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Low birth weight, a form of fetal growth compromise, is a well-established risk factor for Attention-Deficit/Hyperactivity Disorder (AD/HD; Nigg, Nikolas, & Burt, 2010); however, it is unclear how birth weight moderates genetic risk for AD/HD. From a Developmental Origins of Health and Disease (Gluckman & Hanson, 2004) framework, this study investigated if fetal growth compromise moderated relationships between SNPs within angiogenic, dopaminergic and neurotrophic genes and AD/HD symptom severity. A total of 398 youth from two multi-site, family-based studies of AD/HD were included in the current analysis. Results demonstrated that fetal growth compromise moderated associations between SNPs within angiogenic (HIF1A and NRP1) and a neurotrophic gene (NTRK3), but not dopamine genes, and AD/HD symptom severity. The gene x environment interactions remained significant after controlling for SNPs associated with birth weight and adjusting for multiple testing. Taken together, findings may suggest that prenatal ischemia/hypoxia is an environmental pathogen for AD/HD which confers vulnerability for the disorder through regulating the expression of angiogenic and neurotrophic genes.

FETAL GROWTH COMPROMISE MODERATES ASSOCIATIONS BETWEEN SNPS WITHIN ANGIOGENIC AND NEUROTROPHIC GENES AND AD/HD SYMPTOM SEVERITY

by

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To my parents, Leigh and Mary Smith.

APPROVAL PAGE

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CHAPTER I

INTRODUCTION

Most biological theories of attention-deficit/hyperactivity disorder (AD/HD; American Psychiatric Association [APA], 2000) identify dopaminergic dysfunction as the primary pathway to AD/HD (e.g., Levy, 1991). These theories, which suggest that hypodopaminergic functioning in frontal and limbic neural systems underlie AD/HD symptomatology, have had some success in guiding AD/HD etiological research and the development of pharmacotherapies for the disorder (Swanson et al., 2007). Recent evidence from molecular genetic (Franke, Neale, & Faraone, 2009; Poelmans, Pauls, Buitelaar, & Franke, 2011) and environmental studies on AD/HD (see Banerjee, Middleton, & Faraone, 2007 for a review) implicate a broader range of neurodevelopmental processes in the etiology of the disorder. These findings emphasize AD/HD's vast etiological heterogeneity and highlight the limitations of the dopamine hypothesis in accounting for the numerous developmental pathways that result in the disorder.

Two additional neurodevelopmental systems which may confer vulnerability for AD/HD include: 1) neurotrophic factors (Ribasés et al., 2008), which promote nerve survival, differentiation and growth; and 2) angiogenic factors (Jesmin et al., 2004; Weber, Lurschg, & Fahnenstich, 2005), which promote the formation of new blood vessels. To date, there has been inconsistent evidence implicating neurotrophic factors in

the etiology of AD/HD (Gizer, Ficks, & Waldman, 2009; Sanchez-Mora et al., 2009) and limited investigation of angiogenic factors. One explanation for the inconsistency or lack of findings may be the failure to specify an environmental pathogen in vulnerability models for the disorder. Given that both neurotrophic and angiogenic factors are regulated by environmental pathogens (Mill & Petronis, 2008; Schmidt-Kastner, van Os, Steinbusch, & Schmitz, 2006), the impact of neurotrophic and angiogenic factors on vulnerability for AD/HD may be dependent upon environmental risk. Therefore, the purpose of this project is to better understand the interaction of dopaminergic, neurotrophic and angiogenic factors with environmental conditions in conferring risk for AD/HD.

As background, it is first necessary to provide an overview of AD/HD, with an emphasis on the etiology of AD/HD. Next, research examining the genetic and environmental underpinnings of AD/HD will be reviewed. Then, the potential role of dopaminergic, neurotrophic and angiogenic factors in the etiology of AD/HD will be outlined. Finally, the research questions and hypotheses of this study are stated within a Developmental Origins of Health and Disease (DOHaD; Gluckman & Hanson, 2004) framework.

AD/HD Overview

There are five diagnostic criteria that need to be met to be diagnosed with one of the three AD/HD subtypes (Combined Type, Predominantly Inattentive Type, and Predominantly Hyperactive-Impulsive Type; APA, 2000). The five re-ordered DSM-IV criteria (Anastopoulos & Shelton, 2001) are: 1) clear evidence of clinically significant

impairment; 2) impairment in two or more settings related to AD/HD symptoms; 3) evidence for at least 6 out of 9 symptoms of inattention and/or hyperactivity-impulsivity, which have persisted for at least 6 months, and are developmentally deviant; 4) some symptoms causing impairment were present before 7 years of age; and 5) the symptoms cannot be better accounted for by another mental disorder.

When all five diagnostic criteria are applied to representative community samples, the average prevalence rate of AD/HD in children and adolescents is between 5-5.5% (Polanczyk, De Lima, Horta, Biederman, & Rohde, 2007); however, rates vary by gender and age. In terms of gender, DSM-IV (APA, 2000) reports that boys are 4 to 9 times as likely as girls to meet criteria for the disorder; however, these estimates are based largely on clinical samples. Evidence from community samples (Polanczyk et al., 2007) suggests that boys are approximately 2.5 times as likely to meet criteria for the disorder. AD/HD rates tend to decrease with age (Polanczyk et al., 2007) and approximately 50-80% of children diagnosed with AD/HD in childhood continue to display clinically significant symptom levels into adolescence (Barkley, Fischer, Edelbrock, & Smallish, 1990). These symptoms also persist into adulthood, albeit at relatively lower rates (Mannuzza, Klein, Bessler, Malloy, & Hynes, 1997). The reduction in AD/HD prevalence over time may reflect neurodevelopmental normalization over time (Shaw et al., 2006) and/or developmentally inappropriate DSM-IV AD/HD diagnostic criteria for adolescents and adults (McGough & Barkley, 2004).

Approximately 60% of individuals diagnosed with AD/HD meet diagnostic criteria for another psychological disorder (e.g., Pfiffner et al., 1999), including

oppositional defiant disorder, conduct disorder, learning disorders, anxiety disorders, depressive disorders, and tic disorders. Furthermore, individuals with AD/HD are at heightened risk for delays in cognitive, language and motor development (Barkley, 2006). Taken together, the variability in symptomatology, course, comorbid profiles and associated features within AD/HD demonstrates that AD/HD is marked by phenotypic heterogeneity.

Given the magnitude of AD/HD related impairment and the major public-health cost of the disorder (Pelham, Foster, & Robb, 2007), there has been much interest in identifying the etiological underpinnings of AD/HD. Broadening the understanding of the etiology of AD/HD will help to reduce the public health impact of AD/HD both indirectly and directly by: 1) continuing to inform AD/HD taxonomy and classification; and 2) helping to identify malleable environmental pathogens and epigenetic mechanisms that can be targeted to reduce the prevalence of AD/HD.

AD/HD Etiology

AD/HD is a multifactorial disorder arising from a variety of genetic, neurobiological and environmental factors (Nigg, 2006). Most putative etiological factors have been identified by comparing individuals with AD/HD and individuals without AD/HD. Thus, little is known about etiological variability within AD/HD or specific etiological pathways to AD/HD.

Neurotransmission. Most AD/HD etiological theories implicate dysfunctional neurotransmission as the main pathway to AD/HD. For instance, the dopamine hypothesis of AD/HD (Levy, 1991; Swanson et al., 2007) indicates that

hypodopaminergic functioning in specific neural pathways is one major underpinning of AD/HD. Although the dopaminergic system has been the most widely researched neurotransmitter system related to AD/HD, serotonergic, adrenergic, and cholinergic neurotransmitter systems have also been implicated (Biederman & Faraone, 2002; McClernon & Kollins, 2008; Pliszka, McCracken, & Maas, 1996). The neurotransmitter hypotheses have helped to guide etiological research and treatment development for AD/HD; however, neurofunctional and neuroanatomical studies implicate a broader range of neurodevelopmental processes in the etiology of AD/HD.

Neurofunctional deficits. In addition to abnormal neurotransmission, individuals with AD/HD demonstrate hypoactivation in particular neural regions during cognitive tasks. This hypoactivation tends to occur in the prefrontal and limbic regions which are presumed to underlie AD/HD symptoms (Barkley, 1997; Durston, De Zeeuw, & Staal, 2009). Though neurotransmitter deficits are the most widely cited contributor to the observed hypoactivation in these brain regions (e.g., Swanson et al., 2007), other factors may also be implicated. For instance, decreased cerebral blood flow to prefrontal and limbic regions may also underlie the neural hypoactivation (Gustafsson, Thernlund, Ryding, Rosen, & Cederblad, 2000; Kim, Lee, Shin, Cho, & Lee, 2002). Todd and Botteron (2001) have also proposed that deficient astrocyte glucose metabolism may be associated with prefrontal and limbic hypoactivation in AD/HD.

Neuroanatomical structure. Multiple neuroanatomical correlates of AD/HD have also been identified. In general, individuals with AD/HD have reduced overall brain volumes, with an average reduction of approximately 5% (Castellanos et al., 2002).

In addition, multiple studies have identified the largest differences between individuals with AD/HD and controls in the prefrontal cortex, basal ganglia (e.g., caudate and putamen), corpus callosum and cerebellum (Valera, Faraone, Murray, & Seidman, 2007) - all of which are implicated in executive functioning processes which are impaired in many individuals with AD/HD (Barkley, 1997). Furthermore, reduction in volume has been observed in both white and gray matter in the right (Filipek et al., 1997; Overmeyer et al., 2001) and left prefrontal cortices (Kates et al., 2002; Mostofsky, Cooper, Kates, Denckla, & Kaufmann, 2002). These anatomical differences are apparent in childhood and are generally stable into adolescence, which suggests a non-progressive neurodevelopmental deficit (Castellanos et al., 2002). However, Shaw et al. (2006) found that some individuals diagnosed with AD/HD who demonstrated increased cortical thickness over time also showed lagged reduction in AD/HD symptomatology, suggesting that some individuals with AD/HD have neurodevelopmental delays which normalize over time.

Taken together, neurodevelopmental vulnerability for AD/HD is dimensional in nature and gives rise to a range of inattentive and hyperactive-impulsive symptoms (Shaw et al., 2011), which at their extreme meet the symptom criterion for the disorder. Neurodevelopmental risk for AD/HD consists of a myriad of neurotransmitter, neurofunctional, and neuroanatomical abnormalities which likely reflect both stable neurodevelopmental deficits (Castellanos et al., 2002) and neurodevelopmental delays (Shaw et al., 2006). Although the exact origins of neurodevelopmental risk for AD/HD

remain largely unknown, neurodevelopmental risk results from the interplay between genetic and environmental risk factors.

Overview of AD/HD Genetic Studies

Behavioral genetics. Findings from family, adoption, and twin studies suggest that genetic factors play a substantial role in the etiology of AD/HD. For example, in an exanimation of parent-child concordance, offspring of adults diagnosed with AD/HD have approximately a 50% chance of also meeting diagnostic criteria for the disorder (Biederman, Faraone, Mick, & Spencer, 1995). To disentangle the relative influences of genetic and environmental effects on AD/HD, Faraone et al. (2005) analyzed 20 separate twin studies in which AD/HD was defined using parent report. Results suggested that 76% of AD/HD phenotypic heterogeneity in the population is accounted for by genetic factors. Non-shared environmental factors (i.e., factors that make twins different from one another) accounted for roughly a quarter of the phenotypic heterogeneity in parent-reported AD/HD, but shared environmental factors (i.e., factors that make twins more alike) did not account for unique variability in the AD/HD phenotype. This suggests that although the genetic contribution to the etiology of AD/HD is paramount, environmental factors also play a substantial role. These findings have important implications for research examining causal factors of AD/HD.

Molecular genetics. Given the size of the heritability estimate, many molecular genetic studies have attempted to identify specific genes that underlie the AD/HD's genetic vulnerability. Findings from candidate gene studies of AD/HD generally support the neurotransmitter hypotheses of AD/HD. For example, genes associated with

dopaminergic functioning (DAT1, DRD4 and DRD5), serotonergic functioning (5HTT and HT1RB) and cholinergic functioning (CHRN14) were found to be associated with AD/HD. In addition, SNAP25, which is associated with axonal growth and synaptic plasticity, was also found to be associated with AD/HD (Gizer et al., 2009). Though such findings support etiological theories of AD/HD, the magnitude of association between candidate genes and AD/HD has been small and variable across studies. For example, Gizer et al. (2009) recently conducted a meta-analysis of candidate gene studies of AD/HD whichshowed that 11 genetic variants (some within the same gene) had a small to modest association with AD/HD (Odds Ratios ranged from 1.12-1.33).

In response to the inconsistent association between candidate genes and AD/HD, exploratory Genome-Wide Association Studies (GWAS) have attempted to uncover new AD/HD risk genes and replicate those found in previous candidate gene studies. Unfortunately, AD/HD genome-wide association studies have found few regions of overlap between studies (Lasky-Su et al., 2008a; Lasky-Su et al., 2008b; Lesch et al., 2008; Neale et al., 2008a) and little support for classic AD/HD candidate genes (Franke et al., 2009). Instead, evidence across AD/HD genome-wide association studies implicate genes associated with more basic cellular processes including cell-cell communication, cell division, cell adhesion, neuronal migration, and neural plasticity in the etiology of AD/HD (Franke et al., 2009; Poelmans et al., 2011); however, AD/HD genome-wide association studies have yet to detect genes at the level of genome-wide significance.

Together, candidate-gene and genome-wide association studies suggest that both neurotransmission and more basic cellular processes (e.g., plasticity) influence vulnerability for AD/HD. Although a handful of candidate genes are associated with AD/HD, the molecular genetic contribution to AD/HD's heritability estimate is still largely unknown. Multiple sources are likely to underlie AD/HD's "hidden heritability" including rare genetic variants (e.g., Copy Number Variants) that have a large effect in individuals but are uncommon in the population (McCarthy & Hirschhorn, 2008), gene-gene interactions (Derks et al., 2008), gene-environment correlation and gene x environment interactions (GxE; Rutter, Moffitt, & Caspi, 2006). For example, molecular genetic studies that fail to incorporate environmental measures may fail to uncover AD/HD vulnerability genes that are dependent on exposure to an environmental pathogen. Although genetic factors have the largest impact on the etiology of AD/HD, behavioral genetic studies indicate that non-shared environmental factors also play a substantial role in the etiology of the disorder. To better elucidate pathways to AD/HD, etiological models of the disorder need to specify both genetic and environmental factors.

Environmental Factors Associated with AD/HD

Many environmental risk factors have been associated with AD/HD. Although typically referred to as "environmental" risk factors in the psychological literature, many of these risk factors have substantial heritability estimates (e.g., Kendler & Baker, 2007). Thus, observed associations between environmental risk factors and AD/HD may not be entirely "environmental" in nature. Furthermore, environmental risk factors tend to congregate together (e.g., Knopik et al., 2006); therefore, before determining if a causal

relationship between an environmental factor and AD/HD exists, one must rule-out the role of confounding genetic and environmental factors.

The most extensively studied environmental factors associated with AD/HD are relatively uncommon and are believed to confer risk for AD/HD early in life. Specifically, exposure to prenatal teratogens has been a major focus of study, with prenatal exposure to smoking and alcohol garnering the most attention. Findings generally suggest that prenatal exposure to smoking and alcohol is associated with increased risk for AD/HD (Linnet et al., 2003), though the magnitude of association is small. In addition, the route to AD/HD risk from these and other prenatal teratogens is still largely unknown. Exposure to prenatal smoking may covary with genetic risk for AD/HD as parents with AD/HD may be at increased risk to smoke and consume alcohol during pregnancy (Burke, Loeber, & Lahey, 2001). However, after parental AD/HD is statistically controlled for, prenatal exposure to nicotine is still associated with AD/HD (Milberger, Biederman, Faraone, Chen, & Jones, 1996; Milberger, Biederman, Faraone, & Jones, 1998). In addition to early exposure to teratogens, prenatal hypoxia has been associated with increased risk for AD/HD (Ben Amor et al., 2005; Pineda et al., 2007). Exposure to other toxicants (lead, mercury and manganese) and post-natal environmental factors such as environmental deprivation and trauma have been examined to a lesser degree (Banerjee et al., 2007). Interestingly, many of these prenatal environmental factors restrict nutrient availability in utero and are associated with fetal growth compromise (Kramer, 1987). Although many prenatal environmental risk factors increase risk for

AD/HD, it remains relatively unclear how environmental factors confer risk for AD/HD and how genes may moderate environmental risk.

GxE in AD/HD

Investigating the interplay between genes and environment on AD/HD will build on research examining their independent effects and may: 1) account for AD/HD's hidden heritability estimate (Nigg, 2006), 2) explain variability AD/HD outcomes in individuals exposed to environmental risk factors; and 3) further the search of causal pathways to AD/HD. GxE studies in AD/HD (Nigg et al., 2010) have investigated the interaction of classic AD/HD candidate genes with a wide variety of putative environmental risk factors including prenatal smoking exposure (Altink et al., 2008; Becker, El-Faddagh, Schmidt, Esser, & Laucht, 2008; Kahn, Khoury, Nichols, & Lanphear, 2003; Neuman et al., 2007; Todd & Neuman, 2007), prenatal alcohol exposure (Brookes et al., 2008), season of birth (Brookes et al., 2008; Seeger, Schloss, Schmidt, Rüter-Jungfleisch, & Henn, 2004), exposure to psychosocial adversity (Laucht et al., 2007; Retz et al., 2008; Sonuga-Barke et al., 2008; Sonuga-Barke et al., 2009; Waldman, 2007) and birth weight (Langley et al., 2008). Although GxE studies have helped to broaden our understanding of the etiology of AD/HD, inconsistent findings and lack of methodological rigor have limited implications from this body of research (Ficks & Waldman, 2009; Nigg et al., 2010).

Indirect and retrospective measurement of the environmental exposure is one example of lack of methodological rigor in AD/HD GxE studies. For instance, many GxE studies examining prenatal exposure cigarette and alcohol use rely on a mother's report

of her cigarette or alcohol use during pregnancy (Altink et al., 2008; Brookes et al., 2006b; Langley, Holmans, Van Den Bree, & Thapar, 2007; Neuman et al., 2007; Todd & Neuman, 2007), both of which have been shown to have limited reliability(Derauf, Katz, & Easa, 2003). In addition, the precision of AD/HD phenotype measurement is highly variable and many studies conduct group based analyses using arbitrary criteria to define AD/HD and non-AD/HD groups. For example, Laucht et al. (2007) dichotomized their community sample into two groups (one with zero AD/HD symptoms and the other with at least one AD/HD symptom). Such an approach does not match the dimensional nature of AD/HD (Levy, Hay, McStephen, Wood, & Waldman, 1997; Shaw et al., 2011). It is also unclear how reliable such classifications are over time. The loss of reliability in the measure of the environmental factor or outcome may produce false negatives, especially when the studies are underpowered (Moffitt, Caspi, & Rutter, 2006).

Moreover, GxE studies fail to account for the significant heritability components of environmental risk factors (Kendler & Baker, 2007). This suggests that findings of the association between the environmental risk factor and AD/HD may be confounded by a shared genetic liability. Finally, studies often do not provide an explanation of the biological mechanism of action. For example, most AD/HD GxE studies examine interactions between dopamine genes and environmental pathogens; however, little to no rationale is provided for why an environmental pathogen would moderate the effect of a dopaminergic genotype on vulnerability for AD/HD. To improve upon previous studies, it is essential for future studies to provide a framework for how genetic and environmental factors coalesce to influence vulnerability for AD/HD.

Developmental Origins of Health and Disease

The Developmental Origins of Health and Disease hypothesis (DOHaD; Gluckman & Hanson, 2004) provides a framework to conceptualize how genetic and early environmental factors interact to confer vulnerability for AD/HD. Briefly, DOHaD purports that adverse influences, during critical periods of development, lead to fetal growth compromise. In addition to restricting nutrient and oxygen supply in utero, such influences may also lead the organism to make structural and functional adaptations to adverse environmental influences. To the extent that there is a mismatch between the prenatal and postnatal environments, the organism, which has adapted to increase probability of survival during prenatal development, may be ill-equipped to function adaptively in the future. This mismatch confers vulnerability for later disease as the organism is functioning in an environment for which it did not prepare. In addition to predicted adaptive responses, a restricted nutrient supply in utero may also constrain, delay, or disrupt developmental plasticity or developmental processes.

In the case of AD/HD, neurodevelopmental delays or neurodevelopmental disruptions may result from a limited supply of nutrients and oxygen in utero. Within individuals exposed to a restricted nutrient supply, and who subsequently have restricted fetal growth, vulnerability for AD/HD may then be moderated by the individual's genotype, maternal genotype, epigenetic changes, and the postnatal environment (see Figure 1). Such a conceptual model has yet to be tested. Prior to pursuing this line of research, it is first necessary to clarify the relationship between fetal growth compromise and AD/HD.

Fetal Growth Compromise in GxE studies of AD/HD

Moffitt et al. (2006) suggest that an environmental risk factor should be considered for use in a GxE study if: 1) individuals demonstrate a variable response after being exposed to the factor; 2) the environmental risk factor has a plausible effect on pathophysiology of the disorder; and 3) if there is evidence that the environmental risk factor is pathogenic in nature. These criteria help to ensure that the environmental risk factor is causal and that genetic factors are able to moderate the relationship between the environmental risk factor and outcome of interest. For the purpose of this study, the environmental factor under consideration is a restricted nutrient supply in utero; however, in human studies, this factor is rarely measured directly. Instead, nutrient supply in utero is often measured indirectly through fetal growth compromise (Maulik, 2006). Therefore, the following discussion will focus on the relationship between fetal growth compromise, as an indicator for restricted nutrient supply in utero, and AD/HD.

Variability in AD/HD outcome in fetal growth compromise. Across 22 prospective case-control studies examining the association between birth weight and AD/HD, individuals who were Low Birth Weight (LBW), Small for Gestational Age (SGA) or Intrauterine Growth Restricted were at two times greater risk of developing AD/HD compared to control groups with average fetal growth (Smith, Unpublished Manuscript). The magnitude of association between individuals who experienced restricted fetal growth and AD/HD is greater than that of any common candidate genes (Gizer et al., 2009). In addition, AD/HD risk increased as more comprehensive AD/HD assessments were employed (Botting, Powls, Cooke, & Marlow, 1997; Breslau et al.,

1996; Indredavik et al., 2004), suggesting that error in AD/HD assessment reduces the observed association between fetal growth compromise and AD/HD. Similar findings were also reported in retrospective AD/HD case-control studies (e.g., Mick, Biederman, Prince, Fischer, & Faraone, 2002). Finally, evidence from population cohort (Boulet, Schieve, & Boyle, 2009) and twin-studies (van Os et al., 2001) suggest that as fetal growth decreases, risk for AD/HD and externalizing behavior problems increases, respectively. Together, these three lines of evidence suggest that: 1) individuals who experienced fetal growth compromise are at increased risk for AD/HD; and 2) there is variability in AD/HD outcomes in those exposed to fetal growth compromise, regardless of the severity of fetal growth compromise (Hack et al., 2009). Therefore, the variability in AD/HD outcomes among those exposed to fetal growth compromise may be related to individual genetic factors.

Fetal growth compromise on pathophysiology of AD/HD. There is much evidence to suggest that the relationship between fetal growth compromise and AD/HD holds true even after numerous covariates are taken into account. For example, the relationship between birth weight and AD/HD cannot be accounted for by child factors such as sex, season of birth or duration of breast feeding (Elgen, Sommerfelt, & Markestad, 2003; Horwood, Mogridge, & Darlow, 1998) or by parental factors such as marital status, age, maternal education, paternal education, maternal stress, parental psychopathology, substance abuse, maternal smoking, or parental nurturance (Breslau et al., 1996; Elgen et al., 2003; Horwood et al., 1998; Indredavik et al., 2004; Linnet et al., 2006; Zubrick et al., 2000).

Furthermore, there are plausible biological processes that may link fetal growth compromise with AD/HD. For example, the second and third trimesters are critical periods in brain development, most notably for neurogenesis, neural migration, dendrite formation and synapse formation (Rodier, 2004). Therefore, prenatal insults during this period of development have the potential of restricting fetal growth and have lasting effects on neurodevelopment. For instance, growth restricted infants had reductions in overall white and gray matter compared to normally grown infants (Brown et al., 2009; Larroque et al., 2003; Tolsa et al., 2004). Such reductions relate to poorer performance on early measures of attention, more negative neurodevelopmental outcomes (Peterson et al., 2003; Tolsa et al., 2004) and are consistent with findings in AD/HD samples (Filipek et al., 1997; Kates et al., 2002; Mostofsky et al., 2002; Overmeyer et al., 2001). In addition to between group findings, neuroanatomical abnormalities predict increased risk for AD/HD within a fetal growth restricted cohort (Whitaker et al., 1997); however, it remains largely unclear why some individuals who experience fetal growth compromise develop AD/HD while others do not. Together, these findings suggest that youth who have experienced fetal growth compromise tend to display neurodevelopmental abnormalities which are functionally related to AD/HD symptomatology and consistent with findings in AD/HD samples.

Fetal growth compromise as a marker for an environmental pathogen. Evidence suggests that exposure to environmental factors that contribute to fetal growth compromise cause neurodevelopmental deficits consistent with AD/HD. For example, in multiple studies of monozygotic AD/HD discordant twins, the AD/HD affected co-twins

tended to have lower birth weights compared to the unaffected co-twins (Lehn et al., 2007; Sharp et al., 2003). In addition, MRI studies of discordant monozygotic AD/HD twins found that the AD/HD twin had a smaller caudate volume (Castellanos et al., 2003) and reductions of gray and white matter in the dorsolateral prefrontal cortex and corpus callosum (van 't Ent et al., 2007). Although genetic effects cannot be ruled out in the general population, findings from monozygotic discordant twin studies suggest that *environmental* or *non-genetic* factors contribute to differences in neurodevelopmental deficits and AD/HD symptomatology.

