Distribution Agreement

In presenting this dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this dissertation. I retain all ownership rights to the copyright of the dissertation. I also retain the right to use in future works (such as articles or books) all or part of this dissertation.

Signature:

_____ _____

Kelly M. Harrington Date

Evaluating the External Validity of *DSM-IV* ADHD Subtypes and an Alternative Diagnostic Subtyping System

By

Kelly M. Harrington
Doctor of Philosophy

Psychology

_____

Irwin D. Waldman, Ph.D.
Advisor

_____

Stephan Hamann, Ph.D.
Committee Member

_____

Scott O. Lilienfeld, Ph.D.
Committee Member

_____

Donna L. Maney, Ph.D.
Committee Member

_____

Elaine Walker, Ph.D.
Committee Member

Accepted:

_____

Lisa A. Tedesco, Ph.D.
Dean of the Graduate School

_____

Date

Evaluating the External Validity of *DSM-IV* ADHD Subtypes and an Alternative Diagnostic Subtyping System

By

Kelly M. Harrington
B.S., Cornell University, 1999
M.A., Emory University, 2004

Advisor: Irwin Waldman, Ph.D.

An abstract of
A dissertation submitted to the Faculty of the Graduate School of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
in Psychology
2008

Abstract

Evaluating the External Validity of *DSM-IV* ADHD Subtypes and an Alternative Diagnostic Subtyping System

By Kelly M. Harrington

Heterogeneity within the attention-deficit/hyperactivity disorder (ADHD) diagnosis likely accounts for some inconsistent findings in molecular genetic studies of ADHD. The present dissertation evaluated whether an alternative comorbid ADHD phenotype increases the external validity over and above the extant *DSM-IV* diagnostic subtypes by identifying more homogeneous conditions genetically and with regard to sex, age, and overlapping disorders. The sample included 372 children (ages 5 – 18) who were recruited as part of an ongoing study on the genetics of ADHD. Probands, their siblings, and parents were genotyped for a variable number of tandem repeats (VNTR) in exon 3 of the dopamine D4 receptor gene (*DRD4)* and an insertion / deletion polymorphism in the promoter region of the serotonin transporter gene (*5-HTTLPR*). Planned comparisons revealed that the Combined subtype is characterized by a higher proportion of males, lower mean age, and higher symptom levels of oppositional defiant and conduct disorders, depression, and anxiety disorders compared with the Inattentive or Hyperactive-Impulsive subtypes. Genetic association analyses suggested that the *DRD4* 7-repeat allele is more strongly related to the Combined and Inattentive subtypes than the Hyperactive-Impulsive subtype, whereas the *5-HTTLPR* long allele is more strongly related to the Inattentive subtype compared with the other two subtypes. Furthermore, there was evidence suggesting that co-occurring symptoms of conduct disorder (CD) moderated the ADHD-*DRD4* relation, such that the association between ADHD and *DRD4* was stronger in children with both ADHD and elevated CD symptoms. There was also evidence of a moderating influence of co-occurring symptoms of anxiety, whereby the association between ADHD and *5-HTTLPR* was stronger in children with higher levels of anxiety. In contrast, there was no significant evidence of association between the overall ADHD diagnosis and the *DRD4* 7-repeat allele or the *5-HTTLPR* long allele. These findings suggest that when testing a phenotypically heterogeneous condition such as ADHD, undertaking subgroup analyses (i.e., examining either diagnostic subtypes or comorbid subgroups of ADHD) or incorporating continuous measures of overlapping psychopathology as moderators can be a useful strategy for enhancing external validity and our ability to detect genetic associations that might be otherwise obscured.

Evaluating the External Validity of *DSM-IV* ADHD Subtypes and an Alternative Diagnostic Subtyping System

By

Kelly M. Harrington
B.S., Cornell University, 1999
M.A., Emory University, 2004

Advisor: Irwin Waldman, Ph.D.

A dissertation submitted to the Faculty of the Graduate School of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
in Psychology
2008

Acknowledgements

I would first like to thank to my advisor, Irwin Waldman, for his important contributions, insights, and guidance throughout this project. I would also like to thank the other members of my committee, Stephan Hamann, Scott Lilienfeld, Donna Maney, and Elaine Walker, for the helpful suggestions they offered during the development and completion of this project. Finally, I would like to express my heartfelt gratitude towards my husband, parents, sister, and close friends for the tremendous support they provided throughout the process of completing my dissertation. I am particularly indebted to my husband, Daniel Tribuiani, for the unwavering encouragement, patience, and understanding he provided for the duration of my graduate training.

Table of Contents

List of Tables

Paper 1

Paper 2

List of Appendices

Appendix Page

General Introduction

Attention-deficit/hyperactivity disorder (ADHD) is regarded as the most prevalent and widely researched mental disorder of childhood, affecting roughly 3-7 % of school-age children (American Psychiatric Association [APA], 2000). The cardinal features of ADHD include inattention, hyperactivity, and impulsivity, though there has been considerable debate concerning the classification of the core deficit(s) associated with this disorder. The current operationalization of ADHD includes two distinct, but correlated symptom dimensions (viz., inattention and hyperactivity-impulsivity), each consisting of 9 symptoms (APA, 2000). Specifically, the inattentive symptoms described in the *DSM-IV* (Diagnostic and Statistical Manual of Mental Disorders, 4th Ed.) include "often has difficulty sustaining attention in tasks or play activities" and "often fails to give close attention to details or makes careless mistakes" (APA, 2000). The hyperactive-impulsive symptoms include "often fidgets with hands or feet" and "often has difficulty awaiting turn" (APA, 2000).

Children with ADHD are also at heightened risk for several negative sequelae, including decreased educational attainment, lower income, underemployment, and impaired social relationships (Greene et al., 2001; Mannuzza et al., 1993), as well as legal difficulties and substance abuse, largely owing to the frequent overlap between ADHD and conduct disorder (CD) (Biederman et al., 2003; Foley, Carlton, & Howell, 1996). The nature of ADHD symptoms also places individuals at higher risk for motor vehicle accidents, traffic citations, and severe injuries (Barkley et al., 1996; DiScala et al., 1998; Nada-Raja et al., 1997). Given the seriousness of the adverse outcomes associated with ADHD, a solid understanding of its etiology is paramount for improving the prevention and treatment of this impairing condition. Furthermore, accurate and valid characterization of the ADHD

phenotype is crucial before quantitative and molecular genetic methods can be maximally utilized to uncover the genetic and environmental risk factors that give rise to ADHD (Lahey et al., 2004).

Brief Historical Overview of ADHD Taxonomy

The taxonomic history of ADHD has been marked by controversy regarding the best way to define and classify this disorder. Since it was originally described as "Hyperkinetic Reaction of Childhood" in the *DSM-II* (APA, 1968), the conceptualization of ADHD has varied considerably. The criteria presented in *DSM-III* (APA, 1980) constituted the first contemporary definition of ADHD and represented a major conceptual shift from a unidimensional classification system to a multidimensional classification system. The *DSM-III* introduced the diagnostic term "Attention Deficit Disorder" (ADD) and proposed two subtypes of ADD to delineate children who displayed symptoms of inattention, impulsivity, and hyperactivity (ADD/H) from children whose symptom presentation included inattention and impulsivity in the absence of hyperactivity (ADD/WO) (Lahey, Schaughency, Strauss, & Frame, 1984). In the next revision of the ADHD taxonomy (i.e., *DSM-III-R* [APA, 1987]), the diagnostic subtypes were eliminated and replaced by a unidimensional approach to ADHD. The key features of *DSM-III-R* ADHD included inattention, impulsivity, and hyperactivity, but no essential characteristics were designated (i.e., any combination of eight or more symptom criteria were sufficient for the diagnosis). The shift from the three-dimensional *DSM-III* definition to the single dimension *DSM-III-R* definition was met with controversy and stimulated extensive research on the taxonomic structure of ADD symptoms (e.g., the number of symptom dimensions that underlie the disorder).

An influential paper by Lahey et al. (1988) demonstrated via factor analytic methods that a two-factor solution provided a more accurate and parsimonious model of ADHD than the three-factor model implied in *DSM-III*. Lahey et al.'s factor analyses yielded one factor composed of inattention and disorganization symptoms and a second factor consisting of motor hyperactivity and impulsivity symptoms. Subsequent empirical and clinical evidence provided support for a multidimensional model (for reviews, see Lahey & Carlson, 1991; Lahey, Carlson, & Frick, 1997) and led to the reinstatement of a subtyping system for ADHD in *DSM-IV* (APA, 1994, 2000). In the current *DSM-IV* nomenclature, the definition of ADHD includes three subtypes— the Predominantly Inattentive type (IT), the Predominantly Hyperactive-Impulsive type (HT), and the Combined type (CT)—characterized by surpassing thresholds on inattentive and/or hyperactive-impulsive symptom dimensions.

Validity of the DSM-IV ADHD Diagnosis

In addition to ongoing debate regarding how to best classify ADHD, some critics have further challenged whether ADHD is truly a valid disorder that requires medical treatment (e.g., Baughman, 2001; McCubbin & Cohen, 1997). In a recent review, Faraone (2005) evaluated whether ADHD is in fact a valid diagnostic category by applying Robins and Guze's (1970) seminal criteria for establishing the validity of a psychiatric diagnosis. Robins and Guze's validational criteria were based on the fundamental concept that the validity of any diagnosis must derive from empirical research demonstrating the diagnosis is distinguishable from other diagnoses and shows well-defined clinical correlates, a characteristic course and outcome, evidence of heritability from family and genetic studies, distinct neurobiological correlates, and a characteristic response to treatment. Faraone's

review of extant empirical studies revealed compelling evidence that ADHD fulfills all of these criteria for a valid diagnostic category (albeit to varying degrees).

Although there is ample evidence to support the notion that ADHD is a valid psychiatric disorder overall, there nevertheless have been conflicting findings among studies with respect to understanding its epidemiology, etiology, and treatment (Faraone, 2005). Notably, molecular genetic studies of ADHD represent one such domain wherein there is a mixed picture of positive and negative findings, as will be discussed below. Additionally, there have been relatively few tests of external validity conducted on the *DSM-IV* nomenclature for childhood disorders, including ADHD (Lahey et al., 2008). Moreover, evidence supporting the distinct validity of the *DSM-IV* ADHD subtypes is particularly scant (for recent reviews, see Woo & Rey, 2005; Baeyens, Roeyers, & Walle, 2006). Nonetheless, some important differences between the ADHD subtypes have been documented (e.g., in prevalence, mean age, gender ratios, and rates of co-occurring conditions) that to some researchers are suggestive of distinct underlying pathology and etiologies, and these have been summarized in a recent qualitative review (Milich, Ballentine, & Lynam, 2001).

To briefly summarize, significant changes in the classification of ADHD can be largely attributed to ongoing debate concerning the core deficit(s) associated with this childhood disorder, as well as whether ADHD and its components are best conceptualized as one or more symptom dimensions or diagnostic categories (Carlson & Mann, 2002; Lahey & Willcutt, 2002). Despite intense taxonomic study of ADHD, there is ongoing debate among researchers with respect to the best way to classify and diagnose this prevalent childhood disorder. Specifically, the alternative possibilities include

whether ADHD should be conceptualized as a) a unitary disorder, b) a unitary disorder with subtypes, or c) multiple distinct disorders. Thus, there is clearly a need for further external validation studies of *DSM-IV* ADHD, as well as its constituent diagnostic subtypes and symptom dimensions.

Genetic Influences of ADHD

Evidence from twin, family, and adoption studies suggests that substantial genetic influences contribute to ADHD, with heritability estimates ranging from 60 to 90% (Waldman & Rhee, 2002). It is noteworthy that these heritability estimates have shown considerable consistency across studies, regardless of whether ADHD is defined as a discrete diagnostic category or in terms of the continuous inattentive and hyperactive-impulsive symptom dimensions (e.g., Goodman & Stevenson, 1989; Levy et al., 1997). Given strong evidence of genetic influences from quantitative genetic studies, the search for specific susceptibility genes for ADHD would seem to hold great promise. Unfortunately, the fact that ADHD is a genetically complex disorder, like most psychiatric disorders, has significantly complicated this search (Faraone & Biederman, 1998; Kendler, 2005).

Considering the range of complexities involved in the genetic influences underlying complex traits and disorders (e.g., multiple predisposing genes, genetic heterogeneity, risk alleles with high allele frequencies), it appears that there are a multitude of susceptibility genes implicated in their underlying causes, each contributing only a small fraction of the overall risk for the disorder. Indeed, there exists a general consensus among researchers that the etiology of ADHD is polygenic (i.e., caused by the combined effects of three or more genetic loci and environmental influences), whereby

the effects of a single gene are neither necessary nor sufficient for the development of the disorder (Farone & Khan, 2006; Plomin & Crabbe, 2000). These complex inheritance patterns pose considerable challenges when conducting molecular genetic research, and help explain the difficulties in replicating initial significant findings, as well as the small effect sizes (i.e., each gene accounts for less than 5% of the variance associated with a disorder of interest) that are typically observed in candidate gene studies of ADHD (Waldman & Gizer, 2006). Importantly, this pattern of inconsistent findings is not unique to ADHD, rather it is characteristic for candidate gene studies of all psychiatric and complex medical disorders (Ioannidis, Ntzani, Trikalinos, & Contopulos-Ionannidis, 2001).

Candidate Gene Studies of ADHD

Molecular genetic studies of ADHD have typically utilized a ***candidate gene approach*** to identify the specific genes related to the etiology of this disorder (Mick & Faraone, 2008). In well-designed candidate gene studies, the location, function, and etiological relevance of candidate genes are most often known or strongly hypothesized ***a priori***, thus allowing for a targeted test of the role of specific genes in the etiology of the disorder of interest. Genes are selected based on the known or hypothesized involvement of their gene product in the etiology of the trait or disorder, such as its pathophysiological function and etiological relevance (Alsobrook & Pauls, 1998; Waldman & Gizer, 2006). Regarding ADHD, the majority of molecular genetic studies have concentrated on candidate genes underlying various aspects of the dopaminergic, and to a lesser extent the serotonergic and noradrenergic, neurotransmitter pathway on the basis of mounting evidence suggesting these neurotransmitter systems are involved in the etiology and

pathophysiology of ADHD (Waldman & Gizer, 2006). Despite the perennial difficulty of nonreplication in the field of psychiatric genetics, several candidate genes have shown evidence of replicable associations with ADHD, including the dopamine transporter gene (*DAT1*), the dopamine D4 and D5 receptor genes (*DRD4* and *DRD5*, respectively), and the serotonin transporter gene (*5-HTT*) (Faraone & Khan, 2006).

A polymorphism is a sequence of DNA that has more than one form (or allele), each with a frequency of at least 1% in the general population (Speer, 1998). Polymorphisms are the source of genetic variation that result in individual differences, and as such, are of central interest in molecular genetic studies. The current study utilizes two types of polymorphisms to test for genetic association between two candidate genes and ADHD, namely a variable number of tandem repeats (VNTR) polymorphism in exon 3 of *DRD4* and an insertion / deletion polymorphism in the promoter region of *5-HTT*. A VNTR is a polymorphism classified by the length of a sequence consisting of varying numbers of repeats of a DNA sequence (e.g., 2-repeat vs. 4-repeat vs. 7-repeat) (Alsobrook & Pauls, 1998). As its name suggests, an insertion / deletion polymorphism involves the insertion or deletion of DNA into an existing sequence (Speer, 1998). The *5-HTT* promoter polymorphism is characterized by a 44-base pair insertion or deletion involving repeat elements 6 to 8, resulting in two common alleles that differ in length by 44-base pairs (Heils et al., 1996).

Meta-analysis is an increasingly common approach for resolving inconsistent findings across candidate gene studies, allowing an evaluation of the overall strength of evidence for association as well as formal tests for possible evidence of publication bias (Munafo & Flint, 2004). For example, the association between *DRD4* and ADHD has

been examined in numerous independent studies, yielding both positive and negative findings. Notably, the results of 3 recent meta-analyses (Faraone, Doyle, Mick, & Biederman, 2001; Faraone et al., 2005; Maher, Marazita, Ferrell, & Vanyukov, 2002) demonstrated overall strong support for a significant association between the 7-repeat allele of *DRD4* and ADHD, with pooled odds ratios (OR) ranging from 1.4 - 1.9 for case-control studies and from 1.2 – 1.4 for within-family studies (an OR of 1.0 indicates no association). Similarly, studies of the association between the long allele of *5-HTT* and ADHD have revealed mixed results. Nonetheless, a recent meta-analysis by Faraone et al. (2005) showed a weak positive association between the long allele of the *5-HTT* promoter polymorphism and ADHD, with a pooled OR of 1.3. In summary, *DRD4* and *5-HTT* were selected for study in this dissertation based on evidence for 1) their replicable associations with ADHD, 2) the functional relevance of each gene in the pathophysiology of ADHD, and 3) initial support for differentiable patterns of association among the diagnostic subtypes. The rationale for selecting *DRD4* and *5-HTT* will be reviewed in further detail in the individual papers comprising the dissertation.

Relation between ADHD and Common Co-occurring Childhood Disorders

The presence of high rates of psychiatric comorbidity in children with ADHD poses an additional challenge to the existing classification system and has complicated the diagnosis and treatment of this disorder (reviewed in Jensen, Martin, & Cantwell, 1997; Willcutt, Pennington, Chhabildas, Friedman, & Alexander, 1999). The common co-occurrence of ADHD, oppositional defiant disorder (ODD), and conduct disorder (CD) has been consistently reported in epidemiological as well as clinical samples, with an estimated 30 - 45% of children diagnosed with ADHD also having concurrent ODD and 20% having

concurrent CD (Acosta, Arcos-Burgos, & Muenke, 2004; Biederman, Newcorn, & Sprich, 1991). Findings from both family and twin studies suggest that much of this overlap among externalizing disorders can be attributed to a common genetic etiology (e.g., Coolidge, Thede, & Young, 2000; Dick, Viken, Kaprio, Pulkkinen, & Rose, 2005; Nadder, Silberg, Eaves, Maes, & Meyer, 1998; Silberg et al., 1996). In fact, some investigators have found that the genetic correlation between ODD and CD is sufficiently high to argue that these symptoms are part of a joint construct (e.g., Eaves et al., 2000; Nadder, Rutter, Silberg, Maes, & Eaves, 2002), though other studies have provided evidence for the distinction between ODD and CD (e.g., Burns et al., 1997; Loeber, Lahey, & Thomas, 1991; Waldman, Rhee, Levy, & Hay, 2001).

In contrast to the well documented co-occurrence of childhood externalizing disorders (ADHD, ODD, and CD), the co-occurrence of internalizing disorders (depression and anxiety) in children with ADHD has been less recognized and understudied. Nonetheless, both clinical and epidemiological studies have shown that children with ADHD also frequently experience co-occurring symptoms of anxiety and depressive disorders. Many studies of ADHD have shown rates of comorbid anxiety disorders to be as high as 25% (Schatz & Rostain, 2006), whereas the estimated prevalence of depressive disorders in children diagnosed with ADHD ranges from 10 - 20% (Biederman et al., 1991).

