association was observed for the interaction between praise and DAT1 on IA (RRR = 1.80, $p = .24$; 95% CI = [0.68, 2.18]). We then tested the interaction between negativity and praise with DRD4 for IA. The negativity×DRD4 and praise×DRD4 interactions were not significant (RRR = .62, $p = .33$; 95% CI = [0.23, 1.64] and RRR = .83, $p = .73$; 95% CI = [0.29, 2.40], respectively).

Next, we compared the relative risk of HI (relative to non-ADHD). A significant interaction between DAT1 and negativity was detected (RRR = 0.09, $p < .05$; 95% CI = [0.01, 0.71]) where negativity marginally increased risk for HI (RRR = 3.54, $p = .08$; 95% CI = [0.42, 7.53]) among youth with the 9/9 or 9/10 genotypes, but not among youth with the 10/10 genotype (RRR = 1.06, $p = .94$; 95% CI = [0.23, 4.99]). No interaction was detected between praise and DAT1 for HI (RRR = 12.20, $p = .06$; 95% CI = [0.90, 20.10]). The negativity×DRD4

and praise×DRD4 interactions were not significant (RRR = 1.02, p = .98; 95% CI = [0.21, 5.08] and RRR = 1.38, p = .76; 95% CI = [0.18, 10.77], respectively).

Finally, we compared the relative risk for CT vs. being in the non-ADHD group. The interaction between negativity and DAT1 genotype was not significant (RRR = 0.54, p = .33; 95% CI = [0.16, 1.82]). Comparable patterns of non-significance were detected for the interaction between praise and DAT1 for CT (RRR = 12.20, p = .06; 95% CI = [0.90, 20.10]). There were so significant interactions between DRD4 and negativity (RRR = .51, p = .17; 95% CI = [0.19, 1.34]) and between DRD4 and praise (RRR = 1.26, p = .66; 95% CI = [0.45, 3.48]) for CT.

Observed Parenting Behaviors: Dimensional ADHD

Using the same data analytic models as those described above, we examined the association of DAT1, observed parental negativity and praise, and their respective interactions in separate models with the total number of parent-rated ADHD symptoms. Using negative binomial regression, we found that DAT1, parental negativity, and their interaction was each unrelated to the number of parent-rated ADHD symptoms (B = 0.23, SE = 0.22, p = .31; B = 0.51, SE = 0.36, p = .16; and B = -0.45, SE = 0.43, p = .29, respectively). In the same model, but with praise instead of negativity, similar patterns of non-significance were observed for DAT1, praise, and their interaction for the number of parent-rated ADHD symptoms (B = -0.03, SE = 0.24, p = .91 and B = 0.04, SE = 0.26, p = .86; and B = 0.15, SE = 0.34, p = .67, respectively).

For the models with DRD4, there was no main effect of genotype (B = 0.29, SE = 0.22, p = .20), negativity (B = 0.40, SE = 0.24, p = .10) or their interaction (B = -0.32, SE = 0.42, p = .44) with parent-rated ADHD symptoms. There was also no main effect for DRD4 (B = 0.07, SE

= 0.25, p = .78), praise (B = 0.09, SE = 0.19, p = .66), and their interaction (B = 0.15, SE = 0.38, p = .70).

Self-Reported Parenting Behaviors: Categorical ADHD

We analyzed self-reported negative parenting from the APQ, child genotypes, and their interaction on parent-rated ADHD (i.e., subtypes) using multinomial logistic regression. We controlled for age, sex, race-ethnicity, parental depression symptoms, family income, and child ODD diagnosis from the DISC. We then reproduced this model but with positive parenting. First, we examined the risk of being in the IA group, relative to the non-ADHD group. A significant interaction emerged between DAT1 and negative parenting (RRR = 2.96, p < .05; 95% CI = [1.24, 7.03]), but not between DAT1 and positive parenting (RRR = .70, p = .39; 95% CI = [0.32, 1.56]). The association between negative parenting and IA was only significant among youth with the 9/9 and 9/10 genotypes, such that high negative parenting increased the risk of IA group membership by 2.13 (p < .05; 95% CI = [1.11, 4.08]). There was no association between negative parenting and IA among youth with the 10/10 genotype (RRR = .62, p = .19; 95% CI = [0.30, 1.27]). There was also no interaction between DRD4 and negative parenting (RRR = .98, p = .96; 95% CI = [0.41, 2.34]) and between DRD4 and positive parenting (RRR = 2.96, p < .05; 95% CI = [1.24, 7.03]).

We then compared the risk of being in the HI group relative to the non-ADHD group. No interaction emerged between DAT1 and negative parenting (RRR = 2.39, p = .34; 95% CI = [0.40, 14.27]) and between DAT1 and positive parenting (RRR = .84, p = .84; 95% CI = [0.16, 4.36]). The DRD4×negative parenting and DRD4×positive parenting interactions were also not significant for HI (RRR = .25, p = .15; 95% CI = [0.04, 1.69] and RRR = 1.96, p = .42; 95% CI = [0.38, 9.99], respectively).

Finally, we examined the relative risk of CT vs. non-ADHD group. No significant effects emerged for DAT1×negative parenting or DAT1×positive parenting (RRR = 1.45, p = .40; 95% CI = [0.61, 3.42] and RRR = .75, p = .49; 95% CI = [0.33, 1.72], respectively). Similarly, there were no significant effects for the DRD4×negative parenting or DRD4×positive parenting for CT (RRR = .43, p = .07; 95% CI = [0.17, 1.08] and RRR = .78, p = .56; 95% CI = [0.34, 1.80], respectively).

Self-Reported Parenting Behaviors: Dimensional ADHD

We then examined the association of DAT1, self-reported positive and negative parenting behaviors, and their respective interactions in separate models with the total number of parent-rated ADHD symptoms. For the models with DAT1, there was no main effect of genotype (B = -0.04, SE = 0.13, p = .77), negative parenting (B = 0.04, SE = 0.10, p = .72) or their interaction (B = 0.15, SE = 0.13, p = .25) with parent-rated ADHD symptoms. There was also no main effect for DAT1 (B = -0.03, SE = 0.13, p = .79), positive parenting (B = 0.04, SE = 0.09, p = .96), and their interaction (B = -0.02, SE = 0.13, p = .86).

For DRD4, we found that negative parenting was positively associated with counts of ADHD symptoms (B = 0.21, SE = 0.08, p < .001). However, DRD4 and the interaction of DRD4 and negative parenting was unrelated to counts of ADHD symptoms (B = 0.14, SE = 0.13, p = .29 and B = -0.13, SE = 0.14, p = .34, respectively). In the same model, but with positive instead of negative parenting, DRD4, positive parenting, and their interaction were unrelated to the number of parent-rated ADHD symptoms (B = -0.10, SE = 0.13, p = .46; B = -0.04, SE = 0.08, p = .58; and B = 0.07, SE = 0.13, p = .61, respectively).

Follow-Up Analyses for Differential Susceptibility

Follow-up analyses were conducted on statistically significant interactions in order to distinguish differential susceptibility from other interaction effects (e.g., "dual risk"). Differential susceptibility is present when: (1) there is a statistically significant interaction; (2) the susceptibility factor and the predictor are independent (i.e., no gene-environment correlation); (3) the association between susceptibility factor and outcome is independent; (4) there is a cross-over effect in which the slope of the susceptible subgroup is significantly different from zero and concurrently, significantly steeper than the slope for the non-susceptible subgroup; and (5) the model is not replicated when a different susceptibility factor (or outcome) is tested, demonstrating specificity of the effect. Three interactions emerged from our results above: (1) DAT1×observed negativity for IA, (2) DAT1× observed negativity for HI, and (3) DAT1×self-reported negative parenting for IA.

First, we tested steps 2 and 3 by examining main effects of DAT1 on observed negativity, self-reported negative parenting, IA and HI. Controlling for age, sex, and race-ethnicity, offspring DAT1 genotype was unrelated to observed parental negativity (B = .05, SE = .15, p = .76), self-reported negative parenting (B = -.16, SE = .15, p = .29), IA (B = -.21, SE = .38, p = .57) and HI (B = -.14, SE = .99, p = .86), which satisfies steps 2-3 in that DAT1 was independent from the predictors and the outcome (respectively). Furthermore, our *post hoc* analyses demonstrated that the association between parental negativity (and negative parenting) with ADHD outcomes was only significant for the 10/10 group, and not the 9/9 or 9/10 groups (see Figures 1, 2 and 3), which indicates a cross-over interaction required for differential susceptibility in step 4. Finally, to test step 5 (i.e., specificity), we examined the same models in the original analyses but with the serotonin transporter gene (5-HTTLPR) as the susceptibility factor instead of DAT1. The interaction between 5-HTTLPR and observed parental negativity

was not significant for IA (RRR = .87, p = .87; 95% CI = [0.16, 4.82]) and HI (RRR = 1.71, p = .66; 95% CI = [0.16, 18.51]), indicating that the G×E effects were specific to DAT1 and parental negativity. All criteria for differential susceptibility were met for the interactions, indicating that among children with the 10/10 genotype, those with the most exposure to parental negativity (and negative parenting) had the highest risk for ADHD, but concurrently had the lowest risk for ADHD if they had low exposure to parental negativity (and negative parenting).

Discussion

Although parental negativity is reliably correlated with ADHD (Wells et al., 2000; Chronis-Tuscano et al., 2008), few G×E studies examined its interaction with genotype and differentiated negative and positive parenting, despite their separable roles in gene expression (Zhu et al., 2010; Belsky & Pluess, 2009). A significant interaction between observed parental negativity and child DAT1 suggested that negativity increased the risk for Inattentive-type (IA) and Hyperactive-type ADHD (HI) among youth with 9/9 or 9/10 genotypes, but not among youth with the 10/10 genotype. These results were replicated for IA (but not HI) using self-reported negative parenting behaviors. We did not detect any significant interactions between DRD4 and parenting behaviors (observed and self-reported) for ADHD. Furthermore, dimensional ratings of ADHD from parents were not sensitive to the genotype× parenting interactions.

Our findings provide additional support to the emerging literature that child genotype interacts with negative parenting for ADHD (Li & Lee, 2012). However, the association of the 9-repeat allele and ADHD differs from the results produced in Study I, as well as from two other G×E studies of ADHD which implicated the risk of 10-repeat homozygosity, although crucially, these previous studies were based on entirely different environmental conditions (i.e.,

maltreatment, prenatal exposure to teratogens, psychosocial adversity) rather than parenting behavior broadly (Kahn et al., 2003; Laucht et al., 2007). Importantly, the precise biological functionality of the DAT1 genotype is unknown (Brookes et al., 2006). Single photon emission computed tomography demonstrated that DAT1 was associated with DA transporter availability and binding potential, but the direction of these effects was unclear (Heinz et al., 2000). Although the 10-repeat homozygosity was positively correlated with DAT1 mRNA expression in human post-mortem midbrain tissue, comparisons with the 9-repeat allele were prohibitive due to limited sample size (Brookes et al., 2007). Human post-mortem studies based on larger sample sizes (e.g., Fuke et al., 2001; VanNess, Owens & Kilts, 2005) failed to find functional differences, perhaps due to the fact that most human cell lines do not naturally express DA (Brookes et al., 2007). Stronger evidence for functional differences in the DAT1 genotype are suggested by fMRI studies, which reported greater activation in the striatum and basal ganglia (i.e., regions that are rich in DA neurotransmission and are involved in motor and reward pathways) during reward-based, go/no-go inhibition tasks among individuals with at least one 9-repeat allele (Congdon et al., 2009). However, conflicting or null results have also been reported (Caldu et al., 2007). Thus, contrasting findings as those reported here, may suggest that DA expression in the brain is likely to be influenced by additional factors beyond DAT1. They may also reflect the influence of other unmeasured variants that are in linkage disequilibrium with the DAT1 VNTR. Finally, statistical geneticists have proposed that these so called "allele flips" may reflect genuine differences in which both "sets" of findings are valid (Clarke & Cardon, 2010).

We speculate that DA may play an especially crucial role in response to parenting, given that the association between parental negativity and ADHD only applied to youth with the 9-repeat DAT1 allele. Youth with this genotype were *more likely* and *less likely* to have ADHD

depending on whether they were exposed more or less negative parenting, respectively. This finding partially supports the differential susceptibility hypothesis, as we did not detect these effects with genotype and positive parenting. The rate of DA binding may depend on the salience of the environmental stimulus (Tripp & Wickens, 2008). DA neurons exhibit the fastest firing rate prior to a salient reward, but the firing rate falls below baseline in anticipation of a harsh punishment (Mirenowicz & Schultz, 1996). Similarly, alterations in the environment can profoundly influence DA binding and expression: individually housed monkeys in impoverished conditions exhibited increased DA and concurrent down-regulation of D2 receptors levels in the prefrontal cortex (Morgan et al., 2002). Monkeys that were later exposed to social housing (i.e., enriched environment) increased their D2 receptor binding and exhibited "normal" levels synaptic DA, however (Morgan et al., 2002). These findings may also help explain why the G×E effects were specific to parental negativity, given that praise may not be sufficiently salient for DA activation. In our study, parental praise during the parent-child interaction task occurred more frequently than instances of negativity, suggesting that negative parenting behavior may be more strongly associated with childhood ADHD than positive behaviors. This view is consistent with evidence that negative parenting was associated with offspring ADHD, but not positive parenting (Ellis & Nigg, 2009; Li & Lee, 2012). Genotypes may also increase susceptibility to both negative and positive environmental conditions, such that children with a particular genotype would have the most ADHD symptoms under conditions of adversity, but also have the fewest ADHD symptoms under conditions of enrichment (Belsky & Pluess, 2009). Future G×E studies should examine positive and negative environmental conditions to test the validity of differential susceptibility.

In addition to the methodological strengths of this study, the G×E findings have potential clinical implications. Children with the 10/10 genotype were protected against environmental risk, in that exposure to parental negativity did not increase their risk for ADHD compared to children with the 9/9 and 9/10 genotypes. Thus, environmental risks may interact with genes to predict not only vulnerability, but also resilience (Kim-Cohen & Gold, 2009). Incorporating genetics into psychosocial research may help identify the biological factors that promote resilience and prevent psychopathology among individuals who experience severe and/or chronic psychosocial adversity (Kim-Cohen & Gold, 2009). Further, there is evidence that certain genetic polymorphisms may confer differential susceptibility. Kranenberg et al. (2008) showed that a parent training intervention designed to increase parental sensitivity was more effective in reducing externalizing problems in preschool-aged children with the "risk" variant of a dopamine receptor gene than in youth without the "risk" variant, supporting the notion that certain individuals show a strong response to intervention. These investigations suggest important implications for intervention, including targeted populations and interventions that focus on environmental change and enrichment (Belsky et al., 2007).

There were important limitations to our study. First, observations from the parent-child interaction task may not have reflected parenting behavior outside of the lab. However, the use of observational coding of parenting behavior may have provided more objective data given that self-report data may reflect psychopathology or negative attributions about the child (Gardner, 2000). Nonetheless, we supplemented our observational codes of parenting behavior with self-reported parenting behaviors on a questionnaire and produced consistent findings using both methods. Next, our findings were robust to stringent control of potentially confounding variables such as parental depression, SES, and child noncompliance during the parent-child

interaction, thereby suggesting that the G×E was specific to ADHD. Future G×E studies should also attempt to improve effect size, including improving the characterization of the phenotype and the environmental criterion (e.g., latent class analysis of phenotypes; see Li & Lee, 2010). Finally, we note that when dimensional ratings of ADHD were used, the G×E effects were not significant, suggesting that (1) clinically-defined categories of ADHD using careful ascertainment procedures may be more robust in molecular genetic studies and (2) future studies should continue to use both approaches, given that these approaches may tap into qualitatively different phenotypes (Thapar et al., 2006).