Furthermore, both prospective and retrospective case-control studies demonstrate that the relationship between birth weight and AD/HD cannot be accounted for by parental AD/HD or parental psychopathology (e.g., Indredavik et al., 2004; Mick et al., 2002). Population twin studies (van Os et al., 2001; Wichers et al., 2002) also report that a shared genetic variable cannot account for the relationship between birth weight and child behavior problems. Therefore, although genetic effects cannot be entirely ruled out, fetal growth compromise represents a constellation of prenatal environmental risk factors (i.e., non-genetic) which compromise fetal growth and are pathogenic in nature.

Optimizing measurement of fetal growth compromise. Moffitt et al. (2006) also emphasize the importance of accurately and reliably measuring the putative environmental risk factor in GxE research. Many different measures of fetal growth and fetal growth compromise are made both prenatally and at birth. Although LBW (weighing less than 2500 grams at birth) has been the most widely studied fetal growth phenotype in the AD/HD literature, LBW lacks specificity and may identify individuals

who are either constitutionally small or premature, but normally grown. Therefore, measures of asymmetric growth restriction, such as ponderal index which measures weight relative to length, or measures of fetal growth for gestational age, may be more appropriate in identifying individuals at risk for AD/HD, as they are less likely to be influenced by factors that do not limit fetal growth (Maulik, 2006).

Measures of asymmetric growth compromise offer a good option for indirectly measuring a restricted nutrient supply in utero (Gluckman & Hanson, 2004; Maulik, 2006); however, measures of birth length are not always recorded in population birth registries or birth records. In the absence of measures of birth length, measures of fetal growth for gestational age (e.g., small for gestational age) are a reasonable alternative. Given that appropriately grown individuals that are born premature are not at increased risk for AD/HD (Heinonen et al., 2010), SGA is considered a better indicator of risk for AD/HD due to a restricted nutrient supply in utero.

For clinical purposes SGA is typically dichotomized (i.e., $<10^{th}$ percentile = SGA; $\geq 10^{th}$ centile = appropriate for gestational age). A dichotomous measure of SGA, however, is not consistent with the continuous nature of association between fetal growth compromise and AD/HD (Boulet et al., 2009; van Os et al., 2001) and would reduce statistical power in a GxE model. Therefore, in models of AD/HD risk, it is most appropriate to measure SGA continuously, through customized birth weight centiles. Given that birth weight varies by sex, ancestry, and parity, SGA calculations are often customized to account for such differences through the use of appropriate reference groups (e.g., Visser, Eilers, Elferink-Stinkens, Merkus, & Wit, 2009).

One limitation of many current GxE studies is the use of retrospective measurements of the environmental pathogen and the resulting loss in reliability (Moffitt et al., 2006). Although measurement at birth is considered the most reliable time of assessment, birth record review has been shown to be a reliable approach to measuring birth phenotype (Northam & Knapp, 2006). In the absence of medical or birth records, maternal recall of birth weight and gestational age has also been used in epidemiological and clinical research. The reliability of maternal recall of birth weight and gestational age is high, with around 75% of mothers recalling birth weight within 100g of the recorded birth weight and within one week of the recorded gestational age (Seidman, Slater, Ever-Hadani, & Gale, 1987).

Selection of candidate systems to moderate the association between fetal growth compromise and AD/HD. In a DOHaD framework, the association between fetal growth compromise and AD/HD is likely moderated by genotype. There are many different approaches to choosing genes to interact with fetal growth compromise. Most previous GxE studies in AD/HD have chosen candidate genes that have a direct association with the disorder. This approach has a limited conceptual basis and may be related to the inconsistent findings in the AD/HD GxE literature (Ficks & Waldman, 2009). In contrast, Moffitt et al. (2006) suggest choosing candidate polymorphisms based on their functional significance in relation to the environmental risk factor. Consistent with this approach, systems that influence vulnerability for AD/HD and whose expression is regulated by restricted nutrient and oxygen supply include the dopaminergic, neurotrophic and angiogenic pathways.

Dopaminergic system. Hypodopaminergic functioning is a central component to most AD/HD etiological theories (Barkley, 1997; Levy, 1991; Nigg & Casey, 2005; Sagvolden, Johansen, Aase, & Russell, 2005; Sonuga-Barke, 2002). Dopaminergic functioning is highly complex (see Missale, Nash, Robinson, Jaber, & Caron, 1998 for a review) and is influenced by a multitude of factors including dopamine synthesis and delivery, dopamine receptors and dopamine termination. The dopaminergic system is embedded in the larger catecholamine pathway and is involved in, among other things, cognitive, behavioral, and emotional functioning (Missale et al., 1998). Dopamine plays a central role in the regulation of prefrontal cortical neural activity which project to the nucleus accumbens and ventral tegmental area (Sagvolden et al., 2005). The mesocortical, mesolimbic, and nigrostraital neural loops are largely regulated by dopamine and are believed to impact hyperactivity-impulsivity, inattention and motor inhibition, respectively (Sagvolden et al., 2005).

Evidence implicating dopamine in the etiology of AD/HD comes from animal research and human genetic research. For example, common AD/HD animal models (e.g., the Spontaneously Hypertensive Rat, DAT knockout mouse and the SNAP-25 deficient mutant coloboma mouse) have genetic abnormalities which lead to hypodopaminergic functioning and AD/HD like behaviors (Russell, 2011). Such findings are also consistent with results in human genetics studies. For example, polymorphisms within dopamine receptors (DRD4 and DRD5), the dopamine transporter (DAT1) and a factor involved with dopamine delivery (SNAP-25) are all associated with AD/HD (Gizer et al., 2009).

There is substantial support linking hypodopaminergic functioning with AD/HD; however, far less is known about how environmental factors influence dopaminergic functioning. Initial research has shown that intermittent hypoxia or malnutrition has led to reduced extracellular dopamine in the prefrontal cortex in rats (Decker, Jones, Solomon, Keating, & Rye, 2005; Mokler, Torres, Galler, & Morgane, 2007), which may be mediated by hypoxia-inducible factor (HIF) which promotes cell survival in hypoxic conditions (Johansen et al., 2010). In addition, many GxE studies have examined if prenatal environmental risk moderates the association between dopamine genes and AD/HD, but findings have been mixed (Nigg et al., 2010).

Neurotrophic system. The neurotrophin family promotes numerous neuroadaptive functions including neuron survival, neural differentiation, neural plasticity as well as synaptic efficiency in both the central nervous system and the peripheral nervous system. This family consists of four closely related proteins including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4) that are derived from their relative proneurotrophins. These factors bind to and activate one or more of the tyrosine kinase neurotrophin receptors (TrkA, TrkB, and TrkC). In addition, all the mature neurotrophins and the proneurotrophins bind to and activate the low affinity p75 receptor (Reichardt, 2006; see Figure 3). Although other factors (e.g., ciliary neurotrophic factor; glial derived neurotrophic factor) promote neural growth and differentiation, to date, the majority of research has focused on the neurotrophin family. In addition to being expressed in nerve cells, the neurotrophin family is also expressed in endothelial cells in the vasculature.

Lines of evidence from animal and human studies, including genetic and neuroimaging studies, suggest that neurotrophic factors may play a role in the etiology of AD/HD (see Ribasés et al., 2008).

Mouse models suggest that neurotrophic factors play a critical role in survival, neural growth and behavior. For example, homozygous BDNF knockout mice fail to survive past the second postnatal week (Ernfors, Lee, & Jaenisch, 1994) and heterozygous BDNF knockout mice display increased hyperactivity, aggression, decreased learning ability and dysregulated eating behavior (Kernie, Liebl, & Parada, 2000; Linnarsson, Björklund, & Ernfors, 2006; Lyons et al., 1999). In addition, neurotrophic factors are also influenced by exposure to putative environmental pathogens. For example, BDNF expression is upregulated in the hippocampus during conditions of restricted nutrient supply (Schmidt-Kastner et al., 2001). Furthermore, decreases in BDNF expression lead to both hyperactivity and severe learning deficiencies in early but not later life (Monteggia et al., 2004). This suggests that the behavioral sequelae related to BDNF expression resemble AD/HD symptomatology and are temporally dependent.

To date, multiple human studies have investigated the role of genes encoding for neurotrophic factors in the etiology of AD/HD. For example, three recent meta-analyses in both child and adult samples (Forero, Arboleda, Vasquez, & Arboleda, 2009; Gizer et al., 2009; Sanchez-Mora et al., 2009) have investigated the association between the BDNF gene and AD/HD. Although individual studies have found a significant association between the BDNF Val66Met and AD/HD (Kent et al., 2005), NTF and

AD/HD (Syed, Dudbridge, & Kent, 2007), and NTF3, NTRK2 (the BDNF receptor) and AD/HD (Ribasés et al., 2008), meta-analyses found no association between SNPs within BDNF and AD/HD. Such inconsistent results are common in psychiatric genetics. One explanation for such inconsistency is that genetic variation in neurotrophic factors confers risk for AD/HD only under particular adverse environmental exposures. To address this limitation, Lasky-Su and colleagues (2007) found that SNPs in the BDNF gene, including the Val66Met SNP, moderated the association between socio-economic status and AD/HD. Similar to results in animal studies (Schmidt-Kastner et al., 2001), this finding suggests that the role of the neurotrophic family in the etiology of AD/HD may depend on environmental risk.

Neuroimaging studies also provide groundwork to link neurotrophic factors and AD/HD. For example, consistent with findings in AD/HD (Valera et al., 2007), neuroimaging genetic studies suggest that compared to BDNF Val/Val homozygotes, BDNF Val/met heterozygotes had reduced anterior cingulated cortex, dorsolateral prefrontal cortex, hippocampus, and amygdala volumes (Nemoto et al., 2006; Sublette et al., 2008). In addition, the combination of early life stress and the BDNF met allele resulted in greater neuroanatomical deficits (Gatt et al., 2009).

Taken together, findings suggest that to elucidate the role of neurotrophic factors in the development of AD/HD, it may be important to consider the environmental context. Given the high rate of neurodevelopment in the prenatal period, a limited nutrient supply in utero may moderate the expression of neurotrophic genes which would alter neurodevelopmental risk for AD/HD.

Angiogenic system. Angiogenesis, or the creation of blood vessels, is necessary for neural development, neural maintenance and neural function (Shibuya, 2008). In humans, the Vascular Endothelial Growth Factor (VEGF) family is the main regulator of angiogenesis (Shibuya & Claesson-Welsh, 2006). The VEGF family consists of VEGF-A, VEGF-B, VEGF-C, VEGF-D, and platelet-derived growth factor (PIGF), and their receptors VEGFR-1, VEGFR-2, and VEGFR-3. VEGF-A has been the most extensively studied factor and VEGF-A promotes angiogenesis by binding to VEGFR-1 and VEGFR-2. VEGF-B and PIGF bind to VEGFR-1 and also influences angiogenesis, albeit to a lesser extent (Shibuya, 2008). VEGF-C and VEGF-D bind to VEGFR-3 and play a central role in the formation of lymphatic vessels, and therefore will not be further discussed.

VEGF-A homozygote and heterozygote knockout mice fail to survive past the embryonic stage due to maladaptive angiogenesis which suggests that VEGF-A protein provided by both VEGF-A alleles is necessary for survival (Ferrara et al., 1996). In addition, decreases in the VEGF-A protein lead to tissue hypoxia and neural degeneration (Haigh et al., 2003). In terms of gene expression, environmental factors have also been shown to regulate the expression of VEGF and their receptors. For example, hypoxia produces an upregulation of the VEGF-A (Jaakkola et al., 2001). The upregulation of VEGF expression, as well as other genes that promote adaptation in the face of hypoxia, is mediated by the transcription factors of hypoxia inducible factors (HIF-1; Mac Gabhann & Popel, 2008). Considering that hypoxia is a risk factor for

neurodevelopmental disorders, including AD/HD, the route to risk from angiogenic factors to AD/HD may depend on restricted nutrient and oxygen supply in utero.

Animal models also suggest that reduced levels of angiogenic factors may play a role in the pathophysiology of AD/HD. For example, a substrain of the spontaneously hypertensive rat (SHR; Okamoto & Aoki, 1963), which demonstrates vulnerability to stroke (Jesmin et al., 2004), exhibits behaviors consistent with AD/HD. Interestingly, the stroke-prone SHR has reduced VEGF serum levels compared to the SHR and Wistar-Kyoto rat strains, suggesting the VEGF expression may be involved in the pathophysiology of AD/HD (Jesmin et al., 2004). In addition, the stroke-prone SHR also demonstrates abnormal regional cerebral blood flow (rCBF; Jesmin et al., 2004). Together, animal research suggests that the VEGF family is necessary for survival, interacts with the environment to influence VEGF expression, and VEGF may be associated with vulnerability for AD/HD via abnormal rCBF.

To date, the role of the VEGF family in contributing to the pathophysiology of AD/HD in humans has not been examined; however, multiple studies have examined the role of rCBF in individuals diagnosed with AD/HD. For example, findings have suggested that youth with AD/HD have decreased rCBF in prefrontal, limbic and cerebellar regions during resting state compared to controls (Kim et al., 2002). In addition to functional changes in neuroanatomical substrates associated with AD/HD, genetic neuroimaging studies suggest that SNPs within the VEGF-A gene are associated with hippocampal volume (Blumberg et al., 2008).

Additional studies have examined the role of rCBF in mediating the therapeutic effects of methylphenidate. In general, studies suggest that responders to methylphenidate have increases in rCBF in the prefrontal cortex and caudate nucleus (Kim et al., 2002).

Together, such findings have led Jesmin et al. (2004) to suggest that individual variability in VEGF concentration or angiogenic response to prenatal insults, like a reduced nutrient supply in utero, may compromise regional Cerebral Blood Flow (rCBF) in prefrontal and limbic systems which, in turn, increases vulnerability for AD/HD.

Summary and Purpose

Although AD/HD behavioral genetic studies have consistently demonstrated that genetic effects are paramount, non-shared environmental factors play a substantial role in the etiology of AD/HD. To date, molecular genetic studies have produced mostly inconsistent results (e.g., Franke et al., 2009; Gizer et al., 2009) and accounted for only a small proportion of the AD/HD heritability estimate (Nigg, 2006). Failure to include environmental factors within genetic studies of AD/HD may account, in part, for the inconsistent findings and help to explain AD/HD's large heritability estimate. In addition, GxE studies have the potential to broaden our understanding of the etiology of AD/HD and help to uncover causal mechanisms which contribute to the development of AD/HD.

Fetal growth compromise is a promising environmental risk factor for use in AD/HD GxE studies for the following reasons: 1) it is associated with increased risk for AD/HD; 2) it is associated with increased neurodevelopmental vulnerability for AD/HD; and 3) prenatal environmental factors underlie the relationship between fetal growth compromise and AD/HD. Together, this suggests that fetal growth compromise is an

indicator for prenatal environmental risk factors which restrict nutrient supply in utero, compromise fetal growth and are pathogenic in nature.

Two previous studies have investigated fetal growth compromise in the context of an AD/HD GxE study (Langley et al., 2007; Langley et al., 2008). These studies did not provide a rationale for examining interactions between birth weight and dopamine and serotonin genes in predicting AD/HD and did not report any significant findings. This approach is consistent with the majority of AD/HD GxE studies which examine interactions between a variety of environmental risk factors and *classic* AD/HD candidate genes, but do not provide a rationale for predicting the presence of GxE.

Therefore, to address this limitation, the current study was conceptually driven by the DOHaD hypothesis (Gluckman & Hanson, 2004). Given that ischemia/hypoxia is believed to underlie fetal growth compromise and is associated with the upregulation of dopaminergic, neurotrophic and angiogenic genes, there is reason to believe that fetal growth compromise may moderate the relationships between angiogenic, dopaminergic and neurotrophic genotypes and AD/HD. From a DOHaD perspective, in response to a restricted nutrient supply in utero, individual variability in dopaminergic, neurotrophic and angiogenic factors (Cannon, Yolken, Buka, & Torrey, 2008; Fu & Olofsson, 2006) may be associated with neurodevelopmental characteristics associated with AD/HD (Rapoport & Gogtay, 2007; Shaw et al., 2006; Toft, 1999) and give way to the AD/HD behavioral phenotype.

To address this possibility, the purpose of this research project was to examine the interaction of polymorphisms in the dopaminergic, neurotrophic and angiogenic systems

with fetal growth compromise to predict AD/HD symptom severity. Consistent with the DOHaD perspective (Gluckman & Hanson, 2004) the following hypotheses were made:

1. Consistent with pervious findings, lower customized birth weight centiles were expected to be associated with increased AD/HD symptomatology.
2. In an extension of previous research, it was predicted that after controlling for main effects, fetal growth compromise would moderate the relationship between SNPs within dopaminergic, neurotrophic and angiogenic genes and AD/HD symptom severity.

The results of this research project will inform our conceptualization of the etiology of AD/HD by potentially helping to: 1) further our understanding of the role of dopaminergic, neurotrophic and angiogenic genes and vulnerability for AD/HD; 2) explain the variability in AD/HD outcome in individuals who experienced fetal growth compromise (e.g., Mick et al., 2002); and 3) shed light on causal mechanisms underlying the etiology of AD/HD.

CHAPTER II

METHOD

Participants

A total of 398 youth participated in the current study. Participants were drawn from multiple sites within the United States and Europe (see Table 1). Of the 398 total participants, 107 youth were from the North Carolina Genetics of AD/HD Project (NCGAP; PI – Allison Ashley-Koch, 1R01NS049067) with recruitment sites at Duke University and the University of North Carolina at Greensboro (UNCG). The remaining 291 youth were drawn from the International Multisite AD/HD Genetics Project (IMAGE; PI-Stephen Faraone, R01MH081803, R01MH62873) with recruitment sites in Ireland, the Netherlands, and the United Kingdom.

NCGAP is a longitudinal, family-based genetic study of AD/HD and its comorbid features. AD/HD probands, AD/HD affected siblings, and unaffected siblings from NCGAP were included in the current study. NCGAP probands: 1) were between the ages of 5-12 years; 2) met DSM-IV criteria for AD/HD; 3) had a full-scale IQ estimate of > 70 as measured by Block Design and Vocabulary subtests of the Wechsler Intelligence Scale for Children, Fourth Edition (WISC-IV; Wechsler et al., 2004)[1]; 4) had a Clinical Global Impression Scale (CGI; Guy, 1976) of ≥ 3; and 5) had a biological parent was

[1] If participant had an IQ estimate between 70 and 80, then a score > 70 was required on the Vineland Adaptive Behavior Rating Scale, Second Edition (Vineland-II; Sparrow, Cicchetti, & Balla, 2005) composite score.

available to participate. Families were excluded from participating in NCGAP if the identified proband: 1) met diagnostic criteria for a pervasive developmental disorder; 2) displayed significant developmental delays; or 3) had a medical, neurological or genetic disorder that could have accounted for the AD/HD symptomatology.

In NCGAP, parent responses to a diagnostic structured interview and parent and teacher rating scale responses were used to establish Diagnostic and Statistical Manual of Mental Disorders – 4th Edition (DSM-IV) AD/HD diagnostic status. Youth met research criteria for AD/HD if they, when off medication: 1) had a positive diagnosis on the AD/HD module of the Computerized Diagnostic Interview Schedule for Children –IV (C-DISC-IV; National Institute of Mental Health [NIMH], 1997); 2) had *T*-scores ≥ 65 and 60 on the parent and teacher forms of the Conners' Rating Scale-Revised Long Form (CRS R:L; Conners, 1997) DSM-IV inattention and/or hyperactivity-impulsivity dimensions, respectively[2]; and 3) were determined to meet DSM-IV criteria for AD/HD, any subtype, by a panel of three senior investigators and licensed psychologists with expertise in AD/HD. The same criteria and panel review process were used for determining AD/HD status of all siblings participating in the study; however, siblings were not required to meet DSM-IV criteria for AD/HD and could range in age from 5 to 17 years.

The IMAGE study (Brookes et al., 2006a; Kuntsi, Neale, Chen, Faraone, & Asherson, 2006; Neale et al., 2008b) is a family-based AD/HD genetics project with 12 participating sites in Belgium, Germany, Ireland, Israel, Spain, Switzerland and the

[2] The elevated CRS-R:L teacher rating of AD/HD symptoms criteria was waived if the teacher was unable to rate the child behavior when off-medication.

United Kingdom; however, due to the need for birth history data, only youth from Ireland, the Netherlands, and the United Kingdom were included in this study. Families participating in the IMAGE study had a child who: 1) was between the ages of 5-17 and met DSM-IV criteria for AD/HD combined type[3]; 2) had at least one full sibling between the ages of 5-17 available to participate in the study; and 3) had at least one biological parent available to participate in the study. Families were excluded from participating in IMAGE if the proband or sibling(s) had autism, epilepsy, an IQ < 70, or any genetic or medical disorder that could explain the presence of AD/HD symptoms based on clinical history.

Prior to enrollment in IMAGE, all probands received clinical evaluations from a child psychiatrist or pediatrician. The presence of DSM-IV AD/HD was determined by combining parental responses on the Parental Account of Childhood Symptoms (PACS; Taylor, Sandberg, Thorley, & Giles, 1991), a semi-structured clinical interview, and teacher responses to DSM-IV AD/HD symptoms on the CRS-R:L (Conners, 1997). Parental qualitative responses to the PACS were matched to a quantitative and frequency severity score. Scores were then weighted and combined with an algorithm to map onto each AD/HD symptom. PACS symptom item scores were combined with teacher responses on the CRS-R:L using the "either rule" to determine AD/HD diagnostic status (Müller et al., 2011).

[3] Before quality control measures were implemented, some IMAGE participants met criteria for DSM-IV Predominantly Inattentive Type, DSM-IV Predominantly Hyperactive-Impulsive Type, or were one symptom short of a DSM-IV AD/HD diagnosis. These individuals were retained in analyses.

Of the 398 total participants in the current study, there were 360 probands and 38 siblings. The sample had a mean age of 10.7 years (3.02) and was 83% male, which is consistent with the gender differences in AD/HD prevalence among clinical samples (APA, 2000). The sample was 100% Caucasian as genotype imputation procedures were based on a Caucasian reference group.

The total sample consisted of 381 youth meeting criteria for AD/HD and 17 unaffected youth. The current study had a higher percentage of individuals meeting criteria for AD/HD Combined Type (86%), and lower percentages of individuals meeting criteria for AD/HD Predominantly Inattentive Type (11%) and AD/HD Predominantly Hyperactive-Impulsive Type (3%) relative to prevalence rates of AD/HD subtypes in community (Dupaul, Power, Anastopoulos, & Reid, 1998; Gaub & Carlson, 1997) and AD/HD clinical samples (Lahey et al., 1994). In terms of AD/HD symptom count, the overall sample displayed an average of 7.97 (*SD* = 1.32) inattentive symptoms and 7.49 (*SD* = 2.03) hyperactive-impulsive symptoms. In regard to developmental deviance, the average parent CRS-R:L AD/HD Total (*M* = 77.59, *SD* =10.45), DSM-IV Inattention (*M* = 71.98, *SD* = 10.12) and DSM-IV Hyperactivity-Impulsivity score (*M* = 78.73, *SD* =11.38) were all in the clinically significant range. Compared to the NCGAP subsample, the IMAGE subsample had a higher proportion of probands, χ^2 (1, N = 398) = 90.91, $p < .01$, a higher proportion of males, χ^2 (1, N = 398) = 18.62, $p < .01$, and was older, t (396) = -9.44, $p < .01$. A summary of demographic variables appears in Table 2. Consistent with differences in ascertainment (i.e., focus on AD/HD Combined subtype in IMAGE), the IMAGE subsample had more severe AD/HD symptomatology than the NCGAP

subsample. For example, the IMAGE subsample had higher levels of both inattentive $t(120) = -3.02$, $p < .01$) and hyperactive-impulsive symptoms, $t(104) = -7.31$, $p < .01$). Furthermore, The IMAGE subsample had a higher parent CRS-R:L AD/HD DSM-IV Hyperactive-Impulsive score $t(137) = -5.09$, $p < .01$) and AD/HD Total Score $t(135) = -2.81$, $p = .006$) than the NCGAP subsample. See Table 3 for additional AD/HD sample information.

Consistent with previous findings in AD/HD clinical samples (Pfiffner et al., 1999) many youth in the current study also met criteria for comorbid disorders, including oppositional defiant disorder (59.2%), conduct disorder (21.8%), mood disorder (18.0%), bipolar disorder (0.8%), anxiety disorder (41.3%), obsessive-compulsive disorder (4.8%), tic disorder (4.3%), and a substance abuse disorder (1.0%). The IMAGE sample had a significantly higher proportion of oppositional defiant disorder $\chi^2(1, N = 395) = 35.53$, $p < .01$, conduct disorder, $\chi^2(1, N = 395) = 24.74$, $p < .01$, mood disorders, $\chi^2(1, N = 395) = 17.27$, $p < .01$, anxiety disorders, $\chi^2(1, N = 395) = 47.06$, $p < .01$, and obsessive-compulsive disorder, $\chi^2(1, N = 395) = 4.73$, $p = .03$. Differences between samples are likely related to differences in NCGAP and IMAGE ascertainment and assessment procedures. See Table 4 for additional comorbidity information.

In terms of birth characteristics, the samples' mean birth weight ($M = 3389.25$ grams or 7 lbs and 11 oz; $SD = 565$ grams or 1 lb and 4 oz) and gestational age ($M = 39.56$ weeks; $SD = 1.94$ weeks) were in the normal range. Additional pregnancy, birth and delivery information is presented in Table 5. Note, however, that the supplementary

pregnancy, birth and delivery variables (e.g., infant needed oxygen) were not consistently or uniformly measured across sites and should be interpreted with caution.