Approaches to Identifying Homogeneous Phenotypes within the ADHD Diagnosis

The search for specific susceptibility genes underlying ADHD may be further complicated by phenotypic heterogeneity within the ADHD diagnosis. More specifically, diagnostic or phenotypic heterogeneity may represent another source of discrepancy across

candidate gene studies of ADHD. A number of investigators have described ADHD as a heterogeneous condition, with regard to its variability in the presentation of core symptoms, its clinical correlates (e.g., demographic characteristics, rates of comorbidity, outcome), and arguably its etiology (Faraone et al., 1995; Hechtman, 1999; Jensen et al., 1997; Newcorn et al., 2001; Todd, 2000). Despite increasing recognition that the phenotypic heterogeneity within ADHD may compromise the validity of this diagnosis, the majority of molecular genetic studies have investigated the relation between a gene of interest and the categorical ADHD diagnosis (Kirley et al., 2004). Thus, tests of association conducted using a single categorical diagnosis of ADHD might mask a significant effect if the candidate gene is associated only with certain aspects of the ADHD phenotype (e.g., a particular diagnostic subtype or comorbid subgroup).

The process of identifying more clinically homogeneous subgroups within the overall ADHD diagnosis plausibly would aid in the search for specific etiologies of, and the development of more specific treatment approaches for, such subgroups. Ideally, the process of refining phenotypes should strengthen our ability to detect associations and linkages between genes of interest and complex traits and disorders. An obvious point of departure for investigating the extent of heterogeneity within this disorder is to examine the external validity and distinctiveness of the *DSM-IV* defined ADHD subtypes.

Accordingly, the first paper of this dissertation serves as an informative test of the external validity and distinctiveness of the ADHD subtypes as currently classified in the *DSM-IV*. *External validity* refers to the generalizability of research findings across groups. Similar to Robins and Guze's (1970) criteria for establishing a valid psychiatric disorder, Hinshaw (2001) described *external validity* as the differentiation of putatively

distinct syndromes or clusters on the basis of criteria that are removed from the symptoms themselves, such as etiological factors, family history, key correlates, pathophysiology, developmental course, and treatment response. Given the relative paucity of studies examining the external validity of the *DSM-IV* ADHD subtypes, the first paper of this dissertation compares the ADHD subtypes with regard to demographic characteristics (e.g., gender ratios, mean age) and clinical correlates (e.g., symptoms of co-occurring internalizing and externalizing disorders) in a large clinic-referred sample. Additionally, few researchers have examined specific candidate genes as indicators of the specific etiology, and hence external validity, of the ADHD subtype diagnoses. Yet there is reason to be concerned that clinically acceptable definitions of disorders may not represent genetically valid phenotypes. Thus, the first paper of this dissertation also examines the distinctiveness and external validity of the ADHD subtypes using molecular genetic approaches to test whether the strength of associations with *DRD4* and *5-HTT* varies by diagnostic subtype.

The first paper of this dissertation has the potential to make valuable contributions to the extant literature in at least two major ways. First, if the *DSM-IV* ADHD subtypes cannot be differentiated on a majority of the external validity indicators examined in this study, it would call into question the adequacy of the current nomenclature. That is, the validity of the ADHD construct (operationalized in *DSM-IV* as comprising three distinct subtypes) rests in large part on its success in segregating more homogeneous subgroups within the broader diagnosis on the basis of important enduring characteristics (e.g., different demographic configurations, clinical correlates, and underlying etiological mechanisms). If it can be demonstrated that there are unique patterns of ADHD subtypes

and co-occurring conditions with regard to their associated clinical correlates, etiology, course, and outcome, it would provide support for the distinctiveness of the subtypes. Alternatively, if the subtypes are found to have similar patterns of demographic characteristics and clinical correlates, one might argue that ADHD is better conceptualized as a relatively homogeneous diagnosis with shared features and etiologies. Furthermore, if there are unique patterns of co-occurring disorders across the subtypes, it may indicate the need for special treatment considerations for certain comorbid subgroups (Jensen et al., 1997, 2001).

Conversely, if the current subtyping system does not prove to provide a parsimonious and reliable way of differentiating comorbid subgroups within ADHD, this raises the question of whether there are viable alternative representations of the phenotype that may serve to identify homogeneous groups within ADHD in a more effective manner. Considering that the high rates of co-occurrence of other psychiatric disorders in ADHD may reflect underlying genetic heterogeneity (Smalley et al., 2000), methods aimed at reducing heterogeneity in the ADHD phenotype by discriminating among common comorbid subgroups may enhance the power to detect associations between genes and ADHD. In fact, there is initial evidence to suggest that stratifying ADHD groups by the presence of co-occurring conditions indeed may be an effective means of revealing associations between candidate genes and ADHD, that otherwise would remain undetected if the phenotype were solely defined as a unitary diagnostic construct (e.g., Caspi et al., 2008; Holmes et al., 2002; Kirley et al., 2004).

Therefore, the second paper of this dissertation represents an attempt to empirically examine one possible alternative approach to reducing the degree of diagnostic heterogeneity in the ADHD phenotype that may help identify subgroups of children possessing more

similar biological risk factors and pathogenesis. More specifically, composites of co-occurring externalizing and internalizing symptoms are evaluated as continuous moderators of the relation between ADHD and several external validity criteria. Furthermore, the second paper tests whether the proposed alternative comorbid ADHD phenotype enhances the external validity of ADHD, as a function of including co-occurring symptom dimensions, over and above the extant *DSM-IV* subtyping system. Specifically, the *DSM-IV* ADHD subtypes are compared with ADHD accompanied by comorbid conditions on the basis of several external validity indicators, including genetic associations with *DRD4* and *5-HTT*, gender composition, and mean age.

In summary, the current dissertation aims to address two major challenges that clinicians and researchers continue to face concerning the classification, treatment, and etiology of ADHD. First, as highlighted above, researchers lack consensus with respect to the best way to classify and diagnose ADHD, despite intense taxonomic study of this prevalent childhood disorder. Second, although there is strong evidence that genetic influences are an important part of the etiology of ADHD, the search for specific susceptibility genes is complicated by the fact that this condition is a genetically complex disorder and by clinical heterogeneity within the ADHD diagnosis. With these challenges in mind, the initial paper examines the external validity and distinctiveness of the *DSM-IV* ADHD diagnostic subtypes. The second paper evaluates whether an alternative comorbid ADHD phenotype provides increased external validity over and above the extant *DSM-IV* diagnostic subtypes.

Evaluating the External Validity and Distinctiveness of *DSM-IV* Attention-Deficit/Hyperactivity Disorder Subtypes

Kelly M. Harrington and Irwin D. Waldman

Emory University

Abstract

There are inconsistent findings in the child clinical literature regarding the distinctiveness of the *DSM-IV* ADHD subtype diagnoses. Given this, the present study contrasted the ADHD subtypes on several external validity indices, including gender ratio, age, overlapping conditions, and associations with candidate genes. The sample comprised 237 clinic-referred children diagnosed with ADHD who were recruited as part of an ongoing study on the genetics of ADHD. Probands, their siblings, and parents were genotyped for a variable number of tandem repeats (VNTR) in exon 3 of the dopamine D4 receptor gene (*DRD4)* and an insertion / deletion polymorphism in the promoter region of the serotonin transporter gene (*5-HTTLPR*). Regression analyses including planned comparisons and tests of candidate gene associations were conducted to examine whether the ADHD subtypes could be distinguished on the basis of the external correlates. Planned comparisons revealed that the Combined subtype is characterized by a higher proportion of males, lower mean age, and higher symptom levels of oppositional defiant and conduct disorders, depression, and anxiety disorders compared with the Inattentive or Hyperactive-Impulsive subtypes. Genetic association analyses suggested that the 7-repeat allele of *DRD4* is significantly associated with symptoms of inattention (i.e., this allele was over-represented in both the Combined and Inattentive subtypes), but not hyperactivity-impulsivity. Further tests of association revealed that the Inattentive subtype is more strongly related to the long allele of *5-HTT* than the other two subtypes. Overall, findings from the current study are most consistent with the representation of ADHD as a unitary disorder with subtypes and provide modest support for the distinctiveness of the *DSM-IV* ADHD subtypes.

Evaluating the External Validity and Distinctiveness of *DSM-IV* Attention-Deficit/Hyperactivity Disorder Subtypes

Attention-deficit/hyperactivity disorder (ADHD) is one of the most commonly diagnosed childhood disorders, affecting roughly 3%-7% of school-age children (American Psychiatric Association [APA], 2000). According to the ***Diagnostic and Statistical Manual of Mental Disorders – 4th Edition (DSM-IV)*** (APA, 1994), ADHD diagnoses are assigned depending on the degree to which an individual exhibits symptoms of the two underlying symptom dimensions of inattention and hyperactivity/ impulsivity, thereby yielding three subtype diagnoses: Predominantly Inattentive Type (IT), Predominantly Hyperactive-Impulsive Type (HT), and Combined Type (CT). Nonetheless, the conceptualization of ADHD has varied considerably since its initial appearance as "Hyperkinetic Reaction of Childhood" in the second edition of the *DSM* (*DSM-II*; APA, 1968), as reflected in the successive revisions of the *DSM* over time.

Significant changes in the classification of ADHD can be largely attributed to ongoing debate concerning the core deficit(s) associated with this childhood disorder, as well as whether ADHD and its components are best conceptualized as one or more symptom dimensions or diagnostic categories (Carlson & Mann, 2002; Lahey & Willcutt, 2002). Hyperkinetic Reaction of Childhood was initially defined as a unitary diagnostic category in the *DSM-II* (APA, 1968). In contrast, the *DSM-III* (APA, 1980) proposed two subtypes of Attention Deficit Disorder (ADD) to delineate children who displayed symptoms of inattention, impulsivity, and hyperactivity (ADD/H) from children whose symptom presentation included inattention and impulsivity in the absence of hyperactivity (ADD/WO) (Lahey, Schaughency, Strauss, & Frame, 1984). The revised *DSM-III* (*DSM-III-R*; APA,

1987) eliminated the diagnostic subtypes and returned to a unidimensional conceptualization of ADHD wherein no essential characteristics were designated (i.e., any combination of eight or more symptom criteria were sufficient for the diagnosis). A subtyping system for ADHD was reinstated in the *DSM-IV* (APA, 1994), however, based on growing empirical and clinical evidence supporting multidimensionality (Lahey, Carlson, & Frick, 1997). Thus, despite intense taxonomic study of ADHD, there is ongoing debate among researchers with respect to the best way to classify and diagnose this prevalent childhood disorder. Specifically, the alternative possibilities include whether ADHD should be conceptualized as a) a unitary disorder, b) a unitary disorder with subtypes, or c) multiple distinct disorders.

One critical classification problem that may compromise the validity of the overall ADHD diagnosis is the heterogeneity within the disorder. There is growing recognition by researchers that ADHD is a heterogeneous condition, with regard to its variability in the presentation of core symptoms, its clinical correlates (e.g., demographic characteristics, rates of comorbidity), and arguably its etiology (Faraone et al., 1995; Hechtman, 1999; Jensen, Martin, & Cantwell, 1997; Newcorn et al., 2001; Todd, 2000). The process of identifying more clinically homogeneous subgroups within the overall ADHD diagnosis thus would aid in the search for specific etiologies of, and the development of more specific treatment approaches for, such subgroups. An obvious point of departure for investigating the extent of heterogeneity within this disorder is to review the extant literature regarding the distinctiveness of the *DSM-IV* defined ADHD subtypes.

Differences between the ADHD subtypes have been documented (e.g., in prevalence, mean age, gender ratios, and rates of co-occurring conditions) that to some researchers are

suggestive of distinct underlying pathology and etiologies, and these have been summarized in a recent qualitative review (Milich, Ballentine, & Lynam, 2001). Nonetheless, despite an abundance of studies investigating ADHD since the publication of the *DSM-IV*, evidence supporting the validity of the distinction among the *DSM-IV* ADHD subtypes remains scant (for recent reviews, see Woo & Rey, 2005; Baeyens, Roeyers, & Walle, 2006). The present study has the potential to make valuable contributions to the extant literature in at least two major ways. First, if the *DSM-IV* ADHD subtypes cannot be differentiated on a majority of the external validity indicators examined in this study, it would call into question the adequacy of the current nomenclature. That is, the validity of the ADHD construct (operationalized in *DSM-IV* as comprising three distinct subtypes) rests in large part on its success in segregating more homogeneous subgroups within the broader diagnosis on the basis of important enduring characteristics (e.g., different demographic configurations, clinical correlates, and underlying etiological mechanisms). Thus, the first objective of the current study is to compare the ADHD subtypes with regard to demographic characteristics (e.g., gender ratios, mean age) and clinical correlates (e.g., symptoms of co-occurring internalizing and externalizing disorders) in a large clinic-referred sample.

Second, few researchers have examined specific candidate genes as indicators of the specific etiology, and hence external validity, of the ADHD subtype diagnoses. There is reason to be concerned that clinically acceptable definitions of disorders may not represent genetically valid phenotypes. For example, different phenotypic definitions may lead to differences in the associations with candidate genes, such that certain definitions might weaken the genetic signal whereas others might enhance it. Moreover,

accurate and valid characterization of the psychopathological phenotypes is necessary before advances in molecular genetics can be maximally utilized to further our understanding of the genetic and environmental risk factors that give rise to psychiatric disorders (Lahey et al., 2004). Thus, the second major objective of the current study is to examine the distinctiveness and external validity of the ADHD subtypes using molecular genetic approaches. Specifically, the ADHD subtypes will be contrasted in their associations with two candidate genes within the dopaminergic and serotonergic systems, the dopamine D4 receptor gene (*DRD4*) and the serotonin transporter gene (*5-HTT*), respectively.

ADHD Subtype Differences on Demographic and Clinical Characteristics

A review of studies comparing the most commonly diagnosed ADHD subtypes reveals that the CT, relative to the IT, is more likely to be male, to have an earlier age of onset or referral, to show greater global impairment including poorer social functioning, and to have higher rates of comorbid externalizing disorders (e.g., Bauermeister et al., 2005; Carlson & Mann, 2000; Faraone, Biederman, Weber, & Russell, 1998). On the other hand, no distinct pattern of comorbid internalizing disorders (e.g., depression, anxiety) between the two groups has emerged from the literature (Milich et al., 2001; Power, Costigan, Eiraldi, & Leff, 2004). A recent meta-analysis by Harrington & Waldman (2008) revealed greater rates of co-occurring depressive and anxiety disorders for the CT relative to the IT, though the magnitude of this effect was only small-to-moderate (weighted mean effect sizes of $d = .14$ and $d = .26$, for depressive disorders and anxiety disorders, respectively).

Furthermore, review of the extant data has not revealed replicable differences between the IT and the CT on neuropsychological and cognitive tasks (Chhabildas,

Pennington, & Willcutt, 2001; Hinshaw, Carte, Sami, Treuting, & Zupan, 2002; Nigg, Blaskey, Stawicki, & Sachek, 2004), measures of academic achievement (Carlson & Mann, 2000; Faraone et al., 1998), family psychiatric history (Faraone, Biederman, & Friedman, 2000; Stawicki, Nigg, & van Eye, 2006), and treatment response (Barkley, DuPaul, & McMurray, 1991; Stein et al., 2003). Taken together, previous studies have demonstrated modest support for the distinctiveness of *DSM-IV* ADHD/IT and ADHD/CT, but results have been largely inconsistent across samples.

Genetic Influences on ADHD and its Constituent Subtypes

Quantitative genetic studies have consistently shown strong evidence suggesting substantial genetic influences contributing to ADHD, with heritability estimates ranging from 60 to 90% (Waldman & Rhee, 2002). Researchers also have examined the heritability of the inattention and hyperactivity-impulsivity symptom dimensions (i.e., as opposed to discrete diagnostic categories), reporting heritability estimates of approximately 75% and 64% for the two symptom dimensions, respectively (Gjone, Stevenson, & Sundet, 1996; Goodman & Stevenson, 1989; Levy, Hay, McStephen, Wood, & Waldman, 1997).

Given the strong evidence for genetic influences on ADHD, numerous molecular genetic studies have been conducted to search for the specific genes that confer risk for ADHD. The majority of molecular genetic studies of ADHD have concentrated on candidate genes in the dopaminergic, and to a lesser extent, serotonergic and noradrenergic neurotransmitter pathway (Waldman & Gizer, 2006). Numerous studies have focused on the dopamine neurotransmitter system as the primary biological basis of ADHD because psychostimulant medications, commonly prescribed to treat symptoms of

ADHD, exert their primary pharmacological actions by binding to the dopamine transporter and thereby blocking dopamine reuptake (Seeman & Madras, 1998; Solanto, 1984). Additional evidence for the relevance of genes within these neurotransmitter systems comes from "knockout" gene studies in mice, in which the behavioral effects of the deactivation of specific genes are examined (e.g., Rubinstein et al., 1997).

Although candidate gene studies of ADHD have yielded inconsistent findings across studies, several candidate genes have shown evidence of replicable associations with ADHD, including the dopamine transporter gene (*DAT1*), the dopamine D4 and D5 receptor genes (*DRD4* and *DRD5*, respectively), and the serotonin transporter gene (*5-HTT*) (for recent reviews, see Faraone & Khan, 2006 and Waldman & Gizer, 2006). In a recent review of molecular genetic studies of ADHD, Faraone et al. (2005) reported that seven genes (with the same variant) have shown evidence of significant association with ADHD on the basis of pooled odds ratios (OR) across 3 or more studies, with ORs ranging from 1.13 to 1.45. The effect sizes of these associations have been relatively small, however, considering that an OR of 1.0 indicates no association between ADHD and the putative risk allele.

In the current study, two candidate genes were evaluated with respect to their differential genetic associations with the ADHD subtypes, namely *DRD4* and *5-HTT*. The first candidate gene included in this study codes for the dopamine D4 receptor (*DRD4*). The dopamine D4 receptor is expressed in areas of the brain that underlie ADHD including the prefrontal cortex (Paterson, Sunohara, & Kennedy, 1999) and has been demonstrated to heighten the effects of alcohol, cocaine, and amphetamine on locomotor activity (Rubinstein et al., 1997). Initial reports of an association between the

personality trait of novelty seeking and *DRD4* (Ebstein et al., 1996; Benjamin et al., 1996) stimulated further investigations of *DRD4* as a genetic susceptibility factor for ADHD. Additional interest in exploring the relation between *DRD4* and ADHD was generated from "knockout" studies in mice and the effects of psychostimulants. For example, Rubinstein et al. (1997) examined behavioral differences between the *DRD4* "knockout" mice and wild-type mice and found that the "knockout" mice showed decreased locomotor activity relative to the wild-type controls, suggesting a general influence of *DRD4* dysregulation on motor activity.

Following from this evidence, the association and linkage of ADHD with a 48-base pair (bp) variable number of tandem repeats (VNTR) sequence in exon 3 of *DRD4* has been examined in a number of studies. Several independent research groups have reported a significant association between ADHD and the 7-repeat allele of *DRD4*, whereas several studies have failed to replicate this finding. The three most common variants of *DRD4* represent greater than 90% of the observed population allelic diversity, namely, the 2-, 4-, and 7-repeat alleles. Notably, the functional significance of these length/sequence changes in the *DRD4* protein has been studied, revealing that the 7-repeat variant exhibits a blunted ability to reduce cyclic AMP levels, in comparison with that of the common 4-repeat variant (Asghari et al., 1995).

Faraone, Doyle, Mick, and Biederman (2001) re-evaluated the findings from 22 independent studies in attempt to reconcile the conflicting findings regarding the association between *DRD4* and ADHD. Notably, the results of the meta-analysis demonstrated a significant *DRD4*-ADHD association for both case-control (OR = 1.9, $p < .001$) and within-family studies (OR = 1.4, $p = .02$). Similar results were achieved by Li,

Sham, Owen, and He (2006) when they conducted a meta-analysis of 33 independent association studies of the relation between ADHD and *DRD4*, providing further evidence that the 7-repeat allele confers increased risk for ADHD (case-control studies: N = 12, OR = 1.6, $p < .001$; within-family studies: N = 21, OR = 1.2, $p < .001$).