The current study suggests that offspring DAT1 genotype may interact with parental negativity to increase the risk for ADHD. Differential susceptibility was partially implicated in that youth with the 9/9 and 9/10 DAT1 genotypes were sensitive to more and less parental negativity in terms of having a greater and lower risk of ADHD, respectively. However, these associations were not replicated with genotype and positive parenting dimensions for ADHD. Given that DA transmission is influenced by positive and negative aspects of the environment, future G×E studies must avoid narrow definitions of environmental adversity. More importantly, the present findings underscore the unique contributions that are afforded by genetically-sensitive designs that simultaneously incorporate careful measurement of biologically-plausible environmental conditions. Further research is needed on the mechanisms of G×E, which may indicate potential targets for environmental intervention. We conclude by emphasizing that parent-child interaction constitutes and dynamically represents important forms of interplay (i.e., correlation, interaction) between genetic and environmental influences (Jaffee & Price, 2007).

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Appendix: Tables and Figures

Table 6: Descriptive statistics

	Non-ADHD (N = 70)	ADHD (N = 80)	Test Statistic	*p*
Mean age (SD)	7.36 (1.18)	7.51 (1.12)	$F = 1.03$	.31
% Caucasian	54.54	55.96	$X^2 = .04$	.84
% Male	64.89	72.72	$X^2 = 2.07$	.15
Mean WISC-IV FSIQ (SD)	108.85 (15.61)	104.03 (14.51)	$F = 5.62$	<.05
% ODD diagnosis	12.96	46.77	$X^2 = 30.93$	<.001
% CD diagnosis	0	7.26	$X^2 = 8.16$	<.01
% Anxiety (not specific phobia)	8.08	18.18	$X^2 = 4.58$	<.05
% MDD	0	5.65	$X^2 = 6.29$	<.05
% Household income < $70,000	67.32	63.55	$X^2 = .33$	.57
Mean parental BDI (SD)	6.33 (6.08)	7.27 (5.93)	$F = 1.29$	.28
Mean parental negativity/minute (SD)	.30 (.27)	.46 (.42)	$F = 8.53$	<.01
Mean praise/minute (SD)	.51 (.48)	.53 (.44)	$F = .11$	.74
Noncompliance/minute (SD)	.13 (.19)	.25 (.31)	$F = 10.01$	<.01

Note. WISC-IV FSIQ = Wechsler Intelligence Scale for Children, Fourth Edition, Full Scale IQ; ODD = oppositional defiant disorder; CD = conduct disorder; MDD = major depressive disorder; BDI = Beck Depression Inventory

Table 7: Multinomial logistic regression from DISC-IV: Interaction of negativity and DAT1

					95% Conf. Int.	
Group	Variable	RRR	SE	P	Lower	Upper
Inattentive Type	Age	1.22	0.29	0.40	0.77	1.95
(*n* = 35)	Sex	1.78	0.89	0.25	0.67	4.75
	Race-ethnicity	0.93	0.47	0.88	0.34	2.52
	Family income	0.38	0.20	0.06	0.13	1.06
	Parental depression	0.89	0.25	0.68	0.51	1.54
	Noncompliance	1.06	0.37	0.87	0.53	2.09
	Parental negativity	4.60	2.62	0.01	1.51	14.03
	Child DAT1	0.71	0.36	0.49	0.26	1.89
	Negativity x DAT1	0.21	0.13	0.02	0.06	0.74
Hyperactive Type	Age	1.43	0.58	0.39	0.64	3.18
(*n* = 9)	Sex	0.59	0.58	0.60	0.09	4.08
	Race-ethnicity	0.55	0.53	0.54	0.08	3.70
	Family income	1.25	1.08	0.99	0.00	0.87
	Parental depression	2.65	1.17	0.03	1.12	6.28
	Noncompliance	1.55	0.95	0.48	0.47	5.18
	Parental negativity	7.79	6.00	0.01	1.72	35.23
	Child DAT1	0.87	0.85	0.89	0.13	5.99
	Negativity x DAT1	0.09	0.10	0.02	0.01	0.71
Combined Type	Age	0.59	0.14	0.03	0.37	0.95
(*n* = 36)	Sex	1.14	0.56	0.78	0.44	2.96
	Race-ethnicity	0.81	0.39	0.67	0.32	2.08
	Family income	0.49	0.27	0.19	0.17	1.42
	Parental depression	1.01	0.25	0.97	0.62	1.65
	Noncompliance	1.13	0.33	0.69	0.63	2.00
	Parental negativity	2.46	1.40	0.11	0.81	7.52
	Child DAT1	0.87	0.42	0.77	0.33	2.25
	Negativity x DAT1	0.54	0.33	0.32	0.16	1.82

Note. All group comparisons were relative to the non-ADHD group (*n* = 70); RRR = relative risk ratios (relative to non-ADHD group); SE = standard error.

Table 8: Multinomial logistic regression from DISC-IV: Interaction of negativity and DRD4

					95% CI	
Group	Variable	RRR	SE	P	Lower	Upper
Inattentive Type	Age	1.04	0.23	0.86	0.67	1.62
	Sex	1.42	0.68	0.47	0.55	3.62
	Race-ethnicity	0.82	0.40	0.68	0.32	2.13
	Family income	1.04	0.27	0.88	0.63	1.73
	Parental depression	0.31	0.16	0.02	0.12	0.83
	Noncompliance	1.11	0.32	0.70	0.64	1.94
	Parental negativity	1.90	0.61	0.05	1.01	3.58
	Child DRD4	1.09	0.52	0.85	0.43	2.77
	Negativity x DRD4	0.62	0.31	0.33	0.23	1.64
Hyperactive Type	Age	0.87	0.32	0.70	0.42	1.78
	Sex	0.49	0.46	0.45	0.08	3.16
	Race-ethnicity	0.99	0.90	0.99	0.17	5.91
	Family income	2.71	1.10	0.01	1.23	5.98
	Parental depression	1.30	0.25	0.98	0.00	3.20
	Noncompliance	1.30	0.46	0.47	0.65	2.61
	Parental negativity	1.81	0.94	0.26	0.65	5.01
	Child DRD4	3.25	2.98	0.20	0.54	19.60
	Negativity x DRD4	1.02	0.84	0.98	0.21	5.08
Combined Type	Age	0.60	0.13	0.02	0.38	0.92
	Sex	1.02	0.48	0.97	0.40	2.57
	Race-ethnicity	0.97	0.45	0.95	0.39	2.42
	Family income	1.06	0.27	0.82	0.64	1.76
	Parental depression	0.63	0.32	0.36	0.23	1.72
	Noncompliance	1.49	0.36	0.09	0.94	2.39
	Parental negativity	1.65	0.53	0.12	0.88	3.11
	Child DRD4	1.35	0.62	0.51	0.55	3.31
	Negativity x DRD4	0.51	0.25	0.17	0.19	1.34

Note. All group comparisons were relative to the non-ADHD group (n = 70); RRR = relative risk ratios (relative to non-ADHD group); SE = standard error.

Table 9: Multinomial logistic regression of DISC-IV: Interaction of praise and DAT1

					95% Conf. Int.	
Group	Variables	RRR	SE	p	Lower	Upper
Inattentive Type	Age	1.22	0.29	0.39	0.77	1.94
(*n* = 35)	Sex	1.72	0.83	0.26	0.67	4.45
	Race-ethnicity	1.20	0.59	0.71	0.45	3.17
	Family income	0.45	0.23	0.11	0.17	1.20
	Parental depression	1.06	0.27	0.83	0.64	1.75
	Noncompliance	1.39	0.41	0.27	0.77	2.49
	Praise	0.80	0.32	0.57	0.37	1.74
	Child DAT1	0.84	0.40	0.71	0.33	2.11
	Praise x DAT1	1.80	0.90	0.24	0.68	4.77
Hyperactive Type	Age	0.89	0.39	0.79	0.38	2.08
(*n*= 9)	Sex	0.38	0.39	0.35	0.05	2.90
	Race-ethnicity	0.39	0.41	0.37	0.05	3.01
	Family income	1.88	2.40	0.99	0.00	0.99
	Parental depression	2.39	1.04	0.05	1.02	5.61
	Noncompliance	2.50	1.36	0.09	0.86	7.27
	Praise	0.11	0.15	0.09	0.01	1.42
	Child DAT1	1.03	1.14	0.98	0.12	9.01
	Praise x DAT1	12.20	16.26	0.06	0.90	20.10
Combined Type	Age	0.58	0.14	0.03	0.36	0.94
(*n* = 36)	Sex	1.15	0.56	0.78	0.44	2.97
	Race-ethnicity	0.89	0.43	0.81	0.34	2.32
	Family income	0.58	0.31	0.31	0.20	1.66
	Parental depression	1.04	0.26	0.88	0.63	1.69
	Noncompliance	1.35	0.37	0.27	0.79	2.30
	Praise	1.02	0.33	0.96	0.54	1.90
	Child DAT1	1.17	0.54	0.74	0.47	2.89
	Praise x DAT1	0.88	0.40	0.77	0.35	2.16

Note. All group comparisons were relative to the non-ADHD group (*n* = 70); RRR = relative risk ratios (relative to non-ADHD group); SE = standard error.

Table 10: Multinomial logistic regression from DISC-IV: Interaction of praise and DRD4

					95% CI	
Group	Variable	RRR	SE	P	Lower	Upper
Inattentive Type	Age	1.10	0.25	0.67	0.71	1.72
	Sex	1.48	0.69	0.40	0.59	3.71
	Race-ethnicity	1.04	0.49	0.94	0.41	2.63
	Family income	1.09	0.28	0.72	0.67	1.79
	Parental depression	0.35	0.17	0.03	0.13	0.92
	Noncompliance	1.38	0.36	0.23	0.82	2.31
	Praise	1.19	0.31	0.50	0.71	2.00
	Child DRD4	1.08	0.50	0.87	0.43	2.70
	Praise x DRD4	0.83	0.45	0.73	0.29	2.39
Hyperactive Type	Age	0.74	0.30	0.46	0.33	1.65
	Sex	0.45	0.42	0.40	0.07	2.88
	Race-ethnicity	0.93	0.86	0.93	0.15	5.73
	Family income	3.28	1.52	0.01	1.33	8.11
	Parental depression	4.19	2.59	0.98	0.00	3.44
	Noncompliance	1.62	0.59	0.19	0.79	3.31
	Praise	0.55	0.39	0.39	0.14	2.17
	Child DRD4	3.97	3.66	0.13	0.65	24.14
	Praise x DRD4	1.38	1.44	0.76	0.18	10.77
Combined Type	Age	0.61	0.14	0.03	0.39	0.95
	Sex	1.07	0.50	0.88	0.43	2.69
	Race-ethnicity	1.03	0.49	0.95	0.41	2.60
	Family income	1.08	0.27	0.77	0.66	1.78
	Parental depression	0.75	0.38	0.57	0.27	2.04
	Noncompliance	1.76	0.42	0.02	1.10	2.80
	Praise	0.90	0.26	0.71	0.52	1.57
	Child DRD4	1.34	0.61	0.52	0.55	3.28
	Praise x DRD4	1.26	0.65	0.66	0.45	3.48

Note. All group comparisons were relative to the non-ADHD group (n = 70); RRR = relative risk ratios (relative to non-ADHD group); SE = standard error.

Figure 3: Interaction of observed parental negativity and DAT1 genotype on parent-rated Inattentive Type ADHD

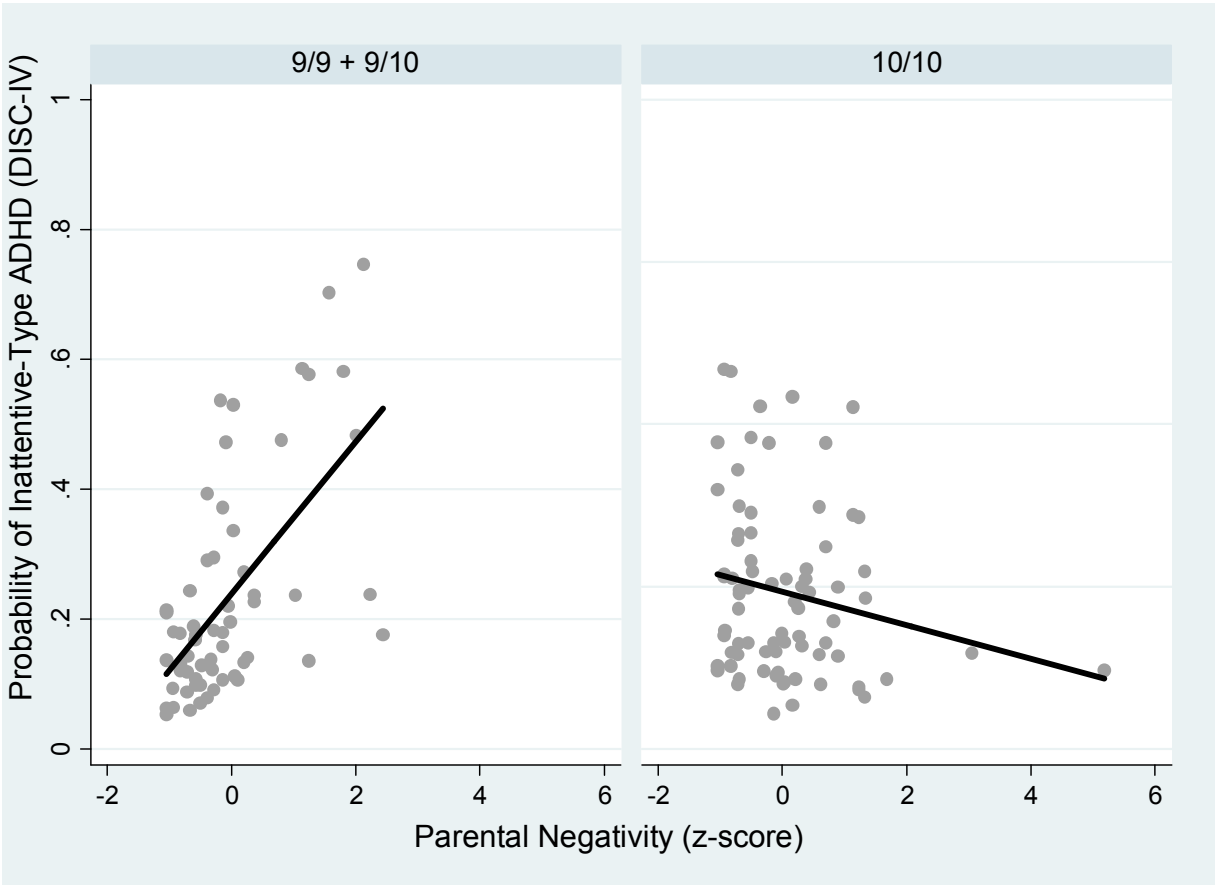


Figure 4: Interaction of observed parental negativity and DAT1 genotype on parent-rated Hyperactive/Impulsive Type ADHD