Measures -NCGAP Ascertainment

Computerized Diagnostic Interview Schedule for Children – IV Parent Version (C-DISC-IV; NIMH, 1997). The C-DISC-IV is a computerized structured diagnostic interview that assesses a broad range of child and adolescent psychopathology based on current DSM-IV criteria. A trained interviewer read each item to the parent, who provided a yes or no response indicating whether or not the item applies to their child. The AD/HD module of the DISC-IV has adequate test-retest reliability in clinic samples (.79; Shaffer, Fisher, Lucas, Dulcan, & Schwab-Stone, 2000) and criterion validity ($\kappa = .72$) with clinician ratings (Schwab-Stone et al., 1996). Parental responses to the C-DISC-IV AD/HD module were used by the diagnostic panel in determining the presence and absence of AD/HD and comorbid conditions of all youth participants.

Conners' Rating Scale-Revised Long Form (CRS-R:L; Conners, 1997). The CRSR:L measures a range of common child psychiatric factors, including DSM-IV AD/HD inattention and hyperactivity-impulsivity symptom dimensions. Parents and teachers rated the extent to which each DSM-IV AD/HD symptom applied to their child on a scale from 0 (not at all true) to 3 (very much true), with higher ratings indicating greater AD/HD symptom severity. The raw scores from the 9-item DSM-IV: AD/HD Inattention and the 9-item DSM-IV: Hyperactive-Impulsive scales were converted to *T*-scores adjusting for age and gender of each participant (Conners, Sitarenios, Parker, & Epstein, 1998). A Conners' Parent Rating Scale (CPRS) *T*-score ≥ 65 and Conners'

Teacher Rating Scale (CTRS) *T*-score ≥ 60 on either the DSM-IV: Inattention or DSM: Hyperactive-Impulsive, were used as indicators of AD/HD symptom developmental deviance and cross-situational pervasiveness.

Clinical Global Impression Scale (CGI; Guy, 1976). The CGI is a standardized clinician rating scale used to measure the severity of an individual's psychiatric disorder from 1 (normal, not ill) to 7 (among the most ill patients). The CGI was used as a severity measure of functional impairment related to AD/HD. Youth that were rated as ≥ 3 (mildly ill) were eligible to be ascertained as NCGAP probands.

Wechsler Intelligence Scale for Children – Fourth Edition (WISC-IV; Wechsler et al., 2004). The WISC-IV is a standardized assessment of a youth's current intellectual functioning. A trained assessor administered the Block Design subtest, which is an indicator of perceptual reasoning, and Vocabulary subtest, which is a measure of verbal reasoning, to youth. The participant's responses were scored and compared to a national sample of similar aged peers to derive an estimate of the participant's current Intellectual Quotient (IQ). Youth were included in NCGAP with an estimated IQ > 70.

Vineland Adaptive Behavior Rating Scale, Second Edition (Vineland-II; Sparrow et al., 2005). The Vineland-II survey interview measures the child's current level of adaptive functioning in multiple domains including communication, daily living skills, socialization, and motor skills. A trained interviewer read each item to the youth's parent and responses were coded, scored, and compared to an age-appropriate normative sample. The Vineland-II was only administered to parents of youth who had an estimated IQ between 70 and 80. Taken together with an estimated IQ between 70 and 80, an

Adaptive Behavior Composite score < 70 was used as exclusionary criteria in the NCGAP study.

Pregnancy, delivery, and infant history. The NCGAP project obtained pregnancy, delivery and infant history through a developmental history form which was typically completed by participants' mothers (See Appendix A).

Measures-IMAGE Ascertainment

Parental Accounts of Childhood Symptoms (PACS; Taylor et al., 1991). The PACS is a standardized, semi-structured diagnostic interview that assesses for DSM-IV child and adolescent psychopathology, including AD/HD. Child psychiatrists and clinical child psychologists trained in the administration of the PACS asked parents to rate the frequency and severity of their child's behavior, across different situations. The interviewer then matched the parent's responses to a behavior frequency or severity category which then were combined and weighted in an algorithm to indicate the presence or absence of corresponding DSM-IV symptoms. Parental responses to the PACS were used, in part, to determine eligibility for inclusion in IMAGE, and to assess for DSM-IV AD/HD and other comorbid disorders.

Conners' Teacher Rating Scale (CRS-R:L; Conners, 1997). The DSM-IV AD/HD total subscale from the CTRS, maps onto the 18 DSM-IV AD/HD symptoms. Teacher symptom ratings of 2 (pretty much true) or 3 (very much true) were coded to indicate the presence of the AD/HD symptom. If either the PACS or CTRS indicated the presence of an AD/HD symptom, then the child was coded as having that symptom. This

process was used to determine if the participant met the symptom frequency criterion for AD/HD.

Pregnancy, Delivery and Infant History Interview. IMAGE obtained pregnancy, delivery and infant history through developmental interviews which were then coded into the same categories found on the NCGAP developmental history form.

GxE Measures

Conners' Parent Rating Scale (CRS-R:L; Conners, 1997). Parent responses to the 18-item DSM-IV AD/HD total score were summed. The raw scores from the DSM-IV AD/HD Total subscale were converted to *T*-scores adjusting for age and gender of each participant (Conners et al., 1998). The resulting *T*-score was a continuous measure of AD/HD symptom severity and served as the outcome measure in this study.

Birth weight centile range. Birth weight centiles were calculated for each participant based on birth weight, gestational age and sex. Given that Dutch children tend to weigh heavier at birth compared to children with other ancestries (Troe et al., 2007), separate normative samples were used to calculate birth weight centiles for the Netherlands, United Kingdom/Ireland, and United States samples. In the current study, birth weight centile is a proxy measure for a restricted nutrient supply in utero and served as the environmental risk factor in the GxE model.

IMAGE. For the Dutch sample, birth weight and gestational age (in weeks) were obtained through parent report. The Netherlands Perinatal Registry reference curves (Visser et al., 2009) were used to calculate birth weight centiles for the Dutch sample. The reference sample consists of 176,000 singleton births in the Netherlands during 2001.

The reference curves provide personalized centile ranges for individuals based on gestational age (in days), sex, ethnicity and parity. Gestational age to the day and parity were not available for individuals in the Dutch sample. Therefore, to offset the slight overestimation of birth weight centile related to using full week gestational age instead of gestational age in days, birth weight centiles were calculated using the multiparous normative sample, which only includes births from women who have delivered two or more babies. Instead of providing individual centiles, the Netherlands Perinatal Registry reference curves (Visser et al., 2009) provide 11 normative references at 2.3, 5, 10, 16, 20, 50, 80, 84, 90, 95, and 97.7 centiles. Therefore, 12 birth weight centile ranges were created (0-2.29, 2.3-4.9, 5-9.9, 10-15.9, 16-19.9, 20-49.9, 50-79.9, 80-83.9, 84-89.9, 90-94.9, 95-97.6, 97.7 -100). Lower scores on the resulting ordinal severity scale of birth weight centile ranges represented higher levels of fetal growth compromise.

Birth weight and gestational age for samples from Ireland and the UK were obtained from retrospective parent report. The UK reference curves (Cole, Williams, & Wright, 2011) and Microsoft excel add-in (http://www.healthforallchildren.co.uk; Pan & Cole, 2010) were used to calculate birth weight centiles for the UK and Ireland samples, based on birth weight, gestational age (in weeks), and sex. The reference curves are based on 9,443 births in the UK between 1983-1993. For consistency, individual birth weight centiles were converted to birth weight centile ranges identical to those created in the Dutch sample.

NCGAP. Birth weight and gestational age for the NCGAP sample was retrieved through medical records, parental report, and state birth registry with help from Marie

Lynn Miranda and Claire Osgood from the Children's Environmental Health Initiative at Duke University. Birth weight centiles for NCGAP were created using all singleton births from 2000-2004 from the CDC National Vital Statistics natality files (www.cdc.gov/nchs/data_access/Vitalstatsonline.htm). Individual birth weight centiles were created using birth weight, week of gestational age and sex. For consistency, individual centiles were then converted to the centile ranges described above.

Given that different methods were employed to assess birth weight and gestational age within and between sites, the level of agreement between parental recall and medical records of birth weight and gestational age was assessed. Consistent with previously reported associations between maternal recall and medical records (Hakim, Tielsch, & See, 1992; Rice et al., 2007) the level of agreement between birth records and maternal recall of birth weight in the NCGAP sample was high, intraclass correlation coefficient, ICC (76) = .99, $p < .01$. Similarly, the agreement between birth records and maternal recall of gestational age was also high, intraclass correlation coefficient, ICC (77) = .84, $p < .01$.

Procedure

NCGAP families were recruited to participate from: 1) two separate university AD/HD specialty clinics; 2) medical clinics in the community; 3) a community AD/HD parent support group; and 4) newspaper and magazine advertisements. Parents and youth from eligible families were scheduled for comprehensive psychological assessments, including structured diagnostic and semi-structured background interviews, self- and other-report ratings scales, and an intelligence assessment screening tool. All data were

collected by graduate-level research assistants or licensed psychologists trained to administer each measure. In addition, a phlebotomist collected blood samples of willing participants. All families received individualized research summary reports and $50 dollars to compensate them for their travel and time. Out of nearly 400 youth who participated in NCGAP, 107 were included in the current study. Only participants that were Caucasian, genotyped using the Illumina Infinium HumanHap300 duo (Illumina, Inc., San Diego, CA), and had all necessary developmental and clinical data were included in the current study.

IMAGE families were recruited from AD/HD specialty centers in 12 European and Asian nations. All participants underwent clinical evaluations including semi-structured clinical interviews and parent- and teacher-rating scales. All evaluations were completed by pediatricians or child psychiatrists, and both existing and new clinic patients were enrolled in the study. The study was approved by the Institutional Review Board of each center which was registered with the National Institute of Health. Of the nearly 1,000 families participating in the IMAGE project, a subset of 291 youth were included in the current study. Only youth from Ireland, the Netherlands, and the United Kingdom participated as birth history data were not collected at other IMAGE sites.

Genotyping. SNP genotyping for the NCGAP subsample was performed using the Illumina Infinium HumanHap300 duo (Illumina, Inc., San Diego, CA). Two Centre d'Etude du Polymorphism Humain (CEPH) controls and blinded duplicates were used for every 94 samples and required to match 100%. Additional quality checks of the genotyping data were examined using PLINK (pgnu.mgh.harvard.edu/~purcell/plink;

Purcell et al., 2007). Call rates exceeded 98% for all individuals. Individuals were excluded due to gender discrepancy and if per-family Mendelian errors were in excess of 1%. SNPs were excluded from analysis if they had Mendelian errors in > 4 families or deviated from Hardy-Weinberg Equilibrium (HWE; $p < 0.000001$).

Genotyping for the IMAGE subsample was performed by Perlegen Sciences on a microarray designed for the Genetic Association Information Network (GAIN). Quality checks were completed by the National Center for Biotechnology Information (NCBI) using GAIN QA/QC (version 0.7.4) created by Goncalo Abecasis and Shyam Gopalakirshnana at the University of Michigan. GAIN QA/QC is available by emailing gopalakr@umich.edu or goncalo@umich.edu. Individuals were excluded due to gender discrepancy and if per-family Mendelian errors were in excess of 2%. SNPs were excluded if the: 1) call rate was < 95%; 2) heterozygosity was > 32%; 3) genotype call quality score was > 10%; or 4) HWE $p < 0.000001$.

SNPs within genes that encode for dopaminergic factors (i.e., COMT, DAT1, DRD2, DRD3, and DRD5), neurotrophic factors (i.e., BDNF, NGF, NT3, NGFR, NTRK2, and NTRK3) and angiogenic factors (VEGFA, VEGFR1, VEGFR2, NRP1, NRP2, HIF1A, and HIF1AN) that passed quality control measures were considered for inclusion in the GxE analysis. In addition, SNPs from NTRK1, a neurotrophic family receptor, were intended to be included in this analysis; however, NTRK1 SNPs were unavailable. Therefore, SNPs from CD1B, a nearby gene, were included instead. CD1B is a member of the CD1 family of transmembrane glycoproteins and is slightly upstream from NTRK1 on chromosome 1.

In addition, SNPs that encode for growth factors (FGF1, FGF2, IGF1, IGF1R, IGF2, IGF2R, and NLN) were considered for inclusion in exploratory analyses.

To increase coverage across candidate genes and to increase genotype overlap across NCGAP and IMAGE, genotype data were imputed with the use of the phased data from the HapMap samples (CEU; build 36, release 22) and MACH (http://www.sph.umich.edu/csg/abecasis/MaCH/download/; Li, Willer, Sanna, & Abecasis, 2009; Li, Willer, Ding, Scheet, & Abecasis, 2010).

A total of 1349 dopaminergic, neurotrophic and angiogenic and 918 growth factor SNPs were submitted for quality checks. To reduce the number of statistical tests conducted, remaining SNPs with a minor allele frequency (MAF) < 0.2 or in linkage disequilibrium (LD; $r^2 \geq .64$) were eliminated. If a SNP, however, was excluded due to LD and has demonstrated functional significance, as determined through F-SNP (http://compbio.cs.queensu.ca/F-SNP/; Lee & Shatkay, 2008), then it was retained. A total of six functional SNPs were retained (i.e., rs20541, rs4934838, rs2228638, rs7993418, rs6265, rs4633). After quality control and multiple testing reduction procedures were completed a total of 97 SNPs in dopaminergic, neurotrophic and angiogenic systems and 53 growth factor SNPS were retained for analysis.

Data Analysis

Bivariate correlations and Pearson product-moment correlation coefficients examined the direction, magnitude, and significance of the relationship between demographic, perinatal, and AD/HD variables. In addition, t-tests and ANOVAs were

conducted to test for differences between demographic groups on perinatal and AD/HD variables. Alpha was set at .01 for these analyses.

Generalized Estimating Equations (GEEs) were conducted to test for main effects of SNP genotype and birth weight centile range, and the interaction between SNP and birth weight centile range in predicting AD/HD symptom severity. GEEs extend generalized linear models to accommodate the analysis of clustered data (Hardin & Hilbe, 2003). Given that within family data are more correlated than between family data, GEEs account for the family correlation among siblings within the sample. For the present study, family was the subject variable and the individual was the within subject variable in the GEEs. An independent working correlation matrix was specified and the model-based robust estimator covariance matrix was selected, which provides a reliable covariance estimate even when the correlation matrix is not correctly specified. The CPRS AD/HD Total score was transformed to have normal skewness (described below) and served as the outcome variable for GEEs.

Three separate sets of linear GEEs were run. First, to examine main effects of SNPs on ADHD severity, the main effects of site, age, and sex were entered into the model as covariates. Next, the SNP main effect was entered into the model to test for effects of SNP genotype on AD/HD symptom severity.

Second, to test the hypothesis that fetal growth compromise is associated with increased AD/HD symptom severity, the main effects of site, age, and sex were first entered into the model as covariates. Next, the main effect of birth weight centile range

was entered into the model to examine the association between birth weight centile range and AD/HD symptom severity. No SNP main effect was included in this model.

Third, to test the hypothesis that fetal growth compromise moderates the relationship between SNP genotype and AD/HD symptom severity, the covariates of site, age, and sex were entered into the model. Next, the main effects of SNP genotype and birth weight centile range were entered into the model. Finally, the interaction of SNP and birth weight centile range was entered into the model.

Wald chi-square tests calculated with Type III sums of squares tested the significance of main and interactive effects. In addition, continuous variables were centered to ease the interpretation of the direction of model effects. For comparison purposes, the above models were also conducted without site, age, and sex covariates. No genetic model (e.g., additive, dominant or recessive) was assumed in this analysis.

In the GEEs, α was set at .05 for *nominally* significant findings. A total of 144 independent GEEs were calculated. The Benjamini-Hochberg False Discovery Rate (FDR; Benjamini & Hochberg, 1995) test was used to adjust for multiple comparisons. The FDR *q*-value threshold was set at .05 to determine statistical significance.

Given that birth size has a substantial heritability estimate, birth weight centile range was regressed onto growth factor SNPs to determine if birth weight centile range was correlated with SNPs within growth factor genes. Forward selection was used to enter SNPs into the stepwise regression model. SNPs were retained in the model if the contribution to R^2 was significant at the .05 level. The birth weight centile range residual from the final model was retained. The residualized birth weight centile range variable

then replaced the non-residualized variable in GEEs containing nominally significant and significant interactions to examine if SNPs associated with fetal growth could account for the observed SNP x birth weight centile range interactions. All analyses were conducted in SPSS version 19.

CHAPTER III

RESULTS

Data Preparation

To examine whether assumptions for GEEs were met, the predictor and response variables were inspected. The *T*-score of the CPRS AD/HD Total scale was non-normally distributed, with a skewness of -.81 (SE = .122) and kurtosis of 1.38 (SE = .24). Separate data transformations were applied to normalize the skewed distribution. The square root of the reflected *T*-score (e.g., $\sqrt{(K - X)}$, where K is a constant and equals the highest score + 1, resulted in the most normally distributed CPRS AD/HD Total score variable, skewness = -.03 (SE = .122), kurtosis = 1.30 (SE = 2.44) and was retained for analysis. Skewness for all other variables included in GEEs was in the normal range; thus, no other variables were transformed. The non-transformed CPRS AD/HD Total score was retained in some analyses for interpretative purposes. In such cases, results were synonymous with the same analysis using the transformed CPRS AD/HD Total score variable.

Demographic and AD/HD Variables

Older youth tended to have higher CPRS AD/HD Total (r = .21; p < .01), DSM-IV: Inattentive (r = .13; p < .01), and DSM-IV: Hyperactive-Impulsive scores (r = .25; p < .01). On average, CPRS AD/HD Total scores were higher for females (M = 81.23) than males (M = 76.82), $t(79.36) = 2.39$, p = .02, at a trend level. CPRS AD/HD Total scores

also varied across data collection sites, $F(5, 397) = 4.09$, $p < .01$. In general, IMAGE samples had higher CPRS AD/HD Total scores compared to the NCGAP samples (Duke, $M = 74.24$, SD = 13.96; UNCG, $M = 75.19$, SD = 14.49; Ireland, $M = 79.27$, SD = 9.26; Netherlands-Amsterdam, $M = 76.35$, SD = 8.22; Netherlands – Nijmegen, $M = 78.32$, SD = 7.82; UK, $M = 81.58$; SD = 8.33).

Demographic, Perinatal, and AD/HD Variables

Older participants tended to have lower birth weight centile range scores ($r = -.14$; $p < .01$). Birth weight was associated with increased birth weight centile range ($r = .755$; $p < .01$) and increased gestational age ($r = .50$; $p < .01$). In addition, maternal smoking during pregnancy was associated with paternal smoking during pregnancy ($r = .72$; $p < .01$) and increased maternal alcohol use during pregnancy ($r = .11$; $p = .03$) at a trend level. See Table 6 for additional correlations between selected demographic, perinatal variables and CPRS AD/HD Total score.

There were no differences between males and females in birth weight centile range $t(396) = .67$, $p = .50$, birth weight $t(396) = .-.707$, $p = .48$, and gestational age $t(396) = .72$, $p = .47$. Birth weight centile range scores, however, varied across data collection sites, $F(5, 397) = 8.34$, $p < .01$ (Duke, $M = 6.92$, SD = .28; UNCG, $M = 7.98$, SD = .34; Ireland, $M = 6.26$, SD = .24; Netherlands – Amsterdam, $M = 5.77$, SD = .22; Netherlands – Nijmegen, $M = 5.88$, SD = .26; UK, $M = 7.24$, SD = .39).

Generalized Estimating Equations (GEEs)

GEEs were used to examine the main effects of SNPs and birth weight centile range, and the interaction effects of SNP x birth weight centile range on AD/HD

symptom severity. Each analysis controlled for family correlation. In addition, although the primary analysis controlled for research site, age at assessment, and sex, separate analyses were also conducted that did not control for covariates for exploratory purposes. Generally, including age, sex and research site as covariates in GEEs did not have a substantial impact on the results. Therefore, only results from the primary analysis including research site, age and sex covariates are summarized below.

Main effects of SNPs on AD/HD symptom severity. Out of the 97 SNPs entered into the independent GEEs, after controlling for site, age, and sex, a total of 8 SNPs had a nominally significant main effect on AD/HD symptom severity (see Table 7). In the dopamine system, rs456774 (Wald = 6.67; $p < .05$) in DAT1 was associated with AD/HD Total Score. In the neurotrophic system, rs7127507 (Wald = 7.34; $p = .03$) and rs6265 (Wald = 6.82; $p = .03$) in BDNF, rs3825885 (Wald = 9.30; $p = .01$) and rs999905 (Wald = 6.67; $p = .04$) in NTRK3, and rs10780796 (Wald = 6.23; $p = .04$) in NTRK2 were associated with AD/HD Total score. In the angiogenic system, rs2104330 (Wald = 7.90; $p = .02$) in VEGFR1 and rs10016788 (Wald = 7.59; $p = .02$) in VEGFR2 were also associated with AD/HD Total score. See Table 8 for a summary of these comparisons. No SNP main effects remained significant after multiple testing corrections.

Main effect of birth weight centile range on AD/HD symptom severity. Contrary to hypotheses, birth weight centile range was not associated with the transformed AD/HD Total score in either the covariate ($b = -.023$, SE = .0198; $p = .243$) or no covariate models ($b = -.012$, SE = .020; $p = .547$).

Interactions between SNPs and birth weight centile range on AD/HD symptom severity. Out of the 97 SNP x birth weight centile range interactions tested, there were 8 nominally significant interactions in the covariate model (see Table 7). Birth weight centile range moderated associations between SNPs within angiogenic (i.e., NRP1, NRP2, and VEGFR1) and neurotrophic genes (i.e., NTRK2 and NTRK3) and AD/HD Total score. In the angiogenic system, birth weight centile range moderated associations between rs9513089 (VEGFR1, Wald = 6.59, p = .04), rs2065364 (NRP1, Wald = 8.05, p = .02), rs734187 (NRP1, Wald = 6.21, p < .05), rs17682318 (NRP2, Wald = 8.27, p = .02), rs10932118 (NRP2, Wald = 7.63, p = .02), and rs12611613 (NRP2, Wald = 6.53, p = .04) and AD/HD Total Score. In the neurotrophic system, birth weight centile range moderated associations between rs11141486 (NTRK2, Wald = 8.92, p = .01) and rs8031510 (NTRK2, Wald = 6.71, p = .04) and AD/HD Total score. Nominally significant interactions appear in Figures 2 and 3. Birth weight centile range did not moderate the relationship between SNPs within dopamine genes and the AD/HD Total score. No interactions remained significant after accounting for multiple testing.

Reexamining nominally significant interactions after statistically controlling for SNPs associated with fetal growth. Given that fetal growth has a substantial heritability estimate, the correlation between SNPs within candidate genes associated with fetal growth was removed from the birth weight centile range variable. Note that these SNPs are within genes that encode for factors associated with fetal growth (Baker, Liu, Robertson, & Efstratiadis, 1993; Hill, Petrik, & Arany, 1998) and are not within the angiogenic, neurotrophin, or dopaminergic systems.

Birth weight centile range was regressed onto 53 SNPs within candidate genes associated with fetal growth (i.e., FGF1, FGF2, IGF1, IGF1R, IGF2, IGF2R, and NLN) using a forward selection method. The final model, $F(3,306) = 6.23$, $p < .01$, included three SNPs within IGF2R (rs4709391) and FGF1 (rs4912870 and rs12523052), and accounted for a small proportion of birth weight centile range variance (adjusted R^2 = .048). Compared to the original birth weight centile range variable, the residualized birth weight centile range is presumably less heritable. Thus, the residualized birth weight centile range variable was included for these GEEs. Residualized birth weight centile range moderated the association between 6 of the 8 previously reported nominally significant interactions including the following SNPs (rs17682318, NRP2, Wald = 8.54, p = .014; rs10932118, NRP2, Wald = 8.48, p = .014; rs11141486, NTRK2, Wald = 7.15,p = .028; rs12611613, NRP2, Wald = 6.57, p = .037; rs734187, NRP1, Wald = 6.07, p = .048; rs9513089, NRP2, Wald = 6.03, p = .049). When using the residualized birth weight centile range variable, two interactions were no longer nominally significant (rs8031510, NTRK3, Wald = 5.41, p = .067; rs2065364, NRP1, Wald = 4.97, p = .083).

Exploratory analyses: Interactions between less prevalent SNPs and birth weight centile range on AD/HD symptom severity. Given that the above analyses focused on SNPs with more common minor allele frequencies, many SNPs within the candidate systems of interest were not examined. Therefore, to expand coverage across candidate systems of interest the minor allele frequency criterion was reduced from .20 to .10 for exploratory analyses. Other inclusion and exclusion criteria remained the same (see page 44) and were reapplied to develop a new list of unique SNPs within candidate

systems of interest (i.e., angiogenic, dopaminergic, and neurotrophic pathways). An additional 47 SNPs were identified for this analysis. Out of the 47 additional SNPs examined, 7 SNPs had nominally significant main effects on the AD/HD Total score. Nominally significant SNPs were within angiogenic, dopamine, and neurotrophic genes. In the angiogenic pathway, variants within NRP1 (rs2776930, Wald = 25.76, p <.01; rs2474712, Wald = 7.59, p = .02) were associated with AD/HD Total score. In the dopaminergic pathway, SNPs within COMT (rs9332377, Wald = 99.87, p < .05), DRD2 (rs4350392, Wald = 7.96, p = .02), and DRD3 (rs324035, Wald = 13.79, p < .01) were associated with AD/HD Total score. Lastly in the neurotrophic pathway, two SNPs within NTRK3 (rs8037291, Wald = 7.92, p = .02; rs7176444, Wald = 6.28, p = .04) were associated with AD/HD Total score. See Table 8 for a summary of these comparisons.