The second candidate gene included in the present study is the serotonin transporter gene (*5-HTT*), which is responsible for the reuptake of serotonin from the synaptic cleft back to the presynaptic neuron. Animal models of ADHD have demonstrated that the paradoxical calming effect of psychostimulants in mice is influenced by serotonergic as well as dopaminergic neurotransmission, suggesting that both neurotransmitter systems are involved in regulating motor activity (Gainetdinov et al., 1999). The serotonin neurotransmitter system also has been hypothesized as a causal factor underlying ADHD given evidence relating serotonergic dysregulation to a variety of behaviors relevant to ADHD, including impulsivity, aggression, and disinhibition (Halperin et al., 1997; LeMarquand et al., 1998; Lucki, 1998; Spivak et al., 1999). Additional studies have demonstrated a positive association between increases in the binding affinity of platelet serotonin transporter and increases in impulsive behavior in children diagnosed with ADHD (Oades, Slusarek, Velling, & Bondy, 2002). Given this evidence, the gene that codes for the serotonin transporter (*5-HTT*) has been investigated as a candidate gene for ADHD.

Several studies of association and linkage between *5-HTT* and ADHD recently have been published, many of which have focused on a 44-bp insertion / deletion in the promoter region of the gene characterized by long and short variants with demonstrated functional consequence. Specifically, the short allele of the *5-HTT*-linked promoter

region polymorphism (*5-HTTLPR*) appears to be associated with decreased serotonin reuptake, whereas the long allele appears to be associated with more rapid serotonin reuptake, thus lower levels of active serotonin (Lesch et al., 1996; Neumeister et al., 2004).

The initial study to examine the association between *5-HTT* and ADHD was conducted by Manor et al. (2001), using a sample of 98 children diagnosed with *DSM-IV* ADHD. They found a statistical trend for lower frequencies of participants homozygous for the short allele of *5-HTT* ($\chi^2(2) = 4.45$, $p = .11$). Notably, when their analyses excluded children with the IT, the association reached statistical significance ($\chi^2(2) = 11.25$, $p = .004$), suggesting that the *5-HTTLPR* may preferentially influence hyperactive-impulsive symptoms (i.e., as opposed to inattentive symptoms). Subsequent attempts to replicate Manor and colleagues' initial positive association between *5-HTTLPR* and ADHD have yielded inconsistent results, however. Faraone et al. (2005) conducted a joint analysis of the *5-HTTLPR*-ADHD association by combining results across all studies published before 2005 and reported that the pooled OR for the long allele was 1.31 (95% CI, 1.09-1.59). Since Faraone and colleagues' pooled analysis, the majority of studies published (i.e., 8 out of 10) have failed to detect statistically significant association between the *5-HTTLPR* and ADHD, with only two exceptions (i.e., Curran et al., 2005 and Li et al., 2007).

There is preliminary evidence indicating that *DRD4* and *5-HTT* may be preferentially related to one symptom dimension or subtype of ADHD, rather than the disorder as a whole. Several studies have suggested a stronger relation between *DRD4* and the inattentive (rather than hyperactive-impulsive) symptoms of ADHD (Lasky-Su et

al., 2008; McCracken et al., 2000; Rowe et al., 1998). Furthermore, other studies have shown *DRD4* to be related to attention problems in the general population (Laucht et al., 2006; Schmidt et al., 2001). In contrast, studies examining the relation between *5-HTT* and ADHD have found *5-HTT* to be preferentially associated with hyperactive-impulsive symptoms and the CT (Manor et al., 2001 and Seeger, Schloss, & Schmidt, 2001, respectively).

Study Hypotheses

Given mixed findings in the extant literature with respect to the distinctiveness of the *DSM-IV* ADHD subtypes, this study examined the external validity of the current diagnostic classification system of ADHD. The following hypotheses were generated and tested on the basis of the evidence reviewed above:

1. Based on previous studies of ADHD using clinically-referred samples, it was hypothesized that the subtypes could be differentiated on demographic characteristics, with IT showing a lower proportion of males and higher mean age relative to CT and HT.
2. It was predicted that CT would exhibit higher rates of both externalizing and internalizing disorder symptoms as compared with IT and HT, consistent with the results of a recent meta-analysis (Harrington & Waldman, 2008).
3. Given preliminary evidence of differential genetic associations between particular ADHD symptom dimensions / subtypes and each of the two selected candidate genes, it was hypothesized that *DRD4* would show stronger association with inattention symptoms and IT and CT, whereas *5-HTT* would

show stronger association with hyperactive-impulsive symptoms and HT and CT.

Method

Participants

The full sample comprised 390 children from 233 families recruited through the Center for Learning and Attention Deficit Disorders (CLADD) at the Emory University School of Medicine in Atlanta, Georgia and through psychiatrists in private practice in Tucson, Arizona. Children were assessed and/or treated for attention-deficit disorders, related behavioral disorders, and/or learning problems at these two clinics. Probands were designated as children who originally brought a family to the attention of this study, regardless of the diagnosis assigned to the children. Whenever possible, both male and female siblings of the probands were also sampled. Any diagnosis assigned to a child remained confidential and did not affect their inclusion in the study.

The study protocol was approved by the institutional review boards of Emory University and the University of Arizona, and appropriate informed consent was obtained for all participants at both sites. While families were being seen at either of the two clinics, they were presented with and given the option to sign a form indicating that they agreed to be contacted for future research. Consenting families were then contacted by phone, presented with details regarding the study, and asked if they would like to participate. All family members were given copies of the informed consent form to read in advance of all assessments. Participating children were given an age-appropriate verbal description of what the study entailed and those children who could read and write also read and signed a written assent form. The parents signed the informed consent forms and

also signed for their children who were too young to read. Families who agreed to participate in the study were assessed in their homes over the course of a single three-hour period. Participating children were comprehensively assessed on lab measures of executive functions and attention and their parents completed questionnaires assessing the demographic characteristics of the family, as well as symptoms associated with commonly diagnosed childhood psychiatric disorders in their children.

Children diagnosed with autism, traumatic brain injury or other neurological conditions (e.g., epilepsy), or an IQ < 75 were excluded from participation in the study. This exclusionary decision was made because these conditions can result in ADHD-like symptoms, although the etiological pathways of these conditions are most likely unique. Only participants who met diagnostic criteria for ADHD (N = 237) were included in the analyses reported in the current paper to ensure that findings of subtype differences were not merely driven by differences from the undiagnosed group. The participants in this study represent an expanded sample that has been previously published on (e.g., Waldman et al., 1998; Rowe et al., 1998).

Demographic characteristics of the current sample are presented in Table 1. The children ranged in age from 5 to 18 years, with an average age of 10.5 years (*SD* = 3.3) at the time of assessment. The overall sample comprised 261 boys (67%) and 129 girls (33%). The racial/ethnic composition of the sample was 78% Caucasian, 9% African-American, 2% Hispanic/Latino, and 11% mixed ancestry. Of the 237 (61%) children who met *DSM-IV* criteria for ADHD, the rates of subtype diagnosis were as follows: 34% IT, 56% CT, and 10% HT. For purposes of comparison, data on demographic characteristics

and ADHD symptoms from a non-disordered control sample (i.e., no ADHD diagnosis) from Atlanta and Tucson also are presented in Table 1.

Assessment Procedures

Parent ratings were obtained for probands and their siblings (whenever possible) using the Emory Diagnostic Rating Scale (EDRS). The EDRS was developed in our lab (Waldman et al., 1998) to assess *DSM-IV* symptoms of the major childhood psychiatric disorders, including disruptive behavior disorders (i.e., ADHD, conduct disorder (CD), and oppositional defiant disorder (ODD)), as well as internalizing disorders such as major depression, dysthymia, and anxiety disorders. Each symptom of a given disorder corresponds to a specific item on the rating scale. Children were rated by their parents on a 0-4 scale, in which a score of 0 indicates that the symptom is "not at all" characteristic of the child and a score of 4 indicates that the symptom is "very much" characteristic of the child. Average symptom dimension scores were calculated for each major childhood disorder by summing the item scores (0-4) comprising each scale and dividing by the total number of scale items. The symptom scales allow quantitative assessments of the disorders, as they distinguish severity and number of symptoms over a broad range.

ADHD diagnoses were then derived from cut-off scores on the continuous symptom dimensions in accordance with *DSM-IV* diagnostic criteria. In the current study, ADHD diagnoses were calculated at a moderate level of symptom severity, wherein each individual symptom was considered present with a score of 2 or higher. Probands and their siblings were assigned an ADHD subtype diagnosis if they surpassed the standard diagnostic thresholds (i.e., $\geq$ 6 of 9 symptoms) on the inattention and/or hyperactivity-impulsivity symptom dimensions at the moderate severity level. That is, children who

were above threshold only on the inattentive symptom dimension were diagnosed with IT, children who were above threshold only for the hyperactive-impulsivity symptom dimension were diagnosed with HT, and children who were above threshold on both symptom dimensions were diagnosed with CT.

All diagnoses were based on mother's symptom ratings, except in cases where mother ratings were unavailable in which case father ratings were substituted. The decision to primarily use mother ratings was largely based on the fact that father ratings were unavailable for a large percentage of the sample and based on evidence demonstrating mothers' superior validity as informants (e.g., Coffman, Guerin, & Gottfried, 2006). In addition, maternal reports of ADHD symptoms have consistently yielded high heritability estimates (Thapar, Holmes, Poulton, & Harrington, 1999). The internal consistency reliabilities of both the inattentive and hyperactive-impulsive symptom dimensions were high (α = .96 and .95, respectively).

DNA Collection, Extraction, and Genotyping Procedures

Probands, their siblings, and parents were genotyped for the 48-bp VNTR in exon 3 of *DRD4* and the *5-HTTLPR* 44-bp insertion / deletion polymorphism. The technique used to collect DNA changed during the course of the study, in order to facilitate the extraction process and increase the yield of DNA. The DNA collection procedures have included the use of sucrose solution washes and buccal brushes to collect buccal cells, as well as Oragene^{TM} DNA self-collection kits to obtain saliva samples (DNA Genotek, Inc.). At the end of the study visit, the DNA samples were immediately refrigerated and transported to the Center for Medical Genomics at Emory University for secure storage and extraction by lab personnel. Buccal cells were pelleted for ten minutes at 2,000 g and

the DNA was extracted using a QIAmp Tissue kit (Qiagen), according to the manufacturer's protocol. The samples were then preserved in TE (10 mM Tris Hcl, 1mM EDTA).

The preserved samples were sent to two laboratories for polymerase chain reactions (PCR) amplification of the *DRD4* and *5-HTTLPR* markers: 1) the University of Arizona's Laboratory of Molecular and Systematic Evolution in Tucson, AZ and 2) the Psychiatric and Neurodevelopmental Genetics Unit (PNGU) in the Center for Human Genetic Research at Massachusetts General Hospital (MGH) in Boston, MA. The 48-bp VNTR in exon 3 of *DRD4* was genotyped by PCR, either according to the protocol originally described by Lichter et al. (1993) in the laboratory at the University of Arizona, or more recently, according to an alternate protocol in the laboratory at MGH (see Appendix for description of alternate protocol). The *5-HTTLPR* polymorphism was genotyped by PCR at MGH, following the procedures outlined in the alternate protocol (see Appendix). After the genotyping procedures were completed at the University of Arizona and MGH, our lab received Microsoft Excel spreadsheets containing the final called genotypes for all samples.

Data Analysis

As shown in Table 2, two sets of ***a priori*** contrasts were utilized throughout the current analyses to help evaluate competing hypotheses regarding whether each dependent variable is more strongly related to 1) inattentive vs. hyperactive-impulsive symptoms or 2) one specific *DSM-IV* ADHD diagnostic subtype. More specifically, the first set of contrasts was used to test the hypothesis that the inattentive ***symptoms*** of ADHD are more strongly associated with the dependent variable of interest by comparing

IT and CT versus HT, and IT versus CT. A second complementary set of contrasts was used to test the hypothesis that the Inattentive *subtype* of ADHD is more strongly related to the dependent variable of interest by comparing IT versus HT and CT, and HT versus CT. Given that all of the study hypotheses described above were directional, one-tailed p-values were reported for all analyses.

The *DSM-IV* ADHD subtypes were first contrasted to determine whether they are distinguishable with respect to demographic characteristics, including gender composition (viz., proportion of males) and mean age. Logistic regression analyses were performed to test for possible gender differences across the ADHD subtypes, using the two sets of aforementioned *a priori* orthogonal contrasts in independent analyses. Child's gender served as the dependent variable and the set of contrasts served as the explanatory variables. Logistic regression yields a Wald χ^2 statistic that tests the significance of each individual predictor in the regression model. More specifically, the Wald χ^2 statistic represents the ratio of the square of the estimate of the regression coefficient to the square of the estimate of its standard error (Cohen, Cohen, West, & Aiken, 2003). The Nagelkerke R^2 and ORs (including their 95% confidence intervals) were reported as measures of effect size.

Analyses of variance (ANOVAs) with planned comparisons were conducted to determine whether the *DSM-IV* ADHD subtypes could be distinguished by mean age at assessment, again using the two sets of *a priori* orthogonal contrasts outlined in Table 2. The child's age at the time of assessment served as the dependent variable and the orthogonal contrasts among the ADHD subtypes served as the independent variable. Similar analyses were used to test for differences in each internalizing and externalizing

symptom dimension as a function of the *a priori* contrasts. For each dependent variable, Levene's tests of homogeneity of variance were performed to examine whether the assumption of equal variances across subtypes was met.

Tests of association were conducted using ordinal logistic regression to examine differences between the *DSM-IV* ADHD subtypes and each of the selected candidate genes (*DRD4* and *5-HTT*) in independent analyses. The number of *DRD4* or *5-HTT* "high-risk" alleles (i.e., 0, 1, or 2) served as the criterion variable and the previously described *a priori* contrasts among the ADHD subtype diagnoses served as the predictor variables. Given the abovementioned findings from previous studies in the human literature suggesting that the 7-repeat allele of the *DRD4* VNTR and the long allele of the *5-HTTLPR* polymorphism are associated with increased risk for ADHD, directional predictions were made designating these variants as the "high-risk" alleles. Accordingly, one-tailed p-values were reported. To control for sex and age differences across the ADHD diagnostic subtypes, sex, age, age^2, and the sex X age, and sex X age^2 interactions were entered as covariates in separate steps of the ordinal logistic regression model prior to performing the genetic analyses. Non-significant sex and age terms were dropped from the model in an iterative fashion until only the most complex sex and age terms that were significant remained in the model.

Although between-family designs (i.e., based on the classic case-control study), are often more powerful statistically than within-family designs (Pericak-Vance, 1998), population stratification is a potential threat to the internal validity of between-family association methods. Two conditions must be true in order for population stratification to occur: 1) the allele frequencies must vary across population subgroups (e.g., ethnic

groups) within the study sample and 2) the ethnic groups that differ in allele frequency must also differ with respect to the outcome variable (e.g., rates of disorder and/or symptom levels) (Hutchinson, Stallings, McGeary, & Bryan, 2004). Hence, spurious associations in candidate gene studies may result if the case-control differences in allele frequencies are due to systematic differences in ancestry rather than the association of genes with disease. Thus, to minimize possible confounding owing to population stratification, participants' parent-reported ethnic backgrounds (i.e., % European-American, % African-American, and % Hispanic) were statistically controlled prior to the primary analysis for all genetic analyses.

Prior to conducting the primary genetic analyses, our lab performed quality control analyses of the genotypes for both genes using crosstab analyses in SPSS version 15 (SPSS, Inc., Chicago, IL). These analyses included estimates of monozygotic twin agreement for genotypes and the concordance of genotypes between genomic and Whole Genome Amplified (WGA) samples, given that genomic as well as WGA DNA samples were available for a subset of subjects (N = 63 and N = 52 for *DRD4* and *5-HTT*, respectively). In addition, the program PEDSTATS (Wigginton & Abecasis, 2006) was used to estimate call rates and exact Hardy Weinberg Equilibrium (HWE) tests.

Results

Analyses of ADHD Subtype Differences in Demographic Variables

Gender ratios. Logistic regression analyses were first conducted to test the hypothesis that the *DSM-IV* ADHD subtypes differ with respect to their gender composition. As shown in Table 3, the analyses revealed that the CT showed a higher proportion of males relative to both the IT (Wald χ^2[N=237] = 3.11, p = .039, OR = 1.32

[95% CI = .97 – 1.80]) and the HT (Wald χ^2[N=237] = 3.01, p = .042, OR = 1.51 [95% CI = .95 – 2.41]). The finding that CT was characterized by a higher male-to-female ratio than IT was consistent with our predictions, whereas the finding that CT had a higher male-to-female ratio than HT was unexpected. The results of contrasts between IT and CT versus HT (Wald χ^2[N=237] = 1.45, p = .115, OR = .83 [95% CI = .62 – 1.12]), as well as IT versus HT and CT (Wald χ^2[N=237] = .183, p = .335, OR = 1.05 [95% CI = .84 – 1.30]), were both non-significant.

Age. We next performed ANOVAs incorporating planned comparisons to examine differences in mean age among the *DSM-IV* ADHD subtypes. The Levene's test was not significant, indicating that equal variances in age can be assumed across the ADHD subtypes (F (2, 234) = 1.19, p = .306). All four contrasts were significant as predicted (also shown in Table 3), revealing that IT is characterized by higher mean age (M = 11.5 years, SD = 3.1), as compared with CT (M = 10.0 years, SD = 2.8) and HT (M = 8.4 years, SD = 2.6). Most notably, the contrast of IT versus HT and CT was highly significant, accounting for approximately 10% of the variance in mean age (t (1, 234) = 5.19, p < .001, η^2 = .10).

Analyses of ADHD Subtype Differences in Rates of Co-occurring Disorders

Results of the planned comparisons contrasting co-occurring internalizing and externalizing symptom levels across the *DSM-IV* ADHD subtypes are shown in Table 4 and revealed that IT could be distinguished from CT on seven internalizing symptom dimensions, including depression (t (1, 190) = - 2.77, p < .001, η^2 = .07), generalized anxiety (t (1, 200) = - 3.70, p < .001, η^2 = .06), obsessions (t (1, 184) = - 3.69, p < .001, η^2 = .07), compulsions (t (1, 198) = - 2.38, p = .009, η^2 = .03), separation anxiety (t (1,

205) = - 3.21, p = .001, η^2 = .05), agoraphobia (t (1, 192) = - 2.81, p = .003, η^2 = .04), and specific phobias (t (1, 203) = - 2.59, p = .005, η^2 = .03). For all significant contrasts, our hypothesis that CT would show ***higher*** mean levels of internalizing symptoms than IT was supported. Further, as predicted CT also exhibited higher mean levels of ODD and CD symptoms when compared with IT (t (1, 230) = - 6.43, p < .001, η^2 = .15, and t (1, 207) = - 4.55, p < .001, η^2 = .09, respectively).

On the other hand, the contrast between HT and CT revealed non-significant differences on the internalizing and externalizing symptom dimensions, with the exception of CT showing significantly higher symptoms of depression (t (1, 40) = 3.72, p < .001, η^2 = .25) and generalized anxiety (t (1, 35) = 2.18, p = .018, η^2 = .12) than HT. As can be seen in Table 4, the results of the contrast between IT versus HT and CT provided confirmation that the HT and CT groups (in combination) are characterized by significantly higher levels of other externalizing symptoms and a range of anxiety symptoms relative to the IT.