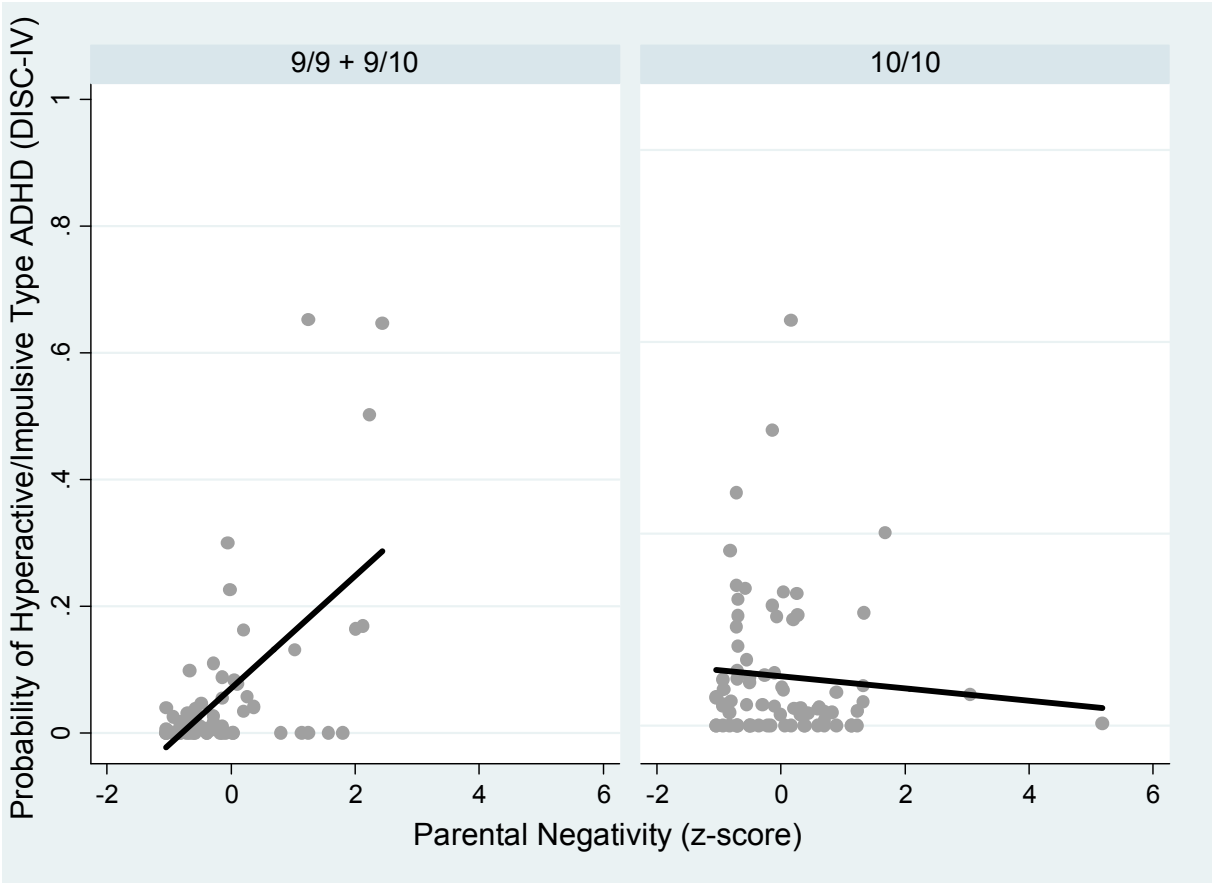
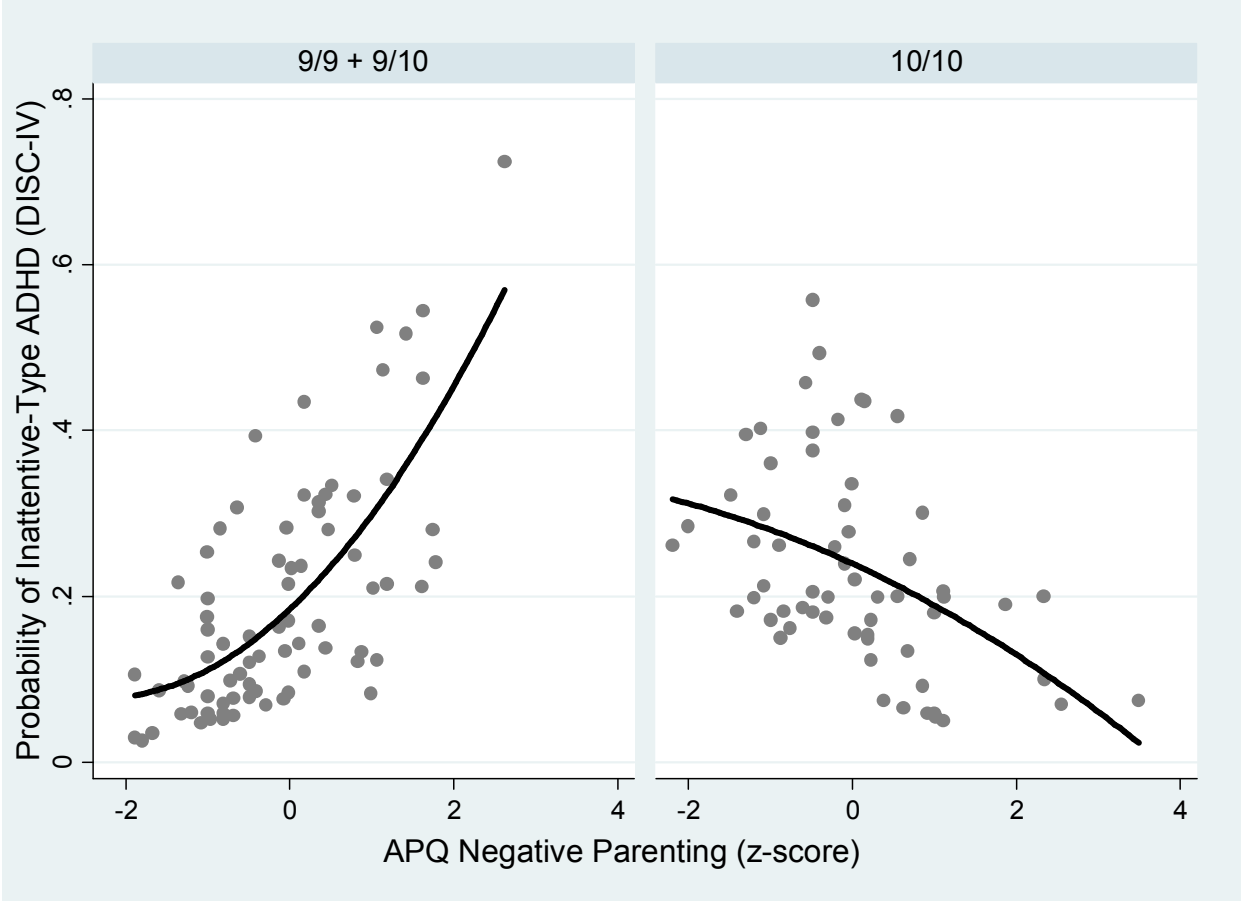


Figure 5: Interaction of self-reported negative parenting and DAT1 genotype on parent-rated Inattentive Type ADHD



Study III: Examining Neurocognitive Endophenotypes for ADHD

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Abstract

Background: Although the dopamine transporter (DAT1) and dopamine D4 receptor (DRD4) genes have been implicated in the etiology of attention-deficit/hyperactivity disorder (ADHD), relatively little is known about what mediates this association. Identifying intermediate markers between the phenotype in question and susceptibility loci may provide some leverage in uncovering the etiology of ADHD. Method: Using a multiple mediation framework, we examined individual differences in neurocognitive functions (NF) [verbal and visual spatial working memory, response inhibition] as mediators of the association of genotype and ADHD in a diverse sample of 150 children. Results: DAT1 was associated with response inhibition (Stroop Interference) and visual spatial working memory (Trails B), such that children with the 10/10 genotype performed better on these tasks than children with the 9/9 or 9/10 genotypes. DRD4 was not associated with any measure of NF. Response inhibition (SSRT) and working memory (Trails B) were inversely associated ADHD, whereby slower speeds on both tasks predicted more ADHD symptoms. However, NF did not mediate either DAT1 or DRD4 for dimensional or subtypes of ADHD. Discussion: We discuss the role of genetic variation and NF dimensions as intermediate traits in the development of ADHD, and provide alternative explanations for the null findings.

Keywords: Neurocognitive functions, ADHD, endophenotype, DAT1, DRD4.

Given the genetic and phenotypic complexity of childhood attention-deficit/hyperactivity disorder (ADHD), the search for replicated susceptibility loci has been elusive (Lesch, 2004). Whereas the previous studies in this dissertation test genetic susceptibility to environmental variation (i.e., G×E) on ADHD, the present study investigates whether the association between genotype and ADHD are mediated by neurocognitive functions (NF) (e.g., response inhibition, working memory). The endophenotype approach is promising because these traits are believed to be more closely connected to the latent substrates of the explicit phenotype, and have the potential to facilitate dissection of complex psychiatric disorders (Gottesman & Gould, 2003; Castellanos & Tannock, 2002). For example, imperfect heritability estimates and small effects sizes for even the most widely studied candidate genes for ADHD suggest gene products may be more proximal to intermediate constructs than to symptoms of the disorder (Waldman, 2005).

Although endophenotypes are conceptualized as mediators of genetic influences on complex phenotypes, formal tests of mediation are exceptionally rare (Munafo, 2006; see Martel et al., 2010 for an exception). Crucially, mediation illustrates *how* genetic variation eventuates in outcome, an important consideration given that psychopathology is likely to reflect multiple distinct causal pathways (Munafo, 2006). However, mediational tests may be hampered by the requirement of a significant effect of genotype on outcome (Collins, Graham, & Flaherty, 1998; Shrout & Bolger, 2002) given that effect sizes for genetic influences on psychopathology are typically modest (Willis-Owen et al., 2005). Therefore, the risk of Type II error is substantial when standard mediational techniques are used. However, there is emerging consensus that the predictor need not necessarily significantly predict outcome (in the absence of the mediator) for mediation to occur (MacKinnon, Krull, & Lockwood, 2000; Preacher & Hayes, 2008). Specifically, because the predictor may first change the mediator, which can subsequently

change the outcome (i.e., "stage sequence" mediation; Collins et al., 1998), skipping this step altogether is justified, particularly when the effects of the predictor on the outcome are distal with modest effect sizes (Shrout & Bolger, 2002). Additionally, classical mediational analyses may be limited by the fact that most outcomes are likely to reflect the simultaneous influence of *several* mediators. To combat this difficulty, multiple mediation (Preacher & Hayes, 2008) procedures disentangle the relative effect of each mediator within the model, and then compare competing models without suffering from biased parameter estimates (Preacher & Hayes, 2008). For example, the association between breast cancer screenings and the effectiveness of education programs to increase screenings is mediated by perceived susceptibility to breast cancer, perceived benefits of the mammogram, perceived consequences of the mammogram, and barriers to obtaining the mammogram (Aiken, West, Woodward, Reno, & Reynolds, 1994) (see Preacher and Hayes (2008) for additional examples of multiple mediation). Emerging findings suggest that NF may affect the relationship between candidate genes and ADHD via mediation (Waldman, 2005; Munafo, 2006), as these constructs (1) frequently co-occur with ADHD, (2) have shown evidence of heritability, and (3) exhibit familial and genetic overlap with ADHD (Martel, Gremillion, Roberts, von Eye, & Nigg, 2010; Almasy & Blangero, 2001).

There are few published molecular genetic studies of psychopathology and putative endophenotypes using traditional mediation methods, let alone multiple mediation. To understand *how* genetic variation results in individual differences in ADHD, the primary aim of this study was to examine simultaneous mediation effects of multidimensional aspects of working memory and response inhibition on the association between genotype and ADHD.

Primary Study Goal

(1) To test moderation and mediation effects of putative endophenotypes on the association of DAT1 and DRD4 genotypes on ADHD.

Method

Participants

We recruited 150 ethnically diverse (67% male; 56% Caucasian, 8% African America, 8% Hispanic or Latino, and 28% Mixed or Other) six to nine year-old children with and without ADHD. The mean age was 7.4 (SD = 1.1). We mailed flyers to local schools and pediatric offices, placed advertisements in public areas, delivered presentations to self-help groups, and received referrals from mental health service providers. Study eligibility required all participants to have a Full Scale IQ above 70, to live with one biological parent at least half the time, and be fluent in English. Exclusion criteria included mental retardation, seizure, autism, or pervasive developmental disorders. ADHD status was based on a fully structured diagnostic interview with the parent. Probands met full diagnostic criteria for any of the three DSM-IV ADHD subtypes. Non-ADHD controls were allowed to meet diagnostic criteria for any disorder other than ADHD according to the same structured diagnostic interview.

Procedures

Eligibility was determined after parents completed an initial telephone screening. Eligible families who were interested in participating were mailed rating scales and then invited to our laboratory for in-person assessments. Following parent consent and child assent, parents completed a structured diagnostic interview of child psychopathology while children completed standardized tests of cognitive ability and academic achievement. Approximately 85% of children were assessed without their medication and parents and teachers were asked to complete

rating scales based on the child's unmedicated behavior. Prior to each interview, all interviewers were blind to the child's diagnostic status. The IRB approved all study procedures.

Genotyping

DNA was extracted from saliva using DNA Genotek Oragene^{TM} Self-Collection Kits (DNA Genotek, Inc., Ottawa, Canada). DA transporter (DAT, SLC6A3) contains a 40-bp VNTR polymorphism in the 3'UTR. The 9-repeat (440 bp) and 10-repeat (480 bp) polymorphisms are the two most common alleles in the population. The primer sequences were: forward, 5'TGTGGTGTAGGGAACGGCCTGAG-3' (fluorescently labeled), and reverse: 5'CTTCCTGGAGGTCACGGCTCAAGG-3'. The DAT1 analyses were conducted by comparing individuals homozygous for the 10-repeat allele (i.e., 10/10) (n = 65; 43.5%) versus individuals with at least a one copy of the 9-repeat allele (i.e., 9/9 + 9/10) (n = 85; 56.5%).

Dopamine D4 Receptor (DRD4) contains a 48-bp VNTR in the third exon. The most common polymorphisms in this VNTR are the 4 and 7 repeats. The primer sequences were: forward, 5'AGGACCCTCATGGCCTTG-3' (fluorescently labeled), and reverse, 5'-GCGACTACGTGGTCTACTCG-3'. Following recommendations, we compared children carrying at least one copy of the 7-repeat allele (i.e., "risk" group) (n = 54; 64.3%) to those who do not have the 7-repeat allele (n = 96; 35.7%) (Loo et al., 2010).

Neuropsychological Measures

Wechsler Intelligence Scale for Children, 4th Edition, Digit Span and Arithmetic Subtests (Wechsler, 2004). In Digit Span, the child is verbally presented with a string of individual numbers and the child must repeat back the same sequence of numbers. The task is repeated again, with different numbers, but the child must repeat the numbers backwards. In Arithmetic, the child is verbally presented with a series of math problems and is asked to provide an answer

(within 30 seconds). Both tests require the ability to store and process verbal information into working memory. Digit Span and Arithmetic have previously demonstrated high internal consistency and test-retest reliability. In the analyses, we used scaled scores and summed both scores to create a composite for "verbal working memory."

Children's Memory Scale, Dot Locations I/II (Cohen, 1997). Children are provided a grid and either 6 (for children age 5-8) or 8 (for children age 9 and above) blue chips. The child is instructed to memorize a target pattern after repeated exposure (three times) and is asked to reproduce the pattern from memory after an interference trial was presented (immediate recall) and after 25 minutes had elapsed (delayed recall). The measure assesses the child's ability to process, learn and recall the spatial location of a dot pattern over three learning trials and a delayed trial (Cohen, 1997). We used the scaled score for the total Dot Locations score, which represented the "visual spatial working memory" component.