In addition, birth weight centile range moderated the association between 12 SNPs and the AD/HD Total score. Nominally significant interactions included SNPs within angiogenic (HIF1A and NRP1) and neurotrophic genes (NTRK3) and CD1B. Furthermore, six SNP x birth weight centile range interactions survived multiple testing corrections. In the angiogenic pathway, birth weight centile range moderated the relationship between SNPs in HIF1A (rs2057482, Wald = 113.70, q < .05; rs2301106, Wald = 124.39, q < .05) and NRP1 (rs11598845, Wald = 29.47, q < .05) and AD/HD Total score. In the neurotrophic pathway, birth weight centile range moderated the relationship between SNPS within NTRK3 (rs71764444, Wald = 45.21, q < .05; rs8037291, Wald = 16.26, q < .05) and AD/HD Total score. Finally, birth weight centile range moderated the association between a SNP in CDIB (rs962879, Wald = 25.53, q <

.05) and AD/HD Total score. Generally, compared to other genotypes, homozygotes for the minor allele had increased AD/HD Total scores as birth weight centile range decreased. A summary of results from these analyses is presented in Table 9. Interactions with *q*-values < .05 appear in Figure 4.

Reexamining significant interactions after statistically controlling for SNPs associated with fetal growth. A residualized birth weight centile range variable was created to account for the relationship between SNPs within fetal growth candidate genes and fetal growth. Birth weight centile range was regressed onto 73 SNPs within FGF1, FGF2, IGF1, IGF1R, IGF2, IGF2R, and NLN using step-wise forward selection. These SNPs are not within angiogenic, neurotrophin, or dopaminergic systems, but rather are associated with fetal growth. SNPs associated with fetal growth were identified using the same criteria as before; however, the minor allele frequency criterion was reduced to .10. Only rs11111272 in IGF1 entered into the model, $F(3,306) = 6.23$, $p < .01$, and the adjusted $R^2 = .011$. To reinvestigate statistically significant interactions, the residualized birth weight centile range variable replaced birth weight centile range in the GEEs. All interactions remained significant ($p < .001$). This may suggest that SNPs within fetal growth candidate cannot account for the observed interactions between SNPs within angiogenic and neurotrophic genes and AD/HD Total score.

CHAPTER IV

DISCUSSION

Behavioral genetic studies demonstrate that genetic factors are paramount in the etiology of AD/HD (Faraone et al., 2005); however, molecular genetic studies have uncovered only a small proportion of AD/HD's heritability estimate (Nigg, 2006). GxE may be a main contributor to AD/HD's hidden heritability, yet there has been a relative lack of AD/HD GxE research. To date, AD/HD GxE research has focused on genes that have demonstrated associations with AD/HD and encode for factors associated with neurotransmission, especially dopamine. This approach has produced mixed findings (Nigg et al., 2010) and studies rarely provide a rationale for why a given environmental risk factor would moderate the association between a selected candidate gene and AD/HD.

Out of many environmental risk factors associated with AD/HD, birth weight centile, or fetal growth compromise is well-suited for inclusion in GxE research as it: 1) has a well-established association with AD/HD (Nigg et al., 2010) which cannot be accounted for by genetic factors (Lehn et al., 2007; Sharp et al., 2003); 2) is associated with neurodevelopmental risk for AD/HD (Brown et al., 2009; Peterson et al., 2003; Tolsa et al., 2004); 3) is a measure of cumulative prenatal environment risk (Kramer, 1987) which restricts nutrient supply in utero; and 4) can be reliably measured (Tomeo et al., 1999). Although the association between fetal growth compromise and AD/HD is

well-established, little is known about how fetal growth compromise moderates the relationship between genetic risk and AD/HD.

GWAS in AD/HD have helped to broaden our understanding of AD/HD's genetic origins. Findings suggest that genes implicated in basic neurodevelopmental processes (Franke et al., 2009; Poelmans et al., 2011) are associated with vulnerability for the disorder. Angiogenic, dopaminergic, and neurotrophic systems have all been implicated in the pathophysiology of AD/HD and are regulated by a restricted nutrient supply in utero. From a DOHaD framework (Gluckman & Hanson, 2004), angiogenic, dopaminergic and neurotrophic factor response to a restricted nutrient supply in utero may modify vulnerability for AD/HD. Therefore, this study examined whether fetal growth compromise moderated associations between SNPs within angiogenic, dopaminergic, and neurotrophic genes and AD/HD symptom severity.

Hypotheses

Contrary to the first hypothesis, fetal growth compromise was not associated with increased AD/HD symptom severity. In general, the literature demonstrates that fetal growth compromise is associated with increased AD/HD symptom severity; however, this finding may vary with sample composition. Research with community samples that exhibit the entire spectrum of AD/HD symptom severity have shown that LBW, birth weight adjusted for gestational age, and ponderal index are associated with increased risk for AD/HD (Bhutta, Cleves, Casey, Cradock, & Anand, 2002; Indredavik et al., 2004; Lahti et al., 2006). In contrast, AD/HD clinical samples have not demonstrated a relationship between fetal growth compromise and AD/HD symptom severity (e.g.,

Langley et al., 2007). The reduced variability in AD/HD symptom severity in case-only or family-based designs may make the relationship between fetal growth and AD/HD difficult to detect. In the absence of a main effect of fetal growth compromise on AD/HD, it is still appropriate to examine for interactions as fetal growth compromise may moderate the relationship between genotype and AD/HD.

In partial support of the second hypothesis, fetal growth compromise moderated the relationship between SNPs within angiogenic and neurotrophic genes, but not dopamine genes, and AD/HD symptom severity. A total of six separate SNP x birth weight centile range interactions were associated with AD/HD. In the observed interactions, homozygosity for the minor allele tended to be associated with higher levels of AD/HD symptom severity, especially at low birth weight centile range scores. All significant interactions were observed for SNPs with minor allele frequencies below .20, suggesting that less common variants in angiogenic and neurotrophic genes may play a role in the etiology of AD/HD.

These findings suggest that angiogenic and neurotrophic factor response to a restricted nutrient supply in utero may be associated with vulnerability for AD/HD. These findings are consistent with research that demonstrates a restricted nutrient supply in utero moderates the expression of genes within angiogenic (Jaakkola et al., 2001; Mac Gabhann & Popel, 2008) and neurotrophic systems (Cannon et al., 2008; Schmidt-Kastner et al., 2001). Together these findings suggest that ischemia/hypoxia regulated variants in angiogenic and neurotrophic pathways interact with a restricted nutrient supply in utero to confer risk for AD/HD. Interestingly, fetal growth compromise did not

moderate the association between SNPs within dopamine genes and AD/HD symptom severity. Although ischemia/hypoxia alters dopaminergic transmission, this change seems to relate to factors that are more directly involved with adaptation to hypoxia, such as HIF1 (Johansen et al., 2010).

In the angiogenic pathway, fetal growth compromise moderates relationships between SNPs within HIF1A and NRP1 and AD/HD, which to date has not been reported in the literature. HIF1A is located on chromosome 14q23.2 and encodes for the alpha subunit of hypoxia-inducible factor 1 (HIF1α). HIF1α is necessary for normal embryonic (Yu et al., 1999), vascular and neural development (Tomita et al., 2003). HIF1α is a primary regulator of the cellular response to hypoxia (Sharp & Bernaudin, 2004) and accumulates in cells during hypoxic conditions (Chávez, Agani, Pichiule, & Lamanna, 2000). HIF1α typically helps organisms adapt to hypoxic conditions by regulating the transcription of a broad range of target genes (Sharp & Bernaudin, 2004) which confer neurological and vascular adaptation (Schmidt-Kastner et al., 2006; Sharp & Bernaudin, 2004). In fact, the two HIF1A SNPs (rs2057482 and rs2301106) which interacted with fetal growth compromise to predict AD/HD symptom severity may be related to HIF1A transcriptional regulation (see Table 10).

Although HIF1α mediates neural and vascular adaptation during prenatal development, increasing cell survival in the moment may lead to increased risk for neurodevelopmental and neurodegenerative disorders in later development (Schmidt-Kastner et al., 2006; Sharp & Bernaudin, 2004). For example, HIF1α confers hypoxic induced protection against future hypoxia, which relates to DNA damage and repair in

cells exposed to hypoxia (Englander, Greeley, Wang, Perez-Polo, & Lee, 1999). From a DOHaD perspective, HIF1α increases cell survival when exposed to prenatal hypoxia; however, the resulting DNA repair response may leave the organism ill prepared to function effectively in postnatal environments with normal oxygen concentration.

Given that hypoxia moderates the expression of HIF1α, and birth weight and hypoxia covary (Apel-Sarid, Levy, Holcberg, & Sheiner, 2010), prenatal hypoxia may be moderating the relationship between HIF1A and AD/HD in nature. Prenatal hypoxia has been shown to be associated with AD/HD (Ben Amor et al., 2005; Pineda et al., 2007); however, it has received little attention in the AD/HD literature.

NRP1 is located on chromosome 10p12 and encodes for a neuropilin-1, a receptor for VEGF-A (Shibuya, 2008) and semaphorin-3A (He & Tessier-Lavigne, 1997). Neuropililin-1 is expressed in the central nervous system, endothelial (Gu et al., 2003) and tumor cells (Chen et al., 2005) and plays an essential role in vascular development and axonal guidance (Gu et al., 2003; Polleux, Morrow, & Ghosh, 2000). NRP1 plays a central role coordinating neuronal migration and guidance of axons that project from the thalamus to the cortex (López-Bendito et al., 2006). Interestingly, these neural pathways are implicated in the pathophysiology of AD/HD (Sagvolden et al., 2005). Previous AD/HD genetic research has also implicated genes involved in neuronal migration in the etiology of the disorder (Franke et al., 2009). Similar to HIF1α, NRP1 is upregulated during prenatal ischemia which in turn disrupts axonal guidance near the ischemic area (Hou et al., 2008). Together, this may suggest that disrupted neuronal migration may be

one route to AD/HD in those exposed to prenatal ischemia/hypoxia and who exhibit vulnerable NRP1 genotypes.

In the neurotrophic pathway, fetal growth compromise moderated the relationship between SNPs within NTRK3 and AD/HD symptom severity. NTRK3 encodes for TrkC, a tropomyosin-related kinase receptor. TrkC is expressed throughout the brain, and is most abundant in the hippocampus (Ernfors, Merlio, & Persson, 1992). Neurotrophin-3 (NT-3) binds to TrkC and TrkA (Lamballe, Klein, & Barbacid, 1991), which promotes neuron survival and synaptic plasticity (Reichardt, 2006). NTRK3 has not been associated with AD/HD by molecular genetic studies; however, NT3 has been associated with AD/HD (Ribasés et al., 2008). Ischemia upregulates TrkC receptors in neurons and microglia (Lin et al., 2006). In addition, TrkC receptors are reduced in the dorsolateral prefrontal cortex of individuals with schizophrenia (Weickert et al., 2005). The dorsolateral prefrontal cortex is also implicated in the pathophysiology of AD/HD (Sagvolden et al., 2005). Together these findings may suggest that when exposed to prenatal ischemia/hypoxia, individuals with vulnerable NTRK3 variants may experience impaired functioning in the hippocampus and dorsolateral prefrontal cortex which increases AD/HD vulnerability.

Fetal growth compromise was not predicted to moderate the association between CD1B SNPs (i.e., rs962879) and AD/HD symptom severity. There is no existing literature on the relationship between CD1B and fetal growth compromise or AD/HD; thus, it is difficult to explain why CD1B may be functionally related to AD/HD. This relationship may be due to rs962879 being in LD with a functional variant in another

gene. As mentioned previously NTRK1 is also located on chromosome 1; however, it is unlikely to account for this relationship as NTRK1 is approximately 1.45 mega base pairs upstream from CD1B. Thus, other nearby variants may be playing a role. Alternatively this unhypothesized result may be a false positive.

With the exception of CD1B, genes involved in the significant interactions are regulated, in part, by ischemia/hypoxia. This may suggest that prenatal ischemia/hypoxia is an environmental pathogen underlying the relationship between fetal growth compromise and AD/HD. If true, the observed SNP x fetal growth compromise interactions are likely mediated by epigenetic processes, such as transcriptional regulation, which alter the expression of ischemia/hypoxia regulated angiogenic and neurotrophic genes. Individual variability in the expression of angiogenic and neurotrophic genes may then be associated with vulnerability for AD/HD (see Figure 5). From a DOHaD perspective, in response to prenatal ischemia/hypoxia, individual variability in angiogenic or neurotrophic gene expression may confer vulnerability for AD/HD in multiple ways. First, insufficient angiogenic or neurotrophic factor response to prenatal ischemia/hypoxia may lead to disrupted cerebral vascular or neural development and increase vulnerability for AD/HD. Second, if angiogenic and neurotrophic factor response to prenatal ischemia/hypoxia is adequate to promote neural and vascular endothelial cell survival, increased risk for future neurodevelopmental problems may arise from the surviving cells repair response to DNA damage (Sharp & Bernaudin, 2004). In addition, early cerebral vascular adaptation to prenatal ischemia/hypoxia may constrain an individual's ability to increase cerebral blood flow during demanding

environmental conditions (Fu & Olofsson, 2006). Therefore, epigenetic processes that upregulate angiogenic and neurotrophic factors in response to prenatal ischemia/hypoxia may not always be associated with decreased vulnerability for AD/HD. Taken together, angiogenic and neurotrophic factor response to prenatal ischemia/hypoxia may confer vulnerability for AD/HD through developmental disruption, predicted adaptive responses, or constrained developmental plasticity.

This model helps to link previous AD/HD molecular genetic (Franke et al., 2009; Poelmans et al., 2011) and environmental research (Banerjee et al., 2007) which suggest that multiple neurodevelopmental factors are implicated in the pathophysiology of the disorder. For example, this study found that fetal growth compromise moderates the association between NRP1 (which is implicated in neuronal migration) and AD/HD. This fits with Poelmans et al. (2011) who found that disrupted axon guidance may be a common neurodevelopmental pathway to AD/HD. This suggests that to elucidate neurodevelopmental pathway to AD/HD, research should examine the interplay between environmental factors and genes that are: 1) functionally related; and 2) associated with more basic neurodevelopmental processes.

Although hypodopaminergic functioning in prefrontal and limbic brain regions may be associated with many AD/HD cases (Levy, 1991), this study did not provide evidence that fetal growth compromise moderated the relationship between dopaminergic factors and AD/HD. This suggests that although hypodopaminergic functioning may be a primary contributor to the pathophysiology of ADHD it is not necessarily the root cause of ADHD, for all individuals. Instead, these findings suggest that hypodopaminergic

functioning: 1) may result from more basic neurodevelopmental delays or disruptions (e.g., disrupted axonal guidance); or 2) is not necessarily implicated in AD/HD's pathophysiology, especially for individuals who develop AD/HD after experiencing prenatal ischemia/hypoxia. Taken together, these findings suggest that multiple developmental pathways to ADHD exist, which originate from the interplay between a broad range of genetic and environmental factors. This interplay confers vulnerability for ADHD by altering basic neurodevelopmental processes which may or may not give way to hypodopaminergic functioning.

Limitations

The findings of this study are promising but need to be considered in light of study limitations. First, youth in the study were either diagnosed with AD/HD or at genetic risk for AD/HD. This resulted in constrained variability in the primary outcome variable, AD/HD symptom severity. Constrained variability in outcome measures reduces statistical power and the likelihood of significant findings. In addition, the average AD/HD symptom severity score was approximately 2.5 standard deviations above the population mean. Although etiological factors would be expected to exert similar influence on AD/HD throughout severity levels (Levy et al., 1997), the main and interactive effects in this study were observed in individuals with high levels of AD/HD symptoms. Therefore, it is unclear how findings would generalize to individuals with low to moderate levels of AD/HD symptom severity.

On a related note, given that AD/HD symptoms vary across context, a multi-informant approach to measuring AD/HD symptom severity is generally preferred, but

was not utilized in this study. Given that ADHD symptoms vary across context (Anastopoulos & Shelton, 2001), it is important to get multiple raters of youth's behavior.AD/HD symptom severity was measured using a parent-report rating scale for consistency across sites and to maximize sample size. In addition, inattention and hyperactive-impulsive symptoms were combined as a measure of overall AD/HD symptom severity, to reduce multiple testing. Although inattentive and hyperactive-impulsive symptom dimensions share similar etiologies (McLoughlin, Ronald, Kuntsi, Asherson, & Plomin, 2007), each symptom dimension also has unique genetic and environmental risk factors (Nikolas & Burt, 2010; Smith, 2010).

Birth weight centile range served as a proxy measure for nutrient supply in utero as no direct measure of nutrient supply in utero was available. Therefore, inferences about the underlying environmental pathogen were made in this study. Furthermore, birth weight has a substantial heritability estimate and it is not completely clear if identified interactions reflect gene-environment interplay and/or epistasis. To limit the probability of epistasis, SNPs within candidate fetal growth genes were controlled for in the analyses; however, other genetic factors may have played a role.

Next, this analysis combined participants from NCGAP and IMAGE. NCGAP and IMAGE had different approaches to ascertainment, genotyping, and assessment of fetal growth. These differences likely contributed to the many demographic, prenatal and phenotypic differences between the samples. In addition, the inclusion of the IMAGE Dutch sample required the use of birth weight centile range scores. This in turn complicated the interpretation of main and interaction effects involving birth weight

centile range. Research site was included as a covariate in the analyses to reduce the impact of site differences on the observed findings. Despite complications related to combining samples, this analysis was able to provide evidence that fetal growth compromise moderates associations between candidate system SNPs and AD/HD. Such findings may have been obscured in a smaller, more homogeneous sample.

When conducting inferential statistical tests there is always a possibility of false positive findings. This study conducted 144 separate GxE interactions and utilized FDR to correct for multiple testing. FDR was chosen to preserve power; however, this approach is less conservative than other multiple testing corrections. Furthermore, many steps were taken to reduce the number of statistical tests conducted, including removing SNPs that were in LD ($r^2 \geq .64$). Although LD SNP exclusion reduced the number of statistical tests conducted, this may have resulted in excluding SNPs that interacted with fetal growth compromise to modify vulnerability for AD/HD.

Due to imputation procedures, samples in psychiatric genetic studies are often racially homogenous. Pooling together samples from multiple sites, including North Carolina, Ireland, the Netherlands, and United Kingdom, allowed for European ancestral diversity within the sample. Due to genotyping procedures, however, this sample was 100% Caucasian. This limits the generalizability of findings to other racial groups. Given that Caucasian race and access to prenatal care is a protective factor for fetal growth (Kramer, 1987), it is unclear how these findings would generalize to individuals of different races or individuals who do not have access to prenatal medical care.

Lastly, the design of this study was unable to rule-out the effect of maternal genotype on the observed SNP x birth weight centile range interactions (see Waldman, 2007). In light of the role of maternal angiogenic genotype in prenatal ischemia/hypoxia (e.g., Nakamura, Okamoto, Nagaya, & Hayashi, 2011), future research should include both maternal and child genotype in etiological models of AD/HD.

Clinical and Public Health Implications

Bearing these limitations in mind, the results of this study have implications for AD/HD assessment and intervention. In terms of assessment, this study supports the notion that AD/HD reflects a continuous trait and that vulnerability for AD/HD is best conceptualized on a spectrum (Levy et al., 1997). For etiological and prognostic considerations (e.g., Molina & Pelham, 2003). 2003), it is important to not only assesses the presence of AD/HD as a category, but also assess the severity of AD/HD symptomatology. Consistent with this notion, Lahey and Willcutt (2010) suggest that DSM-V move towards using continuous symptom counts as diagnostic modifiers for AD/HD. For example, compared to AD/HD subtypes, symptom counts are better predictors of future functional impairment (Lahey & Willcutt, 2010).

In terms of intervention, LBW is a well-established environmental risk factor for AD/HD (Nigg et al., 2010). Therefore, it has been argued that reducing the incidence of LBW would lead to a modest reduction in the prevalence of AD/HD (Mick et al., 2002; Nigg, 2006). Unfortunately, reducing the incidence of LBW has proven difficult in both developing and developed nations (United Nations Children's Fund, 2004). Another approach to decreasing AD/HD risk for individuals who experience fetal growth

compromise is to identify malleable epigenetic and environmental factors that mediate the relationship between fetal growth compromise and AD/HD. Findings from this study may suggest that prenatal ischemia/hypoxia modifies the expression of angiogenic and neurotrophic genes, which in turn influences vulnerability for AD/HD. If true, targeting epigenetic mechanisms that are regulated by prenatal ischemia/hypoxia would provide an opportunity to improve neurodevelopment and decrease AD/HD risk in the face of prenatal ischemia/hypoxia. For example, interventions that increase the availability of HIF1 may help to protect individuals from prenatal ischemia/hypoxia (Bergeron et al., 2000) and reduce risk for AD/HD and other neurodevelopmental disorders. Other research suggests that postnatal factors such as maternal warmth may also help to protect LBW youth from AD/HD (Tully, Arseneault, Caspi, Moffitt, & Morgan, 2004).

Research Implications

This study provides support for candidate pathway approaches to genetic research, especially for studies with modest sample sizes. Candidate pathway GxE interaction studies are theoretically driven, and balance the importance of examining multiple genes that are related to the environmental risk factor and limiting the number of statistical tests. Future GxE studies may look to utilize the candidate pathway approach to manage these competing demands.

In addition, this study identified multiple genetic variants that are associated with AD/HD and many which have not been previously reported. This suggests that in order to elucidate AD/HD vulnerability genes, it is important to investigate GxE, as some genetic variants may only increase risk for the disorder in the presence of an environmental

factor. Including environmental factors in AD/HD genetic research may help to uncover more of AD/HD's heritability estimate. Furthermore, although dopaminergic genes have shown to be reliably associated with AD/HD (Gizer et al., 2009), fetal growth compromise only moderated associations between SNPs within angiogenic and neurotrophic genes and AD/HD. This suggests that the selection of candidate genes for GxE studies should be based on their relationship with the environmental pathogen (Moffitt et al., 2006) rather than the disorder of interest. This is one explanation for the varied findings in the AD/HD GxE literature, as candidate gene selection seems to be based on a genes association with AD/HD.

Future Directions

It is important to replicate the promising findings from this study to further the search for malleable mechanisms linking birth weight with AD/HD. In particular, replication studies may include racially diverse and population-based samples. The majority of replication efforts in GxE studies in psychiatry fail (Duncan & Keller, 2011); however, the odds of positive replication may be greater for this study compared to the extant literature. For example, following recommendations from Moffitt and colleagues (2006), this study started with a well-established environmental risk factor for AD/HD (Nigg et al., 2010). Additionally, candidate gene pathways were selected based on their association with a restricted nutrient supply in utero, the DOHaD hypothesis, and relevance to AD/HD. This is in contrast to many other GxE studies that provide little rationale for predicting the presence of a GxE. In addition, following recommendations from Waldman (2007), attention was paid to the heritability estimate of birth weight.

Variants within birth weight candidate genes were statistically controlled for to reduce the probability that epistatic interactions accounted for the observed GxE findings. Finally, multiple ischmia/hypoxia regulated genes were found to interact with fetal growth compromise to predict AD/HD. This evidence suggests that fetal growth compromise moderates ischemia/hypoxia regulated genes in angiogenic and neurotrophic pathways to predict AD/HD.

If replicated, future research should examine the underpinnings of the observed GxE findings. For example, studies may examine the role of epigenetic mechanisms underlying the observed SNP x birth weight centile range interactions predicting AD/HD. Twin studies may investigate the differences in DNA methylation of angiogenic and neurotropic pathway genes in monozygotic twins who are discordant for AD/HD. Such studies would follow up on previous MZ discordant twin studies in AD/HD (Hultman et al., 2007; Lehn et al., 2007; Pearsall-Jones et al., 2008; Sharp et al., 2003) to examine epigenetic mechanisms linking fetal growth compromise to AD/HD.

Fetal growth compromise, angiogenic genes, and neurotrophic genes are associated with a wide range of psychiatric disorders. Given that the gene and environmental risk factors in this study are not specific to AD/HD, findings may generalize to other neurodevelopmental disorders. For example, identifying how the observed GxE interactions relate to neuroanatomical or neurofunctional outcomes may help to elucidate neurodevelopmental pathways to neurodevelopmental disorders, more generally. Furthermore, multiple genes are regulated by ischmia/hypoxia and a more comprehensive analysis may identify other candidate genes or pathways that interact with

fetal growth compromise to predict AD/HD. For example, Oades (2011) demonstrated that prenatal risk factors were associated with alterations in kynurenine and cytokine metabolism in youth with AD/HD. An expanded analysis of this study also suggests that fetal growth compromise moderates associations between kynurenine variants and AD/HD symptom severity (Smith et al., In preparation). It is also important to identify the true environmental pathogen(s) that moderates the relationship between angiogenic and neurotrophic variants and AD/HD. Animal studies may help to disentangle the effects of prenatal and postnatal environmental factors on neurodevelopment and AD/HD symptomatology. In addition, human studies may look to further refine the measurement of prenatal ischemia/hypoxia by taking direct measurements during fetal development or by taking a latent variable approach using pregnancy, delivery and birth outcome indicators.

Conclusion

The current study examined the role of fetal growth compromise in moderating relationships between SNPs within angiogenic, neurotrophic, and dopamine genes and AD/HD symptom severity. In an extension of previous research, this study suggests that: 1) angiogenic genes play a role in the etiology of AD/HD; and 2) fetal growth compromise moderates associations between SNPs within angiogenic and neurotrophic genes and AD/HD symptom severity. This suggests that angiogenic and neurotrophic factor response to a restricted nutrient supply in utero modifies vulnerability for AD/HD. This study also suggests that the DOHaD hypothesis (Gluckman & Hanson, 2004) is a useful framework to conceptualize how a restricted nutrient supply in utero confers risk

for AD/HD. If replicated, these findings may help to guide the search for epigenetic mechanisms which modify vulnerability for AD/HD and can be targeted by interventions.

REFERENCES

Altink, M. E., Arias-Vasquez, A., Franke, B., Slaats-Willemse, D. I., Buschgens, C. J., Rommelse, N. N., … Buitelaar, J. K. (2008). The dopamine receptor D4 7-repeat allele and prenatal smoking in ADHD-affected children and their unaffected siblings: No gene-environment interaction. *Journal of Child Psychology and Psychiatry, 49*(10), 1053-1060.