Genetic Analyses Examining the Relation between ADHD and DRD4 and 5-HTT

Quality control analyses. We conducted a series of Quality Control (QC) analyses of the ***DRD4*** and ***5-HTT*** genotype data using our family samples prior to analyses of differential association among the ADHD subtypes. The call rate in our sample was 90% and 86% for the ***DRD4*** and ***5-HTTLPR*** markers, respectively. The concordance of genotypes within monozygotic (MZ) twin pairs (N = 31) yielded an allelic discordance rate of approximately 13% and 5% for the ***DRD4*** and ***5-HTTLPR*** markers respectively. Genotypes from genomic and Whole Genome Amplified (WGA)

DNA samples were compared in a subset of individuals (i.e., N = 63 cases for *DRD4* and N = 52 cases for the *5-HTTLPR*) and no discrepancies were revealed for either marker.

Finally, tests of HWE were conducted for the full sample (including ADHD cases) versus founders only (equivalent to the probands' parents in this study). Marginally significant departure from HWE was observed for both the *DRD4* exon 3 VNTR (p = .053) and the *5-HTT* promoter polymorphism (p = .045) in the full sample that included ADHD cases, whereas departure from HWE was not detected in founders only (p = .191 and p = .209, for *DRD4* and *5-HTT*, respectively). Wittke-Thompson, Pluzhnikov, and Cox (2005) demonstrated that a similar pattern of results (i.e., significant HWE departure in cases, but not in controls) suggests that the gene of interest may be a disease susceptibility locus, rather than representing genotyping error. Thus, the HWE results provide initial support for the association between *DRD4* and *5-HTT* and ADHD.

Ordinal logistic regression analyses were next conducted using *a priori*, orthogonal contrasts (shown in Table 2) to examine ADHD subtype differences in the association with the number of *DRD4* and *5-HTT* high-risk alleles (i.e., 0, 1, or 2). A key assumption underlying ordinal regression is that the slope of the regression line is equivalent across levels of the dependent variable. The test of parallel lines was non-significant for both *DRD4* (χ^2(N=211) = 2.20, p = .332) and *5-HTT* (χ^2(N=204) = .958, p = .958), indicating that this assumption was not violated.

For *DRD4*, association was found with three out of the four *a priori* contrasts (see Table 5). The contrasts between IT versus CT and HT versus CT revealed that CT showed a stronger association with the 7-repeat allele of *DRD4* relative to IT (Wald χ^2[N=210] = 3.10, p = .039, OR = 1.36 [95% CI = .97 – 1.92]) as well as HT (Wald

χ^2[N=210] = 4.75, *p* = .014, OR = 3.19 [95% CI = 1.12 – 9.04]). The contrast between IT and CT versus HT was also significant, such that the IT and CT groups showed stronger association with *DRD4* than the HT group (Wald χ^2[N=210] = 3.63, *p* = .028, OR = 1.95 [95% CI = .98 – 3.90]). Taken together, this pattern of results provides further evidence to suggest that *DRD4* is associated more strongly with symptoms of inattention than hyperactivity-impulsivity, given that CT and IT are characterized by elevations in inattentive symptoms whereas HT is not, combined with the fact that the CT showed significantly higher levels of inattentive symptoms than the IT (*t* (1, 234) = - 3.44, *p* < .001, η^2 = .05). When sex and age terms were added into the model as covariates, the interactive effects of sex and age (i.e., sex X age) were found to be significant (Wald χ^2[N=210] = 4.17, *p* = .041). As shown in Table 5, all contrasts remained significant after controlling for the effects of sex and age.

The tests of *a priori* contrasts yielded significant evidence for association between *5-HTT* and ADHD subtypes in two out of the four contrasts (see Table 5). The contrast of IT versus HT and CT revealed a significantly stronger relation between the long allele of *5-HTT* and IT, as compared with the other two subtypes combined (Wald χ^2[N=203] = 4.23, *p* = .020, OR = 1.26 [95% CI = 1.01 –1.56]). There also was a marginally significant finding for the contrast between IT and CT versus HT, with the IT and CT groups demonstrating a stronger association with the long variant of *5-HTT* than the HT group (Wald χ^2[N=203] = 2.22, *p* = .068, OR = 1.26 [95% CI = .93 – 1.72]). In addition, the contrast between IT and CT approached significance, with the IT showing greater association with the long allele of *5-HTT* than the CT (Wald χ^2[N=203] = 2.26, *p* = .066, OR = 1.25 [95% CI = .93 – 1.66]). Notably, the addition of sex and age as covariates

revealed no evidence for a significant association with the *5-HTTLPR*. Therefore, all sex and age terms were subsequently dropped from the regression model.

Discussion

In light of inconsistent findings in the literature on the distinctiveness of the *DSM-IV* subtypes of ADHD, the present study tested the external validity of this diagnostic classification system on the basis of several external correlates. These included subtype differences in sex, age, symptoms of co-occurring externalizing and internalizing disorders, and associations with *DRD4* and *5-HTT*. As predicted, and consistent with previous studies using clinic-referred samples, the current study found that CT is characterized by a significantly higher proportion of males and a lower mean age compared with children in the IT and HT groups. For example, in this study children diagnosed with CT were 1.5 years older on average and 30% more likely to be male relative to children diagnosed with IT.

Although these findings suggest that the three subtypes differ somewhat in their gender and age distributions from a cross-sectional perspective, this may actually reflect different manifestations of ADHD over the course of development (Woo & Rey, 2005). In support of this developmental hypothesis, studies have shown that hyperactive-impulsive symptoms appear earlier (around age 3-4 years), with the inattention symptoms becoming apparent later (at age 5-7 years, around the start of school). Hyperactive-impulsive symptoms show a steeper decline during childhood than do the inattention symptoms (Barkley, 1997; Gjone, Stevenson, & Sundet, 1996; Hart, Lahey, Loeber, Applegate, & Frick, 1995). Furthermore, a recent prospective study conducted by Lahey et al. (2005) reported considerable instability of the *DSM-IV* ADHD subtypes from preschool through

elementary school, particularly for children initially diagnosed with HT who often shift to CT in later years. To avoid the problem of ADHD children shifting from one nominal diagnostic label to another over time, Lahey et al. recommended replacing the current *DSM-IV* nominal subtype classification system with a single diagnostic category of ADHD in *DSM-V*. Importantly, they further suggested the addition of a diagnostic qualifier in *DSM-V* based on continuous ratings of hyperactivity-impulsivity symptoms, such that IT and CT could be distinguished on a continuous rather than a nominal basis.

Consistent with our hypotheses, planned comparisons revealed that CT and IT could be distinguished with respect to rates of co-occurring externalizing and internalizing symptoms, with the CT consistently showing higher levels of ODD, CD, depression, and a variety of anxiety symptoms (i.e., generalized anxiety, separation anxiety, agoraphobia, specific phobias, obsessions, compulsions). It is a well documented finding that children with CT are generally rated higher by parents and teachers on externalizing dimensions (e.g., on measures of aggression, delinquent behavior, and conduct problems), in comparison to children with IT (Milich et al., 2001). Nonetheless, a distinct pattern of ADHD subtype differences in internalizing symptoms, such as depression and anxiety, has not previously been reported and is a novel finding of this study.

Studies of *DSM-III* subtypes of ADHD (attention deficit disorder with hyperactivity [ADD/H] and without hyperactivity [ADD/WO]) suggested a trend for the ADD/WO subtype to demonstrate higher levels of internalizing symptoms than the ADD/H subtype (e.g., Lahey & Carlson, 1991). This pattern does not hold for the corresponding subtypes in *DSM-IV* (i.e., CT and IT), however. On the contrary, there is growing evidence that children with CT tend to be at higher risk for developing both concurrent externalizing

symptoms (ODD, CD) and internalizing symptoms (depression, anxiety) than IT, as demonstrated in a recent meta-analysis (Harrington & Waldman, 2008). The current study provides further support for the hypothesis that children with the CT are at increased risk for developing both co-occurring externalizing and internalizing disorders. Nevertheless, although children with CT may show greater internalizing symptoms compared with children with IT, it is essential to highlight that all three subtypes of ADHD show higher rates of internalizing disorders relative to a non-ADHD comparison group.

Although the patterns of co-occurring psychopathology with HT have been much less studied due to this diagnostic subtype's low prevalence, results from the current study also provide initial evidence that the HT is more similar to the CT than to the IT. With the exception of symptoms of depression and GAD, CT and HT did not differ significantly in levels of ODD, CD, or a range of other anxiety disorders (i.e., separation anxiety, panic, agoraphobia, social phobia, specific phobias, obsessions, and compulsions). In light of recent longitudinal evidence suggesting that the HT is particularly unstable, the HT may be best viewed as a milder form of CT that sometimes remits but often develops into CT later in elementary school as attentional difficulties become more pervasive (Lahey et al., 2005).

Given preliminary evidence suggesting specificity in the relations of the selected candidate genes with a particular subtype or symptom dimension of ADHD (e.g., Lasky-Su et al., 2008 for *DRD4*; Manor et al., 2001 for *5-HTT*), we hypothesized that *DRD4* would show stronger association with inattentive symptoms, IT, and CT, whereas *5-HTT* would show stronger association with hyperactive-impulsive symptoms, HT, and CT. For *DRD4*, the subtype analyses revealed significantly greater association with CT and IT (to a lesser extent) than HT, confirming findings from several previous studies suggesting a

stronger relation between *DRD4* and the inattentive (rather than hyperactive-impulsive) symptoms of ADHD (Lasky-Su et al., 2008; McCracken et al., 2000; Rowe et al., 1998).

The subtype analyses suggested that the long allele of the *5-HTTLPR* is preferentially related to inattentive symptoms, given that the IT showed stronger evidence for association than the CT and HT groups combined. In addition, a trend towards significance emerged for the contrast between IT and CT, wherein the IT showed greater association with the long allele of *5-HTTLPR* than the CT. These findings conflict with earlier studies that reported a specific association between the long allele of *5-HTTLPR* and the CT, as well as hyperactive-impulsive symptoms (e.g., Manor et al., 2001; Seeger et al., 2001).

To our knowledge, this study is the first to find a preferential association between *5-HTTLPR* and the IT or inattentive symptoms. However, the majority of studies investigating the association between ADHD and *5-HTT* have not examined differences in association between the subtypes or symptom dimensions of ADHD. It is well documented that the inattention and hyperactivity-impulsivity symptom dimensions are substantially correlated (e.g., r = .69 in the current sample; r = .67 reported by Lahey et al., 2004). Therefore, it is possible that previous studies reporting a significant association between the *5-HTTLPR* and ADHD may have found evidence for a specific association with inattentive symptoms as well as hyperactive-impulsive symptoms, if analyses of ADHD subtypes or symptom dimensions had been conducted. Further support for a possible role for *5-HTT* in the etiology of attentional problems comes from evidence that some tricyclic antidepressants, known for reducing serotonergic activity, appear to be efficacious in reducing impaired concentration, as well as hyperactivity and impulsiveness in children with ADHD (Spivak et al., 1999). Other possible explanations

for the partial failure to replicate previous reports of association between the long allele of the *5-HTTLPR* and hyperactive-impulsive symptoms (e.g., Manor et al., 2001; Seeger et al., 2001) include variation across studies in subject ascertainment criteria, diagnostic assessment, and genetic heterogeneity.

An important contribution of the present study stems from the finding that CT was distinguishable from IT by greater concurrent externalizing and internalizing symptomatology. This finding suggests that within the heterogeneous diagnostic category of ADHD, the CT represents a more severely affected group of children than the IT. That is, there is increasing evidence that CT is associated with greater severity of overall psychopathology, including higher rates of comorbidity, as well as elevated essential symptoms (i.e.., inattention and hyperactivity-impulsivity) relative to IT. Moreover, a recent study by Gadow et al. (2004) demonstrated that the CT still showed significantly higher levels of anxiety and depression than the IT and HT groups, even after controlling statistically for the severity of the essential ADHD symptoms. Thus, the higher rate of internalizing symptoms exhibited by the CT cannot be solely attributed to the fact that the CT possesses more severe inattentive and hyperactive-impulsive symptoms.

Study Limitations and Directions for Future Research

Findings presented in this study should be considered within the context of several limitations. The first limitation pertains to the relatively modest sample size for undertaking analyses of diagnostic subtypes of ADHD. Dividing a diagnostic category (i.e., ADHD) into its constituent subtypes reduces the sample size per group, which likely led to decreased power in detecting differences among the subgroups of ADHD, particularly for the genetic analyses. This is especially problematic for HT, given that this

subtype consistently represents the smallest proportion of ADHD cases across studies. Consequently, our understanding of HT continues to lag behind that of the more common subtypes of ADHD (i.e., CT and IT), thus this subtype in particular requires further external validation in future studies.

A second limitation concerns the generalizability of the results from the current study to other populations. This limitation is probably most salient for molecular genetic studies of ADHD, considering evidence that differences in phenotypic measurement (e.g., diagnoses based on parent ratings, teacher ratings, clinical interviews, multiple raters, or multiple assessment instruments) appear to influence the degree of genetic variation explained (Curran et al., 2001; Simonoff et al., 1998; Thapar et al., 2000). Further variation across studies stems from the sample type or referral source (viz., community-based vs. clinic-referred samples), owing to factors such as higher rates of comorbidity and functional impairment that are typically found in clinic-referred samples relative to nonreferred samples (Waschbusch, 2002). Therefore, caution should be used in generalizing the results from the current study beyond the methodology described herein (i.e., clinic-referred sample, clinical diagnosis of ADHD based on parents' symptom ratings).

Future studies should focus on exploring ways to reduce phenotypic heterogeneity within the current definition of ADHD. Refinement of the ADHD phenotype is one strategy that should help reduce heterogeneity and bolster our ability to identify genes contributing to ADHD. As several researchers have recently suggested, one under-explored factor contributing to the inconsistency in molecular genetic results for ADHD seems to be diagnostic heterogeneity, which possibly reflects underlying genetic heterogeneity (Lasky-Su

et al., 2008; Todd et al., 2001). One approach to reducing the heterogeneity within ADHD is to examine symptom clusters of other childhood disorders that frequently co-occur with ADHD. Taking into account levels of co-occurring externalizing and internalizing symptoms may help to identify a more genetically homogeneous phenotype, thus increasing our ability to detect candidate genes that confer risk for ADHD (e.g., Faraone, Biederman, & Friedman, 2000; Jensen et al., 1997; Volk et al., 2005).

Lubke et al.'s (2007) recent work represents another promising direction for future research on the validation of ADHD classification, whereby alternative conceptualizations of the ADHD phenotype can be tested simultaneously. They used a novel statistical approach involving factor mixture modeling (FMM), which simultaneously incorporates aspects of both factor analysis and latent class analysis, to determine whether symptoms of inattention, hyperactivity, and impulsivity reflect 1) qualitatively distinct subtypes of ADHD, 2) variants along a single continuum of severity, or 3) severity differences within subtypes. Lubke and colleagues' FMM analysis of an ADHD rating scale revealed that the best fitting model was a hybrid model that separated the non-diagnosed majority from the diagnosed minority, while allowing for severity differences in both inattention and hyperactivity-impulsivity dimensions. Thus, their results support the conceptualization that ADHD is characterized by two moderately correlated continuous dimensions (inattention and hyperactivity-impulsivity), rather than clusters of qualitatively distinct subtypes.

In conclusion, the current study provides modest support for the distinctiveness and external validity of *DSM-IV* ADHD subtypes. The most striking finding was that the CT appears to represent a more severe form of ADHD relative to the other subtypes (IT and HT). Although the ADHD subtypes were found to differ significantly on several

external correlates, their overall symptom profiles appear more similar than different when compared with non-ADHD children. Taken together, the findings from the current investigation are most consistent with the representation of ADHD as a unitary disorder with subtypes. Lubke et al.'s (2007) recent work further suggests that the most parsimonious conceptualization of ADHD is two correlated symptom dimensions that vary in severity. Recent findings from Lahey et al.'s (2005) longitudinal study showed evidence of considerable instability of the *DSM-IV* ADHD subtypes across development, providing further support for the clinical utility of continuous ratings of inattentive and hyperactive-impulsive symptoms, over and above nominal diagnostic categories. This set of findings suggests that additional research is needed to 1) examine the external validity of the ADHD subtypes and symptom dimensions, and 2) investigate novel methods of refining the phenotype that reduce diagnostic heterogeneity and thus bolster our ability to identify genes that contribute to ADHD.

References

American Psychiatric Association. (1968). *Diagnostic and Statistical Manual of Mental Disorders* (2nd ed.). Washington D.C.: American Psychiatric Association.

American Psychiatric Association. (1980). *Diagnostic and Statistical Manual of Mental Disorders* (3rd ed.). Washington D.C.: American Psychiatric Association.

American Psychiatric Association. (1987). *Diagnostic and Statistical Manual of Mental Disorders* (3rd ed., revised). Washington, D.C.: American Psychiatric Association.

American Psychiatric Association. (1994). *Diagnostic and Statistical Manual of Mental Disorders* (4th ed.). Washington, D.C.: American Psychiatric Association.

American Psychiatric Association. (2000). *Diagnostic and Statistical Manual of Mental Disorders* (4th ed., text revision). Washington, D.C.: American Psychiatric Association.

Asghari, V., Sanyal, S., Buchwaldt, S., Paterson, A., Jovanovic, V., & Van Tol, H.H. (1995). Modulation of intercellular cyclic AMP levels by different human dopamine D4 receptor variants. *Journal of Neurochemistry, 65*, 1157–1165.

Baeyens, D. Roeyers, H., & Walle, J.V. (2006). Subtypes of attention-deficit / hyperactivity disorder: Distinct or related disorders across measurement levels? *Child Psychology & Human Development, 36*, 403-417.

Barkley, R.A., (1997). *ADHD and the nature of self-control.* New York: Guilford Press.

Barkley, R.A., DuPaul, G.J., & McMurray, M.B. (1990). Comprehensive evaluation of attention deficit disorder with and without hyperactivity as defined by research criteria. *Journal of Counseling & Clinical Psychology, 58*(6), 775-789.

Barkley, R. A., DuPaul, G.J., & McMurray, M.B. (1991). Attention deficit disorder with

and without hyperactivity: Clinical response to three does of methylphenidate. *Pediatrics, 87*, 519-531.

Bauermeister, J.J., Matos, M., Reina, G., Salas, C.C., Martinez, J.V., Cumba, E., et al. (2005). Comparison of the DSM-IV combined and inattentive types of ADHD in a school-based sample of Latino/Hispanic children. *Journal of Child Psychology and Psychiatry, 46*(2), 166-179.

Benjamin, J., Li, L., Patterson, C., Greenberg, B., Murphy, D., & Hamer, D. (1996). Population and familial association between the D4 dopamine receptor gene and measures of Novelty Seeking. *Nature Genetics, 12*, 81-84.

Carlson, C.L., & Mann, M. (2000). Attention-deficit/hyperactivity disorder, predominantly inattentive subtype. *Child and Adolescent Psychiatric Clinics of North America, 9*(3), 499-510.

Carlson, C.L., & Mann, M. (2002). Sluggish cognitive tempo predicts a different pattern of impairment in the Attention Deficit Hyperactivity Disorder, predominantly inattentive type. *Journal of Clinical Child & Adolescent Psychology, 31*(1), 123-129.

Chhabildas, N., Pennington, B., & Willcutt, E. (2001). A comparison of the neuropsychological profiles of the *DSM-IV* subtypes of ADHD. *Journal of Abnormal Child Psychology, 29*, 529-540.