Trail Making Test (Reitan, 1979). In Trails A, children are instructed to connect a series of numbered circles in order from least to greatest (i.e., 1, 2, 3, etc.). In Trails B, children are presented with a random pattern of lettered and numbered circles on the page. The task requires children to connect the two sets of stimuli in an alternating order (i.e., 1, A, 2, B, 3, C, etc.). The amount of time taken (in seconds) to complete Trails A is believed to measure processing speed (Crowe, 1998), whereas Trails B is believed to measure working memory, processing speed and set-shifting ability (Waldman, 2005). Trails A and B exhibited good psychometric properties; Spreen and Strauss's (1998) review on the Trail Making Test reported test-retest reliability estimates between .50 to .89 for Trails A, and .54 to .86 for Trails B, with higher estimates for psychiatric populations and lower reliability for control populations. For the analyses, we used

the log transformation of the total time (in seconds) for Trails B only, given the focus on working memory for the present study.

Stroop Color-Word Test (Golden, 1978). The Stroop task consists of three subtests. First, children are instructed to read as many words (red, blue, green) as they can on a card in 45 seconds (Word condition). Next, children are instructed to say the names of the color of the ink on the card as quickly as possible in 45 seconds (Color condition). The Word and Color conditions are believed to measure verbal fluency (Golden, 1978). For the final condition, children are instructed to name the color of the printed word on the card, ignoring the word that is actually printed (Color-Word condition). The Color-Word condition measures interference control (i.e., response inhibition) and is the primary variable of interest for the present study. In order to assess functioning on these conditions, we used to total number of items correct within the 45-second limit. Each of the three Stroop conditions exhibit good reliability in children with and without ADHD (see Homack and Riccio, 2004) meta-analysis of the Stroop Task with children). For the present study, we only used the Color-Word interference raw score as a measure of response inhibition ability.

Stop-Signal Task (SST; Logan & Cowan, 1984). We used the STOP-IT program, developed by Verbruggen, Logan, and Stevens (2008) to assess response inhibition reaction time (SSRT). In this computerized task, participants are conditioned to perform responses to a standard two-choice reaction task in which a random stop-signal is presented on 25% of the trials, requiring the inhibition of the response to the target signal. Following the fixation sign (+), the target stimuli were a square and a circle that were presented in the center of the screen in white, on a black background. The stop signal (750 Hz, 75 msec) is presented after the stimulus onset. The primary variables are the SSRT and the coefficient of variability (CV), which was

calculated from the standard deviations of the no-signal trials divided by the mean reaction times of the no-signal trials (Vaurio, Simmonds, & Mostofsky, 2009).

ADHD

Diagnostic Interview Schedule for Children, Version IV (DISC-IV; Shaffer et al., 2000). The DISC-IV is a computer-assisted, fully structured diagnostic interview with the parent used to assess child psychopathology based on the Diagnostic and Statistical Manual, 4th Edition (American Psychiatric Association [APA], 1994). The ADHD module of the DISC-IV has good psychometric properties, including high test-retest reliability (r = .79 after one year) and internal consistency (ICC = .84 for symptoms counts, ICC = .77 for criterion counts) among the parents from a large community sample (see Shaffer et al., 2000). We analyzed ADHD categorically (i.e., by subtypes) and dimensionally using full DSM-IV diagnostic criteria and counts of the total number of DSM-IV ADHD symptoms, respectively.

Analyses

To evaluate multiple mediation, we used bootstrapping procedures recommended by Shrout and Bolger (2002) and Preacher and Hayes (2008). Bootstrapping is a nonparametric re-sampling procedure that repeatedly samples from a dataset *k* number of times, generating an empirical approximation of a sampling distribution of the indirect effect (Preacher & Hayes, 2008). We used the multiple mediator macro provided by Preacher and Hayes (2008), specifying 1,000 bootstrap samples and variables were entered as follows: DAT1 and DRD4 was entered as the independent variables (separately), dimensions of NF (i.e., verbal and visuospatial working memory, SSRT, CV, Trails B, and Stroop - interference) were entered simultaneously as mediators, and subtypes of ADHD as outcomes. We examined subtypes of ADHD in order to parallel the analytic strategies employed in Studies I and II. However, to complement our

subtype (i.e., categorical) approach as well as to characterize the ADHD phenotype with greater precision, we also include the results of these models with total ADHD symptoms as the outcome. For all models, we entered child age, race-ethnicity, sex and oppositional defiant disorder (ODD) diagnosis as covariates. Missing data was excluded via listwise deletion.

Results

Subtype Analyses: DAT1

We tested the potential mediation of DAT1 with ADHD Inattentive Type (IA) (relative to controls) and each component of NF using multinomial logistic regression and linear regression, respectively, controlling for child age, race-ethnicity, sex, and previous diagnosis of ODD. The total effect of DAT1 on IA (relative to control) was not significant (B = -.23, SE = .42, p = .58). DAT1 genotype was marginally associated with Trails B and significantly associated with Stroop-I (B = -.65, SE = .35, p = .06 and B = 2.35, SE = 1.02, p < .05, respectively), such that youth with the 10/10 genotype had faster Trails B scores and more correct responses on the Stroop-I relatively to youth with the 9/9 and 9/10 genotypes. DAT1 genotype was unrelated to SSRT, CV, verbal WM, and visual spatial VM, however. Furthermore, none of the NF components were related to IA. When the effect of DAT1 on IA was assessed through the mediators (i.e., direct effect), this association was also not significant (B = -.13, SE = .47, p = .79). We further assessed the total and specific indirect effects through NF using a bootstrap analysis, specifying 1,000 simulation samples (see Table 11). No significant indirect effects emerged in the mediation model involving DAT1, NF, and IA. Overall, no mediation effects via NF were detected for the association between DAT1 and IA.

Next, we tested the association of DAT1 with ADHD Hyperactive/Impulsive (HI) type through NF. The total effect (not accounting for mediators) of DAT1 with HI was not significant

(*B* = .21, *SE* = .46, *p* = .65). The parameter estimates for DAT1 with NF are identical to the model above for IA. No associations between NF and HI emerged in these models (see Figure 7). Finally, the direct effect of DAT1 on HI (through the mediators) was not significant (*B* = .30, *SE* = .47, *p* = .53). The total and specific indirect effect of each mediator was similarly not significant for HI, indicating no mediation. Finally, we tested the association of DAT1 with ADHD Combined-Type (CT) type through NF. The total effect of DAT1 on CT was not significant (*B* = -.17, *SE* = .46, *p* = .71). Furthermore, the direct effects of NF on HI were similarly not significant (see Figure 8) The direct effect of DAT1 on CT through the mediators was also not significant (*B* = -.10, *SE* = .48, *p* = .83). Bootstrap analysis for the indirect effect of each mediator yielded no significant effects (see Table 11).

Dimensional Analyses: DAT1

We examined the mediating effects of NF on DAT1 and counts of total ADHD symptoms using linear regression, controlling for child age, race-ethnicity, sex, and previous diagnosis of ODD. The total effect of DAT1 on ADHD symptoms was not significant (*B* = .11, *SE* = .78, *p* = .88). DAT1 genotype was associated with Stroop-I, such that youth with the DAT1 10/10 genotype performed better on the task than those with the 9/9 or 9/10 genotypes (*B* = 2.37, *SE* = 1.00, *p* < .01). DAT1 genotype was also modestly associated with Trails B performance, such that youth with the 10/10 genotype performed better on this task than youth with the 9/9 or 9/10 genotypes (*B* = -.62, *SE* = .34, *p* = .07). There was no association between DAT1 and SSRT, CV, verbal WM, and visual spatial WM (*B* = -14.02, *SE* = 16.20, *p* = .39, *B* = .005, *SE* = .01, *p* = .72, *B* = .08, *SE* = .81, *p* = .92, and *B* = .42, *SE* = .51, *p* = .42, respectively). SSRT and Trails B were significantly and marginally associated with ADHD symptoms, respectively (*B* = .008, *SE* = .004, *p* < .05 and *B* = .39, *SE* = .21, *p* = .06, respectively). Slower reaction times on the SST

and Trails B predicted more ADHD symptoms. However, CV, verbal WM, visual spatial WM, and Stroop-I were unrelated with ADHD symptoms (B = 5.88, SE = 5.11, p = .25, B = -.03, SE = .09, p = .76, B = -.01, SE = .13, p = .91, B = -.04, SE = .07, p = .52, respectively). The direct effect of DAT1 on ADHD symptoms, through the mediators, was not significant (B = .56, SE = .77, p = .47). The bootstrap results of the indirect effects for each mediator are presented on Table 13.

Subtype Analyses: DRD4

Using parallel analytic strategies as those performed for DAT1, we then tested the potential mediation of DRD4 with ADHD Inattentive type (IA) (relative to controls) through NF using multinomial logistic regression and linear regression, respectively, controlling for child age, race-ethnicity, sex, and previous diagnosis of ODD. The total effect of DRD4 on IA was not significant (B = -.02, SE = .36, p = .96). DRD4 was not associated with any NF measure (see Figure 9). However, Trails B was marginally associated with IA, such that youth who were slower on completing Trails B were more likely to have IA (B = .20, SE = .11, p = .06). SSRT, CV, verbal WM, visual spatial WM, and Stroop-I were not associated with IA. Also, the direct effect of DRD4 on IA through the mediators was not significant (B = .03, SE = .37, p = .93). We reported the bootstrap analyses reported in Table 12; these analyses did not yield any significant indirect effects.

We then tested the association DRD4 with Hyperactive/Impulsive (HI) type and NF. The total effect of DRD4 on HI was not significant (B = .73, SE = .46, p = .12). Similarly, none of the components of NF was associated with HI (see Figure 10). The direct effect of DRD4 on HI through the mediators was also not significant (B = .75, SE = .48, p = .12). Bootstrap analyses are reported in Table 12 and did not yield any significant indirect effects. For Combined type

(CT), the total effect and direct effects were not significant (B = .57, SE = .47, p = .22 and B = .62, SE = .48, p = .20, respectively). The direct effects of NF on CT were all non-significant (see Figure 11). Finally, the bootstrap analyses did not yield any significant indirect effects (Table 12).

Dimensional Analysis: DRD4

Finally, we tested the mediating effects of NF on DRD4 and counts of ADHD symptoms, controlling for child age, race-ethnicity, sex, and ODD. The total effect of DRD4 on ADHD was not significant (B = .65, SE = .78, p = .41). Furthermore, DRD4 was not associated with any aspect of NF (all $p > .10$). We did detect a significant association between SSRT and Trails B performance with ADHD. ADHD was positively associated with slower reaction times on the SST and Trails B (B = .01, SE = .004, $p < .05$ and B = .43, SE = .20, $p < .05$, respectively). The other aspects of NF were unrelated to ADHD, however ($p > .10$). Finally, there was no direct effect of DRD4 on ADHD, through the mediators (B = .73, SE = .76, p = .34). The indirect effects of NF on ADHD did not yield any significant mediating effects (see bootstrap analyses on Table 13).

Discussion

Previous research has established impaired working memory and response inhibition in ADHD (Gregoire, Rivalan, Le Moine, & Dellu-Hegadorn, 2012). Deficits within these domains may serve as plausible endophenotypes for ADHD, given that their functions are influenced by dopamine activity and are similarly associated with polymorphic variants in DAT1 and DRD4. We used a multiple mediation framework to examine whether working memory and response inhibitions served as endophenotypes between the association of genotype and ADHD. Our

results did not support a mediating role for working memory and response inhibition for the association of either DAT1 or DRD4 on ADHD.

Despite the fact that endophenotypes are often conceptualized as mediators of genetic influences on complex phenotypes, the present study is unique by virtue of being among the first to interrogate the endophenotype hypothesis for ADHD using a mediational framework. Molecular genetic studies of ADHD involving NF endophenotypes have emerged in recent years, although these studies did not employ mediation as the driving methodology. For instance, Bellgrove et al. (2005) found that ADHD children with the 10/10 DAT1 genotype had greater response variability and spatial attentional asymmetry, as well as lower sustained attention compared to non-10 repeat homozygotes and healthy controls (with either genotype), suggesting possible mediation of the DAT1 10-repeat allele on NF impairment in ADHD. For adults, it was the 9-repeat DAT1 carriers that were not only more likely to have ADHD, but also performed more poorly on measures of working memory compared to 10-repeat homozygotes (Brown et al., 2011). Regarding DRD4, a recent meta-analytic review found that reaction time variability was associated with 7-repeat absence in ADHD, while processing speed, inhibition and set shifting were not (Kebir & Joober, 2011). However, this same review also concluded that the vast majority of studies of DRD4 and DAT1 reported no association with NF in the context of ADHD, and that these genes may have a modulating rather than a mediating effect on NF with ADHD (Kebir & Joober, 2011). For instance, while Trails A response time mediated the effects of DRD4 on ADHD status, Trails B moderated these effects (see Waldman, 2005). Indeed, genetic moderation *and* mediation is rarely addressed in the current literature and is a promising analytic framework for future genetic studies involving endophenotypes.

It is also possible that environmental influences contribute endophenotypic variability or in ADHD. Environmental risk factors, such maternal smoking during pregnancy, are implicated in both ADHD and NF deficits in offspring and may potentially confound endophenotype studies. In a recent study, paternal (and not maternal) smoking during pregnancy was positively associated with poorer attentional control in offspring with ADHD, and this effect was mediated by paternal DAT1 and DRD4 genotypes, such that the association was reduced to non-significance if fathers carried the DRD4 7-repeat allele and the DAT1 intron 9 6/6 genotype (Altink et al., 2009). This finding suggests the possibility of epigenetic transmission, such that the effect of paternal smoking may be a proxy for common genetic risk factors for ADHD and related disorders transmitted to the offspring. In fact, environmental factors themselves may influence gene expression directly (Jaenisch & Bird, 2003; Wong et al., 2010). Hillemacher and colleagues (2008) studied 76 patients undergoing detoxification treatment (and were previously diagnosed with alcohol dependence) and reported significant DNA hypermethylation in the DAT1-promoter amongst patients compared to 35 healthy controls. Thus, environmental effects (i.e., detoxification treatment) directly affected DAT1 gene expression. These early findings illuminate the importance of environmental factors in examining potential mechanisms between susceptibility loci and a particular phenotype.

Several study limitations demand consideration. First, our mediation models were based on cross-sectional data. In the absence of longitudinal data, which affords temporal ordering, we are unable to authoritatively disentangle NF dimensions from ADHD. That is, interpreting mediation effects without temporal separation between the putative cause (i.e., NF deficts) and its effect (i.e., ADHD) is ambiguous (Maxwell & Cole, 2007; Collins et al., 1998). Future genetic studies of mediation must use longitudinal designs (e.g., autoregressive models) to

establish temporal separation of each construct (Maxwell & Cole, 2007), although genotype obviously precedes all relevant phenotypes. Second, some common co-occurring disorders with ADHD were not accounted for and may have influenced NF performance and genetic associations. Although ODD status was controlled in each of our models, we could not rule out the influence of specific learning disorders which are known to affect estimates of NF. Rommelse et al. (2009) examined the association of motor and executive endophenotypes with ADHD alone versus ADHD plus co-morbid conditions (e.g., anxiety, mood, ODD). Individuals with ADHD and another co-occurring disorder generally exhibited a wider range and greater severity of NF deficits compared to individuals with ADHD alone. Thus, although disentangling endophenotypes may improve genetic studies in helping to understand the etiology of complex phenotypes, the reality is that the variance explained by endophenotypes may or may not be greater than the variance explained by candidate genes alone, especially given the clinical heterogeneity of phenotypes such as ADHD. As an example, a genome-wide association study found SNPs that explained 6% of the variance for Type II diabetes (Zeggini et al., 2008) whereas SNPs associated for fasting glucose levels (believed to be an endophenotype for Type II diabetes) was only 1.5% (Prokopenko et al., 2009). This suggests that, in some cases, endophenotypes themselves may be more genetically complex than the phenotype in question.