American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders:DSM-IV-TR* (4th ed.). Washington, DC: American Psychiatric Association.

Anastopoulos, A. D., & Shelton, T. L. (2001). *Assessing attention-deficit/hyperactivity disorder*. New York, NY: Kluwer Academic/Plenum Publishers.

Apel-Sarid, L., Levy, A., Holcberg, G., & Sheiner, E. (2010). Term and preterm (< 34 and< 37 weeks gestation) placental pathologies associated with fetal growth restriction. *Archives of Gynecology and Obstetrics, 282*(5), 487-492.

Baker, J., Liu, J. P., Robertson, E. J., & Efstratiadis, A. (1993). Role of insulin-like growth factors in embryonic and postnatal growth. *Cell, 75*(1), 73-82.

Banerjee, T. D., Middleton, F., & Faraone, S. V. (2007). Environmental risk factors for attention-deficit hyperactivity disorder. *Acta Paediatrica, 96*(9), 1269-1274.

Barkley, R. (2006). *Attention-deficit hyperactivity disorder: A handbook for diagnosis and treatment* (3rd ed.). New York, NY: Guilford Press.Barkley, R. A. (1997).

Behavioral inhibition, sustained attention, and executive functions: constructing a unifying theory of ADHD. *Psychological Bulletin, 121*(1), 65-94.

Barkley, R. A., Fischer, M., Edelbrock, C. S., & Smallish, L. (1990). The adolescent outcome of hyperactive children diagnosed by research criteria: An 8-year prospective follow-up study. *Journal of the American Academy of Child and Adolescent Psychiatry*, *29*(4), 546-557.

Becker, K., El-Faddagh, M., Schmidt, M., Esser, G., & Laucht, M. (2008). Interaction of dopamine transporter genotype with prenatal smoke exposure on ADHD symptoms. *The Journal of Pediatrics, 152*(2), 263-269.

Ben Amor, L., Grizenko, N., Schwartz, G., Lageix, P., Baron, C., Ter-Stepanian, M., … Joober, R. (2005). Perinatal complications in children with attention-deficit hyperactivity disorder and their unaffected siblings. *Journal of Psychiatry and Neuroscience*, *30*(2), 120-126.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological), 57*(1), 289-300.

Bergeron, M., Gidday, J. M., Yu, A. Y., Semenza, G. L., Ferriero, D. M., & Sharp, F. R. (2000). Role of hypoxia-inducible factor-1 in hypoxia-induced ischemic tolerance in neonatal rat brain. *Annals of Neurology*, *48*(3), 285-296.

Bhutta, A. T., Cleves, M. A., Casey, P. H., Cradock, M. M., & Anand, K. J. (2002). Cognitive and behavioral outcomes of school-aged children who were born

preterm: a meta-analysis. *Journal of the American Medical Association, 288*(6), 728-737.

Biederman, J., & Faraone, S. V. (2002). Current concepts on the neurobiology of Attention-Deficit/Hyperactivity Disorder. *Journal of Attention Disorders, 6* (1), S7-16.

Biederman, J., Faraone, S. V., Mick, E., & Spencer, T. (1995). High risk for attention deficit hyperactivity disorder among children of parents with childhood onset of the disorder: A pilot study. *The American Journal of Psychiatry, 152*(3), 431-435.

Blumberg, H., Wang, F., Chepenik, L., Kalmar, J., Edmiston, E., Duman, R., & Gelernter, J. (2008). Influence of vascular endothelial growth factor variation on human hippocampus morphology. *Biological Psychiatry, 64*(10), 901-903.

Botting, N., Powls, A., Cooke, R., & Marlow, N. (1997). Attention deficit hyperactivity disorders and other psychiatric outcomes in very low birthweight children at 12 years. *Journal of Child Psychology and Psychiatry, 38*(8), 931-942.

Boulet, S. L., Schieve, L. A., & Boyle, C. A. (2009). Birth weight and health and developmental outcomes in US children, 1997-2005. *Maternal Child Health Journal, 15*(7), 836-844.

Breslau, N., Brown, G., DelDotto, J., Kumar, S., Ezhuthachan, S., Andreski, P., & Hufnagle, K. (1996). Psychiatric sequelae of low birth weight at 6 years of age. *Journal of Abnormal Child Psychology, 24*(3), 385-400.

Brookes, K., Xu, X., Chen, W., Zhou, K., Neale, B., Lowe, N., Aneey, R., … Ebstein, R. (2006a). The analysis of 51 genes in DSM-IV combined type attention deficit

hyperactivity disorder: association signals in DRD4, DAT1 and 16 other genes. *Molecular Psychiatry, 11*(10), 934-953.

Brookes, K. J., Mill, J., Guindalini, C., Curran, S., Xu, X., Knight, J., … Asherson, P. (2006b). A common haplotype of the dopamine transporter gene associated with attention-deficit/hyperactivity disorder and interacting with maternal use of alcohol during pregnancy. *Archives of General Psychiatry, 63*(1), 74-81.

Brookes, K. J., Neale, B., Xu, X., Thapar, A., Gill, M., Langley, K., …Asherson, P. (2008). Differential dopamine receptor D4 allele association with ADHD dependent of proband season of birth. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 147B*(1), 94-99.

Brown, N., Inder, T., Bear, M., Hunt, R., Anderson, P., & Doyle, L. (2009). Neurobehavior at term and white and gray matter abnormalities in very preterm infants. *The Journal of Pediatrics, 155*(1), 32-38.

Burke, J. D., Loeber, R., & Lahey, B. B. (2001). Which aspects of ADHD are associated with tobacco use in early adolescence? *Journal of Child Psychology and Psychiatry, 42*(4), 493-502.

Cannon, T., Yolken, R., Buka, S., & Torrey, E. (2008). Decreased neurotrophic response to birth hypoxia in the etiology of schizophrenia. *Biological Psychiatry, 64*(9), 797-802.

Castellanos, F., Sharp, W., Gottesman, R., Greenstein, D., Giedd, J., & Rapoport, J. (2003). Anatomic brain abnormalities in monozygotic twins discordant for

attention deficit hyperactivity disorder. *American Journal of Psychiatry, 160*(9), 1693-1694.

Castellanos, F. X., Lee, P. P., Sharp, W., Jeffries, N. O., Greenstein, D. K., Clasen, L. S., … Rapoport, J. L. (2002). Developmental trajectories of brain volume abnormalities in children and adolescents with attention-deficit/hyperactivity disorder. *Journal of the American Medical Association, 288*(14), 1740-1748.

Chávez, J. C., Agani, F., Pichiule, P., & LaManna, J. C. (2000). Expression of hypoxia-inducible factor-1α in the brain of rats during chronic hypoxia. *Journal of Applied Physiology, 89*(5), 1937-1942.

Chen, C., Li, M., Chai, H., Yang, H., Fisher, W. E., & Yao, Q. (2005). Roles of neuropilins in neuronal development, angiogenesis, and cancers. *World Journal of Surgery, 29*(3), 271-275.

Cole, T. J., Williams, A. F., & Wright, C. M. (2011). Revised birth centiles for weight, length and head circumference in the UK-WHO growth charts. *Annals of Human Biology, 38*(1), 7-11.

Conners, C. K. (1997). Conners rating scales (revised), technical manual. *Toronto: MHS*.

Conners, C. K., Sitarenios, G., Parker, J. D. A., & Epstein, J. N. (1998). The revised Conners' parent rating scale (CPRS-R): Factor structure, reliability, and criterion validity. *Journal of Abnormal Child Psychology, 26*(4), 257-268.

Decker, M. J., Jones, K. A., Solomon, I. G., Keating, G. L., & Rye, D. B. (2005). Reduced extracellular dopamine and increased responsiveness to novelty:

Neurochemical and behavioral sequelae of intermittent hypoxia. *Sleep, 28*(2), 169-176.

Derauf, C., Katz, A. R., & Easa, D. (2003). Agreement between maternal self-reported ethanol intake and tobacco use during pregnancy and meconium assays for fatty acid ethyl esters and cotinine. *American Journal of Epidemiology, 158*(7), 705-709.

Derks, E. M., Hudziak, J. J., Dolan, C. V., van Beijsterveldt, T. C., Verhulst, F. C., & Boomsma, D. I. (2008). Genetic and environmental influences on the relation between attention problems and attention deficit hyperactivity disorder. *Behavior Genetics, 38*(1), 11-23.

Duncan, L. E., & Keller, M. C. (2011). A critical review of the first 10 years of candidate gene-by-environment interaction research in psychiatry. *American Journal of Psychiatry, 168*(10), 1041-1049.

DuPaul, G., Power, T., Anastopoulos, A., & Reid, R. (1998). *ADHD Rating Scale-IV: Checklists, norms, and clinical interpretation.* New York, NY: Guilford Press.

Durston, S., de Zeeuw, P., & Staal, W. G. (2009). Imaging genetics in ADHD: A focus on cognitive control. *Neuroscience and Biobehavioral Reviews, 33*(5), 674-689.

Elgen, I., Sommerfelt, K., & Markestad, T. (2003). Population based, controlled study of behavioural problems and psychiatric disorders in low birthweight children at 11 years of age. *Obstetrical & Gynecological Survey, 58*(2), 128-132.

Englander, E. W., Greeley, G. H., Wang, G., Perez-Polo, J. R., & Lee, H. M. (1999). Hypoxia-induced mitochondrial and nuclear DNA damage in the rat brain. *Journal of Neuroscience Research, 58*(2), 262-269.

Ernfors, P., Lee, K., & Jaenisch, R. (1994). Mice lacking brain-derived neurotrophic factor develop with sensory deficits. *Nature, 368*, 147-150.

Ernfors, P., Merlio, J. P., & Persson, H. (1992). Cells expressing mRNA for neurotrophins and their receptors during embryonic rat development. *European Journal of Neuroscience, 4*(11), 1140-1158.

Faraone, S., Perlis, R., Doyle, A., Smoller, J., Goralnick, J., Holmgren, M., & Sklar, P. (2005). Molecular genetics of attention-deficit/hyperactivity disorder. *Biological Psychiatry, 57*(11), 1313-1323.

Ferrara, N., Carvermoore, K., Chen, H., Dowd, M., Lu, L., Oshea, K., … Moore, M. (1996). Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature, 380*(6573), 439-442.

Ficks, C. A., & Waldman, I. D. (2009). Gene-environment interactions in attention-deficit/hyperactivity disorder. *Current Psychiatry Reports, 11*(5), 387-392.

Filipek, P. A., Semrud-Clikeman, M., Steingard, R. J., Renshaw, P. F., Kennedy, D. N., & Biederman, J. (1997). Volumetric MRI analysis comparing subjects having attention-deficit hyperactivity disorder with normal controls. *Neurology, 48*(3), 589-601.

Forero, D., Arboleda, G., Vasquez, R., & Arboleda, H. (2009). Candidate genes involved in neural plasticity and the risk for attention-deficit hyperactivity disorder: A

meta-analysis of 8 common variants. *Journal of Psychiatry and Neuroscience, 34*(5), 361-366.

Franke, B., Neale, B. M., & Faraone, S. V. (2009). Genome-wide association studies in ADHD. *Human Genetics, 126*(1), 13-50.

Fu, J., & Olofsson, P. (2006). Restrained cerebral hyperperfusion in response to superimposed acute hypoxemia in growth-restricted human fetuses with established brain-sparing blood flow. *Early Human Development, 82*(3), 211-216.

Gatt, J., Nemeroff, C., Dobson-Stone, C., Paul, R., Bryant, R., Schofield, P., … Williams, L. (2009). Interactions between BDNF Val66Met polymorphism and early life stress predict brain and arousal pathways to syndromal depression and anxiety. *Molecular Psychiatry, 14*(7), 681-695.

Gaub, M., & Carlson, C. L. (1997). Behavioral characteristics of DSM-IV ADHD subtypes in a school-based population. *Journal of Abnormal Child Psychology, 25*(2), 103-111.

Gizer, I. R., Ficks, C., & Waldman, I. D. (2009). Candidate gene studies of ADHD: A meta-analytic review. *Human Genetics, 126*(1), 51-90.

Gluckman, P. D., & Hanson, M. A. (2004). Living with the past: evolution, development, and patterns of disease. *Science, 305*(5691), 1733-1736.

Gu, C., Rodriguez, E. R., Reimert, D. V., Shu, T., Fritzsch, B., Richards, L. J., Kolodkin, A. L., & Ginty, D. D. (2003). Neuropilin-1 conveys semaphorin and VEGF signaling during neural and cardiovascular development. *Developmental Cell, 5*(1), 45-57.

Gustafsson, P., Thernlund, G., Ryding, E., Rosen, I., & Cederblad, M. (2000). Associations between cerebral blood-flow measured by single photon emission computed tomography (SPECT), electro-encephalogram (EEG), behaviour symptoms, cognition and neurological soft signs in children with attention-deficit hyperactivity disorder (ADHD). *Acta Paediatrica*, *89*(7), 830-835.

Guy, W. (1976). ECDEU Assessment Manual for Psychopharmacology —Revised (pp. 218-222). Rockville, MD: U.S. Department of Health, Education, and Welfare, Public Health Service, Alcohol, Drug Abuse, and Mental Health Administration, NIMH Psychopharmacology Research Branch, Division of Extramural Research Programs.

Hack, M., Taylor, H., Schluchter, M., Andreias, L., Drotar, D., & Klein, N. (2009). Behavioral outcomes of extremely low birth weight children at age 8 years. *Journal of Developmental & Behavioral Pediatrics*, *30*(2), 122-130.

Haigh, J., Morelli, P., Gerhardt, H., Haigh, K., Tsien, J., Damert, A., … Ferrara, N. (2003). Cortical and retinal defects caused by dosage-dependent reductions in VEGF-A paracrine signaling. *Developmental Biology*, *262*(2), 225-241.

Hakim, R. B., Tielsch, J. M., & See, L. C. (1992). Agreement between maternal interview-and medical record based gestational age. *American Journal of Epidemiology*, *136*(5), 566-573.

Hardin, J. W., & Hilbe, J. M. (2003). *Generalized Estimating Equations (GEE)*. Boca Raton: Chapman & Hall/CRC.

He, Z., & Tessier-Lavigne, M. (1997). Neuropilin is a receptor for the axonal chemorepellent Semaphorin III. *Cell, 90*(4), 739-751.

Heinonen, K., Raikkonen, K., Pesonen, A. K., Andersson, S., Kajantie, E., Eriksson, J. G., … Lano, A. (2010). Behavioural symptoms of attention deficit/hyperactivity disorder in preterm and term children born small and appropriate for gestational age: A longitudinal study. *BMC Pediatrics, 10*(1), 91-99.

Hill, D. J., Petrik, J., & Arany, E. (1998). Growth factors and the regulation of fetal growth. *Diabetes Care, 21*, B60.

Horwood, L., Mogridge, N., & Darlow, B. (1998). Cognitive, educational, and behavioural outcomes at 7 to 8 years in a national very low birthweight cohort. *Archives of Disease in Childhood. Fetal and Neonatal Edition, 79*(1), F12-20.

Hou, S. T., Keklikian, A., Slinn, J., O'Hare, M., Jiang, S. X., & Aylsworth, A. (2008). Sustained up-regulation of semaphorin 3A, Neuropilin1, and doublecortin expression in ischemic mouse brain during long-term recovery. *Biochemical and Biophysical Research Communications, 367*(1), 109-115.

Hultman, C., Torrång, A., Tuvblad, C., Cnattingius, S., Larsson, J., & Lichtenstein, P. (2007). Birth weight and attention-deficit/hyperactivity symptoms in childhood and early adolescence: A prospective Swedish twin study. *Journal of the American Academy of Child and Adolescent Psychiatry, 46*(3), 370-377.

Indredavik, M., Vik, T., Heyerdahl, S., Kulseng, S., Fayers, P., & Brubakk, A. (2004). Psychiatric symptoms and disorders in adolescents with low birth weight. *Archives of Disease in Childhood Fetal and Neonatal Edition, 89*(5), F445-450.

Jaakkola, P., Mole, D., Tian, Y., Wilson, M., Gielbert, J., Gaskell, S., Kriegsheim, A., Hebestreit, H., Mukherji, M., & Schofield, C. (2001). Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. *Science, 292*(5516), 468-472.

Jesmin, S., Togashi, H., Mowa, C., Ueno, K., Yamaguchi, T., Shibayama, A., Miyauchi, T., Sakuma, I., & Yoshioka, M. (2004). Characterization of regional cerebral blood flow and expression of angiogenic growth factors in the frontal cortex of juvenile male SHRSP and SHR. *Brain Research, 1030*(2), 172-182.

Johansen, J. L., Sager, T. N., Lotharius, J., Witten, L., Mørk, A., Egebjerg, J., & Thirstrup, K. (2010). HIF prolyl hydroxylase inhibition increases cell viability and potentiates dopamine release in dopaminergic cells. *Journal of Neurochemistry, 115*(1), 209-219.

Kahn, R. S., Khoury, J., Nichols, W. C., & Lanphear, B. P. (2003). Role of dopamine transporter genotype and maternal prenatal smoking in childhood hyperactive-impulsive, inattentive, and oppositional behaviors. *The Journal of Pediatrics, 143*(1), 104-110.

Kates, W. R., Frederikse, M., Mostofsky, S. H., Folley, B. S., Cooper, K., Mazur-Hopkins, P., … Kaufmann, W. E. (2002). MRI parcellation of the frontal lobe in boys with attention deficit hyperactivity disorder or Tourette syndrome. *Psychiatry Research, 116*(1-2), 63-81.

Kendler, K. S., & Baker, J. H. (2007). Genetic influences on measures of the environment: a systematic review. *Psychological Medicine, 37*(5), 615-626.

Kent, L., Green, E., Hawi, Z., Kirley, A., Dudbridge, F., Lowe, N., … Craddock, N. (2005). Association of the paternally transmitted copy of common Valine allele of the Val66Met polymorphism of the brain-derived neurotrophic factor (BDNF) gene with susceptibility to ADHD. *Molecular Psychiatry, 10*(10), 939-943.

Kernie, S., Liebl, D., & Parada, L. (2000). BDNF regulates eating behavior and locomotor activity in mice. *The EMBO Journal, 19*(6), 1290-1300.

Kim, B. N., Lee, J. S., Shin, M. S., Cho, S. C., & Lee, D. S. (2002). Regional cerebral perfusion abnormalities in attention deficit/hyperactivity disorder. Statistical parametric mapping analysis. *European Archives of Psychiatry and Clinical Neuroscience, 252*(5), 219-225.

Knopik, V. S., Heath, A. C., Jacob, T., Slutske, W. S., Bucholz, K. K., Madden, P. A., … Martin, N. G. (2006). Maternal alcohol use disorder and offspring ADHD: disentangling genetic and environmental effects using a children-of-twins design. *Psychological Medicine, 36*(10), 1461-1471.

Kramer, M. (1987). Determinants of low birth weight: methodological assessment and meta-analysis. *Bulletin of the World Health Organization, 65*(5), 663.

Kuntsi, J., Neale, B. M., Chen, W., Faraone, S. V., & Asherson, P. (2006). The IMAGE project: methodological issues for the molecular genetic analysis of ADHD. *Behavioral and Brain Functions, 2*(1), 27-39.

Lahey, B. B., Applegate, B., McBurnett, K., Biederman, J., Greenhill, L., Hynd, G.W., … Richters, J. (1994). DSM-IV field trials for attention deficit hyperactivity disorder in children and adolescents. *American Journal of Psychiatry, 151*(11), 1673-1685.

Lahey, B. B., & Willcutt, E. G. (2010). Predictive validity of a continuous alternative to nominal subtypes of attention-deficit/hyperactivity disorder for DSM–V. *Journal of Clinical Child and Adolescent Psychology, 39*(6), 761-775.

Lahti, J., Räikkönen, K., Kajantie, E., Heinonen, K., Pesonen, A., Järvenpää, A., & Strandberg, T. (2006). Small body size at birth and behavioural symptoms of ADHD in children aged five to six years. *Journal of Child Psychology and Psychiatry, 47*(11), 1167-1174.

Lamballe, F., Klein, R., & Barbacid, M. (1991). TrkC, a new member of the trk family of tyrosine protein kinases, is a receptor for neurotrophin-3. *Cell, 66*(5), 967-979.

Langley, K., Holmans, P. A., van den Bree, M. B. M., & Thapar, A. (2007). Effects of low birth weight, maternal smoking in pregnancy and social class on the phenotypic manifestation of attention deficit hyperactivity disorder and associated antisocial behaviour: investigation in a clinical sample. *BMC Psychiatry, 7*(1), 1-8.

Langley, K., Turic, D., Rice, F., Holmans, P., van den Bree, M., Craddock, N., … Thapar, A. (2008). Testing for gene × environment interaction effects in attention deficit hyperactivity disorder and associated antisocial behavior. *American Journal of Medical Genetics: Part B: Neuropsychiatric Genetics, 147*(1), 49–53.

Larroque, B., Marret, S., Ancel, P., Arnaud, C., Marpeau, L., Supernant, K., … Cambonie, G. (2003). White matter damage and intraventricular hemorrhage in very preterm infants: The EPIPAGE study. *The Journal of Pediatrics, 143*(4), 477-483.

Lasky-Su, J., Anney, R. J., Neale, B. M., Franke, B., Zhou, K., Maller, J. B., … Faraone, S. V. (2008a). Genome-wide association scan of the time to onset of attention deficit hyperactivity disorder. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 147B*(8), 1355-1358.

Lasky-Su, J., Faraone, S., Lange, C., Tsuang, M., Doyle, A., Smoller, J., … Biederman, J. (2007). A study of how socioeconomic status moderates the relationship between SNPs encompassing BDNF and ADHD symptom counts in ADHD families. *Behavior Genetics, 37*(3), 487-497.

Lasky-Su, J., Neale, B. M., Franke, B., Anney, R. J., Zhou, K., Maller, J. B., … Faraone, S. V. (2008b). Genome-wide association scan of quantitative traits for attention deficit hyperactivity disorder identifies novel associations and confirms candidate gene associations. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 147B*(8), 1345-1354.

Laucht, M., Skowronek, M. H., Becker, K., Schmidt, M. H., Esser, G., … Rietschel, M. (2007). Interacting effects of the dopamine transporter gene and psychosocial adversity on attention-deficit/hyperactivity disorder symptoms among 15-year-olds from a high-risk community sample. *Archives of General Psychiatry, 64*(5), 585-590.

Lee, P. H., & Shatkay, H. (2008). F-SNP: computationally predicted functional SNPs for disease association studies. *Nucleic Acids Research, 36*(suppl 1), D820-D824.

Lehn, H., Derks, E., Hudziak, J., Heutink, P., van Beijsterveldt, T., & Boomsma, D. (2007). Attention problems and attention-deficit/hyperactivity disorder in

discordant and concordant monozygotic twins: evidence of environmental mediators. *Journal of the American Academy of Child and Adolescent Psychiatry, 46*(1), 83-91.

Lesch, K. P., Timmesfeld, N., Renner, T. J., Halperin, R., Roser, C., Nguyen, T.T., … Jacob, C. (2008). Molecular genetics of adult ADHD: converging evidence from genome-wide association and extended pedigree linkage studies. *Journal of Neural Transmission, 115*(11), 1573-1585.

Levy, F. (1991). The dopamine theory of attention deficit hyperactivity disorder (ADHD). *Australian and New Zealand Journal of Psychiatry, 25*(2), 277-283.

Levy, F., Hay, D., McStephen, M., Wood, C., & Waldman, I. (1997). Attention-deficit hyperactivity disorder: a category or a continuum? Genetic analysis of a large-scale twin study. *Journal of the American Academy of Child and Adolescent Psychiatry, 36*(6), 737-744.

Li, Y., Willer, C., Sanna, S., & Abecasis, G. (2009). Genotype imputation. *Annual Review of Genomics and Human Genetics, 10,* 387-406.

Li, Y., Willer, C. J., Ding, J., Scheet, P., & Abecasis, G. R. (2010). MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genetic Epidemiology, 34*(8), 816-834.

Lin, C. H., Cheng, F. C., Lu, Y. Z., Chu, L. F., Wang, C. H., & Hsueh, C. M. (2006). Protection of ischemic brain cells is dependent on astrocyte-derived growth factors and their receptors. *Experimental Neurology, 201*(1), 225-233.

Linnarsson, S., Björklund, A., & Ernfors, P. (2006). Learning deficit in BDNF mutant mice. *European Journal of Neuroscience, 9*(12), 2581-2587.

Linnet, K., Wisborg, K., Agerbo, E., Secher, N., Thomsen, P., & Henriksen, T. (2006). Gestational age, birth weight, and the risk of hyperkinetic disorder. *British Medical Journal, 91*(8), 655-660.

Linnet, K. M., Dalsgaard, S., Obel, C., Wisborg, K., Henriksen, T. B., Rodriguez, A., … Jarvelin, M. R. (2003). Maternal lifestyle factors in pregnancy risk of attention deficit hyperactivity disorder and associated behaviors: review of the current evidence. *American Journal of Psychiatry, 160*(6), 1028-1040.

López-Bendito, G., Cautinat, A., Sánchez, J. A., Bielle, F., Flames, N., Garratt, A. N., … Marín, O. (2006). Tangential neuronal migration controls axon guidance: a role for neuregulin-1 in thalamocortical axon navigation. *Cell, 125*(1), 127-142.

Lyons, W., Mamounas, L., Ricaurte, G., Coppola, V., Reid, S., Bora, S., … Tessarollo, L. (1999). Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. *Proceedings of the National Academy of Sciences of the United States of America, 96*(26), 15239-15244.

Mac Gabhann, F., & Popel, A. (2008). Systems biology of vascular endothelial growth factors. *Microcirculation, 15*(8), 715-738.