Coffman, J.K, Wright, G., & Gottfried, A.W. (2006). Reliability and validity of the parent-child relationship inventory (PCRI): Evidence from a longitudinal cross-informant investigation. *Psychological Assessment, 18*(2), 209-214.

Cohen, J., Cohen, P., West, S.G., & Aiken, L.S. (Eds.). (2003). *Applied multiple regression / correlation analysis for the behavioral sciences* (3rd ed.). Mahwah, N.J.: Lawrence Erlbaum Associates, Publishers.

Curran, S., Mill, J., Sham, P., Rijsdijk, F., Marusic, K., Taylor, E., et al. (2001). QTL association analysis of the DRD4 exon 3 VNTR polymorphism in a population sample of children screened with a parent rating scale for ADHD symptoms. *American Journal of Medical Genetics (Neuropsychiatric Genetics), 105*, 387-393.

Curran, S., Purcell, S., Craig, I., Asherson, P., & Sham, P. (2005). The serotonin transporter gene as a QTL for ADHD. *American Journal of Medical Genetics Part B (Neuropsychiatric Genetics), 134B*, 42-47.

Ebstein, R., Novick, O., Umansky, R., Priel, B., Osher, Y., Blaine, D., et al. (1996). Dopamine D4 receptor (DRD4) exon III polymorphism associated with the human personality trait of Novelty Seeking. *Nature Genetics, 12*, 78-80.

Eisenberg, J., Zohar, A., Mei-Tal, G., Steinberg, A., Tartakovsky, E., Gritsenko, I., Nemanov, L, & Ebstein, R.P. (2000). A haplotype relative risk study of the dopamine D4 receptor (DRD4) exon III repeat polymorphism and attention deficit hyperactivity disorder (ADHD). *American Journal of Medical Genetics, (96)*3, 258-261.

Faraone, S.V., Biederman, J., Chen, W.J., Milberger, S., Warburton, R., & Tsuang, M. (1995). Genetic heterogeneity in attention-deficit hyperactivity disorder (ADHD): Gender, psychiatry comorbidity, and maternal ADHD. *Journal of Abnormal Psychology, 104*(2), 334-345.

Faraone, S.V., Biederman, J., & Friedman, D. (2000). Validity of DSM-IV subtypes of Attention-deficit/hyperactivity disorder: A family study perspective. *Journal of the American Academy of Child & Adolescent Psychiatry, 39*(3), 300-307.

Faraone, S.V., Biederman, J., Weber, W., & Russell, R.L. (1998). Psychiatric, neuropsychological, and psychosocial features of DSM-IV subtypes of Attention-deficit/hyperactivity disorder: Results from a clinically referred sample. *Journal of the American Academy of Child & Adolescent Psychiatry, 37*(2), 185-193.

Faraone, S. V., Doyle, A. E., Mick, E., & Biederman, J. (2001). Meta-analysis of the association between the 7-repeat allele of the dopamine D(4) receptor gene and attention deficit hyperactivity disorder. *American Journal of Psychiatry, 158,* 1052-1057.

Faraone, S., Perlis, R., Doyle, A., Smoller, J., Goralnick, J., Holmgren, M., et al. (2005). Molecular genetics of Attention-Deficit/Hyperactivity Disorder. *Biological Psychiatry, 57,* 1313-1323.

Faraone, S.V. & Khan, S.A. (2006). Candidate gene studies of attention-deficit/hyperactivity disorder. *Journal of Clinical Psychiatry, 67*(Suppl 8), 13-20.

Gadow, K.D., Drabick, D.A.G., Loney, J., Sprafkin, J., Salisbury, H., Azizian, A., et al. (2004). Comparison of ADHD symptom subtypes as source-specific syndromes. *Journal of Child Psychology & Psychiatry, 45*(6), 1135-1149.

Gainetdinov, R.R., Wetsel, W.C., Jones, S.R., Levin, E.D., Jaber, M., & Caron, M.G. (1999). Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. *Science, 283,* 397-401.

Gjone, H., Stevenson, J., & Sundet, J.M. (1996). Genetic influence on parent-reported

attention-related problems in a Norwegian general population twin sample. *Journal of the American Academy of Child & Adolescent Psychiatry, 35,* 588-596.

Goodman, R., & Stevenson, J. (1989). A twin study of hyperactivity, II: The aetiological role of genes, family relationships and perinatal adversity. *Journal of Child Psychology & Psychiatry, 30,* 691-709.

Halperin, J. M., Newcorn, J. H., Schwartz, S. T., Sharma, V., Siever, L. J., Koda, V. H., et al. (1997). Age-related changes in the association between serotonergic function and aggression in boys with ADHD. *Biological Psychiatry, 41,* 682-689.

Harrington, K.M., & Waldman, I.D. (2008). Differences among the attention-deficit / hyperactivity disorder subtypes in their overlap with internalizing and externalizing disorders: A meta-analytic review. Manuscript in preparation.

Hart, E.L., Lahey, B.B., Loeber, R., Applegate, B., & Frick, P.J. (1995). Developmental change in Attention-Deficit Hyperactivity Disorder in boys: A four-year longitudinal study. *Journal of Abnormal & Child Psychology, 23,* 729-749.

Hechtman, L. (1999). Predictors of long-term outcome in children with attention-deficit/ hyperactivity disorder. *Pediatric Clinics of North America, 46*(5), 1039-1052.

Hinshaw, S.P., Carte, E.T., Sami, N., Treuting, J.J., & Zupan, B.A. (2002). Preadolescent girls with attention-deficit/hyperactivity disorder: II. Neuropsychological performance in relation to subtypes and individual classification. *Journal of Consulting and Clinical Psychology, 70*, 1099-1111.

Holmes, J., Payton, A., Barrett, J., Hever, T., Fitzpatrick, H., Trumper, A. L., et al. (2000). A family-based and case-control association study of the dopamine D4 receptor gene and dopamine transporter gene in attention deficit hyperactivity

disorder. *Molecular Psychiatry, 5,* 523-530.

Hutchison, K.E., Stallings, M., McGeary, J., & Bryan, A. (2004). Population stratification in the candidate gene study: Fatal threat or red herring. *Psychological Bulletin, 130*(1), 66-79.

Jensen, P.S., Martin, D., & Cantwell, D.P. (1997). Comorbidity in ADHD: Implications for research, practice, and *DSM-IV*. *Journal of the American Academy of Child & Adolescent Psychiatry, 36*(8), 1065-1079.

Lahey, B. A., & Carlson, C. L. (1991). Validity of the diagnostic category of attention deficit disorder without hyperactivity: A review of the literature. *Journal of Learning Disabilities, 24,* 110-120.

Lahey, B.B., Carlson, C.L., & Frick, P.J. (1997). Attention-deficit disorder without hyperactivity. In Widiger, T.A., Frances, A.J., Pincus, H.A, et al. (Eds): *DSM-IV source book* (Vol. 3, pp.163-188). Washington, D.C.: American Psychiatric Association.

Lahey, B.B., Pelham, W.E., Loney, J., Lee, S.S., & Willcutt, E. (2005). Instability of the *DSM-IV* subtypes of ADHD from preschool through elementary school. *Archives of General Psychiatry, 62,* 896-902.

Lahey, B.B., Pelham, W.E., Schaughency, E.A., Atkins, M.S., Murphy, A., Hynd, G., Russo, M., Hartagen, S., & Lorys-Vernon, A. (1988). Dimensions and types of attention deficit disorder. *Journal of the American Academy of Child & Adolescent Psychiatry, 27*(3), 330-335.

Lahey, B.B., Pelham, W.E., Stein, M.A., Loney, J., Trapani, C., Nugent, K., et al. (1998). Validity of DSM-IV attention-deficit/hyperactivity disorder for younger children.

Journal of the American Academy of Child & Adolescent Psychiatry, 37(7), 695-702.

Lahey, B.B., Schaughency, E.A., Hynd, G.W., Carlson, C., & Niever, C. (1987). Attention deficit disorder with and without hyperactivity: Comparison of behavioral characteristics of clinic referred children. *Journal of the American Academy of Child & Adolescent Psychiatry, 26,* 718-723.

Lahey, B.B., Schaughency, E.A., Strauss, C.C., & Frame, C.L. (1984). . *Journal of the American Academy of Child & Adolescent Psychiatry, 23*(3), 302-309.

Lahey, B.B., Waldman, I.D., Hankin, B.L., Applegate, B., Loft, J.D., & Rick, J. (2004). The structure of child and adolescent psychopathology: Generating new hypotheses. *Journal of Abnormal Psychology, 113*(3), 358-385.

Lahey, B.B. & Willcutt, E. (2002). The validity of attention-deficit/hyperactivity disorder among children and adolescents. In P.S. Jensen & J.R. Cooper (Eds.), *Diagnosis and treatment of attention deficit hyperactivity disorder: An evidence-based approach.* New York: Civic Research Institute, pp. 1-23.

Lasky-Su, J., Lange, C., Biederman, J., Tsuang, M., Doyle, A.E., Smoller, J.W., et al. (2008). Family-based association analysis of a statistically derived quantitative traits for ADHD reveal an association in DRD4 with inattentive symptoms in ADHD individuals. *American Journal of Medical Genetics Part B (Neuropsychiatric Genetics), 147B,* 100-106.

Laucht, M., Becker, K., Schmidt, M.H. (2006). Visual exploratory behaviour in infancy and novelty seeking in adolescence: two developmentally specific phenotypes of DRD4? *Journal of Child Psychology & Psychiatry,47*(11), 1143-1151.

LeMarquand, D.G., Pihl, R.O., Young, S.N., Tremblay, R.E., Seguin, J.R., Palmour, R.M., & Benkelfat, C. (1998). Tryptophan depletion, executive functions, and disinhibition in aggressive, adolescent males. *Neuropsychopharmacology, 19*, 333-341.

Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science, 274,* 1527-1531.

Levy, F., Hay, D.A., 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 American Academy of Child & Adolescent Psychiatry, 36,* 737-744.

Li, D., Sham, P.C., Owen, M.J., & He, L. (2006). Meta-analysis shows significant association between dopamine system genes and attention deficit hyperactivity disorder (ADHD). *Human Molecular Genetics, 15*(14), 2276-2284.

Li, J., Wang, Y., Zhou, R., Zhang, H., Yang, L., Wang, B., & Faraone, S.V. (2007). Association between polymorphisms in serotonin transporter gene and Attention Deficit Hyperactivity Disorder in Chinese Han subjects. *American Journal of Medical Genetics Part B, 144B*, 14-19.

Lichter, J.B., Barr, C.L., Kennedy, J.L., Van Tol, H.H., Kidd, K.K., & Livak, K.J. (1993). A hypervariable segment in the human dopamine receptor D4 (DRD4) gene. *Human Molecular Genetics, 2*, 767-773.

Lubke, G.H., Muthen, B., Moilanen, I.K., McGough, J.J., Loo, S.K., Swanson, J.M., et al. (2007). Subtypes versus severity differences in attention-deficit / hyperactivity

disorder in the northern Finnish birth cohort. *Journal of American Academy of Child & Adolescent Psychiatry, 46*(12), 1584-1593.

Lucki, I. (1998). The spectrum of behaviors influenced by serotonin. *Biological Psychiatry, 44*, 151-162.

Manor, I., Eisenberg, J., Tyano, S., Sever, Y., Cohen, H., Ebstein, R. P., et al. (2001). Family-based association study of the serotonin transporter promoter region polymorphism (5-HTTLPR) in attention deficit hyperactivity disorder. *American Journal of Medical Genetics, 105,* 91-95.

McCracken, J.T., Smalley, S.L., McGough, J.J., Crawford, L., Del'Homme, M., Cantor, R.M., et al. (2000). Evidence for linkage of a tandem duplication polymorphism upstream of the dopamine D4 receptor gene (DRD4) with attention deficit hyperactivity disorder (ADHD). *Molecular Psychiatry,*5(5), 531-536.

Milich, R., Balentine, A.C., & Lynam, D.R. (2001). ADHD combined type and ADHD predominantly inattentive type are distinct and unrelated disorders. *Clinical Psychology: Science & Practice, 8*(4), 463-506.

Neumeister, A., Young, T., & Stastny, J. (2004). Implications of genetic research on the role of serotonin in depression: emphasis on the serotonin type 1_A receptor and the serotonin transporter. *Psychopharmacology, 174*, 512-524.

Newcorn, J.H., Halperin, J.M., Jensen, P.S., Abikoff, H.B., Arnold, E., Cantwell, D.P., et al. (2001). Symptom profiles in children with ADHD: Effects of comorbidity and gender. *Journal of the American Academy of Child & Adolescent Psychiatry, 40*(2), 137-146.

Nigg, J.T., Blaskey, L., Stawicki, J., & Sachek, J. (2004). Evaluating the endophenotype

model of ADHD neuropsychological deficit: results for parents and siblings of children with ADHD combined and inattentive subtypes. *Journal of Abnormal Psychology, 113,* 614-625.

Oades, R. D., Slusarek, M., Velling, S., & Bondy, B. (2002). Serotonin platelet-transporter measures in childhood attention-deficit/hyperactivity disorder (ADHD): clinical versus experimental measures of impulsivity. *World Journal of Biological Psychiatry, 3,* 96-100.

Paterson, A.D., Sunohara, G.A., & Kennedy, J.L. (1999). Dopamine receptor gene: Novelty or nonsense? *Neuropsychopharmacology, 21,* 3-16.

Pericak-Vance, M.A. (1998). Linkage disequilibrium and allelic association. In: Haines JL, Pericak-Vance MA (Eds) *Approaches to Gene Mapping in Complex Human Diseases.* Wiley: New York, 1998, pp 323-334.

Power, T.J., Costigan, T.E., Eiraldi, R.B., & Leff, S.S. (2004). Variations in anxiety and depression as a function of ADHD subtypes defines by DSM-V: Do subtype differences exist or not? *Journal of Abnormal Child Psychology, 32*(1), 27-37.

Rowe, D.C., Stever, C., Giedinghagen, L.N., Gard, J.M., Cleveland, H.H., Terris, S.T., et al. (1998). Dopamine DRD4 receptor polymorphism and attention deficit hyperactivity disorder. *Molecular Psychiatry, 3,* 419-426.

Rubinstein, M., Phillips, T. J., Bunzow, J. R., Falzone, T. L., Dziewczapolski, G., Zhang, G., et al. (1997). Mice lacking dopamine D4 receptors are supersensitive to ethanol, cocaine, and methamphetamine. *Cell, 90,* 991-1001.

Schmidt, L., Fox, N., Perez-Edgar, K., Hu, S., & Hamer, D. (2001). Association of DRD4 with attention problems in normal childhood development. *Psychiatric Genetics, 11*, 25-29.

Seeger, G., Schloss, P., & Schmidt, M. H. (2001). Functional polymorphism within the promotor of the serotonin transporter gene is associated with severe hyperkinetic disorders. *Molecular Psychiatry, 6*, 235−238.

Seeman, P., & Madras, B.K. (1998). Anti-hyperactivity medication: Methylphenidate and amphetamine. *Molecular Psychiatry, 3,* 386-396.

Simonoff, E., Pickles, A., Hervas, A., Silberg, J. L., Rutter, M., & Eaves, L. (1998). Genetic influences on childhood hyperactivity: Contrast effects imply parental rating bias, not sibling interaction. *Psychological Medicine, 28*, 825-837.

Solanto, M. V. (1984). Neuropharmacological basis of stimulant drug action in attention deficit disorder with hyperactivity: A review and synthesis. *Psychological Bulletin, 95*, 387−409.

Spivak, B., Vered, Y., Yoran-Hegesh, R., Averbuch, E., Mester, R., Graf, E., et al. (1999). Circulatory levels of catecholamines, serotonin and lipids in attention deficit hyperactivity disorder. *Acta Psychiatrica Scandinavica, 99*, 300-304.

Stawicki, J.A., Nigg, J.T., & van Eye, A. (2006). Family psychiatric history evidence on the nosological relations of DSM-IV ADHD combined and inattentive subtypes: new data and meta-analysis. *Journal of Child Psychology and Psychiatry, 47*(9), 935-945.

Stein, M.A., Sarampote, C.S., Waldman, I.D., Robb, A.S., Conlon, C., Pearl, P.L., et al. (2003). A dose-response study of OROS methylphenidate in children with

attention-deficit / hyperactivity disorder. *Pediatrics, 112*(5), e404-e413.

Thapar, A.M., Harrington, R.F., Ross, K.M., & McGuffin, P.F. (2000). Does the definition of ADHD affect heritability? *Journal of the American Academy of Child & Adolescent Psychiatry, 39*(12), 1528-1536.

Thapar, A., Holmes, J., Poulton, K., & Harrington, R. (1999). Genetic basis of attention deficit and hyperactivity. *British Journal of Psychiatry, 174,* 105-111.

Todd, R.D. (2000). Genetics of attention deficit / hyperactivity disorder: Are we ready for molecular genetic studies? *American Journal of Medical Genetics, 96,* 241-243.

Todd, R.D., Neuman, R.J., Lobos, E.A., Jong, Y.I., Reich, W., & Heath, A.C. (2001). Lack of association of dopamine D4 receptor gene polymorphism with ADHD subtypes in a population sample of twins. *American Journal of Medical Genetics Part B (Neuropsychiatric Genetics), 105*, 432-438.

Volk, H.E., Neuman, R.J., & Todd, R.D. (2005). A systematic evaluation of ADHD and comorbid psychopathology in a population-based twin sample. *Journal of the American Academy of Child & Adolescent Psychiatry, 44*, 768-775.

Waldman, I.D., & Gizer, I.R. (2006). The genetics of attention deficit hyperactivity disorder. *Clinical Psychology Review, 26*, 396-432.

Waldman, I. D., & Rhee, S. H. (2002). Behavioral and molecular genetic studies. In S. Sandberg (Ed.), *Hyperactivity and attention disorders of childhood, 2nd Ed.* (pp. 290-335). New York: Cambridge University Press.

Waldman, I. D., Rowe, D. C., Abramowitz, A., Kozel, S. T., Mohr, J. H., Sherman, S. L., et al. (1998). Association and linkage of the dopamine transporter gene and attention-deficit hyperactivity disorder in children: heterogeneity owing to

diagnostic subtype and severity. *American Journal of Human Genetics, 63,* 1767-1776.

Waschbusch, D.A. (2002). A meta-analytic examination of comorbid hyperactive-impulsive-attention problems and conduct problems. *Psychological Bulletin, 128*(1), 118-150.

Wigginton, J.E. & Abecasis, G.R. (2005). PEDSTATS: descriptive statistics, graphics and quality assessment for gene mapping data. *Bioinformatics, 21*(16), 3445-3447.

Wittke-Thompson, J., Pluzhnikov, A., & Cox, N. (2005). Rational inferences about departures from Hardy-Weinberg Equilibrium. *The American Journal of Human Genetics, 76*(6), 967-986.

Woo, S.C., & Rey, J.M. (2005). The validity of the DSM-IV subtypes of attention-deficit/ hyperactivity disorder. *Australian & New Zealand Journal of Psychiatry, 39,* 344-353.

Table 1. *Sample Demographic Characteristics and Mean ADHD Symptom Levels for ADHD Subtypes and Normal Controls*

	ADHD/IT	ADHD/CT	ADHD/HT	Normal controls
Participants, N	82	132	23	215
ADHD subtype diagnoses (%)	34%	56%	10%	--
Gender, N (%) male	55 (67%)	103 (78%)	14 (61%)	155 (72%)
Mean age at assessment (SD)	11.5 (3.0)	10.0 (2.8)	8.4 (2.5)	11.3 (4.0)
Mean inattention sx (SD)	2.81 (.62)	3.10 (.56)	1.40 (.66)	.67 (.54)
Mean hyp-imp. sx (SD)	.95 (.68)	2.92 (.63)	2.77 (.58)	.49 (.48)

Note. ADHD = Attention-Deficit/Hyperactivity Disorder; ADHD/IT = Inattentive Type; ADHD/CT = Combined Type; ADHD/HT = Hyperactive-Impulsive Type; sx = symptoms; hyp-imp. = hyperactive-impulsive.