The present study is significant by virtue of having formally tested mediation using a biologically-plausible and theoretically-derived set of putative endophenotypes for ADHD. Our approach utilized multiple mediation to account for the simultaneous influence of several NF dimensions, including for working memory and response inhibition. Decomposing endophenotypes by means of neurocognitive assessment may improve diagnostic assessment and treatment efficacy (Finke et al., 2011), but our findings did not support the NF endophenotype

hypothesis for ADHD in the context of two biologically-plausible candidate genes. Future studies should test genetic moderation and mediation on putative endophenotypes with ADHD, and integrate parental and/or environmental influences to account for possible epigenetic transmission of the endophenotype.

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Appendix: Tables and Figures

Table 11: Bootstrap analysis: Indirect Effects of NF on DAT1 and ADHD subtypes

			95% CI	
	Point Est.	SE	Lower	Upper
Inattentive Type				
TOTAL	-.17	.25	-0.70	.31
SSRT	-.03	.07	-.37	.04
CV	.02	.09	-.12	.25
Verbal WM	-.002	.06	-.14	.11
Visual Spatial WM	.008	.05	-.05	.23
Trails B	-.07	.13	-.45	.09
Stroop Interference	-.09	.15	-.46	.17
Hyperactive Type				
TOTAL	-.08	.19	-.46	.34
SSRT	-.03	.08	-.32	.03
CV	-.002	.05	-.11	.10
Verbal WM	.0007	.05	-.11	.11
Visual Spatial WM	.01	.07	-.07	.26
Trails B	-.05	.10	-.31	.12
Stroop Interference	-.002	.13	-.25	.30
Combined Type				
TOTAL	-.08	.23	-.51	.38
SSRT	-.04	.10	-.37	.05
CV	-.003	.07	-.13	.17
Verbal WM	.001	.06	-.12	.15
Visual Spatial WM	.007	.06	-.07	.22
Trails B	-.06	.12	-.39	.11
Stroop Interference	.02	.15	-.26	.35

Note. Point est. = point estimate of the indirect effect; SE = standard error; BCa bootstrap CI = bias corrected and accelerated confidence intervals; SSRT = stop signal response time; CV = stop signal coefficient of variability; Verbal WM = composite score from WISC-IV arithmetic and digit span; Visual spatial WM = total score from CMS dot locations.

Table 12: Bootstrap analysis: Indirect Effects of NF on DRD4 and ADHD subtypes

			95% CI	
	Point Est.	SE	Lower	Upper
Inattentive Type				
TOTAL	-0.04	0.18	-0.41	0.30
SSRT	0.02	0.07	-0.08	0.25
CV	-0.02	0.08	-0.20	0.15
Verbal WM	0.00	0.04	-0.10	0.09
Visual Spatial WM	0.00	0.04	-0.08	0.11
Trails B	-0.07	0.09	-0.31	0.06
Stroop Interference	0.03	0.07	-0.08	0.21
Hyperactive Type				
TOTAL	-0.03	0.19	-0.44	0.31
SSRT	0.02	0.08	-0.07	0.28
CV	0.00	0.06	-0.20	0.09
Verbal WM	0.00	0.07	-0.12	0.21
Visual Spatial WM	0.00	0.06	-0.11	0.13
Trails B	-0.03	0.08	-0.29	0.06
Stroop Interference	-0.01	0.11	-0.27	0.18
Combined Type				
TOTAL	-0.04	0.20	-0.47	0.37
SSRT	0.02	0.10	-0.13	0.29
CV	0.00	0.07	-0.14	0.08
Verbal WM	0.00	0.08	-0.17	0.19
Visual Spatial WM	0.00	0.07	-0.17	0.14
Trails B	-0.05	0.09	-0.35	0.05
Stroop Interference	-0.02	0.11	-0.33	0.19

Note. Point est. = point estimate of the indirect effect; SE = standard error; BCa bootstrap CI = bias corrected and accelerated confidence intervals; SSRT = stop signal response time; CV = stop signal coefficient of variability; Verbal WM = composite score from WISC-IV arithmetic and digit span; Visual spatial WM = total score from CMS dot locations.

Table 13: Bootstrap analysis: Indirect Effects of NF on DAT1/DRD4 and ADHD (continuous)

			95% CI	
	Point Est.	SE	Lower	Upper
DAT1				
TOTAL	-0.44	0.35	-1.21	0.20
SSRT	-0.12	0.17	-0.66	0.10
CV	0.02	0.09	-0.08	0.32
Verbal WM	0.00	0.07	-0.19	0.13
Visual Spatial WM	0.00	0.08	-0.28	0.12
Trails B	-0.24	0.19	-0.82	0.01
Stroop Interference	-0.10	0.17	-0.59	0.17
DRD4				
TOTAL	-0.07	0.33	-0.74	0.58
SSRT	0.06	0.17	-0.25	0.50
CV	0.02	0.08	-0.26	0.11
Verbal WM	0.00	0.06	-0.13	0.13
Visual Spatial WM	-0.01	0.08	-0.29	0.10
Trails B	-0.14	0.17	-0.61	0.10
Stroop Interference	0.04	0.13	-0.13	0.42

Note. Point est. = point estimate of the indirect effect; SE = standard error; BCa bootstrap CI = bias corrected and accelerated confidence intervals; SSRT = stop signal response time; CV = stop signal coefficient of variability; Verbal WM = composite score from WISC-IV arithmetic and digit span; Visual spatial WM = total score from CMS dot locations.

Figure 6: Multiple mediator model of the ADHD Inattentive-Type by NF measures and DAT1 (beta coefficients)

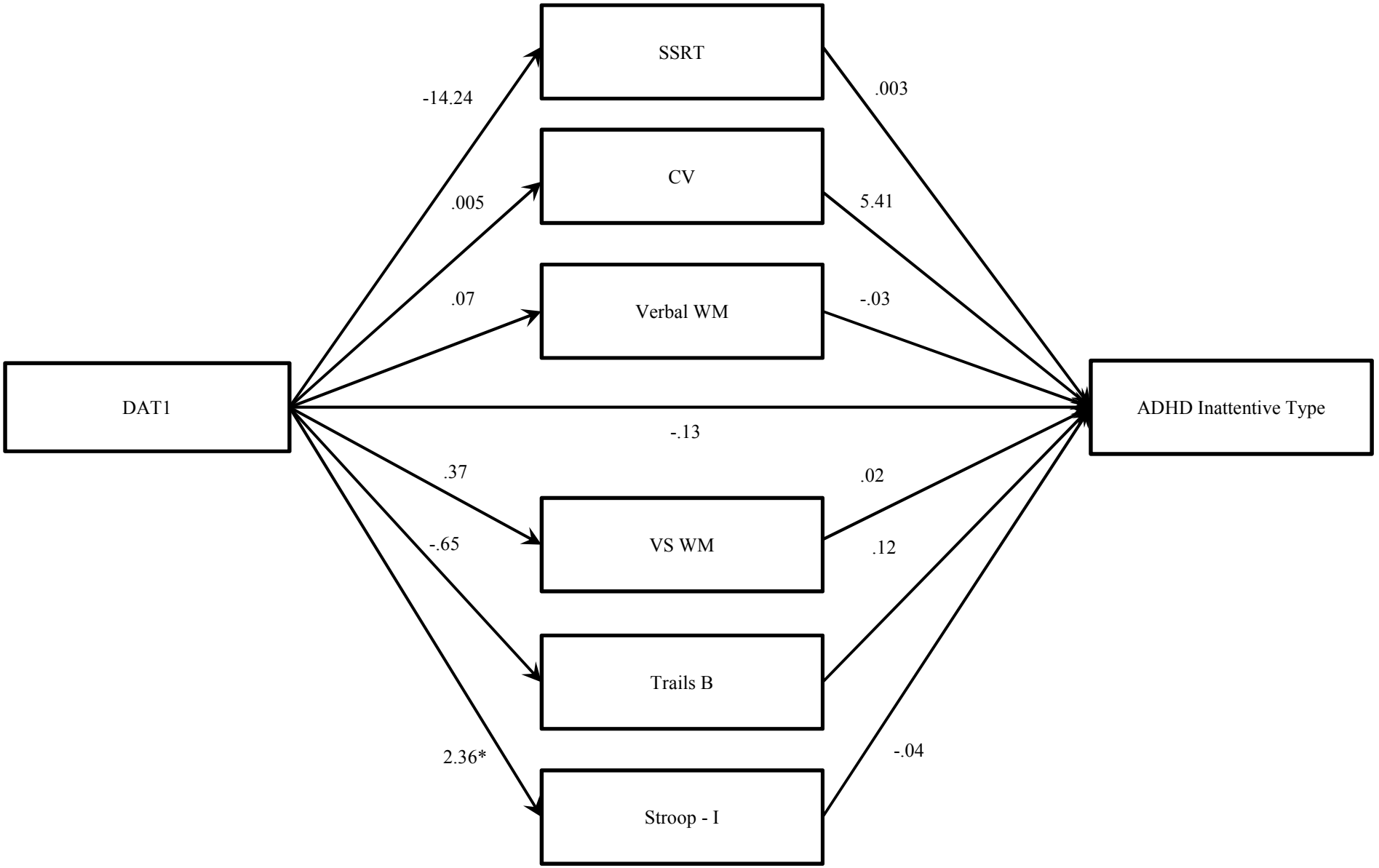


Figure 7: Multiple mediator model of the ADHD Hyperactive/Impulsive Type by NF measures and DAT1 (beta coefficients)

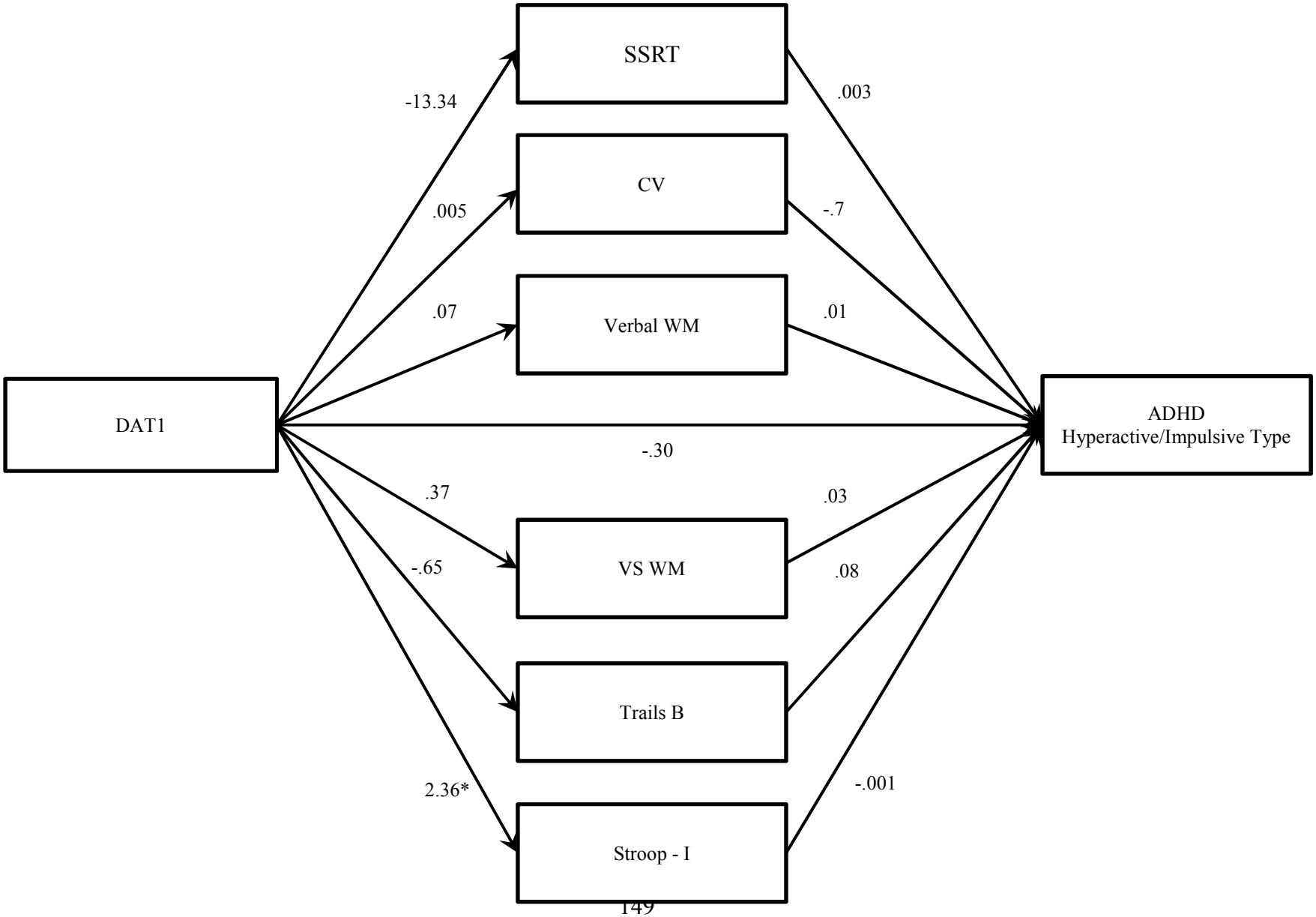


Figure 8: Multiple mediator model of the ADHD Combined Type by NF measures and DAT1 (beta coefficients)

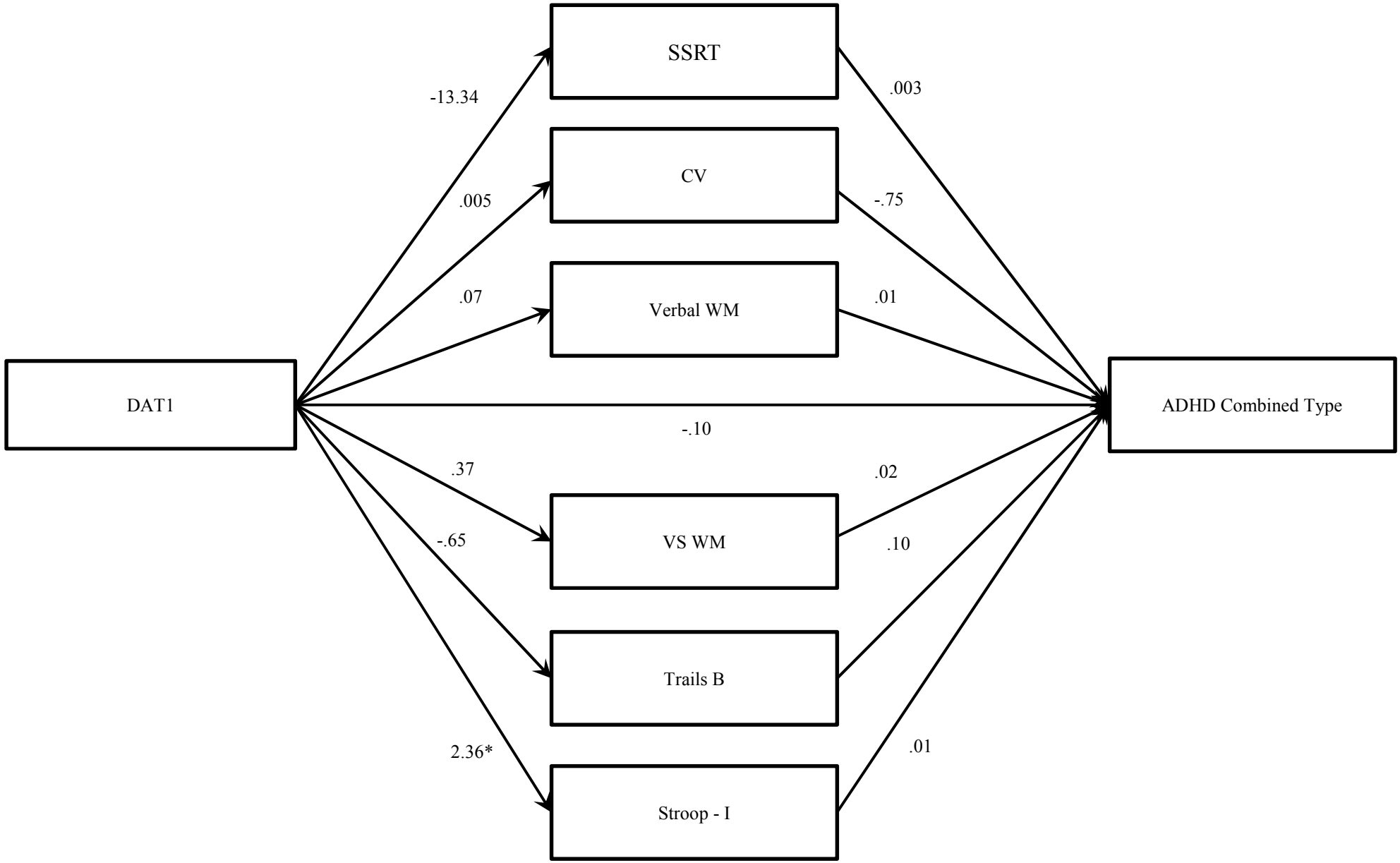


Figure 9: Multiple mediator model of the ADHD Inattentive Type by NF measures and DRD4 (beta coefficients)