Mannuzza, S., Klein, R. G., Bessler, A., Malloy, P., & Hynes, M. E. (1997). Educational and occupational outcome of hyperactive boys grown up. *Journal of the American Academy of Child and Adolescent Psychiatry, 36*(9), 1222-1227.

Maulik, D. (2006). Fetal growth compromise: Definitions, standards, and classification. *Clinical Obstetrics and Gynecology, 49*(2), 214-218.

McCarthy, M. I., & Hirschhorn, J. N. (2008). Genome-wide association studies: Potential next steps on a genetic journey. *Human Molecular Genetics, 17*(R2), R156-165.

McClernon, F. J., & Kollins, S. H. (2008). ADHD and smoking: From genes to brain to behavior. *Annals of the New York Academy of Sciences, 1141*, 131-147.

McGough, J. J., & Barkley, R. A. (2004). Diagnostic controversies in adult attention deficit hyperactivity disorder. *American Journal of Psychiatry, 161*(11), 1948-1956.

McLoughlin, G., Ronald, A., Kuntsi, J., Asherson, P., & Plomin, R. (2007). Genetic support for the dual nature of attention deficit hyperactivity disorder: Substantial genetic overlap between the inattentive and hyperactive–impulsive components. *Journal of Abnormal Child Psychology, 35*(6), 999-1008.

Mick, E., Biederman, J., Prince, J., Fischer, M. J., & Faraone, S. V. (2002). Impact of low birth weight on attention-deficit hyperactivity disorder. *Journal of Developmental and Behavioral Pediatrics, 23*(1), 16-22.

Milberger, S., Biederman, J., Faraone, S. V., Chen, L., & Jones, J. (1996). Is maternal smoking during pregnancy a risk factor for attention deficit hyperactivity disorder in children? *Am J Psychiatry, 153*(9), 1138-1142.

Milberger, S., Biederman, J., Faraone, S. V., & Jones, J. (1998). Further evidence of an association between maternal smoking during pregnancy and attention deficit

hyperactivity disorder: Findings from a high-risk sample of siblings. *Journal of Clinical Child Psychology, 27*(3), 352-358.

Mill, J., & Petronis, A. (2008). Pre- and peri-natal environmental risks for attention-deficit hyperactivity disorder (ADHD): The potential role of epigenetic processes in mediating susceptibility. *Journal of Child Psychology and Psychiatry, 49*(10), 1020-1030.

Missale, C., Nash, S. R., Robinson, S. W., Jaber, M., & Caron, M. G. (1998). Dopamine receptors: From structure to function. *Physiological Reviews, 78*(1), 189-225.

Moffitt, T., Caspi, A., & Rutter, M. (2006). Measured gene-environment interactions in psychopathology. *Perspectives on Psychological Science, 1*(1), 5-27.

Mokler, D. J., Torres, O. I., Galler, J. R., & Morgane, P. J. (2007). Stress-induced changes in extracellular dopamine and serotonin in the medial prefrontal cortex and dorsal hippocampus of prenatally malnourished rats. *Brain Research, 1148*, 226-233.

Molina, B. S. G., & Pelham, W. E. (2003). Childhood predictors of adolescent substance use in a longitudinal study of children with ADHD. *Journal of Abnormal Psychology, 112*(3), 497.

Monteggia, L., Barrot, M., Powell, C., Berton, O., Galanis, V., Gemelli, T., … Nestler, E. (2004). Essential role of brain-derived neurotrophic factor in adult hippocampal function. *Proceedings of the National Academy of Sciences of the United States of America, 101*(29), 10827-10832.

Mostofsky, S. H., Cooper, K. L., Kates, W. R., Denckla, M. B., & Kaufmann, W. E. (2002). Smaller prefrontal and premotor volumes in boys with attention-deficit/hyperactivity disorder. *Biological Psychiatry, 52*(8), 785-794.

Müller, U., Asherson, P., Banaschewski, T., Buitelaar, J., Ebstein, R., Eisenberg, J., … Oades, R. (2011). The impact of study design and diagnostic approach in a large multi-centre ADHD study. Part 1: ADHD symptom patterns. *BMC Psychiatry, 11*(1), 54-73.

Nakamura, E., Okamoto, T., Nagaya, K., & Hayashi, T. (2011). Maternal deletion allele of angiotensin-converting enzyme gene is associated with fetal growth restriction. *Pediatric and Developmental Pathology, in press, Epub ahead of print retrieved December 9, 2011 from http://www.pedpath.org/doi/abs/10.2350/11-09-1092-OA.1*.

Neale, B. M., Lasky-Su, J., Anney, R., Franke, B., Zhou, K., Maller, J. B., … Faraone, S. V. (2008a). Genome-wide association scan of attention deficit hyperactivity disorder. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 147B*(8), 1337-1344.

Neale, B. M., Sham, P. C., Purcell, S., Banaschewski, T., Buitelaar, J., Franke, B., … Faraone, S. V. (2008b). Population differences in the International Multi-Centre ADHD Gene Project. *Genetic Epidemiology, 32*(2), 98-107.

Nemoto, K., Ohnishi, T., Mori, T., Moriguchi, Y., Hashimoto, R., Asada, T., & Kunugi, H. (2006). The Val66Met polymorphism of the brain-derived neurotrophic factor gene affects age-related brain morphology. *Neuroscience Letters, 397*(1-2), 25-29.

Neuman, R., Lobos, E., Reich, W., Henderson, C., Sun, L., & Todd, R. (2007). Prenatal smoking exposure and dopaminergic genotypes interact to cause a severe ADHD subtype. *Biological Psychiatry, 61*(12), 1320-1328.

Nigg, J., & Casey, B. (2005). An integrative theory of attention-deficit/hyperactivity disorder based on the cognitive and affective neurosciences. *Development and Psychopathology, 17*(03), 785-806.

Nigg, J., Nikolas, M., & Burt, S. A. (2010). Measured Gene-by-Environment Interaction in Relation to Attention-Deficit/Hyperactivity Disorder. *Journal of the American Academy of Child and Adolescent Psychiatry, 49*(9), 863-873.

Nigg, J. T. (2006). *What causes ADHD? : Understanding what goes wrong and why*. New York: Guilford Press.

Nikolas, M. A., & Burt, S. A. (2010). Genetic and environmental influences on ADHD symptom dimensions of inattention and hyperactivity: A meta-analysis. *Journal of Abnormal Psychology, 119*(1), 1-17.

National Institue of Mental Health. (1997). *Computerized Diagnostic Interview Schedule for Children, IV*. New York: Columbia University.

Northam, S., & Knapp, T. (2006). The reliability and validity of birth certificates. *Journal of Obstetric, Gynecologic, and Neonatal Nursing, 35*(1), 3-12.

Oades, R. D. (2011). An exploration of the associations of pregnancy and perinatal features with cytokines and tryptophan/kynurenine metabolism in children with attention-deficit hyperactivity disorder (ADHD). *Attention Deficit and Hyperactivity Disorders*, 1-18.

Okamoto, K., & Aoki, K. (1963). Development of a strain of spontaneously hypertensive rats. *Japanese Circulation Journal, 27*(3), 282-293.

Overmeyer, S., Bullmore, E. T., Suckling, J., Simmons, A., Williams, S. C., Santosh, P. J., & Taylor, E. (2001). Distributed grey and white matter deficits in hyperkinetic disorder: MRI evidence for anatomical abnormality in an attentional network. *Psychological Medicine, 31*(8), 1425-1435.

Pan, H., & Cole, T. J. (2010). LMSgrowth, a microsoft excel add-in to access growth references based on the LMS method, Version 2.69., from http://www.healthforallchildren.co.uk/

Pearsall-Jones, J., Piek, J., Martin, N., Rigoli, D., Levy, F., & Hay, D. (2008). A monozygotic twin design to investigate etiological factors for DCD and ADHD. *Journal of Pediatric Neurology, 6*(3), 209-219.

Pelham, W., Foster, E., & Robb, J. (2007). The economic impact of attention-deficit/ hyperactivity disorder in children and adolescents. *Ambulatory Pediatrics, 7*(1), 121-131.

Peterson, B., Anderson, A., Ehrenkranz, R., Staib, L., Tageldin, M., Colson, E., Gore, J., Duncan, C., Makuch, R., & Ment, L. (2003). Regional brain volumes and their later neurodevelopmental correlates in term and preterm infants. *Pediatrics, 111*(5), 939-948.

Pfiffner, L., McBurnett, K., Lahey, B., Loeber, R., Green, S., Frick, P., & Rathouz, P. (1999). Association of parental psychopathology to the comorbid disorders of

boys with attention deficit-hyperactivity disorder. *Journal of Consulting and Clinical Psychology, 67*, 881-893.

Pineda, D. A., Palacio, L. G., Puerta, I. C., Merchán, V., Arango, C. P., Galvis, A. Y., … Arcos-Burgos, M. (2007). Environmental influences that affect attention deficit/hyperactivity disorder. *European Child and Adolescent Psychiatry, 16*(5), 337-346.

Pliszka, S. R., McCracken, J. T., & Maas, J. W. (1996). Catecholamines in attention-deficit hyperactivity disorder: Current perspectives. *Journal of the American Academy of Child and Adolescent Psychiatry, 35*(3), 264-272.

Poelmans, G., Pauls, D. L., Buitelaar, J. K., & Franke, B. (2011). Integrated genome-wide association study findings: Identification of a neurodevelopmental network for attention deficit hyperactivity disorder. *American Journal of Psychiatry, 168*(4), 365-377.

Polanczyk, G., de Lima, M. S., Horta, B. L., Biederman, J., & Rohde, L. A. (2007). The worldwide prevalence of ADHD: A systematic review and metaregression analysis. *American Journal of Psychiatry, 164*(6), 942-948.

Polleux, F., Morrow, T., & Ghosh, A. (2000). Semaphorin 3A is a chemoattractant for cortical apical dendrites. *Nature, 404*(6778), 567-573.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., … Daly, M. J. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *The American Journal of Human Genetics, 81*(3), 559-575.

Rapoport, J., & Gogtay, N. (2007). Brain neuroplasticity in healthy, hyperactive and psychotic children: insights from neuroimaging. *Neuropsychopharmacology, 33*(1), 181-197.

Reichardt, L. (2006). Neurotrophin-regulated signalling pathways. *Philosophical Transactions of the Royal Society B: Biological Sciences, 361*(1473), 1545-1564.

Retz, W., Freitag, C. M., Retz-Junginger, P., Wenzler, D., Schneider, M., Kissling, C., … Rosler, M. (2008). A functional serotonin transporter promoter gene polymorphism increases ADHD symptoms in delinquents: interaction with adverse childhood environment. *Psychiatry Research, 158*(2), 123-131.

Ribasés, M., Hervás, A., Ramos-Quiroga, J., Bosch, R., Bielsa, A., Gastaminza, X., … Valero, S. (2008). Association study of 10 genes encoding neurotrophic factors and their receptors in adult and child attention-deficit/hyperactivity disorder. *Biological Psychiatry, 63*(10), 935-945.

Rice, F., Lewis, A., Harold, G., van den Bree, M., Boivin, J., Hay, D., … Thapar, A. (2007). Agreement between maternal report and antenatal records for a range of pre and peri-natal factors: The influence of maternal and child characteristics. *Early Human Development, 83*(8), 497-504.

Rodier, P. (2004). Environmental causes of central nervous system maldevelopment. *Pediatrics, 113*(4), 1076-1083.

Russell, V. A. (2011). Overview of animal models of attention deficit hyperactivity disorder (ADHD). *Current Protocols in Neuroscience*, *9*(35), 1-25.

Rutter, M., Moffitt, T., & Caspi, A. (2006). Gene-environment interplay and psychopathology: multiple varieties but real effects. *Journal of Child Psychology and Psychiatry, 47*(3-4), 226-261.

Sagvolden, T., Johansen, E. B., Aase, H., & Russell, V. A. (2005). A dynamic developmental theory of attention-deficit/hyperactivity disorder (ADHD) predominantly hyperactive/impulsive and combined subtypes. *Behavioral and Brain Sciences, 28*(3), 397-419.

Sanchez-Mora, C., Ribases, M., Ramos-Quiroga, J. A., Casas, M., Bosch, R., Boreatti-Hummer, A., … Cormand, B. (2009). Meta-analysis of brain-derived neurotrophic factor p.Val66Met in adult ADHD in four European populations. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 153B*(2), 512-523.

Schmidt-Kastner, R., Truettner, J., Lin, B., Zhao, W., Saul, I., Busto, R., & Ginsberg, M. (2001). Transient changes of brain-derived neurotrophic factor (BDNF) mRNA expression in hippocampus during moderate ischemia induced by chronic bilateral common carotid artery occlusions in the rat. *Molecular Brain Research, 92*(1-2), 157-166.

Schmidt-Kastner, R., van Os, J., Steinbusch, H., & Schmitz, C. (2006). Gene regulation by hypoxia and the neurodevelopmental origin of schizophrenia. *Schizophrenia Research, 84*(2-3), 253-271.

Schwab-Stone, M., Shaffer, D., Dulcan, M., Jensen, P., Fisher, P., Bird, H., Goodman, S., Lahey, B., Lichtman, J., & Canino, G. (1996). Criterion validity of the NIMH

Diagnostic Interview Schedule for Children version 2.3 (DISC-2.3). *Journal of the American Academy of Child and Adolescent Psychiatry, 35*(7), 878-888.

Seeger, G., Schloss, P., Schmidt, M., Rüter-Jungfleisch, A., & Henn, F. (2004). Gene–environment interaction in hyperkinetic conduct disorder (HD+ CD) as indicated by season of birth variations in dopamine receptor (DRD4) gene polymorphism. *Neuroscience Letters, 366*(3), 282-286.

Seidman, D., Slater, P., Ever-Hadani, P., & Gale, R. (1987). Accuracy of mothers' recall of birthweight and gestational age. *British Journal of Obstetrics and Gynaecology, 94*(8), 731-735.

Shaffer, D., Fisher, P., Lucas, C., Dulcan, M., & Schwab-Stone, M. (2000). NIMH Diagnostic Interview Schedule for Children Version IV (NIMH DISC-IV): Description, differences from previous versions, and reliability of some common diagnoses. *Journal of the American Academy of Child and Adolescent Psychiatry, 39*(1), 28-38.

Sharp, F. R., & Bernaudin, M. (2004). HIF1 and oxygen sensing in the brain. *Nature Reviews Neuroscience, 5*(6), 437-448.

Sharp, W., Gottesman, R., Greenstein, D., Ebens, C., Rapoport, J., & Castellanos, F. (2003). Monozygotic twins discordant for attention-deficit/hyperactivity disorder: Ascertainment and clinical characteristics. *Journal of the American Academy of Child and Adolescent Psychiatry, 42*(1), 93-97.

Shaw, P., Gilliam, M., Liverpool, M., Weddle, C., Malek, M., Sharp, W., … Giedd, J. (2011). Cortical development in typically developing children with symptoms of

hyperactivity and impulsivity: support for a dimensional view of attention deficit hyperactivity disorder. *American Journal of Psychiatry, 168*(2), 143-151.

Shaw, P., Lerch, J., Greenstein, D., Sharp, W., Clasen, L., Evans, A., … Rapoport, J. (2006). Longitudinal mapping of cortical thickness and clinical outcome in children and adolescents with attention-deficit/hyperactivity disorder. *Archives of General Psychiatry, 63*(5), 540-549.

Shibuya, M. (2008). Vascular endothelial growth factor-dependent and -independent regulation of angiogenesis. *BMB Reports, 41*(4), 278-286.

Shibuya, M., & Claesson-Welsh, L. (2006). Signal transduction by VEGF receptors in regulation of angiogenesis and lymphangiogenesis. *Experimental Cell Research, 312*(5), 549-560.

Smith, T. F. (2010). Meta-analysis of the heterogeneity in association of DRD4 7-repeat allele and AD/HD: Stronger association with AD/HD combined type. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 153*(6), 1189-1199.

Smith, T.F. [The role of low birthweight in the etiology of AD/HD]. Unpublished data.

Smith, T.F. et al. [Fetal growth compromise moderates the associations between SNPs within angiogenic, kynurenine, and neurtrophic genes and AD/HD symptom severity]. Manuscript in preparation.

Sonuga-Barke, E. J., Lasky-Su, J., Neale, B. M., Oades, R., Chen, W., Franke, B., … Faraone, S. V. (2008). Does parental expressed emotion moderate genetic effects in ADHD? An exploration using a genome wide association scan. *American*

Journal of Medical Genetics Part B: Neuropsychiatric Genetics, *147B*(8), 1359-1368.

Sonuga-Barke, E. J., Oades, R. D., Psychogiou, L., Chen, W., Franke, B., Buitelaar, J., … Faraone, S. V. (2009). Dopamine and serotonin transporter genotypes moderate sensitivity to maternal expressed emotion: The case of conduct and emotional problems in attention deficit/hyperactivity disorder. *Journal of Child Psychology and Psychiatry*, *50*(9), 1052-1063.

Sonuga-Barke, E. J. S. (2002). Psychological heterogeneity in AD/HD--a dual pathway model of behaviour and cognition. *Behavioural Brain Research*, *130*(1-2), 29-36.

Sparrow, S., Cicchetti, D., & Balla, D. (2005). Vineland adaptive behavior scales: (Vineland II), survey interview form/caregiver rating form. *Livonia, MN: Pearson Assessments*.

Sublette, M., Baca-Garcia, E., Parsey, R., Oquendo, M., Rodrigues, S., Galfalvy, H., … Mann, J. (2008). Effect of BDNF val66met polymorphism on age-related amygdala volume changes in healthy subjects. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *32*(7), 1652-1655.

Swanson, J. M., Kinsbourne, M., Nigg, J., Lanphear, B., Stefanatos, G. A., Volkow, N., … Wadhwa, P. D. (2007). Etiologic subtypes of attention-deficit/hyperactivity disorder: Brain imaging, molecular genetic and environmental factors and the dopamine hypothesis. *Neuropsychology Review*, *17*(1), 39-59.

Syed, Z., Dudbridge, F., & Kent, L. (2007). An investigation of the neurotrophic factor genes GDNF, NGF, and NT3 in susceptibility to ADHD. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 144*(3), 375-378.

Taylor, E., Sandberg, S., Thorley, G., & Giles, S. (1991). *The epidemiology of childhood hyperactivity. Maudsley Monographs 33*: Oxford, Oxford University Press.

Todd, R. D., & Botteron, K. N. (2001). Is attention-deficit/hyperactivity disorder an energy deficiency syndrome? *Biological Psychiatry, 50*(3), 151-158.

Todd, R. D., & Neuman, R. J. (2007). Gene-environment interactions in the development of combined type ADHD: evidence for a synapse-based model. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 144B*(8), 971-975.

Toft, P. (1999). Prenatal and perinatal striatal injury: a hypothetical cause of attention-deficit-hyperactivity disorder? *Pediatric Neurology, 21*(3), 602-610.

Tolsa, C., Zimine, S., Warfield, S., Freschi, M., Sancho, R., Lazeyras, F., … Huppi, P. (2004). Early alteration of structural and functional brain development in premature infants born with intrauterine growth restriction. *Pediatric Research, 56*(1), 132-138.

Tomeo, C., Rich-Edwards, J., Michels, K., Berkey, C., Hunter, D., Frazier, A., … Buka, S. (1999). Reproducibility and validity of maternal recall of pregnancy-related events. *Epidemiology*, 774-777.

Tomita, S., Ueno, M., Sakamoto, M., Kitahama, Y., Ueki, M., Maekawa, N., Sakamoto, H., Gassmann, M., Kageyama, R., & Ueda, N. (2003). Defective brain

development in mice lacking the HIF-1 alpha gene in neural cells. *Molecular and Cellular Biology*, *23*(19), 6739-6749.

Troe, E., Raat, H., Jaddoe, V., Hofman, A., Looman, C., Moll, H., Steegers, E., Verhulst, F., Witteman, J., & Mackenbach, J. (2007). Explaining differences in birthweight between ethnic populations. The Generation R Study. *British Journal of Obstetrics and Gynaecology, 114*(12), 1557-1565.

Tully, L., Arseneault, L., Caspi, A., Moffitt, T., & Morgan, J. (2004). Does maternal warmth moderate the effects of birth weight on twins' attention-deficit/hyperactivity disorder (ADHD) symptoms and low IQ? *Journal of Consulting and Clinical Psychology, 72*(2), 218-226.

United Nations Children's Fund. (2004). *Low Birthweight: Country, Regional and Global Estimates.* Retrieved from http://www.unicef.org/publications/index_24840.html

Valera, E. M., Faraone, S. V., Murray, K. E., & Seidman, L. J. (2007). Meta-analysis of structural imaging findings in attention-deficit/hyperactivity disorder. *Biological Psychiatry, 61*(12), 1361-1369.

van 't Ent, D., Lehn, H., Derks, E. M., Hudziak, J. J., Van Strien, N. M., Veltman, D. J., … Boomsma, D. I. (2007). A structural MRI study in monozygotic twins concordant or discordant for attention/hyperactivity problems: evidence for genetic and environmental heterogeneity in the developing brain. *Neuroimage, 35*(3), 1004-1020.

van Os, J., Wichers, M., Danckaerts, M., Van Gestel, S., Derom, C., & Vlietinck, R. (2001). A prospective twin study of birth weight discordance and child problem behavior. *Biological Psychiatry, 50*(8), 593-599.

Visser, G. H. A., Eilers, P. H. C., Elferink-Stinkens, P. M., Merkus, H. M. W. M., & Wit, J. M. (2009). New Dutch reference curves for birthweight by gestational age. *Early Human Development, 85*(12), 737-744.

Waldman, I. D. (2007). Gene-environment interactions reexamined: does mother's marital stability interact with the dopamine receptor D2 gene in the etiology of childhood attention-deficit/hyperactivity disorder? *Development and Psychopathology, 19*(4), 1117-1128.

Weber, P., Lurschg, J., & Fahnenstich, H. (2005). Cerebral hemodynamic changes in response to an executive function task in children with attention-deficit hyperactivity disorder measured by near-infrared spectroscopy. *Journal of Developmental and Behavioral Pediatrics, 26*(2), 105-111.

Wechsler, D., Kaplan, E., Fein, D., Kramer, J., Morris, R., Delis, D., & Maerlender, A. (2004). Wechsler Intelligence Scale for Children Fourth Edition—Integrated: Technical and interpretative manual. *San Antonio: The Psychological Corporation*.

Weickert, C., Ligons, D., Romanczyk, T., Ungaro, G., Hyde, T., Herman, M., … Kleinman, J. (2005). Reductions in neurotrophin receptor mRNAs in the prefrontal cortex of patients with schizophrenia. *Molecular Psychiatry*, *10*(7), 637-650.

Whitaker, A., Van Rossem, R., Feldman, J., Schonfeld, I., Pinto-Martin, J., Tarre, C., Shaffer, D., & Paneth, N. (1997). Psychiatric outcomes in low-birth-weight children at age 6 years: Relation to neonatal cranial ultrasound abnormalities. *Archives of General Psychiatry, 54*(9), 847-856.

Wichers, M., Purcell, S., Danckaerts, M., Derom, C., Derom, R., Vlietinck, R., & van Os, J. (2002). Prenatal life and post-natal psychopathology: Evidence for negative gene–birth weight interaction. *Psychological Medicine, 32*(07), 1165-1174.

Yu, A. Y., Shimoda, L. A., Iyer, N. V., Huso, D. L., Sun, X., McWilliams, R., … Sylvester, J. (1999). Impaired physiological responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1a. *Journal of Clinical Investigation, 103*(5), 691-696.

Zubrick, S., Kurinczuk, J., McDermott, B., McKelvey, R., Silburn, S., & Davies, L. (2000). Fetal growth and subsequent mental health problems in children aged 4 to 13 years. *Developmental Medicine and Child Neurology, 42*(01), 14-20.

APPENDIX A:

TABLES AND FIGURES

Table 1

Sample Size by Site

Site	n	% of total sample
NCGAP Subtotal	107	26.9
Duke	65	16.3
UNCG	42	10.6
IMAGE Subtotal	291	73.1
Ireland	84	21.1
Netherlands-Amsterdam	101	25.4
Netherlands-Nijmegen	73	18.3
United Kingdom	33	8.3
Total Sample	398	100

Note. NCGAP = North Carolina Genetics of ADHD Project. UNCG = University of North Carolina at Greensboro. IMAGE = International Multisite ADHD Genetics Project

Table 2

Sample Characteristics

	NCGAP		IMAGE		Total	
	M	SD	M	SD	M	SD
Age	8.59	2.54	11.50	2.80	10.72	3.02
	Percentage	N	Percentage	N	Percentage	N
Male	30.84	74	87.62	255	82.66	329
Female	69.16	33	12.37	36	17.34	38
Proband	67.29	72	98.97	288	360	90.45
Sibling	32.71	35	1.03	3	38	9.55

Note. NCGAP = North Carolina Genetics of ADHD Project. IMAGE = International Multisite ADHD Genetics Project

Table 3

AD/HD Clinical Characteristics

	IMAGE		NCGAP		Total	
	M	SD	M	SD	M	SD
CPRS ADHD Total	78.68	8.49	74.61	14.11	77.59	10.45
CPRS DSM-IV IA	72.44	8.47	70.75	13.60	71.98	10.12
CPRS DSM-IV HI	80.80	9.08	73.08	14.69	78.73	11.38
CTRS ADHD Total	69.98	11.75	62.86	11.66	66.75	11.91
CTRS DSM-IV IA	64.84	10.09	63.10	12.14	64.42	10.62
CTRS DSM-IV HI	68.08	13.29	59.76	12.79	66.09	13.63
IA Symptoms	8.11	1.15	7.54	1.70	7.97	1.32
HI Symptoms	8.03	1.29	5.80	2.85	7.49	2.03

Note. CPRS = Conners Parent Rating Scale. CTRS = Conners Teacher Rating Scale. IA = Inattention. HI = Hyperactive-Impulsive. CTRS data on 286 IMAGE and 90 NCGAP youth.