Table 2. *A Priori Orthogonal Contrasts for Comparing DSM-IV ADHD Subtypes*

Contrast set 1	Contrast 1	Contrast 2
ADHD diagnostic subtype		
Inattentive	1	1
Hyperactive-impulsive	-2	0
Combined	1	-1
Contrast set 2	**Contrast 1a**	**Contrast 2a**
ADHD diagnostic subtype		
Inattentive	2	0
Hyperactive-impulsive	-1	-1
Combined	-1	1

Table 3. *Contrasts among the DSM-IV ADHD Subtypes on Gender Ratios and Mean Age*

Contrast set 1	ADHD/IT + CT vs. ADHD/HT		ADHD/IT vs. ADHD/CT		Significant pairwise group differences
	p	*OR*[a] / η^{2b}	*p*	*OR*[a] / η^{2b}	
Child's sex (% of males)	.114	1.20[a]	.039*	1.32[a]	CT > IT
Mean age	< .001***	.056[b]	< .001***	.060[b]	IT + CT > HT; IT > CT
Contrast set 2	**ADHD/IT vs. ADHD/HT + CT**		**ADHD/HT vs. ADHD/CT**		**Significant pairwise group differences**
	p	*OR*[a] / η^{2b}	*p*	*OR*[a] / η^{2b}	
Child's sex (% of males)	.334	1.05[a]	.041*	1.51[a]	CT > HT
Mean age	< .001***	.103[b]	.008**	.025[b]	IT > HT + CT; CT > HT

Note. ADHD/IT = Attention-Deficit/Hyperactivity Disorder, Inattentive Type; ADHD/CT = Combined Type; ADHD/HT = Hyperactive-Impulsive Type.

[a] Odds Ratio (OR); [b] Eta-squared (η^2) = effect size calculated for contrasts.

*p < .05; **p < .01; ***p < .001.

Table 4. *Contrasts among the DSM-IV ADHD Subtypes on Essential and Overlapping Symptom Scales*

Symptom scales	ADHD/IT *Mean (SD)*	ADHD/CT *Mean (SD)*	ADHD/HT *Mean (SD)*	Omnibus F *p-value*	Significant pairwise group differences
Inattention	2.81 (.62)	3.10 (.56)	1.40 (.66)	< .001	IT + CT > HT; IT < CT; IT > HT + CT; HT < CT
Hyp-imp.	.95 (.68)	2.92 (.63)	2.77 (.58)	< .001	IT + CT < HT; IT < CT; IT < HT + CT
CD	.27 (.31)	.51 (.45)	.41 (.37)	< .001[a]	IT < CT; IT < HT + CT
ODD	1.36 (1.00)	2.31 (1.04)	2.31 (1.11)	< .001	IT + CT < HT; IT < CT; IT < HT + CT
MDD	.45 (.49)	.73 (.59)	.36 (.41)	< .001[a]	IT + CT > HT; IT < CT; HT < CT
GAD	.52 (.53)	.84 (.70)	.55 (.56)	< .001[a]	IT < CT; IT < HT + CT; HT < CT
SAD	.18 (.40)	.39 (.57)	.27 (.39)	.006[a]	IT < CT; IT < HT + CT
Agoraphobia	.08 (.23)	.20 (.41)	.12 (.33)	.020[a]	IT < CT; IT < HT + CT
Panic	.03 (.08)	.03 (.11)	.05 (.12)	.299	---
Specific phobia	.28 (.33)	.41 (.43)	.31 (.37)	.022[a]	IT < CT; IT < HT + CT (*Trend*)
Social phobia	.56 (.66)	.59 (.77)	.60 (.83)	.472	---
Obsessions	.14 (.29)	.37 (.59)	.26 (.31)	.002[a]	IT < CT; IT < HT + CT
Compulsions	.08 (.27)	.19 (.40)	.20 (.35)	.038[a]	IT < CT; IT < HT + CT

Note. ADHD/IT = Attention-Deficit/Hyperactivity Disorder, Inattentive Type; ADHD/CT = Combined Type; ADHD/HT = Hyperactive-Impulsive Type; Hyp-imp. = hyperactive-impulsive symptoms; CD = Conduct Disorder; ODD = Oppositional Defiant Disorder; MDD = Major Depressive Disorder; GAD = Generalized Anxiety Disorder; SAD = Separation Anxiety Disorder.

[a] indicates significant Levene's test; thus, results of contrast tests were adjusted based on a model that does not assume equal variances.

Table 5. *Tests of Association among DSM-IV ADHD Subtypes and DRD4 and 5-HTT*

Contrast set 1	ADHD/IT + CT vs. ADHD/HT		ADHD/IT vs. ADHD/CT		
	p	OR	*p*	OR	Significant planned comparisons
DRD4 (controlling for ethnicity only)	.028**	1.95	.039**	1.36	IT + CT > HT; CT > IT
DRD4 (controlling for ethnicity, sex X age)	.035**	1.90	.013**	1.52	IT + CT > HT; CT > IT
5-HTT (controlling for ethnicity only [a])	.068*	1.26	.066*	1.25	IT + CT > HT; IT > CT
Contrast set 2	**ADHD/IT vs. ADHD/HT + CT**		**ADHD/HT vs. ADHD/CT**		
	p	OR	*p*	OR	Significant planned comparisons
DRD4 (controlled for ethnicity only)	.182	1.20	.014**	3.19	CT > HT
DRD4 (controlled for ethnicity, sex X age)	.294	1.12	.014**	3.24	CT > HT
5-HTT (controlled for ethnicity only [a])	.020**	1.26	.160	1.27	IT > HT + CT

Note. [a] When sex, age, age^2, and sex X age, and sex X age^2 interactions were tested as covariates in the *5-HTT*-ADHD association analyses, none of the sex and age terms were significant, and thus, were subsequently dropped from the model.

*$p < .10$; **$p < .05$.

Reexamining the Associations of *DRD4* and *5-HTT* with ADHD Using an Alternative Comorbid ADHD Phenotype

Kelly M. Harrington and Irwin D. Waldman

Emory University

Abstract

Heterogeneity within the attention-deficit/hyperactivity disorder (ADHD) diagnosis likely accounts for some inconsistent findings in molecular genetic studies of ADHD. One approach to reducing diagnostic heterogeneity is to differentiate ADHD on the basis of comorbid conditions. The objective of this study was to examine the external validity of an alternative ADHD phenotype based on co-occurring symptoms of externalizing and internalizing disorders. The sample included 372 children (ages 5 – 18) who were recruited as part of an ongoing study on the genetics of ADHD. Probands, their siblings, and parents were genotyped for a variable number of tandem repeats (VNTR) in exon 3 of dopamine D4 receptor gene (*DRD4)* and an insertion / deletion polymorphism in the promoter region of the serotonin transporter gene (*5-HTTLPR*). Ordinal logistic regression analyses revealed no significant evidence of association between the overall ADHD diagnosis and the *DRD4* 7-repeat allele (p = .398) or the *5-HTTLPR* long allele (p = .219). In contrast, there was significant evidence suggesting that co-occurring symptoms of conduct disorder moderated the ADHD-*DRD4* relation, such that a stronger association between ADHD and *DRD4* (p = .036, OR = 2.9) was found in children with both ADHD and elevated conduct disorder symptoms. There was also significant evidence of a moderating influence of co-occurring symptoms of anxiety, whereby a stronger association between ADHD and *5-HTTLPR* (p = .044, OR = 1.5) was found in children with higher levels of anxiety. These findings suggest that co-occurring symptoms of conduct disorder and anxiety may bolster detection of associations between ADHD and candidate genes such as *DRD4* and *5-HTTLPR*.

Reexamining the Associations of *DRD4* and *5-HTT* with ADHD Using an Alternative Comorbid ADHD Phenotype

Attention-deficit/hyperactivity disorder (ADHD) is a prevalent childhood-onset psychiatric condition characterized by impairing symptoms of inattention, overactivity, and impulsiveness. The *Diagnostic and Statistical Manual of Mental Disorders – 4th Edition (DSM-IV)* (APA, 1994) defines three subtypes of ADHD based on the degree to which an individual exhibits symptoms of the two underlying symptom dimensions of inattention and hyperactivity-impulsivity, namely the Predominantly Inattentive Type (IT), Predominantly Hyperactive-Impulsive Type (HT), and Combined Type (CT).

Family, twin, and adoption studies have consistently shown strong evidence to suggest that substantial genetic influences are contributing to ADHD, with heritability estimates ranging from 60 to 90% (Waldman & Rhee, 2002). Despite strong and consistent evidence for genetic influences on ADHD, candidate gene studies of ADHD have yielded mixed findings across studies. Furthermore, though several candidate genes have shown evidence of replicable associations with ADHD, including the dopamine transporter gene (*DAT1*), the dopamine D4 and D5 receptor genes (*DRD4* and *DRD5*, respectively), and the serotonin transporter gene (*5-HTT*), the effect sizes of these associations have been relatively small (Faraone & Khan, 2006; Waldman & Gizer, 2006). In a recent review of molecular genetic studies of ADHD, for example, Faraone et al. (2005) reported that seven genes have shown replicable evidence of significant association with ADHD on the basis of pooled odds ratios (OR) across 3 or more studies, with ORs ranging from 1.1 to 1.4 (an OR of 1.0 indicates no association between ADHD and the putative risk allele).

Before advances in molecular genetics can be maximally utilized to further our understanding of the genetic and environmental risk factors that give rise to child psychiatric disorders, accurate and valid characterization of the psychopathological phenotypes is necessary (Lahey et al., 2004). A number of investigators have described ADHD as a heterogeneous condition with regard to its variability in the presentation of core symptoms, its clinical correlates (e.g., demographic characteristics, rates of comorbidity, outcome), and arguably its etiology (Faraone et al., 1995; Hechtman, 1999; Jensen, Martin, & Cantwell, 1997; Newcorn et al., 2001; Todd, 2000). Although there is increasing recognition that the phenotypic heterogeneity within ADHD may compromise the validity of this diagnosis and complicate its treatment (Jensen et al., 1997), the majority of molecular genetic studies have investigated the relation between a gene of interest and the categorical ADHD diagnosis (Kirley et al., 2004). Thus, tests of association conducted using just the categorical diagnosis of ADHD might mask a significant effect if the candidate gene is associated only with certain aspects of the ADHD phenotype (e.g., a particular diagnostic subtype or comorbid subgroup).

Notably, there is preliminary evidence suggesting that some candidate genes for ADHD may be preferentially related to one symptom dimension or diagnostic subtype, rather than the disorder as a whole. For example, several studies have suggested a stronger relation between *DRD4* and the IT as well as with inattentive (rather than hyperactive-impulsive) symptoms (Lasky-Su et al., 2008; McCracken et al., 2000; Rowe et al., 1998). In contrast, studies examining the relation between *5-HTT* and ADHD have found *5-HTT* to be preferentially associated with hyperactive-impulsive symptoms and the CT (Manor et al., 2001; Seeger, Schloss, & Schmidt, 2001).

An alternative approach for reducing the degree of diagnostic heterogeneity in the ADHD phenotype is to stratify ADHD groups by the presence of co-occurring disorders, such as oppositional defiant disorder (ODD), conduct disorder (CD), depressive disorders, and anxiety disorders (e.g., Faraone et al., 1995). There is a growing body of research focused on identifying more homogeneous subgroups of children with ADHD based on differing profiles of comorbid conditions (e.g., Faraone, Biederman, & Friedman, 2000; Hinshaw, 1987; Jensen et al., 1997; Neuman et al., 2001; Newcorn et al., 2001). Furthermore, the high rates of co-occurrence of other psychiatric disorders in ADHD may reflect underlying genetic heterogeneity (Smalley et al., 2000). Thus, it seems axiomatic that methods aimed at reducing heterogeneity in ADHD by discriminating among common comorbid conditions would enhance the power to detect associations between genes and ADHD.

There is initial evidence to suggest that stratifying ADHD groups by the presence of co-occurring conditions indeed may be an effective means of revealing associations between candidate genes and ADHD that otherwise would remain undetected if the phenotype were solely defined as a unitary diagnostic construct (e.g., Caspi et al., 2008; Holmes et al., 2002; Kirley et al., 2004). First, Holmes et al. (2002) reported a significant association between the 7-repeat allele of *DRD4* and ADHD with comorbid "conduct problems" (i.e., broadly defined by ODD and at least one symptom of CD), whereas analyses of the total ADHD sample yielded negative results. In an expanded subset of the original sample described by Holmes and colleagues, Kirley et al. (2004) also found a significant association between the *DRD4* 7-repeat allele and ADHD with comorbid ODD, whereas no association was detected between *DRD4* and the overall ADHD sample.

Most recently, Caspi et al. (2008) examined the relation between antisocial behavior and the catchol O-methyltransferase gene (*COMT*) in three independent samples of children diagnosed with ADHD. Caspi and colleagues did not find evidence for significant association between the *COMT* valine/methionine (Val/Met) polymorphism and antisocial behavior among children without ADHD, nor between the Val/Met variant and ADHD. In contrast, there was evidence for significant association between antisocial behavior and the Val/Met variant among children diagnosed with ADHD, suggesting the association between antisocial behavior and genotype was conditional on ADHD diagnosis. Thus, the authors concluded that the *COMT* Val/Met variant influenced phenotypic variation within children diagnosed with ADHD and helped to identify a subset of these children who exhibit antisocial behavior.

Given preliminary evidence that the identification of more phenotypically homogeneous subgroups within the overall ADHD diagnosis may enhance our ability to detect associations with candidate genes, the primary aim of the current study is to examine an alternative ADHD phenotype based on co-occurring symptoms of externalizing and internalizing disorders. Furthermore, this study tests whether the proposed comorbid ADHD phenotype enhances the external validity of ADHD, as a function of including co-occurring symptom dimensions, over and above the extant *DSM-IV* subtyping system. Specifically, the *DSM-IV* ADHD subtypes were compared with ADHD accompanied by comorbid conditions on the basis of several external validity indicators, including genetic associations with *DRD4* and *5-HTT*, gender composition, and mean age. Three "comorbid conditions" were designated as follows: 1) *ADHD* X *ODD/CD*, 2) *ADHD* X *Distress* (i.e., major depressive disorder (MDD), generalized

anxiety disorder (GAD), and social phobia), and 3) *ADHD* X *Fear* (i.e., separation anxiety disorder (SAD), specific phobias, obsessions, compulsions).

The rationale for using composite variables comprised of externalizing and internalizing symptoms largely stems from recent research on the factor structure of psychopathology (Krueger, 1999; Lahey et al., 2004) and related behavior genetic findings (Kendler et al., 2003). Krueger (1999) conducted a series of confirmatory factor analyses (CFAs) of *DSM-III-R* (APA, 1987) diagnoses in the National Comorbidity Study dataset (i.e., a population-based sample of adults). The CFA revealed that the model that best fit the data contained three factors, consisting of an *Externalizing* dimension (alcohol dependence, drug dependence, antisocial personality disorder), an *Anxious-Misery* dimension (MDD, dysthymia, GAD), and a *Fear* dimension (panic disorder, agoraphobia, social phobia, simple phobia). The latter two factors were strongly correlated and therefore defined a high-order *Internalizing* dimension. Similarly, Krueger and Markon (2006) labeled these separate, but highly correlated internalizing factors as *Distress* and *Fear*. More specifically, the authors delineated *Distress* as a liability to major depression, dysthymia, and GAD versus *Fear* as a liability to panic disorder and the phobic disorders.

Lahey et al. (2004) examined the structure of psychopathology in a large, representative child and adolescent sample using principal factor analysis. Lahey and colleagues' findings were largely consistent with the adult studies, most notably indicating that certain facets of anxiety (SAD, fears, obsessions, and compulsions) are reasonably distinct from depression, whereas other types of anxiety (GAD and perhaps

social anxiety) are so highly correlated with depression that they appear to be part of the same dimension.

Furthermore, Kendler et al. (2003) conducted behavior genetic analyses using data from the Virginia Twin Registry to examine the structure of genetic and environmental risk factors that give rise to common psychiatric disorders. Kendler et al.'s findings supported the phenotypic structure of adult psychiatric disorders proposed by Krueger, wherein the structural model of genetic risk factors also suggested the presence of three underlying dimensions (i.e., *Externalizing*, *Anxious-Misery* (*or Distress*), and *Fear*). For the purposes of the current study, the critical finding from Kendler and colleagues' work is that a common set of genetic influences underlie MDD and GAD, whereas a different set of genetic influences underlie other anxiety disorders (e.g., specific phobias). Notably, panic disorder did not load strongly with phobic disorders as predicted by Krueger's (1999) CFA results.

The first candidate gene included in this study codes for the dopamine D4 receptor (*DRD4*). Initial reports of an association between the personality trait of novelty seeking and *DRD4* (Ebstein et al., 1996; Benjamin et al., 1996) stimulated further investigations of *DRD4* as a genetic susceptibility factor for ADHD (see Schinka et al., 2002 for meta-analysis of *DRD4*-novelty seeking association). Neuroimaging and neuropsychological studies have implicated frontal-subcortical pathways in the pathophysiology of ADHD, pathways that control attention and motor behavior and in which dopamine D4 receptors are prevalent (Faraone & Biederman, 1998). The association and linkage of ADHD with a 48-bp variable number of tandem repeats (VNTR) sequence in exon 3 of *DRD4* has been examined in numerous studies. Despite

conflicting findings regarding this association across independent studies, three recent meta-analyses have demonstrated overall strong support for a significant association between the *DRD4* 7-repeat allele and ADHD (Faraone, Doyle, Mick, & Biederman, 2001; Faraone et al., 2005; Maher, Marazita, Ferrell, & Vanyukov, 2002). Specifically, all three meta-analyses yielded statistically significant results for the association between ADHD and *DRD4*, with pooled ORs ranging from 1.4 – 1.9 for case-control studies and from 1.2 – 1.4 for within-family studies.

As reviewed above, there is also preliminary evidence suggesting that the *DRD4* 7-repeat allele may confer even greater risk for children with ADHD who also have concurrent symptoms of ODD and CD (e.g., Holmes et al., 2002; Kirley et al., 2004). Considering the well documented associations between *DRD4* and the disruptive behavior disorders, as well as the personality trait of novelty seeking, a significant relation between the *DRD4* 7-repeat allele and internalizing behaviors (e.g., anxiety, depression, social withdrawal) might seem counterintuitive. In fact, studies investigating possible associations between *DRD4* and depressive and anxiety disorders have yielded largely negative results (e.g., see Hamilton et al., 2000 and Kennedy et al., 2001 for anxiety disorders; see Frisch et al., 1999 and Serretti et al., 2002 for depressive disorders). In addition, a recent meta-analysis by López León et al. (2005) examined the association between MDD and the *DRD4* 7-repeat allele, revealing significant *undertransmission* of the 7-repeat allele in depressed subjects (pooled OR = .70, p = .02).