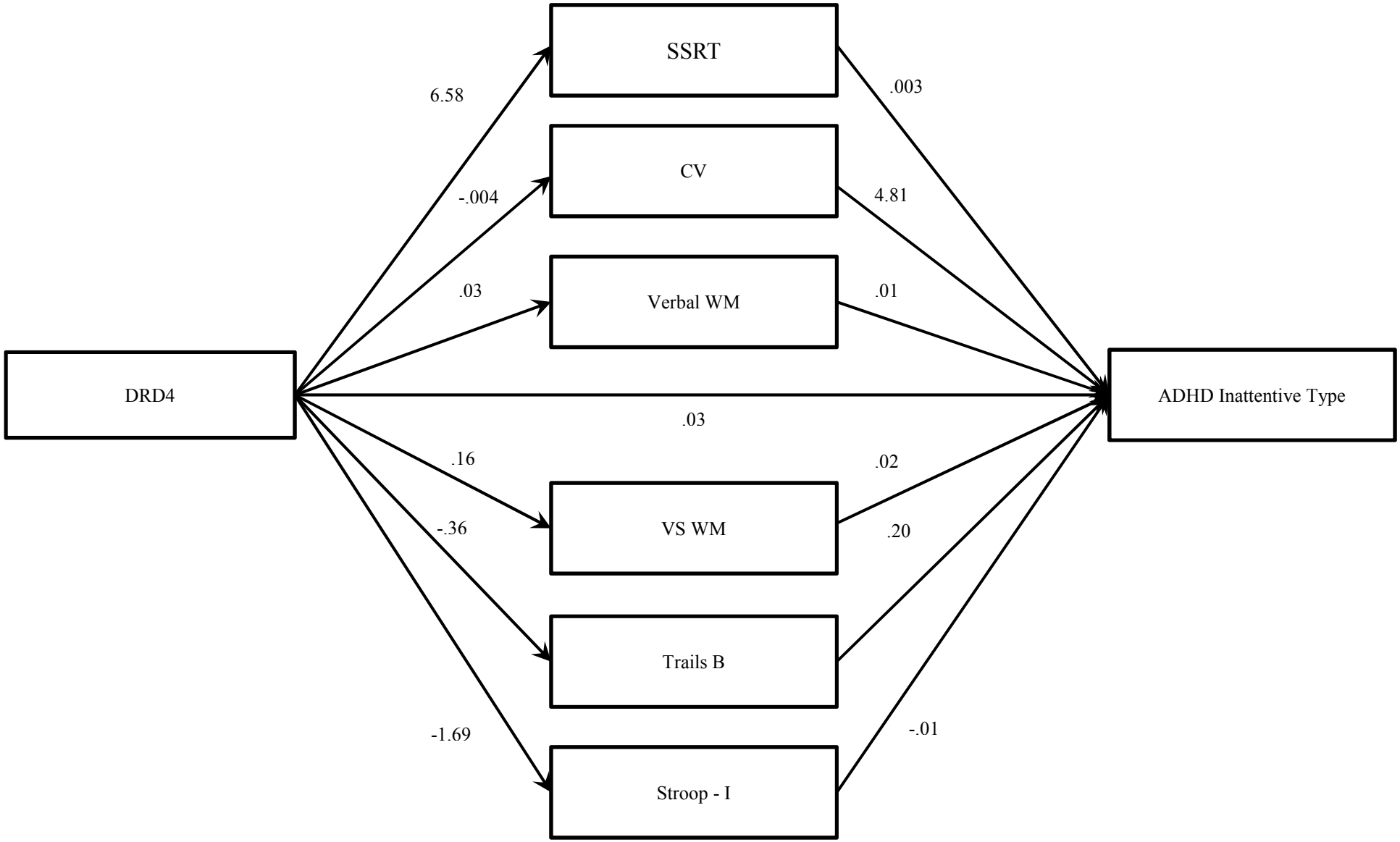


Figure 10: Multiple mediator model of the ADHD Hyperactive/Impulsive Type by NF measures and DRD4 (beta coefficients)

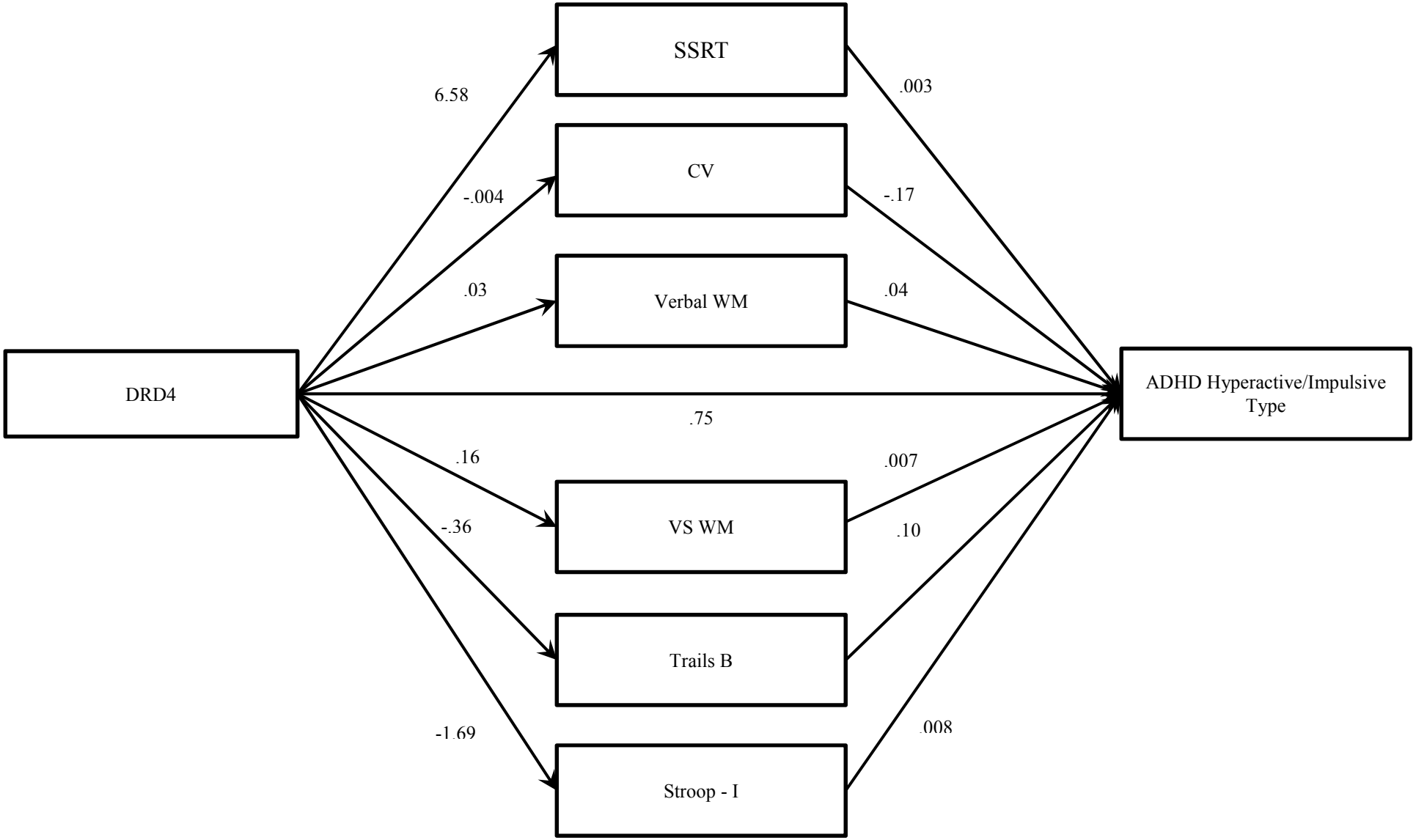
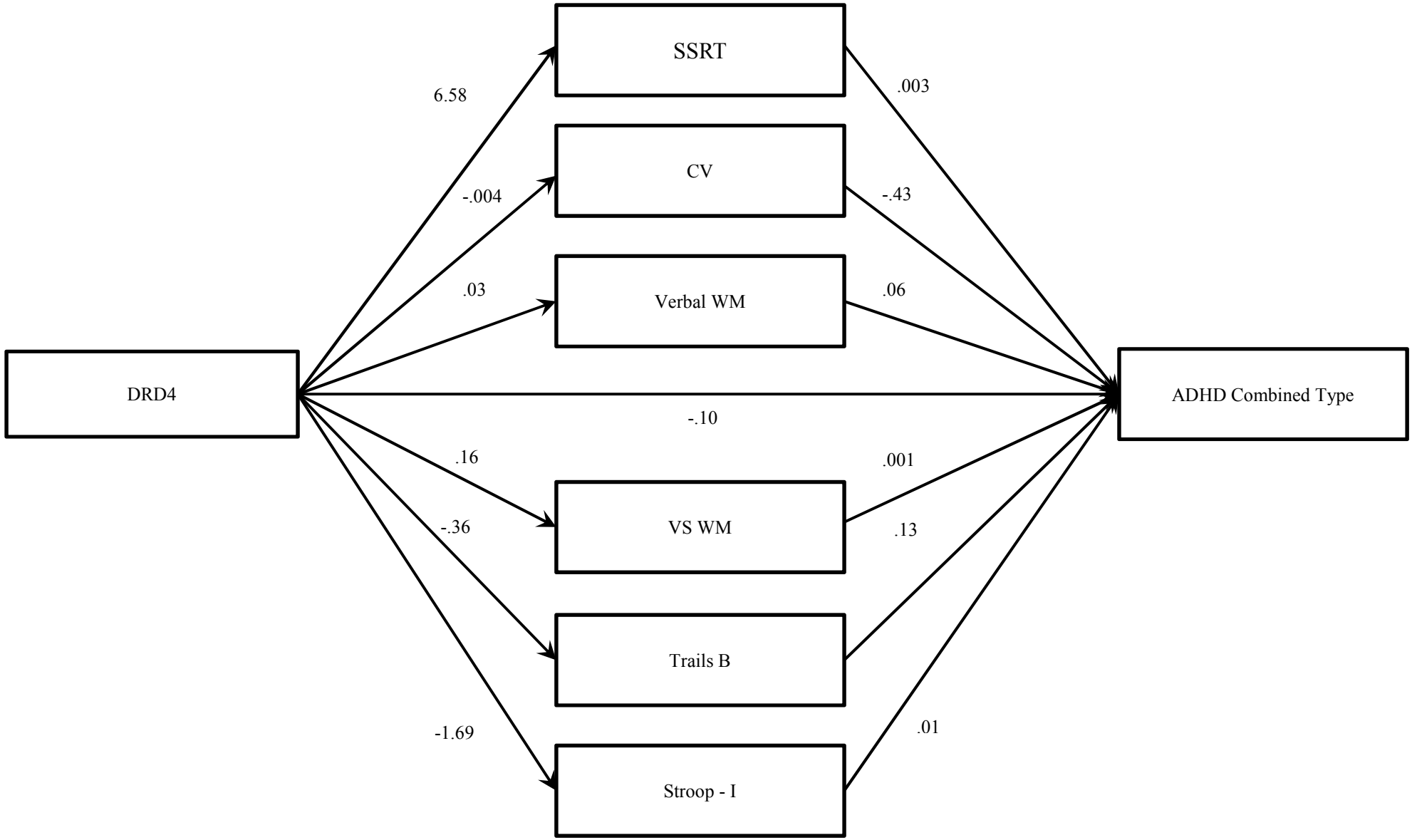


Figure 11: Multiple mediator model of the ADHD Combined Type by NF measures and DRD4 (beta coefficients)



Study IV: Experimental Test of Gene-Environment Interaction for ADHD

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Abstract

Objective: We tested the differential susceptibility theory by examining the interaction between child genotypes and experimentally manipulated parenting behaviors on offspring deviant behavior during the experimental task, offspring task performance, and coder-rated ADHD symptoms during the task. Method: Ninety-three 8-11 year-old children with and without ADHD were ascertained. The parent and child engaged in a dyadic activity consisting of giving directions on a map and writing a poem together. Parents were assigned to high and low involvement parenting conditions (counterbalanced). Objective counts of child behavior were coded using the dyadic parent-child interaction coding system (DPICS). Task performance was assessed by the number of correct items on each task and ratings of ADHD were provided by the video coders. The 40 base-pair VNTR in DAT1 and the 48-bp VNTR in DRD4 were genotyped. Results: There was a marginal interaction between DAT1 and parenting condition on child deviant behavior on the map task, such that the high involvement condition was positively associated with offspring deviant behavior among the 9/9 and 9/10 DAT1 genotype group, but not among children with the 10/10 DAT1 genotype. There was no interaction between DAT1 and condition for task performance or ADHD symptoms on either the map or poem tasks. Furthermore, there was no interaction between DRD4 and condition for deviant behavior, task performance, or ADHD symptoms for the map or poem tasks. Conclusions: Experimental methods have the potential to elucidate the biological significance of G×E. However, important limitations need to be addressed first, including designing more analogous environmental factors with the experimental manipulation.

Keywords: Parenting, gene-environment interaction, DAT1, DRD4.

The association between environmental exposure (i.e., positive or negative parenting) and child development may be a function of an unmeasured third variable that can influence both variables (Belsky, 2009). For example, genes not only contribute to the disorder in question, but may also elicit environmental experiences (e.g., gene-environment correlation), thereby confounding G×E interpretations (Jaffee, & Price, 2007). Given that there are likely multiple genes (of small effect sizes) that contribute to any given complex trait, most molecular genetic studies of ADHD may be limited by the possibility of this confound. Experimental studies can illuminate causal influences between genes and psychopathology by examining its effect across experimentally manipulated levels (Hinshaw, 2002). The goal of Study IV is to explicitly test the differential susceptibility theory by experimentally manipulating the environmental variable (i.e., parenting) and examining its effect on offspring behavior and performance on the task.