Table 4

AD/HD and Comorbidity

	IMAGE		NCGAP		Total	
	%	N	%	N	%	N
AD/HD Subtype						
Combined Type	94.50	275	48.60	52	82.16	327
Predominantly IA Type	3.09	9	30.84	33	10.55	42
Predominantly HI Type	1.72	5	6.54	7	3.02	12
Unaffected	.69	2	14.02	15	4.27	17
Oppositional Defiant Disorder	68.17	197	34.91	37	59.24	234
Conduct Disorder	28.03	81	4.72	5	21.77	86
Mood Disorder	22.84	66	4.72	5	17.97	71
Bipolar Disorder	1.04	3	0	0	.76	3
Anxiety Disorder	51.56	149	13.21	14	41.27	163
Obsessive-Compulsive Disorder	6.23	18	.09	1	4.8	19
Tic Disorder	3.80	11	5.66	6	4.30	17
Substance Abuse Disorder	1.38	4	0	0	1.01	4

Note. Comorbidity N=395. IA = Inattentive; HI = Hyperactive-Impulsive.

Table 5

Pregnancy, Birth and Delivery Characteristics

	IMAGE		NCGAP		Total		Differences Between Studies		
	%	N	%	N	%	N	χ^2	*p*-value	% Missing
Maternal Smoking	22.3	65	7.8	8	18.6	73	10.49	<.01	1.3
Paternal Smoking	38.8	100	22.2	22	34.2	122	8.70	<.01	10.3
Maternal Alcohol Use	25.5	73	2.0	2	19.4	75	26.19	<.01	3.0
Anemia	6.9	20	8.7	6	7.2	26	.28	.60	9.5
High Blood Pressure	7.9	23	11.2	10	8.7	33	.95	.33	4.5
Kidney Disorder	0	0	1.1	1	.3	1	3.32	.07	4.8
Toxemia	1.4	4	7.9	7	2.9	11	10.21	<.01	4.5
Rh Incompatibility	0	0	9.0	8	2.1	8	26.72	<.01	4.5
Cesarean Section	4.1	12	30.3	27	10.3	39	50.85	<.01	4.5
Premature Labor	.3	1	14	12	3.4	13	36.93	<.01	5.3
Delivery Induced	9.3	27	32.6	29	14.7	56	29.46	<.01	4.5
Forceps	2.4	7	11.5	10	4.5	17	12.88	<.01	5.0
Breech Delivery	.7	2	2.3	2	1.1	4	1.63	.20	4.8
Trouble Breathing	6.9	20	10.1	9	7.6	29	1.02	.31	4.5
Needed Oxygen	4.1	12	10.5	9	5.6	21	5.08	.02	5.3
Cyanotic	4.5	13	8.0	7	5.3	20	1.64	.20	4.8
Jaundiced	3.8	11	25.8	23	8.9	34	40.72	<.01	4.5
Infection	0	0	5.6	5	1.3	5	16.57	<.01	4.5
Seizures	0	0	0	0	0	0	-	-	4.5
Given Medication	0	0	8.0	7	1.8	7	23.58	<.01	4.8
Hospital for > 6 days	0	0	5.7	5	1.3	5	16.76	<.01	4.8

Note. NCGAP = North Carolina Genetics of ADHD Project. IMAGE = International Multisite ADHD Genetics Project.

Table 6

Correlations Between Selected Demographic, ADHD, and Perinatal Variables

		Age	CPRS ADHD Total	Birth Weight Centile Range	Birth Weight (g)	Gestational Age	Maternal Smoking	Paternal Smoking	Maternal Alcohol Use
Age	*r*	1	.21*	-.14*	-.08	.08	-.04	-.05	-.03
	N	398	398	398	398	398	398	365	398
CPRS ADHD Total	*r*	.21*	1	.02	.04	.10	-.09	-.19*	-.04
	N	398	398	398	398	398	398	365	398
Birth Weight Centile Range	*r*	-.14*	.02	1	.76*	-.09	-.04	-.00	-.05
	N	398	398	398	398	398	398	365	398
Birth Weight (g)	*r*	-.08	.04	.76*	1	.50*	-.10	-.05	-.06
	N	398	398	398	398	398	398	365	398
Gestational Age	*r*	.08	.10	-.09	.50*	1	-.10	-.07	-.05
	N	398	398	398	398	398	398	365	398
Maternal Smoking	*r*	-.04	-.09	-.04	-.10	-.10	1	.72*	.11
	N	398	398	398	398	398	398	365	398
Paternal Smoking	*r*	-.05	-.19*	-.00	-.05	-.07	.72*	1	.06
	N	365	365	365	365	365	365	365	365
Maternal Alcohol Use	*r*	-.03	-.04	-.05	-.06	-.05	.11	.06	1
	N	398	398	398	398	398	398	365	398

Note. CPRS = Conners' Parent Rating Scale. Maternal smoking, paternal smoking and maternal alcohol use are binary variables for where 1 = any reported use during the pregnancy and 0 = no reported use during the pregnancy. * = correlation is significant at the 0.01 level (2-tailed).

Table 7

Main Effects of SNPs and Interactions between SNPs and Birth Weight Centile Range Predicting the Transformed CPRS ADHD Total Score

System	Gene	NCBI36	SNP	Allele (Freq)	# of Youth	*p*-values (no covariate model)		*p*-values (covariate model)	
						SNP Main Effect	Interaction	SNP Main Effect	Interaction
Neurotrophic	NTRK2	86522947	rs11141486	G (0.31)	397	0.697	0.084	0.862	0.012
Angiogenic	NRP2	206285589	rs17682318	C (0.28)	392	0.904	0.018	1.000	0.016
Angiogenic	NRP1	33634008	rs2065364	T (0.28)	396	0.691	0.043	0.487	0.018
Angiogenic	NRP2	206272281	rs10932118	T (0.45)	397	0.441	0.013	0.442	0.022
Neurotrophic	NTRK3	86265616	rs8031510	C (0.22)	384	0.992	0.074	0.838	0.035
Angiogenic	VEGFR1	27835419	rs9513089	A (0.36)	396	0.270	0.023	0.150	0.037
Angiogenic	NRP2	206279650	rs12611613	G (0.39)	398	0.882	0.018	0.958	0.038
Angiogenic	NRP1	33524702	rs734187	A (0.22)	396	0.875	0.006	0.599	0.045
Dopaminergic	DRD3	115345577	rs963468	A (0.42)	398	0.905	0.014	0.937	0.056
Angiogenic	NRP1	33585470	rs10827221	C (0.32)	398	0.830	0.063	0.947	0.065
Dopaminergic	DRD3	115340891	rs2134655	T (0.26)	398	0.885	0.142	0.551	0.078
Dopaminergic	DAT1	1481135	rs10053602	C (0.23)	397	0.437	0.055	0.581	0.085
Neurotrophic	BDNF	27671460	rs7127507	C (0.31)	396	0.018	0.384	0.025	0.088
Neurotrophic	BDNF	27636492	rs6265	T (0.20)	398	0.070	0.324	0.033	0.090
Angiogenic	VEGFR1	27904847	rs3794405	C (0.24)	397	0.812	0.096	0.471	0.100
Dopaminergic	DRD2	112834984	rs7131056	A (0.48)	396	0.273	0.284	0.156	0.104
Neurotrophic	NTRK3	86391497	rs1948066	T (0.33)	397	0.149	0.089	0.091	0.109
Dopaminergic	DAT1	1485202	rs456774	C (0.22)	393	0.059	0.124	0.047	0.127
Neurotrophic	NGFB	115655119	rs556723	T (0.3)	398	0.320	0.105	0.263	0.132
Angiogenic	NRP1	33587815	rs3780867	A (0.50)	398	0.812	0.171	0.796	0.143
Neurotrophic	BDNF	27656701	rs7103411	C (0.21)	397	0.125	0.324	0.067	0.147

System	Gene	NCBI36	SNP	Allele (Freq)	# of Youth	*p*-values (no covariate model)		*p*-values (covariate model)	
						SNP Main Effect	Interaction	SNP Main Effect	Interaction
Angiogenic	NRP1	33613812	rs7079372	T (0.20)	398	0.980	0.068	0.947	0.150
Neurotrophic	NTRK3	86364875	rs999905	C (0.34)	398	0.030	0.146	0.036	0.159
Angiogenic	NRP1	33593263	rs2073320	A (0.38)	397	0.538	0.192	0.510	0.161
Angiogenic	NRP2	206344344	rs849511	G (0.50)	395	0.952	0.289	0.936	0.186
Dopaminergic	DRD2	112823183	rs4245146	T (0.45)	398	0.087	0.366	0.802	0.188
Angiogenic	NRP2	206301921	rs13018627	G (0.45)	384	0.701	0.484	0.779	0.234
Neurotrophic	NTRK2	86661581	rs452723	T (0.41)	387	0.202	0.321	0.723	0.237
Angiogenic	HIF1AN	102285826	rs2295778	G (0.27)	383	0.049	0.342	0.182	0.250
Angiogenic	NRP2	206370927	rs11678877	G (0.48)	396	0.666	0.464	0.742	0.260
Neurotrophic	NTRK2	86673280	rs1952348	A (0.44)	395	0.360	0.265	0.148	0.279
Neurotrophic	NTRK3	86336921	rs4887346	C (0.44)	396	0.381	0.280	0.447	0.281
Neurotrophic	NTRK3	86289432	rs1369426	A (0.49)	398	0.351	0.353	0.167	0.284
Angiogenic	VEGFR1	27839060	rs7995976	A (0.24)	398	0.986	0.401	0.556	0.286
Angiogenic	NRP1	33608278	rs2776925	G (0.36)	398	0.712	0.467	0.806	0.298
Angiogenic	VEGFA	43855555	rs3025010	C (0.32)	398	0.676	0.639	0.580	0.299
Neurotrophic	NTRK3	86459135	rs4887362	C (0.3)	386	0.410	0.288	0.814	0.319
Neurotrophic	NTRK2	86500477	rs10868456	A (0.37)	390	0.115	0.607	0.172	0.359
Neurotrophic	NTRK3	86518284	rs7179806	C (0.28)	394	0.862	0.316	0.865	0.372
Neurotrophic	NTRK3	86262610	rs1347424	T (0.34)	398	0.052	0.566	0.059	0.398
Neurotrophic	NTRK3	86303546	rs8038245	G (0.46)	396	0.237	0.338	0.171	0.417
Dopaminergic	COMT	18326686	rs4646316	T (0.26)	392	0.751	0.762	0.619	0.420
Angiogenic	NRP1	33618294	rs4934858	C (0.36)	398	0.496	0.433	0.738	0.421
Neurotrophic	NTRK3	86519246	rs13329385	C (0.23)	397	0.495	0.629	0.655	0.436
Angiogenic	VEGFR1	27867458	rs2104330	C (0.47)	395	0.084	0.909	0.019	0.443
Neurotrophic	NTRK3	86538332	rs4887381	T (0.22)	386	0.328	0.147	0.511	0.447
Neurotrophic	BDNF	27656893	rs7103873	C (0.48)	398	0.234	0.749	0.459	0.453

System	Gene	NCBI36	SNP	Allele (Freq)	# of Youth	*p*-values (no covariate model)		*p*-values (covariate model)	
						SNP Main Effect	Interaction	SNP Main Effect	Interaction
Neurotrophic	NTRK3	86294898	rs7178071	G (0.21)	385	0.400	0.703	0.488	0.476
Neurotrophic	NTRK2	86588285	rs10119168	A (0.46)	386	0.119	0.062	0.166	0.496
Angiogenic	NRP1	33638553	rs12358711	A (0.44)	392	0.353	0.408	0.069	0.505
Dopaminergic	DRD2	112786283	rs6279	G (0.3)	396	0.830	0.659	0.622	0.510
Neurotrophic	NTRK3	86403845	rs3825885	C (0.32)	396	0.015	0.379	0.010	0.525
Neurotrophic	NTRK3	86266972	rs1007533	C (0.43)	386	0.424	0.594	0.320	0.571
Angiogenic	VEGFR1	27871621	rs9319428	A (0.31)	374	0.876	0.545	0.767	0.578
Angiogenic	NRP2	206263877	rs10194604	G (0.45)	386	0.954	0.490	0.988	0.579
Neurotrophic	NTRK3	86520227	rs4887376	G (0.47)	391	0.544	0.572	0.467	0.584
Neurotrophic	NTRK3	86390889	rs3784432	T (0.34)	385	0.207	0.080	0.244	0.593
Neurotrophic	NTRK2	86485592	rs7860382	C (0.36)	396	0.191	0.378	0.489	0.597
Angiogenic	HIF1AN	102303597	rs11292	G (0.21)	398	0.721	0.686	0.521	0.608
Dopaminergic	DAT1	1489408	rs420422	C (0.45)	386	0.172	0.537	0.187	0.627
Neurotrophic	NTRK2	86651130	rs7033669	T (0.26)	394	0.183	0.433	0.389	0.635
Neurotrophic	NTRK2	86597888	rs10514832	A (0.26)	398	0.832	0.262	0.300	0.639
Neurotrophic	NTRK2	86677554	rs10780796	T (0.40)	397	0.058	0.293	0.044	0.643
Angiogenic	VEGFR1	27905143	rs9513113	T (0.38)	397	0.504	0.930	0.705	0.646
Dopaminergic	DRD2	112810524	rs12364051	A (0.43)	393	0.428	0.603	0.525	0.652
Angiogenic	NRP1	33515288	rs2228638	T (0.11)	398	0.868	0.744	0.813	0.667
Neurotrophic	NTRK3	86241794	rs1435402	C (0.37)	396	0.356	0.567	0.241	0.704
Angiogenic	NRP1	33645638	rs2768420	G (0.3)	396	0.696	0.754	0.611	0.708
Neurotrophic	NTRK2	86670391	rs4878017	A (0.37)	397	0.092	0.764	0.208	0.718
Angiogenic	VEGFR2	55675376	rs6832059	C (0.49)	394	0.450	0.959	0.348	0.721
Neurotrophic	NTRK2	86542525	rs962658	C (0.43)	398	0.935	0.944	0.982	0.723
Angiogenic	VEGFR2	55646483	rs2067951	C (0.49)	398	0.026	0.583	0.077	0.750
Neurotrophic	NTRK2	86482260	rs10512176	C (0.29)	397	0.190	0.806	0.342	0.750

System	Gene	NCBI36	SNP	Allele (Freq)	# of Youth	*p*-values (no covariate model)		*p*-values (covariate model)	
						SNP Main Effect	Interaction	SNP Main Effect	Interaction
Angiogenic	NRP1	33607139	rs10827228	T (0.48)	397	0.966	0.580	0.996	0.759
Neurotrophic	NTRK3	86447926	rs16941255	G (0.53)	391	0.146	0.445	0.105	0.762
Angiogenic	NRP1	33602908	rs9299707	T (0.24)	397	0.960	0.855	0.827	0.765
Angiogenic	VEGFR2	55681048	rs4317261	T (0.31)	398	0.207	0.552	0.103	0.765
Angiogenic	VEGFR1	27894418	rs9319434	T (0.27)	385	0.928	0.688	0.993	0.775
Neurotrophic	NTRK2	86649401	rs7048294	C (0.27)	397	0.340	0.483	0.640	0.789
Neurotrophic	NTRK2	86547033	rs10746782	A (0.35)	398	0.848	0.512	0.750	0.794
Neurotrophic	NTRK2	86604868	rs994029	C (0.40)	398	0.048	0.590	0.099	0.810
Neurotrophic	NTRK3	86530483	rs1346164	C (0.32)	384	0.481	0.958	0.907	0.813
Dopaminergic	DRD3	115373505	rs6280	C (0.31)	398	0.010	0.927	0.065	0.829
Neurotrophic	NTRK3	86595863	rs4887400	C (0.35)	398	0.197	0.387	0.084	0.830
Neurotrophic	NTRK2	86497392	rs7858590	T (0.40)	388	0.544	0.673	0.774	0.869
Angiogenic	HIF1A	61276301	rs2301113	C (0.21)	385	0.998	0.937	0.731	0.870
Angiogenic	VEGFR1	27781061	rs7993418	G (0.20)	397	0.746	0.489	0.843	0.885
Angiogenic	NRP1	33550669	rs4934838	A (0.25)	397	0.985	0.724	0.910	0.887
Angiogenic	VEGFR1	27918256	rs7330109	T (0.31)	398	0.365	0.614	0.461	0.890
CD1	CD1B	155116945	rs716221	T (0.21)	397	0.599	0.891	0.718	0.903
Dopaminergic	COMT	18324789	rs4633	C (0.49)	398	0.315	0.847	0.506	0.904
Angiogenic	VEGFR2	55660296	rs10016788	G (0.45)	393	0.033	0.895	0.023	0.940
CD1	CD1B	155108702	rs10797007	G (0.33)	395	0.636	0.969	0.672	0.947
Angiogenic	NRP1	33565842	rs927099	T (0.48)	396	0.341	0.758	0.256	0.974
Neurotrophic	NTRK3	86470167	rs1426300	C (0.52)	388	0.286	0.961	0.134	0.974
Angiogenic	VEGFR1	27956068	rs12858139	A (0.45)	396	0.627	0.758	0.384	0.979
Angiogenic	NRP1	33583935	rs1319013	T (0.46)	396	0.855	0.608	0.377	0.983

Table 8

Mean CPRS AD/HD Total Score by Minor Allele Genotype for Nominally Significant Main Effect SNPs

Gene	SNP	Minor Allele	0	1	2
BDNF	rs6265	T	78.18 (10.21) n = 255	77.11 (10.33) n = 130	70.69 (13.94) n = 13
BDNF	rs7127507	C	76.27 (11.14) n = 189	78.20 (9.83) n = 169	81.03 (8.53) n = 38
COMT	rs9332377	T	77.90 (10.37) n = 286	76.49 (9.65) n = 93	84.50 (0.71) N = 2
DAT1	rs456774	C	76.94 (10.88) n = 245	78.85 (9.27) n = 125	80.96 (7.95) n = 23
DRD2	rs4350392	A	77.01 (10.96) n = 297	78.80 (8.62) n = 93	84.14 (6.47) n = 7
DRD3	rs324035	A	77.73 (10.18) n = 267	76.79 (11.13) n = 123	84.43 (2.88) n = 7
NRP1	rs2474712	C	78.20 (10.09) n = 255	77.16 (10.41) n = 127	69.93 (13.73) n = 15
NRP1	rs2776930	C	77.49 (10.77) n = 304	77.49 (9.42) n = 88	86.00 (4.58) n = 3
NTRK2	rs10780796	T	75.99 (11.25) n = 142	78.62 (10.11) n = 195	78.40 (8.73) n = 60
NTRK3	rs999905	C	78.67 (10.03) n = 182	77.51 (10.20) n = 165	73.98 (11.99) n = 51
NTRK3	rs3825885	C	77.59 (10.82) n = 184	78.84 (8.91) n = 169	72.91 (13.20) n = 43
NTRK3	rs7176444	G	77.71 (10.66) n = 329	77.68 (8.11) n = 57	88.50 (2.12) n = 2
NTRK3	rs8037291	G	77.51 (10.00) n = 268	79.93 (9.66) n = 104	74.00 (8.80) n = 9
VEGFR1	rs2104330	C	79.05 (8.88) n = 104	76.48 (10.95) n = 210	79.21 (8.45) n = 81
VEGFR2	rs10016788	G	79.42 (8.80) n = 104	76.07 (11.79) n = 196	78.65 (8.58) n = 78

Note. Standard Deviation in ().

Table 9

Main Effect of SNPs with MAF between .10 and .20 and Interaction between SNPs and Birth Weight Centile Range Predicting the Transformed CPRS ADHD Total Score

						p - value		FDR *q* -value	
System	Gene	NCBI36	SNP	Allele (Freq)	# of Youth	SNP Main Effect	Interaction	SNP Main Effect	Interaction
Angiogenic	HIF1A	61283601	rs2057482	T (0.13)	390	0.2105	<0.0001	0.6589	<0.0001
Angiogenic	HIF1A	61236316	rs2301106	C (0.11)	398	0.2645	<0.0001	0.7053	<0.0001
Neurotrophic	NTRK3	86558642	rs7176444	G (0.08)	388	0.0434	<0.0001	0.4803	<0.0001
Angiogenic	NRP1	33589567	rs11598845	C (0.14)	390	0.2519	<0.0001	0.7113	<0.0001
CD1	CD1B	155113379	rs962879	C (0.12)	398	0.8905	<0.0001	1.0018	0.0001
Neurotrophic	NTRK3	86308935	rs8037291	G (0.16)	381	0.0190	0.0003	0.4558	0.0071
Neurotrophic	NTRK3	86426100	rs17755717	A (0.2)	378	0.4665	0.0030	0.9077	0.0616
Neurotrophic	NTRK3	86355556	rs1017757	G (0.13)	391	0.0977	0.0064	0.5864	0.1148
Angiogenic	NRP1	33627887	rs2776930	C (0.12)	395	<0.0001	0.0151	0.0002	0.2176
Angiogenic	NRP1	33636052	rs4934901	T (0.17)	396	0.6243	0.0157	0.9268	0.2059
Angiogenic	NRP1	33645682	rs2804493	A (0.14)	396	0.2964	0.0298	0.7358	0.2862
Neurotrophic	NTRK3	86465797	rs2114251	A (0.17)	385	0.3781	0.0463	0.8249	0.3332
Angiogenic	NRP2	206266851	rs13419677	C (0.16)	396	0.0719	0.0679	0.5179	0.4254
Dopaminergic	DRD3	115351544	rs324035	A (0.17)	397	0.0010	0.0685	0.0487	0.4110
Angiogenic	VEGFR1	27937214	rs622227	C (0.14)	398	0.6350	0.0986	0.9331	0.4895
Angiogenic	NRP1	33588382	rs10490938	T (0.18)	398	0.1953	0.1007	0.6393	0.4679
Dopaminergic	DRD2	112787300	rs1124491	A (0.16)	395	0.8959	0.1360	1.0001	0.5441
Angiogenic	NRP1	33566833	rs12765284	A (0.11)	394	0.5927	0.1492	0.9378	0.5509
Neurotrophic	NTRK3	86254661	rs6496455	A (0.16)	382	0.7647	0.1699	0.9921	0.5691
Angiogenic	NRP1	33587471	rs3780869	T (0.16)	398	0.7930	0.1715	0.9929	0.5614
Angiogenic	VEGFR1	27866510	rs17537653	A (0.13)	378	0.9525	0.2181	0.9940	0.6683

System	Gene	NCBI36	SNP	Allele (Freq)	# of Youth	*p* - value		FDR *q* -value	
						SNP Main Effect	Interaction	SNP Main Effect	Interaction
Dopaminergic	DRD2	112840927	rs4350392	A (0.13)	397	0.0186	0.2281	0.5370	0.6844
Angiogenic	NRP1	33511136	rs2506145	C (0.12)	398	0.1941	0.2350	0.6499	0.6767
Neurotrophic	NTRK3	86265806	rs1836592	A (0.21)	392	0.2878	0.2857	0.7270	0.7217
Angiogenic	NRP1	33597285	rs10827224	C (0.16)	398	0.6040	0.2977	0.9352	0.7267
Angiogenic	VEGFA	43859053	rs3025033	G (0.14)	398	0.1070	0.3961	0.5315	0.8776
Angiogenic	NRP1	33569034	rs2269091	T (0.19)	393	0.1675	0.4304	0.6346	0.8853
Angiogenic	NRP1	33508175	rs2506143	G (0.14)	394	0.3567	0.4710	0.8026	0.9044
Neurotrophic	NTRK3	86358314	rs1350799	C (0.12)	390	0.2345	0.5061	0.7185	0.9225
Angiogenic	NRP1	33639848	rs2776934	G (0.16)	388	0.7144	0.5084	0.9891	0.9152
Angiogenic	NRP1	33603899	rs2243668	A (0.12)	397	0.8573	0.5993	0.9876	0.9696
Angiogenic	NRP1	33560360	rs11009311	A (0.17)	395	0.9109	0.6025	0.9937	0.9641
Angiogenic	NRP1	33652767	rs10827234	C (0.16)	396	0.9492	0.6212	0.9977	0.9722
Angiogenic	VEGFR2	55672161	rs6554217	T (0.18)	385	0.5472	0.6527	0.9271	0.9493
Neurotrophic	NTRK2	86664490	rs1576161	T (0.17)	398	0.2458	0.6711	0.7079	0.9568
Angiogenic	NRP2	206278364	rs10432438	T (0.17)	391	0.1013	0.7697	0.5609	0.9809
Angiogenic	NRP1	33506572	rs1044268	T (0.12)	398	0.4080	0.7823	0.8514	0.9795
Neurotrophic	NTRK3	86454485	rs3784415	C (0.12)	392	0.1487	0.7850	0.6691	0.9745
Angiogenic	NRP1	33574886	rs10490939	A (0.15)	397	0.2686	0.8120	0.7034	0.9744
Angiogenic	VEGFR1	27807835	rs7982251	C (0.14)	395	0.5508	0.8187	0.9223	0.9664
Angiogenic	HIF1A	61259284	rs4899056	T (0.12)	381	0.6531	0.8247	0.9405	0.9656
Dopaminergic	DRD2	112792088	rs2440390	T (0.14)	395	0.8285	0.8370	0.9779	0.9565
Angiogenic	NRP1	33551053	rs2273466	C (0.18)	398	0.2966	0.8890	0.7238	0.9772
Dopaminergic	COMT	18330246	rs9332377	T (0.13)	381	<0.0001	0.9364	<0.0001	0.9988
CD1	CD1B	155107015	rs10908647	G (0.11)	396	0.2823	0.9468	0.7258	0.9952
Dopaminergic	DRD2	112811975	rs4436578	C (0.11)	394	0.1515	0.9550	0.6415	0.9893
Angiogenic	NRP1	33537715	rs2474712	C (0.2)	397	0.0224	0.9713	0.4040	0.9990

Table 10

Location and Function of SNPs Involved in Significant GxE Interactions

System	Chromosome Location	Gene	SNP	Location in Gene	Function
Angiogenic	14q21-24	HIF1A	rs2057482	3' UTR	Transcriptional regulation
Angiogenic	14q21-24	HIF1A	rs2301106	Intron	Transcriptional regulation
Neurotrophic	15q25	NTRK3	rs7176444	Intron	Transcriptional regulation
Angiogenic	10p12	NRP1	rs11598845	Intron	No none function
CD1	1q21-q22	CD1B	rs962879	Intron	Transcriptional regulation
Neurotrophic	15q25	NTRK3	rs8037291	Intron	No none function

Note. SNP function reported from F-SNP (http://compbio.cs.queensu.ca/F-SNP/; Lee & Shatkay, 2008).