The second candidate gene included in the present study is the serotonin transporter gene (*5-HTT*), which is responsible for the reuptake of serotonin from the synaptic cleft back to the presynaptic neuron. The serotonin neurotransmitter system has

been hypothesized as a causal factor underlying ADHD given evidence relating serotonergic dysregulation to a variety of behaviors relevant to ADHD, including impulsivity, aggression, and disinhibition (Halperin et al., 1997; LeMarquand et al., 1998; Lucki, 1998; Spivak et al., 1999). Several studies of association and linkage between *5-HTT* and ADHD recently have been published, many of which have focused on a 44-bp insertion / deletion in the promoter region of the gene characterized by long and short variants with demonstrated functional consequence. Specifically, the short allele of the *5-HTT*-linked promoter region polymorphism (*5-HTTLPR*) appears to be associated with decreased serotonin reuptake, whereas the long allele appears to be associated with more rapid serotonin reuptake, thus lower levels of active serotonin (Lesch et al., 1996; Neumeister et al., 2004). As is typical in the field of psychiatric genetics, studies of the association between the *5-HTTLPR* and ADHD have yielded inconsistent results. Nonetheless, Faraone et al.'s (2005) meta-analysis of the association between *5-HTTLPR* and ADHD revealed a significant but weak positive association, wherein the pooled OR for the long allele was 1.3 (95% CI, 1.09-1.59).

In addition, there is initial evidence providing support for a significant association between the *5-HTTLPR* and CD or antisocial behavior, though the findings have been somewhat inconsistent. Seeger et al. (2001) found evidence of significant over-representation of the long allele of the *5-HTTLPR* in children diagnosed with ICD-10 (World Health Organization, 1992) hyperkinetic disorder (HD) with comorbid CD, as compared with controls. More importantly, Seeger et al. demonstrated a stronger association of the homozygous long (LL) genotype with HD *without* comorbid CD relative to HD with CD, suggesting that ADHD symptoms are predominantly driving this

association (Retz et al., 2008). Similarly, in a pediatric sample selected for high levels of aggression, Beitchman et al. (2003) found that those children diagnosed with ADHD were significantly more likely to possess one or two copies of the long allele as compared with those without ADHD. There was no evidence for a significant association between the *5-HTTLPR* and aggression, however. Similarly, Sakai et al. (2007) also failed to detect a significant association between the *5-HTTLPR* and conduct problems or delinquency.

In contrast, several recent studies have reported an association between the *short* allele of the *5-HTTLPR* and violence, aggression, or conduct problems (Gerra et al., 2005; Haberstick et al., 2006; Liao et al., 2004; Retz et al., 2004; Sakai et al., 2006). Interestingly, Retz et al. (2004) found an overall slight excess of the long allele of the *5-HTTLPR* in a forensic sample of adults who reported a childhood history of ADHD, but there was a relative overtransmission of the *short* allele among individuals with a history of recurrent violent behavior as compared with those without such a history. This pattern of findings suggests that the long and short variants of the *5-HTTLPR* may confer risk for different psychopathological outcomes, such that the long allele is associated with greater risk for ADHD and related legal problems, whereas the short allele is associated with greater risk for violent criminal offences (Retz et al., 2004).

The internalizing disorders and related personality traits have been the focus of a large number of *5-HTTLPR* association studies, given that *5-HTT* is the target site for the selective serotonin reuptake inhibitors (SSRIs) which are commonly prescribed for the treatment of depression and anxiety. A recent meta-analysis of 10 studies by Lotrich and Pollock (2004) showed a slight positive association between the SS genotype and MDD

in adult samples (OR = 1.2, $p < .05$). Overall, the evidence for a direct association between *5-HTT* and MDD has been inconsistent, but several recent studies of gene-environment interactions have yielded promising findings. There is mounting evidence suggesting that *5-HTTLPR* variation moderates the relation between stressful life events and depression, such that the short allele confers a marked vulnerability to depression only in individuals with histories of significant recent environmental stress (e.g., Caspi et al., 2003; Kaufman et al., 2004; Kendler et al., 2005; Zalsman et al., 2006).

The results of studies examining the association between the *5-HTTLPR* and specific anxiety disorders have been largely negative, with a few exceptions. As an example, McDougle et al. (1998) reported a significant association between the long allele of the *5-HTTLPR* and OCD ($p < .03$) and You et al. (2005) found a greater frequency of the SS genotype among GAD patients as compared with control subjects (OR = 2.3, $p < .05$). Interestingly, several recent studies have investigated the relation of *5-HTT* and fear-related traits in children, including shyness (e.g., Arbelle et al., 2003; Battaglia et al., 2005; Hayden et al., 2007; Jorm et al., 2000), behavioral inhibition (Fox et al., 2005), and fearfulness (Auerbach et al., 2001; Hayden et al., 2007). These initial investigations have yielded conflicting findings with respect to the role of the *5-HTTLPR* in regulating fear-related traits in childhood, with several studies suggesting the short allele is associated with higher levels of fearfulness and inhibition (Battaglia et al., 2005; Fox et al., 2005; Hayden et al., 2007), whereas other studies have suggested stronger association with the long allele (Arbelle et al., 2003; Auerbach et al., 2001; Jorm et al., 2000).

The common co-occurrence of ADHD, ODD, and CD has been consistently reported in epidemiological as well as clinical samples, with an estimated 30 - 45% of children diagnosed with ADHD also having concurrent ODD and 20% having concurrent CD (Acosta, Arcos-Burgos, & Muenke, 2004; Biederman, Newcorn, & Sprich, 1991). Findings from both family and twin studies suggest that much of this overlap among externalizing disorders can be attributed to a common genetic etiology (e.g., Coolidge, Thede, & Young, 2000; Dick, Viken, Kaprio, Pulkkinen, & Rose, 2005; Faraone et al., 1998; Nadder, Silberg, Eaves, Maes, & Meyer, 1998; Silberg et al., 1996). In fact, some investigators have found that the genetic correlation between ODD and CD is sufficiently high to argue that these symptoms are part of a joint construct (e.g., Eaves et al., 2000; Nadder, Rutter, Silberg, Maes, & Eaves, 2002), though other studies have provided evidence for the distinction between ODD and CD (e.g., Burns et al., 1997; Loeber, Lahey, & Thomas, 1991; Waldman, Rhee, Levy, & Hay, 2001).

The extant literature is mixed as to whether the subgroup of individuals with both ADHD and comorbid conduct disorder (ADHD + CD) represents a quantitative co-occurrence or an etiologically distinct subtype (e.g., Faraone et al., 1997). The ICD-10 (World Health Organization, 1992) already classifies children in a separate diagnostic category (i.e., "hyperkinetic conduct disorder") if they fulfill diagnostic criteria for both hyperkinetic disorder and conduct disorder. There is some clinical support for this distinction, based on evidence suggesting that ADHD + CD is a more severe condition with a worse outcome than either disorder alone (e.g., Barkley et al., 1990; Jensen et al., 1997; Kuhne et al., 1997). Additionally, results from family studies have suggested that the co-occurrence of ADHD + CD represents a distinct familial type of ADHD (Faraone et al., 2000). In contrast,

Thapar, Harrington, and McGuffin (2001) used a liability threshold model approach to examine how the ADHD + CD group is genetically related to ADHD in a population-based twin study. Their results provided support for the notion that ADHD + CD is a quantitative variant of ADHD, associated with higher genetic loading and higher clinical severity, rather than a distinct subtype of ADHD.

In contrast to the well documented co-occurrence of childhood externalizing disorders (ADHD, ODD, and CD), the co-occurrence of internalizing disorders (depression and anxiety) with ADHD has been less recognized and understudied. Nonetheless, both clinical and epidemiological studies have shown that children with ADHD also frequently experience co-occurring symptoms of anxiety and depressive disorders, though prevalence estimates have varied considerably across studies (Bauermeister et al., 2007). Many studies of ADHD have shown rates of comorbid anxiety disorders to be as high as 25% (Schatz & Rostain, 2006), whereas the estimated prevalence of depressive disorders in children diagnosed with ADHD ranges from 10 - 20% (Biederman, Newcorn, & Sprich, 1991).

Several family genetic studies have examined the inheritance pattern of ADHD and anxiety disorders (Biederman, Faraone, Keenan, Steingard, & Tsuang, 1991; Biederman et al., 1992; Perrin & Last, 1996). Results from these studies revealed that the relatives of ADHD probands with anxiety disorders showed a similar risk for ADHD but an elevated risk for anxiety disorders, as compared with the relatives of ADHD probands without anxiety disorders. The degree of ADHD-anxiety disorder comorbidity among relatives of probands diagnosed with both ADHD and anxiety disorder did not exceed levels expected by chance, however, suggesting that the two disorders are independently

transmitted in families (Biederman et al., 1992). Although these findings are inconsistent with the notion that ADHD + anxiety disorder represents a distinct familial subtype, Jensen et al. (1997) highlighted evidence that suggests the co-occurrence of ADHD and anxiety is indeed associated with qualitatively different patterns of treatment response, severity, and outcome. In support of Jensen and colleagues' argument for the addition of an "ADHD, anxious subtype," laboratory studies have demonstrated that children with ADHD and anxiety tend to exhibit less off-task and hyperactive behavior as well as longer reaction times than children with ADHD only (Pliska et al., 1999). Thus, several investigators have proposed that a subgroup of children with concurrent ADHD and anxiety may experience decreased impulsivity but increased difficulties on tasks requiring attention, as compared with children with ADHD only (reviewed in Schatz & Rostain, 2006).

Biederman and colleagues have also examined the shared familial risk for ADHD and depressive disorders using the family genetic study design (Biederman, Faraone, Keenan, & Tsuang, 1991; Biederman et al., 1992). Their familial risk analyses revealed that relatives of ADHD probands with and without MDD showed significantly elevated risk for both ADHD and MDD, when compared with relatives of control subjects. Among the relatives of children diagnosed with ADHD and MDD, however, different relatives accounted for the risks for each disorder. This set of findings is most consistent with the hypothesis that ADHD and MDD share common familial etiologic factors, though the disorders appear to be transmitted independently.

Study Goals and Hypotheses

As previously mentioned, the overarching goal of the current study is to test whether the proposed alternative comorbid ADHD phenotype (*ADHD* X *ODD/CD*, *ADHD* X *Distress*, *ADHD* X *Fear*) increases the external validity of ADHD over and above the extant *DSM-IV* subtyping system. Therefore, the first specific aim of this study is to evaluate how putatively homogenous composites of co-occurring externalizing and internalizing symptoms moderate the association between ADHD and two candidate genes (viz., *DRD4* and *5-HTT*). Relatedly, the second specific aim involves comparing these two representations of the ADHD phenotype with regard to their corresponding strength of association with *DRD4* and *5-HTT*. Thus, the magnitude of genetic association serves as an external validity criterion on which to compare the *DSM-IV* ADHD diagnostic subtypes versus the proposed comorbid conditions. Finally, the third specific aim evaluates whether or not the proposed comorbid conditions can be differentiated on the basis of additional external validity indicators, including gender ratios and mean age. Following from the specific aims outlined above, three sets of ***a priori*** hypotheses were tested:

Hypothesis 1. We hypothesized that the magnitude of the association between ADHD and the *DRD4* 7-repeat allele would be (a) strengthened by the inclusion of moderating levels of *ODD/CD* (e.g., Holmes et al., 2002; Kirley et al., 2004) and (b) unaffected or weakened by the inclusion of moderating levels of *Distress* (e.g., López León et al., 2005) and *Fear* (e.g., Hamilton et al., 2000; Kennedy et al., 2001). It was further predicted that the magnitude of the association between ADHD and the long allele of the *5-HTTLPR* would be strengthened with the inclusion of moderating levels of

ODD/CD (e.g., Seeger et al., 2001; Cadoret et al., 2003), as well as *Fear* (e.g., Arbelle et al., 2003; Auerbach et al., 2001; Jorm et al., 2000; McDougle et al., 1998). On the other hand, it was hypothesized that the magnitude of the association between ADHD and the long allele of the *5-HTTLPR* would be weakened or unchanged with the inclusion of moderating levels of *Distress*, given evidence suggesting that MDD and GAD are more strongly related to the *short* variant of *5-HTTLPR* (e.g., Lotrich & Pollock, 2004; You et al., 2005).

Hypothesis 2. We hypothesized that the proposed alternative comorbid conditions (*ADHD* X *ODD/CD*, *ADHD* X *Distress*, *ADHD* X *Fear*) would show distinct patterns of association with *DRD4* and *5-HTT* that are *at least* as distinguishable as the *DSM-IV* ADHD subtypes, if not more so. In addition, we predicted that differences in genetic association among the *DSM-IV* ADHD subtypes would be strengthened with the addition of moderating influences of the externalizing and internalizing composite variables.

Hypothesis 3. It was predicted that children with the *ADHD* X *ODD/CD* comorbid condition would show a higher proportion of males compared with the *ADHD* X *Distress* and *ADHD* X *Fear* comorbid conditions, based on developmental evidence demonstrating higher rates of externalizing disorders in boys than girls and roughly equivalent rates of internalizing disorders in boys and girls prior to adolescence (Crick & Zahn-Waxler, 2003). It was further predicted that children with the *ADHD* X *Distress* and *ADHD* X *Fear* comorbid conditions would show a higher mean age as compared with *ADHD* X *ODD/CD*, based on evidence documenting that externalizing problems are referred for assessment/treatment at an earlier age than internalizing problems due to their more disruptive nature (Gaub & Carlson, 1997; Gilliom & Shaw, 2004).

Method

Participants

The full sample included 372 children from 233 families recruited through the Center for Learning and Attention Deficit Disorders (CLADD) at the Emory University School of Medicine in Atlanta, Georgia and through psychiatrists in private practice in Tucson, Arizona. Children (i.e., probands) were assessed and/or treated for attention-deficit disorders, related behavioral disorders, and/or learning problems at these two sites. Both male and female siblings of the probands were also sampled whenever possible.

The institutional review boards of Emory University and the University of Arizona approved the study protocol, and appropriate informed consent was obtained for all participants at both sites. Families were presented with and given the option to sign a form indicating that they agreed to be contacted for future research while they were being seen at either of the two clinics. Families who consented were then contacted by phone, presented with details regarding the study, and asked if they would like to participate. Prior to all assessments, all family members were given copies of the informed consent form to read. All participating children were given an age-appropriate verbal description of what the study entailed and those children who could read and write also read and signed a written assent form. The parents signed the informed consent forms and also signed for their children who were too young to read. Families who agreed to participate were assessed in their homes over the course of a single three-hour period. Probands and their siblings were comprehensively assessed on lab measures of executive functions and attention while their parents completed questionnaires assessing the demographic

characteristics of the family, as well as symptoms associated with commonly diagnosed childhood psychiatric disorders in their children.

Children diagnosed with autism, traumatic brain injury or other neurological conditions (e.g., epilepsy), or an IQ < 75 were excluded from participation in the study. The participants in this study represent an expanded sample that has been previously published on (e.g., Waldman et al., 1998; Rowe et al., 1998). Demographic characteristics of the current sample are presented in Table 1. The children ranged in age from 5 to 18 years, with an average age of 10.5 years (*SD* = 3.3) at the time of assessment. The overall sample included 254 boys (68%) and 118 girls (32%). The racial/ethnic composition of the sample was 78% Caucasian, 9% African-American, 2% Hispanic/Latino, and 11% mixed ancestry. Of the 237 (64%) children who met *DSM-IV* criteria for ADHD, the rates of subtype diagnosis were as follows: 34% IT, 56% CT, and 10% HT.

Assessment Procedures

Parent ratings were obtained for probands and their siblings (whenever possible) using the Emory Diagnostic Rating Scale (EDRS), which was developed in our lab (Waldman et al., 1998) to assess *DSM-IV* symptoms of the major childhood psychiatric disorders. These include symptoms of the disruptive behavior disorders (i.e., ADHD, CD, and ODD), as well as internalizing disorders such as major depression, dysthymia, and anxiety disorders. Each symptom of a given disorder corresponds to a specific item on the rating scale. Children were rated by their parents on a 0-4 scale, in which a score of 0 indicates that the symptom is “not at all” characteristic of the child and a score of 4 indicates that the symptom is “very much” characteristic of the child. Average symptom

dimension scores were calculated for each major childhood disorder by summing the item scores (0-4) comprising each scale and dividing by the total number of scale items. The symptom scales allow quantitative assessments of the disorders, as they distinguish severity and number of symptoms over a broad range.

ADHD diagnoses were derived from cut-off scores on the continuous symptom dimensions in accordance with *DSM-IV* diagnostic criteria. Probands and their siblings were assigned an ADHD subtype diagnosis if they surpassed the standard diagnostic thresholds (i.e., $\geq$ 6 of 9 symptoms) on the inattention and/or hyperactivity-impulsivity symptom dimensions at the moderate severity level (i.e., a score of 2 or higher on each individual symptom). Specifically, children who were above threshold only on the inattentive symptom dimension were diagnosed with IT, children who were above threshold only for the hyperactive-impulsive symptom dimension were diagnosed with HT, and children who were above threshold on both symptom dimensions were diagnosed with CT.

All diagnoses were based on mother's symptom ratings, except in cases where mother ratings were unavailable in which case father ratings were substituted. The decision to primarily use mother ratings was largely based on the fact that father ratings were unavailable for a large percentage of the sample and based on evidence demonstrating mothers' superior validity as informants (e.g., Coffman, Guerin, & Gottfried, 2006). Furthermore, maternal reports of ADHD symptoms have consistently yielded high heritability estimates (Thapar, Holmes, Poulton, & Harrington, 1999). The internal consistency reliabilities of both the inattentive and hyperactive-impulsive symptom dimensions were high (α = .96 and .95, respectively).

Definition of composite variables. Given evidence that major depression, dysthymia, GAD, and perhaps social phobia share common etiologies (Kendler et al., 2003; Lahey et al., 2004), along with the fact that the base rates of these co-occurring disorders are relatively low in this clinic-referred ADHD sample, a decision was made to create several composite variables to bolster the power of the analyses. As described above, the composite variables were formed on the basis of the dimensional structure indicated by Krueger (1999), Kendler et al. (2003), Krueger & Markon (2006), and Lahey et al.'s (2004) findings. Thus, the "Distress" composite consists of average symptom counts of MDD, GAD, and social phobia. The "Fear" composite consists of average symptom counts of four anxiety disorder symptom dimensions, viz., SAD, specific phobias, obsessions, and compulsions. As its name suggests, the "ODD/CD" composite consists of average symptom counts of ODD and CD. The internal consistency reliabilities of all three composite variables were high (α = .90 for the "Distress" composite, α = .91 for the "Fear" composite, and α = .93 for the "ODD/CD" composite).

The rationale for using continuous co-occurring symptom dimensions rather than categorical diagnoses in these analyses rests on the fact that considerable information would be lost if individuals were only classified as "affected" or "unaffected" for each co-occurring disorder. Furthermore, using a dimensional approach to examine the moderating effects of co-occurring symptoms of externalizing and internalizing disorders should enhance the power to detect significant differences in the strength of association between ADHD and the selected candidate genes, particularly for the internalizing disorders which have relatively low base rates in this sample.

DNA Collection, Extraction, and Genotyping Procedures

Probands, their siblings, and parents were genotyped for the 48-bp VNTR in exon 3 of *DRD4* and the *5-HTTLPR* 44-bp insertion / deletion polymorphism. The technique used to collect DNA changed during the course of the study, in order to facilitate the extraction process and increase the yield of DNA. The DNA collection procedures have included the use of sucrose solution washes and buccal brushes to collect buccal cells, as well as Oragene™ DNA self-collection kits to obtain saliva samples (DNA Genotek, Inc.). At the end of the study visit, the DNA samples were immediately refrigerated and transported to the Center for Medical Genomics at Emory University for secure storage and extraction by lab personnel. Buccal cells were pelleted for ten minutes at 2,000 g and the DNA was extracted using a QIAmp Tissue kit (Qiagen), according to the manufacturer's protocol. The samples were then preserved in TE (10 mM Tris Hcl, 1mM EDTA).