In addition to employing a novel experimental paradigm to measure environmental variation, the present study complements traditional G×E studies in that child outcomes (i.e., compliance, performance on task) will be measured *in-vivo*, which further minimizes the possibility of an unmeasured gene-environment correlation or other environmental influences. Specifically, this study will examine whether DA gene expression can influence differential susceptibility immediately following the experimental induction, given evidence that exposure to acute stress increase extracellular DA levels in the prefrontal cortex and subcortical regions of the brain within a short period of time (Finlay & Zigmond, 1997). For example, Imperato et al. (1992) found that rats exposed to prolonged restraint stress (i.e., placed in a small plexiglass box) exhibited the most significant increase of DA in the prefrontal cortex, nucleus accumbens and hippocampus immediately following the stress exposure. Similar results have been reported in other brain structures involved in behavior and emotion. DA levels in the amydala reached a maximum level of 200% of the basal value, immediately following exposure to tactile stress

(Inglis & Moghaddam, 1999). Concurrently, exposure to highly positive stimuli may also elicit a similar neurochemical response (Holroyd & Coles, 2002), as DA neurons exhibit the highest firing rate prior to a salient reward, but the firing rate falls below baseline in anticipation of a harsh punishment (Mirenowicz & Schultz, 1996). For example, individually housed monkeys (i.e., impoverished condition) exhibited increased DA and concurrent down-regulation of dopamine D2 receptors levels in the prefrontal cortex (Morgan et al., 2002). However, monkeys that were later exposed to social housing (i.e., enriched environment) increased their D2 receptor binding and exhibited "normal" levels synaptic DA (Morgan et al., 2002). Given the rapid release in DA following stress induction and the corresponding changes in animal behavior, these studies suggest the possibility that gene-environment interplay may exert immediate (in addition to developmental) effects on the phenotype in question. Although dopamine levels are not assayed in the current investigation, this experimental parenting study will examine whether offspring genetic variability accounts for differential task performance and behavioral response to negative and positive parenting (i.e., differential susceptibility).

Primary Study Goal

(1) To explicitly test whether variation in the 40-bp VNTR of DAT1 and 48-bp VNTR in DRD4 is associated with task performance and behavior in children in response to experimentally-induced parenting quality (i.e., high vs. low involvement parenting).

Methods

Participants

Ninety-three families participated as part of a larger ongoing longitudinal study of child development. Children are between 8-11 years old (see *Method* section in Study II and III for additional details on the study population). Study procedures were virtually identical to those

already mentioned in Study II and III, but some procedures have been changed according to developmental theory (e.g., parent-child task in follow-up includes task demands and coding procedures that reflect more verbal exchanges between parent and child relative to younger children). Eligibility was determined after parents completed a telephone screening and eligible families who were interested in returning were mailed a packet rating scales to complete prior to their laboratory visit. Families were then invited to our laboratory for in-person assessments. Following parent consent and child assent, parents completed the DISC and other measures related to parenting, child behavior, family functioning, personality, and their own psychopathology. During that same time, children completed standardized test of cognitive ability and academic achievement. Whenever possible, children were assessed without medication. Similarly, parents and teachers were asked to complete rating scales based on the child's unmedicated behavior. All interviewers were blind to the child's diagnostic status. The Institutional Review Board approved all study procedures.

Genotyping

DNA was extracted from saliva using DNA Genotek Oragene^{TM} Self-Collection Kits. Details regarding DAT1 and DRD4 genotyping can be found in the Method section of Study II and III. Paralleling the aforementioned studies, the DAT1 analyses in the present study were conducted by comparing individuals homozygous for the 10-repeat allele (i.e., 10/10) (n = 55; 56.2%) versus individuals with at least a one copy of the 9-repeat allele (i.e., 9/9 + 9/10) (n = 38; 43.8%). For the DRD4 analyses, we compared children carrying at least one copy of the 7-repeat allele (i.e., "risk" group) (n = 34; 36%) to those who do not have the 7-repeat allele (n = 59; 64%).

Procedures

The parent-child interaction task was adapted from Grolnick, Gurland, DeCourcey, and Jacob (2002). At the start of the task, parents were told that they are participating in a project that studies how parents and children work on schoolwork together. While the child was working on questionnaires and neurocognitive measures in a separate room, the parent was informed about the tasks they will be engaged in with their child (with order of tasks counterbalanced). The parent was then given one of two experimental inductions according to his/her random assignment: "high-involvement" vs. "low-involvement."

The following were read aloud to parents in "high involvement" condition: "We'd like you to play the role of a highly involved parent in this task. Really stress to your child that he/she will have to do a good job on this activity and that he/she will be judged against other children. Try to take over the task completely by physically doing the task for your child. Accept very little input from your child. Resist all his/her efforts to complete the task alone. The whole idea is for your child to think that he/she is meant to complete the task alone, but for you to send out the message that it is too difficult for him/her and that it would be better if you did it instead. We will be testing him/her after to make sure that he/she performs well enough."

For the "low involvement" condition, parents were read the following instructions: "Now, we'd like you to keep your level of involvement in this exercise low; only provide encouragement, praise, and support. Only intervene if your child insists on needing help, and even then keep your input minimal. The whole idea is for you to send the message to your child that he/she is capable of completing the task alone. Be very supportive and encouraging, without being over-involved. We will be asking him/her some questions afterwards, but there is no particular level at which he/she needs to perform."

Following the experimental induction, the dyad was told to enter the room together to complete a worksheet with questions related to the task. They were given five minutes to

complete the task together, during which time the interaction was video-recorded. After the experimental induction, the parent was asked to leave the room and the child was asked to complete a test of the task they just completed on their own. While the child was testing (for which they will be given as much time as necessary to complete), the parent completed the Parent-Child Interaction Questionnaire to assess parental fidelity to the experimental induction. Finally, this procedure was repeated with the new task (map or poem) and a different experimental induction (low involvement vs. high involvement).

Map Task

The dyad was provided a large map that had several landmarks (e.g., school, post office, and restaurant) and a worksheet with the task directions. The directions called for the child to use the map to give directions to someone using three pieces of information: street names, directions, and names of the streets they need to cross over. The worksheet had four questions, including three fill-in-the-blanks and an open-ended question (i.e., "how would you go from Adam's house to the airport using the three pieces of information?"). The parent and child worked on these questions for five minutes together. Afterwards, the child was administered the test question to be completed alone, which is similar to the open-ended question. The child had as much time as needed to complete the test question. The task was scored according to the correct number of the responses on the worksheet.

Poem Task

The dyad was given instructions on how to identify the rhyming pattern of a quatrain poem (a four-lined poem with a rhyming pattern). Afterwards, the dyad was asked to write their own quatrain poem together and identify the rhyming patterns of several sample quatrains. After five minutes, the child composed his/her own quatrain poem (using any rhyming scheme) alone, without the help of his/her parent. The child had as much time as needed to complete the

composition. The final task (quatrain alone) was scored by awarding one point each for: (1) having four lines, (2) specifying/identifying the rhyming pattern, and (3) having lines rhyming according to the quatrain pattern (Grolnick et al., 2002).

Questionnaires

Parent-child interaction questionnaire. The parent was asked to fill out a 4-item questionnaire regarding his/her thoughts and feelings immediately following each task (map and poem task). The questionnaire was administered to assess whether parents exhibited different affective reactions following each induction, as well as to assess parental fidelity to the experimental conditions. The questions are: "How pressured did you feel working with your child? How controlling do you feel you were on this activity? How supportive and encouraging did you feel you were? How well do you do think your child performed on this activity?" Parents were asked to rate each item on a 3-point likert scale: (1) not well, (2) somewhat well, and (3) very well. The items were adapted from the Affective Questionnaire (Grolnick et al., 2002).

Coding

We used the DPICS (Eyberg et al., 2005) to code videotapes of the task. Coding procedures were previously described under the Methods section in Study II. We coded several child behavioral categories, including verbalizations (negative talk, command, questions, and prosocial talk), vocalizations (yell, whine), and responses (compliance, noncompliance). A composite measure of total child deviance was created by combining discrete counts of whining, yelling, negative talk, and noncompliance for each task, following previously used strategies (i.e., Chronis et al., 2007). These categories were scored by tallying the frequency counts (i.e., number of times child uses negative talk) of each behavior during 10 second intervals, with a total of 5 minutes for the task. 20% of the videos were randomly selected and coded by two

separate raters to estimate reliability. The intra-class correlation for our composite category was excellent (ICCs between = .86 and.92).

Coders also rated the frequency on ADHD symptoms during the tasks using the *Disruptive Behavior Disorder Rating Scale* (DBDRS; Pelham, Gnagy, Greenslade, & Milich, 1992).The DBDRS is a 25-item rating scale adapted from DSM-III-R symptoms for disruptive behavior disorders. Coders rated on the frequency on symptoms for each task immediately after coding the video. There are four-response options for each symptom: "Not at All" (0), "Just a Little" (1), "Pretty Much" (2), or "Very Much" (3). We only analyzed the DBDRS dimensionally (i.e., total number of ADHD symptoms). Cronbach's alpha for each condition ranged from good to excellent (map: .95, poem: .74).

Statistical Analysis

Regression models were fit according to the distribution of the dependent variable: zero-inflated negative binomial regressions were used to measure deviant child behaviors and counts of ADHD symptoms and linear regressions were used to predict task performance. For each model, we estimated the influence of each condition of parenting (high vs. low involvement), child DAT1 and DRD4 genotype, and their interactions on 1) performance on each task and 2) frequency of child deviant behaviors (the sum of noncompliance, negative talk, and negative vocalizations). As with each of the previous studies in the dissertation, child age, sex, and race-ethnicity were statistically controlled.

Results

Preliminary Analyses

The means and standard deviations for each DPICS category, the composite variable for child deviant behaviors, performance on each task, and coder-rated DBD ratings of ADHD

symptoms are reported in Table 14, stratified by parenting condition. No significant mean differences between the high and low involvement condition emerged for any of the dependent variables.

Child Deviant Behaviors

Map Task. We first tested the effects of parenting condition, DAT1 genotype, and their interaction on child deviant behaviors for the map task, controlling for age, sex and race-ethnicity (Table 15). There was no main effect for DAT1 (B = .26, SE = .45, p = .56), although high involvement parenting was positively associated with child deviant behavior on the map task (B = 1.16, SE = .43, p < .01). Furthermore, the interaction between DAT1 and condition was marginally significant (B = -1.01, SE = .59, p = .09), such that the high involvement condition was positively associated with deviant behavior among the 9/9 and 9/10 DAT1 genotype group (B = 1.27, SE = .41, p < .01), but not among children with the 10/10 DAT1 genotype (B = .29, SE = .40, p = .48). No significant effects emerged for DRD4 (B = -.42, SE = .49, p = .38), condition (B = .26, SE = .35, p = .45), or the DRD4 x condition interaction (B = .76, SE = .65, p = .25).

Poem Task. Next, we tested the interaction of parenting condition, DAT1 genotype, and their interaction on child deviant behaviors for the poem task, controlling for age, sex and race-ethnicity (Table 15). Once again, there were no effects for DAT1 (B = -.07, SE = .40, p = .86), condition (B = -.05, SE = .43, p = .91), or their interaction (B = .23, SE = .58, p = .70). Similarly, no effects emerged for DRD4 (B = -.06, SE = .39, p = .88), condition (B = .18, SE = .34, p = .61), or their interaction (B = -.30, SE = .66, p = .65) for deviant behavior on the poem task.

Child Performance on Tasks

Map Task. We regressed the parenting condition, DAT1 genotype and their interaction on child performance on the map task, controlling for age, sex, and race-ethnicity, using linear regression (Table 16). There were no effects for DAT1 (B = -.60, SE = .71, p = .40), condition (B

= -1.19, *SE* = .82, *p* = .16), or their interaction (*B* = 1.27, *SE* = 1.05, *p* = .23) in predicting map task performance. A similar pattern of results emerged for the model with DRD4 (*B* = -.81, *SE* = .76, *p* = .29), condition (*B* = -.53, *SE* = .62, *p* = .40), and their interaction (*B* = .69, *SE* = 1.08, *p* = .52) in predicting map task performance.

Poem Task. Controlling for age, sex, and race-ethnicity, there was no association between DAT1 (*B* = .71, *SE* = .88, *p* = .42), condition (*B* = .75, *SE* = .91, *p* = .41), or their interaction (*B* = -1.09, *SE* = 1.17, *p* = .35) in predicting poem task performance. There was also no association between DRD4 (*B* = .15, *SE* = .82, *p* = .85), condition (*B* = .50, *SE* = .68, *p* = .46) and their interaction (*B* = -1.14, *SE* = 1.18, *p* = .34) for poem task performance.

ADHD ratings on DBDRS

Map Task. We used zero-inflated negative binomial regression to predict total counts of ADHD symptoms (as rated by the DPICS coders) from child DAT1, condition, and their interaction controlling for age, sex, and race-ethnicity (Table 17). A main effect for DAT1 emerged (*B* = 1.36, *SE* = .41, *p* < .001), such that the 10/10 DAT1 group was positively associated with ADHD symptoms. There was no association for condition (*B* = .35, *SE* = .85, *p* = .68) or for the DAT1 x condition interaction (*B* = -1.00, *SE* = .91, *p* = .28) in predicting counts of ADHD symptoms. With respect to the same model but with DRD4 instead of DAT1, there was effect of DRD4 (*B* = .02, *SE* = .54, *p* = .96), condition (*B* = -.32, *SE* = .36, *p* = .36), or their interaction (*B* = .60, *SE* = .70, *p* = .39).

Poem Task. There was no association of DAT1 (*B* = -1.65, *SE* = .69, *p* = .34), condition (*B* = -.59, *SE* = .69, *p* = .46), and their interaction (*B* = .84, *SE* = .86, *p* = .33) with counts of ADHD symptoms, controlling for age, sex, and race-ethnicity. Similarly, there was no association of DRD4 (*B* = -.11, *SE* = .56, *p* = .84), condition (*B* = -.09, *SE* = .72, *p* = .90), and their interaction (*B* = .06, *SE* = .82, *p* = .94) with counts of ADHD symptoms.

Discussion

The goal of Study IV was to examine the interaction between genotype and parenting behavior on deviant offspring behavior, task performance, and ADHD during an experimental task in which parent behavior was experimentally manipulated. Under two experimental conditions, in which the parents were told to engage in either highly involved vs. lowly involved parenting strategies, the parent and offspring engaged in a poem and map task that was designed to elicit a range of child behaviors. There was a marginal interaction between DAT1 and parenting condition on child deviant behavior on the map task, such that the high involvement condition was positively associated with offspring deviant behavior among the 9/9 and 9/10 DAT1 genotype group, but not among children with the 10/10 DAT1 genotype. There was no interaction between DAT1 and condition for task performance or ADHD symptoms on either the map or poem tasks. Furthermore, there was no interaction between DRD4 and condition for deviant behavior, task performance, or ADHD symptoms for the map or poem tasks.

Several limitations should be mentioned in light of the results. First, there were no statistically significant differences in child behavior, task performance, or ADHD symptoms in the high involvement vs. low involvement conditions, suggesting that parents may not have been fully engaged in each of the conditions. Parents did not have much time to practice each condition prior to the task, despite the fact they were given scripts with prompts to read during the task. Furthermore, we asked all parents to switch from the high-to-low (or low-to-high) control conditions, which may have been difficult for parents to do (we counterbalanced these conditions to account for any confounds related to ordering, however). We speculate that parents likely resorted to their natural parenting behaviors after the first few minutes of each task and condition, given their lack of practice and familiarity with the goals experiment. Second, the high vs. low involvement conditions may not have been analogous to negative and positive parenting

behaviors, at least compared to how they were operationalized in the previous studies of this dissertation. Real environmental pathogens, especially harsh or negative parenting behaviors, are prohibitive in experimental studies involving human participants due to the ethical concerns of exposing children to such risks (Caspi & Moffitt, 2006). Third, there may have been some notable differences between the poem and map tasks that pulled for different parenting strategies. The map task may have elicited more involved parenting behaviors because it is more structured and has actual correct answers whereas the poem task is less structured and more reliant on the creativity of the child (Grolnick et al., 2002). These differences may have influenced parenting behaviors and task performance, independent of the experimental manipulation. Finally, the laboratory experiment lasted only five minutes per task. Caspi and Moffitt (2006) argue that harm from "naturally occurring" environmental pathogens typically accumulates over months or years (i.e., as a chronic stressor). Alternatively, the parenting behaviors elicited by each condition may not be sufficiently salient (i.e., as an acute stressor) for dopamine (DA) activation, thus blunting the potential for G×E. These concerns may be alleviated in future studies by focusing on experimental designs that elicit stronger stress responses (e.g., parental separation).