Figure 1. Model of the complex interplay between genetic and environmental factors underlying the association between AD/HD and low birth weight. Intergen. = Intergenerational; Environ. = Environment; LBW = Low Birth Weight; ND = Neurodevelopmental.

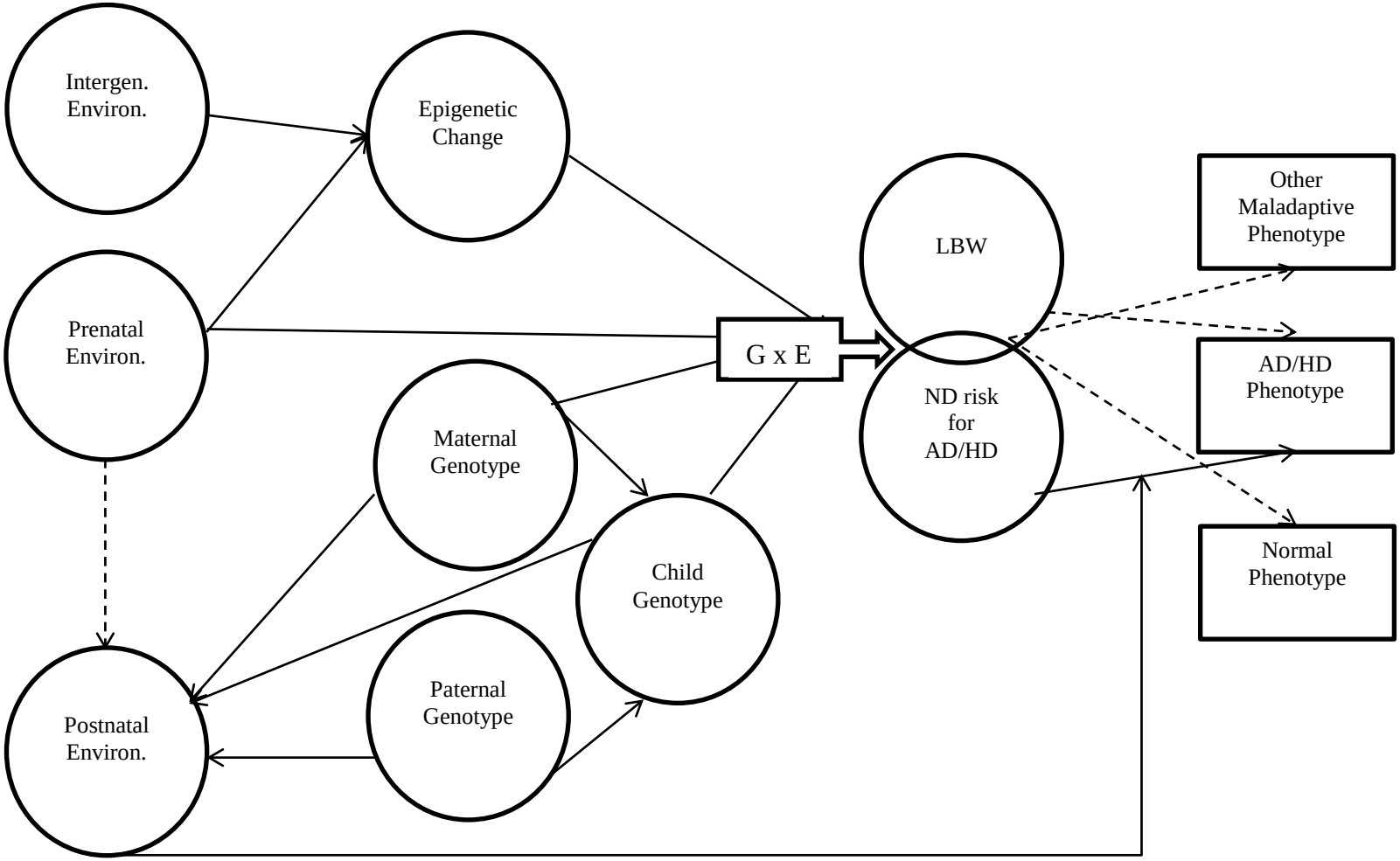


Figure 2. Nominally significant SNP within angiogenic genes by birth weight centile range interactions predicting ADHD symptom severity. p -value for each interaction < .05 after controlling for age, sex, research site and main effects of SNP and birth weight centile range.

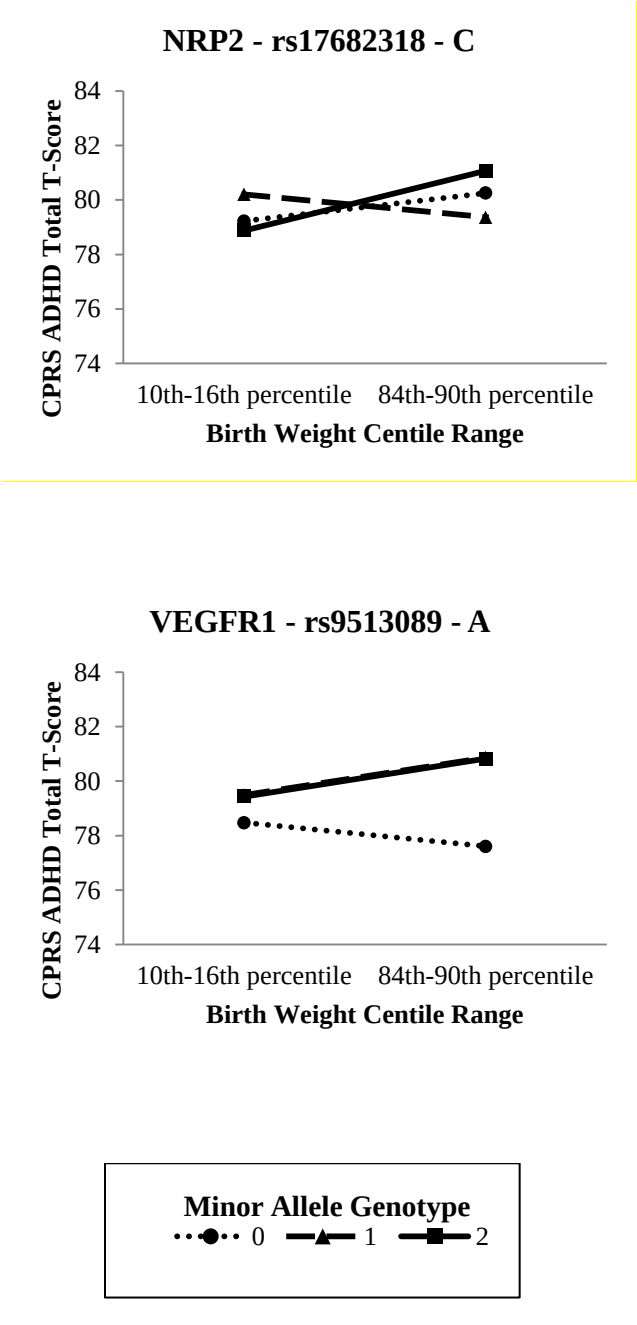
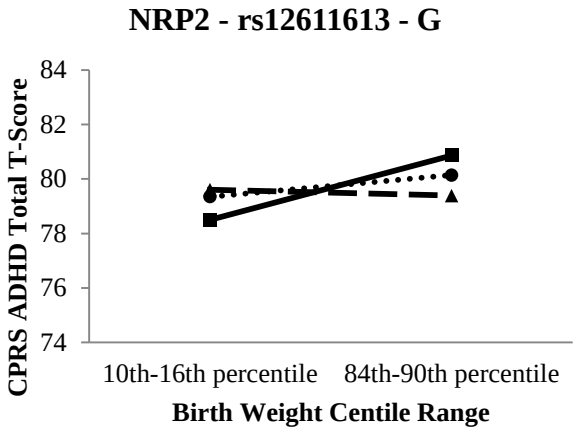
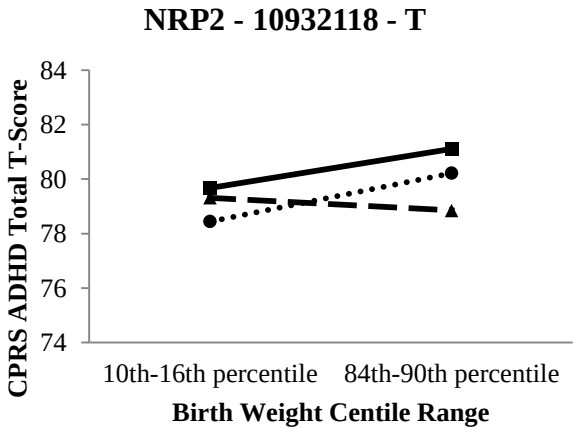


Figure 2 (Continued). Nominally significant SNP within angiogenic genes by birth weight centile range interactions predicting ADHD symptom severity. *p* -value for each interaction < .05 after controlling for age, sex, research site and main effects of SNP and birth weight centile range.





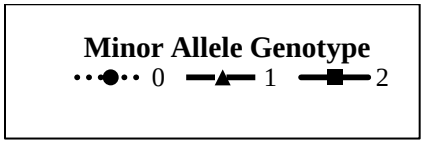
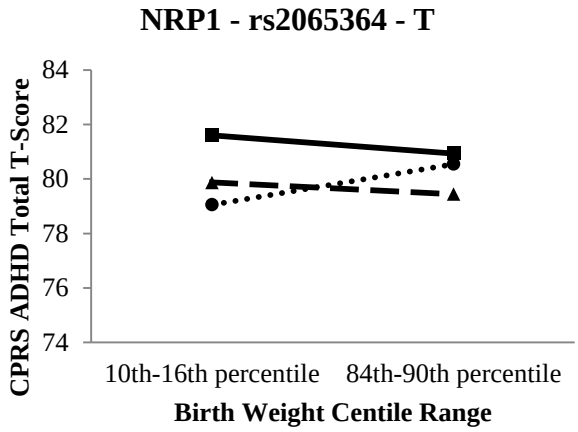
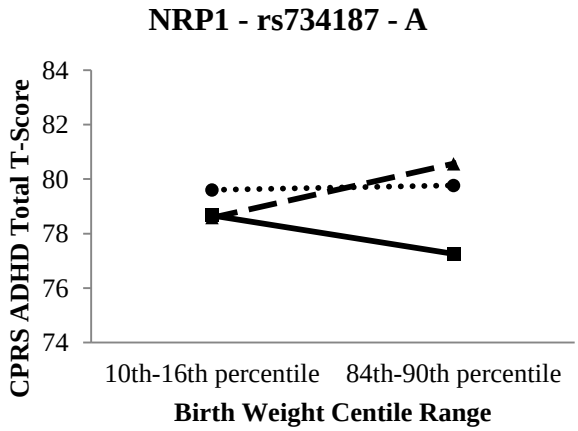


Figure 2 (Continued). Nominally significant SNP within angiogenic genes by birth weight centile range interactions predicting ADHD symptom severity. *p* -value for each interaction < .05 after controlling for age, sex, research site and main effects of SNP and birth weight centile range.





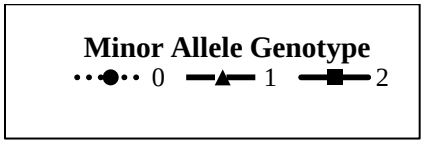
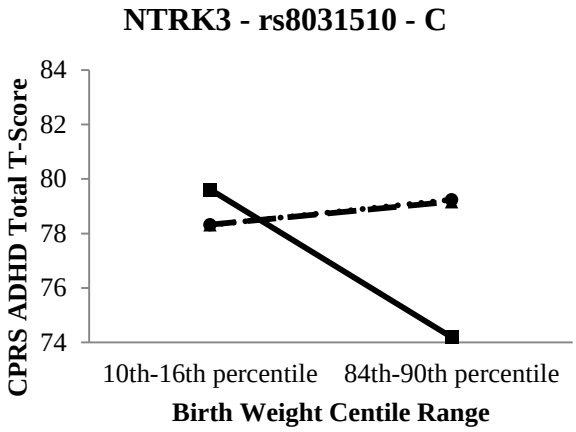
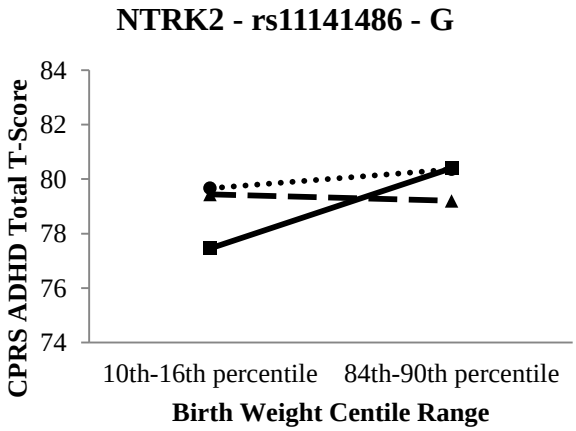


Figure 3. Nominally significant SNP within neurotrophic genes by birth weight centile range interactions predicting ADHD symptom severity. p-value for each interaction < .05 after controlling for age, sex, research site and main effects of SNP and birth weight centile range.





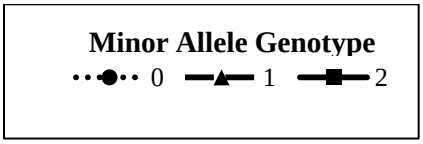
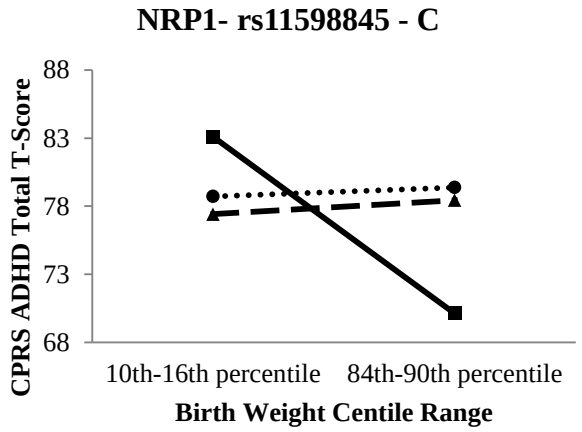
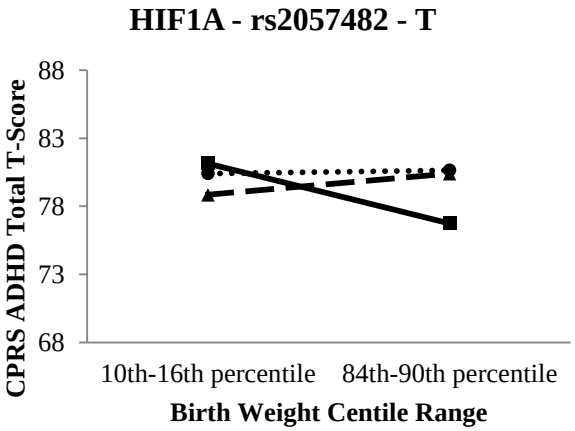


Figure 4. SNP minor allele genotype by birth weight centile range interaction predicting ADHD symptom severity. FDR adjusted p-value for each interaction < .05 after controlling for research site, age, and sex, and main effects of SNPs and birth weight centile range.





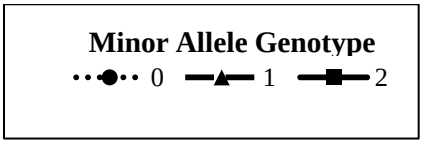
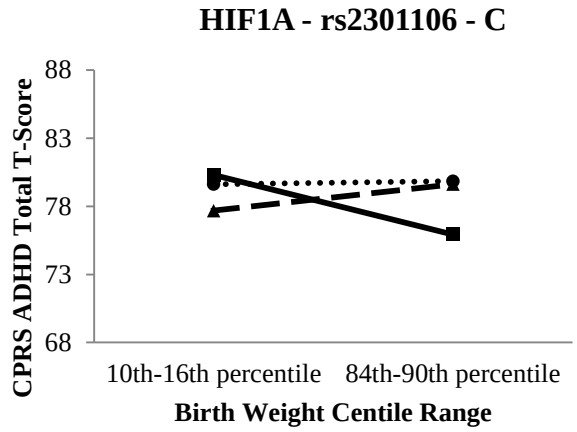
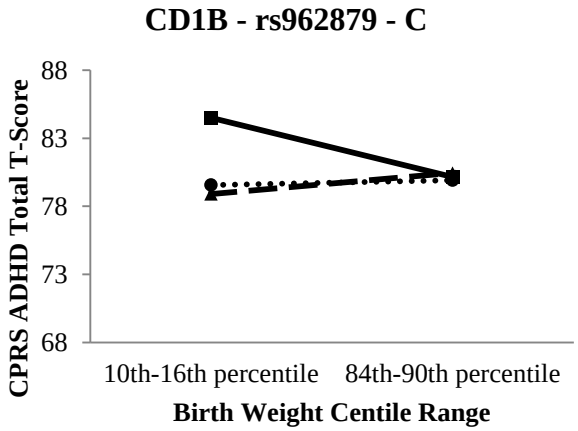


Figure 4 (Continued). SNP minor allele genotype by birth weight centile range interaction predicting ADHD symptom severity. FDR adjusted p-value for each interaction < .05 after controlling for research site, age, and sex, and main effects of SNPs and birth weight centile range.





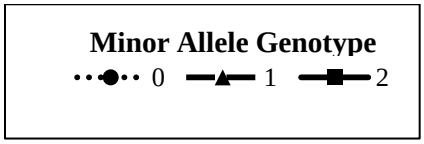
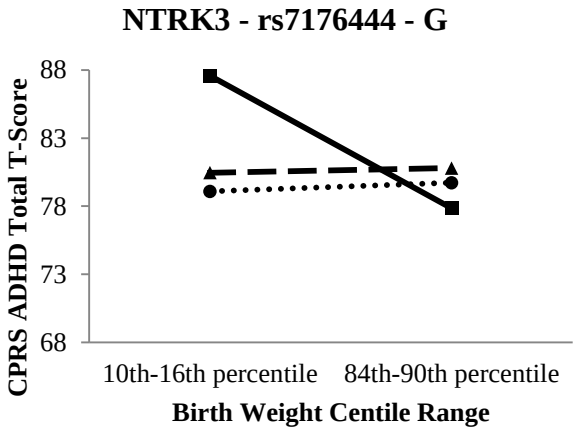
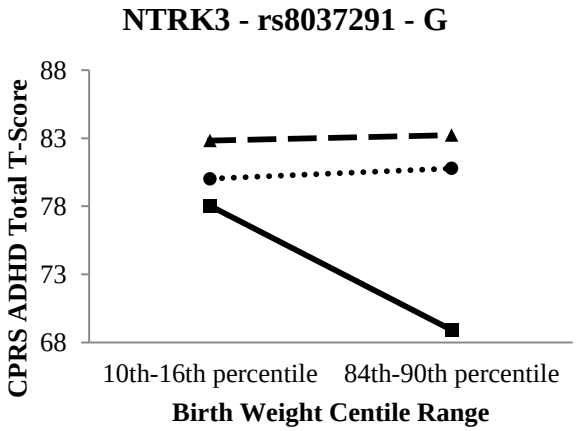


Figure 4 (Continued). SNP minor allele genotype by birth weight centile range interaction predicting ADHD symptom severity. FDR adjusted p-value for each interaction < .05 after controlling for research site, age, and sex, and main effects of SNPs and birth weight centile range.





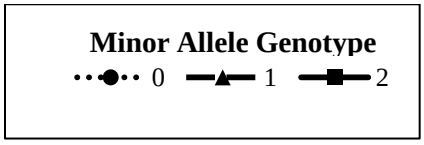
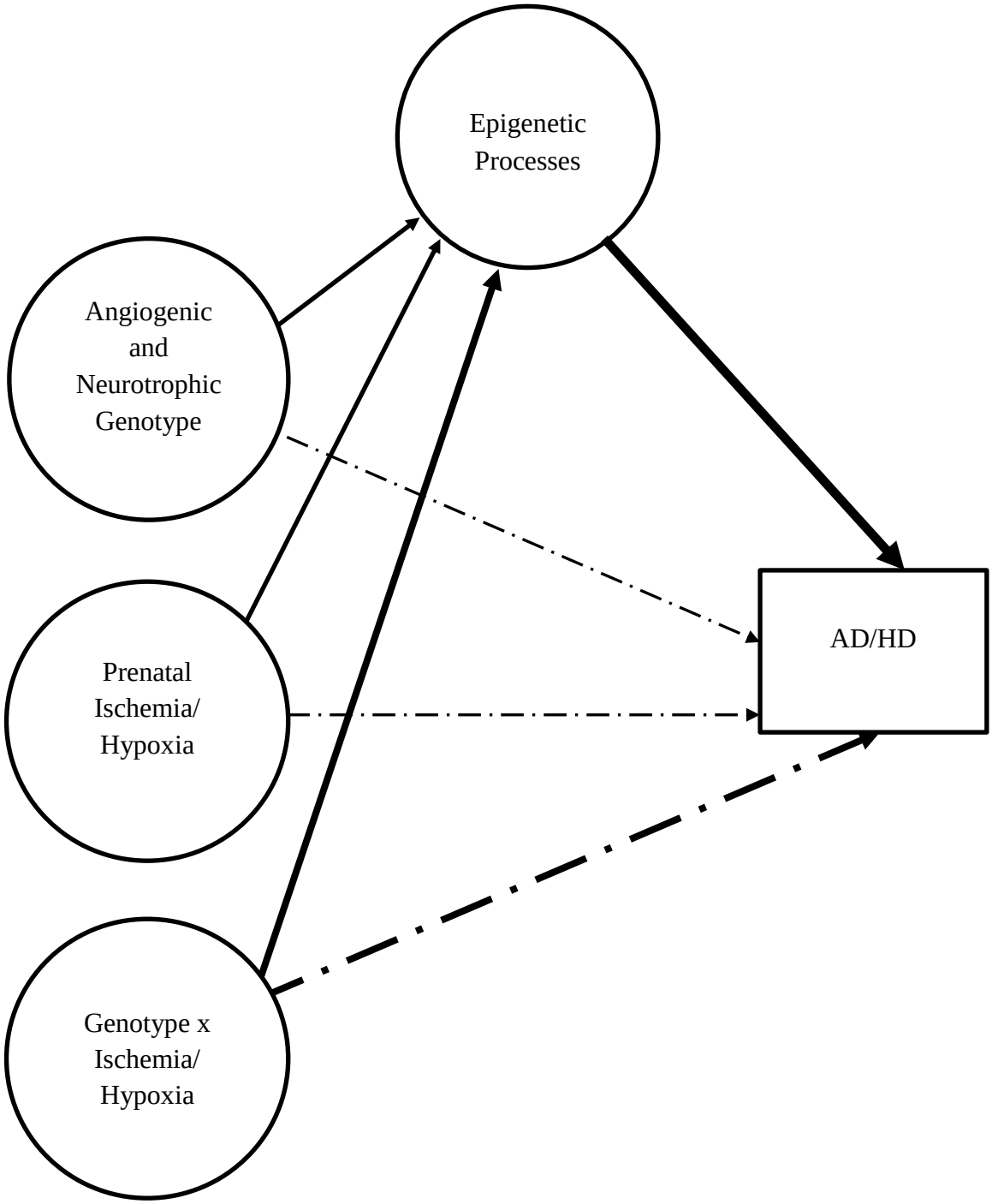


Figure 5. Proposed model of epigenetic processes mediating the relationship between gene-environment interaction and AD/HD.



APPENDIX B:

MEASURES

DEVELOPMENTAL HISTORY

I. PREGNANCY AND DELIVERY **Child's Name:_____**

Length of pregnancy (e.g., full term or 40 weeks, 32 weeks, etc.) _____
Length of delivery (number of hours from initial labor pains to birth) _____
Mother's age when child was born _____
Was the pregnancy with this child under a doctor's care? _____
Was the pregnancy a multiple birth (twins, triplets, etc)? _____

Did any of the following conditions occur during pregnancy/delivery?

	NO	YES
Anemia		
High blood pressure		
Swollen ankles		
Kidney disease		
Bleeding		
Excessive weight gain (more than 30 pounds)		
Toxemia/Preeclampsia		
Rh factor incompatibility		
Frequent nausea or vomiting		
Measles		
German Measles		
Flu		
Strep throat		
Other illness or injury		
Took prescription medication If YES, name of medication:		
Took illegal drugs		
Used alcoholic beverages If YES, approximate number of drinks per week:		
Smoked cigarettes If YES, approximate number of cigarettes per day:		
Was given medication to ease labor pains:		
Threatened miscarriage		
Premature labor		
Delivery was induced		
Had a breech delivery		
Had a Cesarean section delivery		

Severe emotional problems		
Emotional distress		
Other problems….Please describe:		

Did any of the following affect your child during delivery or within the first few days after birth?

	NO	**YES**
Injured during delivery		
Cardiopulmonary distress during deliver		
Delivered with cord around neck		
Had trouble breathing following delivery		
Needed oxygen		
Was cyanotic, turned blue		
Was jaundiced, turned yellow		
Had an infection		
Had seizures		
Was given medications		
Born with congenital defect		
Was in hospital more than seven days		