Although the results from this study did not support the hypothesis that children with the "risk genotype" would be the most sensitive to both high and low involvement parenting conditions, the use of an experimental paradigm in the context of G×E is innovative and advantageous over traditional methods for a number of reasons. First, environmental risks are often correlated with negative outcomes, but their exposure does not always generate disorder (Caspi & Moffitt, 2006). Studies that use observational or questionnaire data are often confounded by co-occurring environmental risks and outcomes. This is especially true with cross-sectional designs because without longitudinal data, temporal ordering of causal influences cannot be established. Thus, the use of experimental studies allow for greater control of the

environmental stimulus (e.g., parenting quality), allowing for more precise and reliable measurement of the environmental exposure. Greater experimental control and measurement precision means that false-positive findings will also be reduced (McClelland & Judd, 1993). A second advantage of using an experimental approach in G×E is the reduced possibility of a gene-environment correlation, which can confound interpretations of G×E. These concerns are ruled out when subjects are randomly assigned to different environmental conditions. Finally, using experimental methods provides evidence that the G×E is a *biological or developmental* phenomenon rather than a statistical one (i.e., an artifact) (Rutter, Thapar, Pickles, 2009; Tabery, 2007). An experimental paradigm provides one window into functional importance of G×E because some genes are expressed only in-response to *specific* environmental influences (i.e., epigenetics). Several well-known examples come from animal experimentation studies (see review by Meaney, 2010), including the study by Barr et al. (2004) that found that variation in the promoter region of the serotonin transporter gene (5-HTTLPR) moderated the association between limbic hypothalamic-pituitary-adrenal axis response to stress and early-rearing experience in infant rhesus macaques. Specifically, 5-HTTLPR heterozygote macaques had higher adrenocorticotropic hormone and cortisol levels when they were separated from their home cage (i.e., separation stress) compared to L allele homozygotes, but only when they were reared by peers and not when they were maternally reared (Barr et al., 2004). Through experimental manipulation of the macaque's rearing experience, the study provided crucial evidence that the genotype-by-early rearing experience interaction had direct biological consequences. These types of studies may help *explain* why certain individuals with a genetic constitution are at higher risk of psychopathology in the face of stressful life events.

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Appendix: Tables and Figures

Table 14. Means and standard deviations of each dependent variable by low and high condition

		Poem (n = 93)			Map (n = 93)		
	Condition	Mean	SD	p	Mean	SD	p
NTA	Low	1.34	1.88	.83	1.37	2.10	.78
	High	1.44	2.64		1.54	3.53	
CM-CO	Low	1.36	1.52	.64	1.60	1.56	.59
	High	1.22	1.31		1.43	1.41	
CM-NC	Low	0.70	1.61	.15	0.65	1.27	.29
	High	1.36	2.56		1.04	2.07	
CM-NOC	Low	2.00	2.21	.03	3.53	3.84	.49
	High	3.42	3.71		3.00	3.39	
QU	Low	3.26	2.35	.69	3.88	3.89	.80
	High	3.49	3.17		3.70	2.87	
QU-AN	Low	4.66	3.36	.65	6.53	5.06	.29
	High	5.09	5.52		5.48	4.35	
QU-NA	Low	1.47	1.72	.99	1.47	1.44	.50
	High	1.47	1.88		1.87	3.63	
QU-NOA	Low	1.45	1.87	.45	2.16	2.47	.72
	High	1.80	2.59		2.37	3.00	
PRO	Low	13.17	8.09	.52	11.35	7.85	.58
	High	12.00	9.20		12.28	8.07	
YE	Low	0.43	1.12	.67	0.33	0.94	.28
	High	0.53	1.33		0.70	2.03	
WH	Low	0.40	0.93	.22	0.84	1.94	.37
	High	0.82	2.14		0.54	1.00	
LA	Low	1.28	2.13	.98	0.86	2.47	.22
	High	1.29	2.06		0.38	0.83	
Tot. negative behaviors	Low	4.40	4.43	.40	4.04	5.53	.23
	High	5.48	7.54		5.80	8.02	
Performance	Low	7.21	2.66	.81	5.14	2.32	.69
	High	7.33	2.36		4.94	2.62	
DBDRS	Low	0.56	1.03	.43	0.92	1.72	.57
	High	0.82	1.67		0.72	1.19	

Note. NTA = negative talk, CM-CO = commands – compliance, CM-NC = commands – non-compliance, CM-NOC = no opportunity for compliance, QU = question, QU-AN = question-answer, QU-NA = question-no answer, QU-NOA = question no opportunity to answer, PRO = prosocial talk, YE = yells, WH = whines, LA = laughter, Tot. negative behaviors = composite of NTA + CM-NC + QU-NA + YE + LA.

Table 15. Zero-inflated negative binomial regressions for child deviant behavior

Genotype Model	Variable	b	SE	*p*
		Map Task		
DAT1	Age	0.10	0.14	0.48
	Sex	-0.30	0.32	0.35
	Race-ethnicity	-0.43	0.29	0.14
	DAT1	0.26	0.45	0.56
	Condition	1.16	0.43	0.01
	DAT1 x condition	-1.01	0.59	0.09
DRD4	Age	0.06	0.14	0.63
	Sex	0.13	0.36	0.72
	Race-ethnicity	-0.32	0.28	0.25
	DRD4	-0.42	0.49	0.38
	Condition	0.26	0.35	0.45
	DRD4 x condition	0.76	0.65	0.25
		Poem Task		
DAT1	Age	-0.09	0.14	0.51
	Sex	-0.09	0.32	0.78
	Race-ethnicity	-0.32	0.28	0.26
	DAT1	-0.07	0.40	0.86
	Condition	-0.05	0.43	0.91
	DAT1 x condition	0.23	0.58	0.70
DRD4	Age	-0.10	0.12	0.44
	Sex	0.06	0.35	0.87
	Race-ethnicity	-0.27	0.27	0.32
	DRD4	-0.06	0.39	0.88
	Condition	0.18	0.34	0.61
	DRD4 x condition	-0.30	0.66	0.65

Table 16. Linear regressions for map/poem task performance

Genotype Model	Variable	b	SE	*p*
	Map Task			
DAT1	Age	0.97	0.25	0.00
	Sex	-0.19	0.58	0.75
	Race-ethnicity	0.13	0.51	0.80
	DAT1	-1.19	0.82	0.16
	Condition	-0.60	0.71	0.40
	DAT1 x condition	1.27	1.05	0.23
DRD4	Age	1.03	0.24	0.00
	Sex	0.21	0.60	0.73
	Race-ethnicity	-0.08	0.50	0.88
	DRD4	-0.53	0.62	0.40
	Condition	-0.81	0.76	0.29
	DRD4 x condition	0.69	1.08	0.52
	Poem Task			
DAT1	Age	0.42	0.28	0.13
	Sex	0.33	0.65	0.61
	Race-ethnicity	0.18	0.57	0.76
	DAT1	0.75	0.91	0.41
	Condition	0.71	0.88	0.42
	DAT1 x condition	-1.09	1.17	0.35
DRD4	Age	0.33	0.27	0.22
	Sex	0.54	0.65	0.41
	Race-ethnicity	0.16	0.55	0.77
	DRD4	0.50	0.68	0.46
	Condition	0.15	0.82	0.85
	DRD4 x condition	-1.14	1.18	0.34

Table 17. Zero-inflated negative binomial regressions for counts of ADHD symptoms from the DBDRS

Genotype Model	Variable	b	SE	*p*
	Map Task			
DAT1	Age	0.31	0.17	0.06
	Sex	-0.09	0.37	0.80
	Race-ethnicity	0.48	0.35	0.17
	DAT1	0.35	0.85	0.68
	Condition	1.36	0.41	0.00
	DAT1 x condition	-1.00	0.91	0.28
DRD4	Age	0.21	0.16	0.18
	Sex	0.12	0.38	0.76
	Race-ethnicity	0.49	0.41	0.24
	DRD4	-0.32	0.36	0.37
	Condition	0.02	0.54	0.97
	DRD4 x condition	0.60	0.70	0.39
	Poem Task			
DAT1	Age	-0.15	0.25	0.55
	Sex	-0.01	0.46	0.98
	Race-ethnicity	-0.53	0.49	0.28
	DAT1	-0.65	0.69	0.34
	Condition	-0.51	0.69	0.46
	DAT1 x condition	0.84	0.86	0.33
DRD4	Age	-0.23	0.19	0.21
	Sex	-0.13	0.48	0.80
	Race-ethnicity	-0.67	0.54	0.22
	DRD4	-0.11	0.56	0.84
	Condition	-0.09	0.72	0.90
	DRD4 x condition	0.06	0.82	0.94

Future Directions

This dissertation provides the groundwork for several future studies. Here are three research ideas I am proposing to follow up the findings from my dissertation. In line with the themes of my dissertation, the overarching goal of the following studies is to improve the precision of measurement through the use of more rigorous statistical and methodological approaches in understanding ADHD and G×E effects.

Examining ADHD Symptoms with Item Response Theory

The results from Study I demonstrated the value of using empirically-driven approaches (i.e., latent class analysis; LCA) to elucidate the underlying structure of a phenotype in a large, population-based sample, over traditional categories defined by the DSM-IV. By creating symptomatically-homogenous subgroups, the LCA yielded greater precision in detecting a gene-environment interaction, which was found to be specific to one relatively small group of individuals (i.e., girls with severe combined type symptoms). Given concerns about the diagnostic stability and validity of the current DSM-IV subtypes of ADHD in clinical and research studies (Valo & Tannock, 2010), one question that remains is whether there is utility (clinically or otherwise) in using LCA-derived subtypes of ADHD. However, an even more crucial consideration regarding the utility of the latent approach to classification is the validity of symptoms themselves. Although many studies have been conducted on the reliability and validity of ADHD symptoms, almost all of these studies have used principles and procedures that were based on classical test theory (CTT; Lord & Novick, 1968), which can artificially inflate psychometric characteristics by having a large item pool, highly specific study population, and overlapping items (e.g., “blurts out” vs. “interrupts”). Thus, the symptoms that constitute the two ADHD dimensions (inattention and hyperactivity/impulsivity) have been largely unchanged

since the field trials leading up to DSM-III (Faraone, Biederman, & Friedman, 2000). The goal of the next study is to rigorously examine the psychometric properties of each ADHD symptom using item response theory (IRT), which is an alternative to CTT that examines the relationship between an individual item and its latent trait (e.g., inattention) by disentangling how well each symptom functions in relation to the others (Embretson & Reise, 2000). For example, Gomez (2008) conducted an IRT on an ADHD questionnaire administered to parents and teachers of 1,475 school-aged Australian children and found significant variation in ADHD symptoms with respect to discrimination values, particularly at the low-end of the ADHD trait. This suggests that the validity of ADHD questionnaires might be limited in epidemiological or studies in which low-levels of ADHD are most prevalent. The validity of studies of ADHD, whether they rely on empirically-derived methods like LCA or theoretically-derived models based on DSM-IV, depend on how well the symptoms measure the construct. Ultimately, the goal of using statistical approaches like IRT and LCA is to create a more refined and precise definition of the ADHD phenotype, which will significantly improve the power to detect meaningful effects in etiological studies of ADHD.

Neurobiological Endophenotypes of ADHD

In Study III, we examined two potential neurocognitive endophenotypes that may explain the co-variation between DAT1 and DRD4 with ADHD. The results of that study were largely non-significant. However, our null findings may not be surprising given the relatively small sample size and the fact that the endophenotypes we examined, working memory and response inhibition, were also psychological constructs themselves that are genetically-complex and likely influenced by other lower-order mechanisms (Doyle et al., 2005; Castellanos & Tannock, 2002). Furthermore, constructs like response inhibition and working memory are often defined by

multiple operational definitions, which lead to the same issues of heterogeneity and lack of precision as the psychiatric outcome in question. Perhaps a more compelling endophenotype may be neurobiological. Epigenetic factors, such as DNA methylation, may be more proximal to genetic influences on psychopathology by influencing *gene expression* (Wong et al., 2010). DNA methylation involves the binding of methyl groups to C-phosphate-G (CpG) sites (overexpressed in the promoter regions of many genes), which modulates the transcriptional efficiency and plasticity of the gene (Jaenisch & Bird, 2003; Mill & Petronis, 2008). Unlike the DNA sequence, which is stable and strongly conserved post-embryogenesis, DNA methylation is developmentally regulated, highly dynamic, and susceptible to environmental factors (Mill & Petronis, 2008). Thus, aberrant methylation signatures are hypothesized to be associated with pathology (Mill & Petronis, 2008). Human epigenetic studies for psychopathology are still in their infancy, although recent findings are promising. Van IJzendoorn and colleagues (2010) found that higher methylation levels at the promoter region of the serotonin transporter (5-HTTLPR) was associated with increased risk of experiencing trauma and unresolved loss, but only among individuals homozygous for the 5-HTTLPR long allele. Hillemacher and colleagues (2009) studied 76 patients undergoing detoxification treatment (and were previously diagnosed with alcohol dependence) and reported significant hypermethylation in the DAT1-promoter amongst patients compared to 35 healthy controls. These early findings illuminate the potential *causal neurobiological mechanisms* between susceptibility loci and a particular phenotype, namely, that hypermethylation may mediate the association of genotype and psychopathology. This innovative approach is a promising avenue for future investigation because it may shed light on biological G×E, rather than a purely statistical one.

Clinical Applications of G×E

The final proposal is an extension of the theoretical ideas of Belsky and Pluess (2009) regarding differential susceptibility and its applicability in the clinical domain. G×E findings have potential clinical implications: environmental risks may interact with genes to predict not only vulnerability, but also resilience (Kim-Cohen & Gold, 2009). Incorporating genetics into psychosocial research may help identify the biological factors that promote resilience and prevent psychopathology among individuals who experience severe and/or chronic psychosocial adversity (Kim-Cohen & Gold, 2009). Developmentally sensitive designs for G×E are especially crucial, given that ADHD symptoms often fluctuate across development and that gene expression may be environmentally sensitive across the lifespan (Mill & Petronis, 2008). The next study will utilize an empirically-supported behavioral intervention (e.g., parent training) for the treatment of ADHD, in which participants are stratified by their genotype and their behaviors are measured across time (i.e., treatment effects and follow up effects). There is already evidence that a parent training intervention designed to increase parental sensitivity was more effective in reducing externalizing problems in preschool-aged children with the "risk" variant of DRD4 than in youth without the "risk" variant, supporting the notion that certain individuals show a stronger response to intervention than others (Bakermans-Kranenberg et al., 2008). This finding has important implications for intervention, including targeted populations and interventions that focus on environmental change and enrichment (Belsky et al., 2007). Unique contributions can be afforded by genetically-sensitive designs that simultaneously incorporate careful measurement of biologically-plausible environmental conditions, including intervention.

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