The preserved samples were sent to two laboratories for polymerase chain reactions (PCR) amplification of the *DRD4* and *5-HTTLPR* markers: 1) the University of Arizona's Laboratory of Molecular and Systematic Evolution in Tucson, AZ and 2) the Psychiatric and Neurodevelopmental Genetics Unit (PNGU) in the Center for Human Genetic Research at Massachusetts General Hospital (MGH) in Boston, MA. The 48-bp VNTR in exon 3 of *DRD4* was genotyped by PCR, either according to the protocol originally described by Lichter et al. (1993) in the laboratory at the University of Arizona, or more recently, according to an alternate protocol in the laboratory at MGH (see Appendix for description of alternate protocol). The *5-HTTLPR* polymorphism was genotyped by PCR at MGH, following the procedures outlined in the alternate protocol (see Appendix). After the genotyping procedures were completed at the University of

Arizona and MGH, our lab received Microsoft Excel spreadsheets containing the final called genotypes for all samples.

Analyses

Specific Aim 1. The strength of association between ADHD and each candidate gene (*DRD4, 5-HTT*) was evaluated after moderating symptoms of co-occurring internalizing and externalizing disorders were taken into account. Tests of association were conducted by using ordinal logistic regression to predict the number of *DRD4* and *5-HTT* high-risk alleles, dependent on 1) whether a child is diagnosed with ADHD or not, and 2) the level of continuous co-occurring symptoms. The child's number of high-risk alleles (i.e., 0, 1, or 2 copies) served as the criterion variable, ADHD diagnostic status served as the predictor variable, and the continuous co-occurring symptom dimension composites served as moderating variables (i.e., "*ODD/CD*," "*Distress*," and "*Fear*" composite variables). As recommended by Cohen, Cohen, West, and Aiken (2003, p. 267), the continuous symptom dimensions were centered prior to the creation of the composite variables and the interaction terms, in order to eliminate nonessential multicollinearity and to simplify interpretation of the results.

The interaction terms, represented by the product of ADHD diagnostic status and each co-occurring symptom dimension composite, as well as their constituent main effects, were included in the regression model. Logistic regression yields a Wald's χ^2 statistic that tests the significance of each individual predictor in the regression model. Wald's χ^2 also was evaluated for the interaction terms, providing a test of the moderation over and above the main effects model. The Nagelkerke R^2 and Odds Ratios (ORs) and their 95% confidence intervals were reported as measures of effect size.

Specific Aim 2. The proposed comorbid conditions (ADHD X *ODD/CD*, ADHD X *Distress*, ADHD X *Fear*) were next compared with the *DSM-IV* ADHD diagnostic subtypes with regard to their patterns of association with *DRD4* and *5-HTT*. This was tested using ordinal regression as described above in Specific Aim 1, except that the predictor variable consisted of *a priori* contrasts among the ADHD subtypes rather than a single contrast of ADHD vs. No ADHD diagnosis. This allowed for a direct comparison of the strength of association between each candidate gene and the *DSM-IV* ADHD subtypes versus ADHD accompanied by comorbid conditions. Two sets of *a priori* orthogonal contrasts were tested and are displayed in Table 2. These *a priori* contrasts helped to evaluate competing hypotheses regarding whether the candidate genes and moderating co-occurring symptoms are more strongly related to 1) ADHD as a unitary diagnostic category, 2) inattentive vs. hyperactive-impulsive symptoms, or 3) one specific *DSM-IV* subtype.

Specific Aim 3. Finally, we evaluated whether or not the proposed alternative comorbid conditions could be differentiated on the basis of additional external validity indicators, including gender and age. First, logistic regression analyses using the *a priori* contrasts outlined in Table 2 were performed to examine possible differences in the gender composition of the comorbid ADHD conditions. Of primary importance, tests of moderation were conducted between the first contrast (i.e., ADHD/IT + HT + CT versus No ADHD diagnosis) and the ADHD comorbid composite variables, with child's sex serving as the criterion variable. Second, planned comparisons were incorporated in Analysis of Variance (ANOVA) to identify possible age differences among the comorbid ADHD conditions. Interaction terms between the first contrast (i.e., ADHD/IT + HT +

CT versus No ADHD diagnosis) and the comorbid composite variables were used to predict differences in child's age. Given the directional predictions, one-tailed significance tests were reported. With respect to the genetic analyses, specific predictions about the direction of association (including the designation of the high-risk allele) were based on replicable findings of association with ADHD and comorbid conditions in the human literature as well as findings from knockout studies in the animal literature, rather than on known functionality of the alleles.

One possible threat to the internal validity of between-family association methods is population stratification. In order for population stratification to occur, two conditions must occur: 1) the allele frequencies must vary across population subgroups (e.g., ethnic groups) within the study sample and 2) the ethnic groups that differ in allele frequency must also differ significantly with respect to the outcome variable (e.g., rates of disorder and/or symptom levels) (Hutchinson, Stallings, McGeary, & Bryan, 2004). Therefore, spurious associations in candidate gene studies may result if the case-control differences in allele frequencies are due to systematic differences in ancestry rather than the association of genes with disease. Thus, for all genetic analyses, participants' parent-reported ethnic backgrounds (i.e., % European-American, % African-American, and % Hispanic) were statistically controlled prior to the primary analysis to minimize possible confounding owing to population stratification.

Quality control analyses of the genotyping for both genes were conducted using crosstab analyses in SPSS version 15 (SPSS, Inc., Chicago, IL). These analyses included estimates of monozygotic twin agreement for genotypes and the concordance of genotypes between genomic and Whole Genome Amplified (WGA) samples, given that

genomic as well as WGA DNA samples were available for a subset of subjects (N = 63 and N = 52 for *DRD4* and *5-HTT*, respectively). Further, call rates and exact Hardy Weinberg Equilibrium (HWE) tests were estimated using the program PEDSTATS (Wigginton & Abecasis, 2006).

Results

Quality Control Analyses

Prior to conducting the association analyses, we performed a series of Quality Control (QC) analyses of the *DRD4* and *5-HTT* genotype data using our family samples. The call rate in our sample was 90% and 86% for the *DRD4* and *5-HTT* markers, respectively. The concordance of genotypes within monozygotic (MZ) twin pairs (N = 31) yielded an allelic discordance rate of approximately 13% and 5% for the *DRD4* and *5-HTT* markers respectively. Genotypes from genomic and Whole Genome Amplified (WGA) DNA samples were compared in a subset of individuals (i.e., N = 63 cases for *DRD4* and N = 52 cases for the *5-HTTLPR*) and no discrepancies were revealed for either marker.

Finally, HWE tests were evaluated for the full sample (including ADHD cases) versus founders only (equivalent to the probands' parents in this study). Marginally significant departure from HWE was observed for both the *DRD4* Exon 3 VNTR (p = .053) and the *5-HTT* promoter polymorphism (p = .045) in the full sample that included ADHD cases, whereas HWE departure was not detected in founders only (p = .191 and p = .209, for *DRD4* and *5-HTT*, respectively). Wittke-Thompson, Pluzhnikov, and Cox (2005) demonstrated that a similar pattern of results (i.e., significant HWE departure in cases, but not in controls) suggests that the gene of interest may be a disease

susceptibility locus rather than a result of genotyping error. Thus, the HWE results provide initial support for the association between *DRD4* and *5-HTT* and ADHD.

Correlations among Constituent Symptom Dimensions of the Composite Variables

Correlation analyses were conducted to examine the degree of association among the symptom dimensions within each composite variable (i.e., *ODD/CD*, *Distress*, and *Fear*). As shown in Table 3, the correlation between the ODD and CD symptom dimensions was high (r = .71). The correlations among the symptom dimensions comprising the *Distress* composite ranged from moderate to high, with the strongest relation between MDD and GAD (r = .68) and the weakest relation between MDD and social phobia (r = .34). Finally, the correlations among the constituent symptom dimensions of the *Fear* composite ranged from low to moderately high, such that SAD and obsessions showed the strongest association (r = .52), whereas specific phobia and compulsions showed the weakest association (r = .22).

Moderating Effects of Co-occurring Disorders on the Association between ADHD and DRD4 and 5-HTT

First, ordinal logistic regression was used to test the overall association between ADHD and *DRD4* and *5-HTT* (see Table 4). There was no evidence of significant association between the categorical ADHD diagnosis and the 7-repeat allele of *DRD4* (Wald χ^2 [N=336] = .07, p = .398, Nagelkerke R^2 = .01, OR = 1.07 [95% CI = .65 – 1.76]) or the long allele of *5-HTT* (Wald χ^2 [N=324] = .60, p = .219, Nagelkerke R^2 = .02, OR = 1.18 [95% CI = .77 – 1.80]).

Tests of moderation were next conducted to examine whether symptoms of co-occurring externalizing and internalizing disorders influence the magnitude of

associations between ADHD and *DRD4* and *5-HTT*. As predicted, co-occurring symptoms of *ODD/CD* significantly moderated the relation between *DRD4* and ADHD, such that the association was strengthened among children with ADHD who also exhibited elevated symptoms of ODD and CD (Wald χ^2 [N=333] = 3.23, p = .036, Nagelkerke R^2 = .02, OR = 2.90 [95% CI = .91 – 9.26]). As shown in Table 4, tests of the moderating effects of co-occurring internalizing symptoms on the *DRD4*-ADHD association were nonsignificant as expected (p = .366 for *Distress*; p = .487 for *Fear*). There were no significant main effects for ADHD diagnostic status, *ODD/CD*, *Distress*, or *Fear* composites.

Consistent with our prediction, a test of the moderating influence of the *Fear* composite revealed a statistical trend, whereby the association between *5-HTT* and ADHD was enhanced for ADHD children with higher symptoms of SAD, specific phobias, and OCD (Wald χ^2 [N=324] = 1.95, p = .081, Nagelkerke R^2 = .03, OR = .31 [95% CI = .06 – 1.61]). It is noteworthy, however, that the ADHD children with concurrent elevated symptoms of *Fear* were more likely to possess 1 or 2 copies of the *short* variant of *5-HTT*, rather than the putative high risk variant for ADHD (i.e., the long allele). Ordinal logistic regression analyses did not yield evidence of moderation by co-occurring symptoms of *ODD/CD* (p = .470) or *Distress* (p = .252) of the relation between *5-HTT* and ADHD (also shown in Table 4). These latter two findings only partially supported our hypotheses, as *ODD/CD* was expected to show a moderating effect on the *5-HTT*-ADHD association based on Seeger et al.'s (2001) previous findings.

For *5-HTT*, there was no significant main effect for ADHD diagnostic status or *ODD/CD*. In contrast, a significant main effect was found for the *Fear* composite, such

that the short allele of the *5-HTTLPR* was associated with higher levels of SAD, specific phobias, obsessions, and compulsions (Wald χ^2 [N=324] = 4.12, p = .021, Nagelkerke R^2 = .03, OR = .42 [95% CI = .18 – .97]). There was also a marginally significant main effect for the *Distress* composite, wherein the short allele of the *5-HTTLPR* was associated with higher levels of depression, GAD, and social phobia (Wald χ^2 [N=323] = 2.26, p = .066, Nagelkerke R^2 = .02, OR = .68 [95% CI = .41 – 1.12]).

Associations between DRD4 and 5-HTT and ADHD Comorbid Conditions versus DSM-IV ADHD Subtypes

Further ordinal logistic regression analyses were performed to directly compare the proposed ADHD comorbid conditions versus the *DSM-IV* ADHD diagnostic subtypes with regard to their patterns of association with *DRD4* and *5-HTT*. Two sets of *a priori* contrasts (shown in Table 2) were used to test competing models of the specific nature of association between each candidate gene and the *DSM-IV* ADHD subtypes. The key function of the first set of contrasts was to test the hypothesis that the inattentive *symptoms* of ADHD are more strongly associated with *DRD4* and *5-HTT* by comparing IT and CT versus HT, and IT versus CT. A second complementary set of contrasts was used to test the hypothesis that the Inattentive *subtype* of ADHD is more strongly related to *DRD4* and *5-HTT* by comparing IT versus HT and CT, and HT versus CT.

DRD4. The results of the first set of contrasts (shown in Table 5) revealed evidence of significant association with the 7-repeat allele of *DRD4*, whereby IT and CT showed a stronger relation with *DRD4* than HT (Wald χ^2 [N=336] = 3.96, p = .023, Nagelkerke R^2 = .05, OR = 2.01 [95% CI = 1.01 – 4.01]) and CT showed a stronger relation to *DRD4* than IT (Wald χ^2 [N=336] = 4.07, p = .022, Nagelkerke R^2 = .05, OR =

.71 [95% CI = .50 – .99]). Notably, the contrast between "Any ADHD diagnosis" versus "No ADHD diagnosis" yielded only a statistical trend towards association with *DRD4* (Wald χ^2 [N=336] = 2.10, p = .073, Nagelkerke R^2 = .05, OR = 1.16 [95% CI = .95 – 1.43]). The second set of contrasts also yielded evidence for a significant association, such that CT showed a stronger relation to *DRD4* compared with HT (Wald χ^2 [N=336] = 5.30, p = .010, Nagelkerke R^2 = .05, OR = 3.40 [95% CI = 1.20 – 9.62]). On the other hand, the contrast between IT versus HT and CT was nonsignificant, suggesting that these two groups do not differ in their association with *DRD4* (p = .187). Overall, this set of findings suggests that the ADHD subtypes differ significantly in their association with *DRD4*, despite modest evidence for significant association between *DRD4* and the categorical ADHD diagnosis. In addition, these results provide further support for the hypothesis that *DRD4* is preferentially associated with inattentive rather than hyperactive-impulsive symptoms, given evidence that CT and IT (though to a lesser extent) consistently showed a stronger relation to *DRD4* than HT.

Tests of moderation were subsequently conducted within the same analytic framework (i.e., ordinal logistic regression with *a priori* contrasts) as was used to examine ADHD subtype differences in their association with *DRD4*, with the addition of the moderator variables (*ODD/CD, Distress, and Fear*). As previously stated, this approach allowed for a direct comparison between the *DSM-IV* ADHD subtypes and the proposed ADHD comorbid conditions. As can be seen in Table 5, there was marginally significant evidence for a moderating influence of *ODD/CD* symptoms on the relation between *DRD4* and ADHD. Consistent with the initial moderation analyses, the *DRD4*-ADHD association was strengthened among children with elevated symptom levels of

ODD and CD (Wald χ^2 [N=333] = 2.44, p = .059, Nagelkerke R^2 = .07, OR = 1.52 [95% CI = .90 – 2.57]). Again, there was no evidence for moderating effects of *Distress* and *Fear* on the association between *DRD4* and ADHD (p = .252 and p = .271, respectively). Examination of potential moderating effects of the co-occurring externalizing and internalizing composites on the association between the *DSM-IV* ADHD subtypes and *DRD4* revealed that the addition of each moderating variable significantly worsened the fit of the model (see Table 5).

5-HTT. As shown in Table 6, the results of the first set of contrasts among the *DSM-IV* ADHD subtypes yielded evidence of significant association with the long allele of *5-HTT*, whereby IT and CT showed a stronger relation to *5-HTT* than HT (Wald χ^2 [N=324] = 2.87, p = .045, Nagelkerke R^2 = .03, OR = 1.30 [95% CI = .96 – 1.77]). There was also a statistical trend for the IT to show a stronger association with *5-HTT* than the CT (Wald χ^2 [N=324] = 1.67, p = .098, Nagelkerke R^2 = .03, OR = 1.20 [95% CI = .91 – 1.60]). The findings from the second set of contrasts suggested that the IT is more strongly associated with *5-HTT* relative to the HT and CT (Wald χ^2 [N=324] = 4.62, p = .019, Nagelkerke R^2 = .03, OR = 1.25 [95% CI = 1.01 – 1.55]). A statistical trend was also detected, such that the CT showed greater association with *5-HTT* than the HT (Wald χ^2 [N=324] = 1.60, p = .103, Nagelkerke R^2 = .03, OR = 1.36 [95% CI = .84 – 2.18]). This pattern of results suggests that the IT is preferentially associated with the long allele of *5-HTT* as compared with the other two subtypes (CT and HT).

Moderation analyses were conducted using *a priori* contrasts of ADHD subtypes, yielding a similar pattern of results as initially reported for *5-HTT*. As shown in Table 6, the only significant finding was a moderating influence of *Fear* on the association

between *5-HTT* and ADHD (Wald χ^2 [N=324] = 2.89, p = .044, Nagelkerke R^2 = .05, OR = .65 [95% CI = .40 – 1.07]). There was no evidence for significant moderating effects of *ODD/CD* (p = .497) or *Distress* (p = .195) on the *5-HTT*-ADHD association. In addition, analyses of the potential moderating effects of the *ODD/CD*, *Distress*, or *Fear* composites on the association between the *DSM-IV* ADHD subtypes and *5-HTT* showed no improvement in the fit of the model with the addition of these moderating variables (see Table 6).

Analyses of Differences in Gender Composition and Mean Age for ADHD Comorbid Conditions

As a final step, the proposed alternative comorbid conditions were examined with respect to whether they could be differentiated on the basis of the additional external validity indicators gender and age. Logistic regression analyses did not suggest any differences in the gender composition of the comorbid ADHD conditions (see Table 7). It is noteworthy, however, that there was a statistical trend for children diagnosed with ADHD to be more likely to be male (73%) than those without an ADHD diagnosis (61%) (Wald χ^2 [N=372] = 2.20, p = .069, Nagelkerke R^2 = .04, OR = 1.10 [95% CI = .97 – 1.23]). Planned comparisons in ANOVA did not yield any significant differences in mean age among the comorbid ADHD conditions (shown in Table 7). Nonetheless, there was a significant difference in mean age between children diagnosed with ADHD compared with those without an ADHD diagnosis (t [1, 372] = - 2.19, p = .014, η^2 = .01). On average, children with ADHD were younger (M = 10.3, SD = 3.0) than children without ADHD (M = 10.8, SD = 3.5).

Post-hoc logistical regression analyses were conducted to examine whether there are gender differences in average levels of co-occurring symptoms. Results of the post-hoc analysis revealed that, on average, boys demonstrated significantly higher ODD and CD symptom levels, as expected (Wald χ^2 [N=372] = 4.01, p = .023, Nagelkerke R^2 = .02, OR = 1.38 [95% CI = 1.01 – 1.90]). In contrast, boys and girls exhibited comparable levels of internalizing symptoms (p = .373 for *Distress* and p = .254 for *Fear*).

Discussion

There is growing recognition that the ADHD diagnosis subsumes a rather heterogeneous group of children, characterized by variability in the presentation of core symptoms, clinical correlates (e.g., demographic characteristics, rates of comorbidity, outcome), and possibly etiological pathways. Thus, efforts to refine the ADHD phenotype that aim to reduce diagnostic heterogeneity seem to hold promise for improving our ability to identify the genes that contribute to ADHD. Accordingly, the primary goal of this study was to evaluate the validity of one approach to phenotypic refinement that sought to characterize more putatively homogeneous conditions within the ADHD phenotype on the basis of co-occurring symptoms of externalizing and internalizing disorders (i.e., *ADHD* X *ODD/CD*, *ADHD* X *Distress*, and *ADHD* X *Fear*). Assuming that some true associations may be obscured if a given candidate gene is associated only with certain aspects of the ADHD phenotype (e.g., a comorbid subgroup), we hypothesized that symptoms of co-occurring externalizing and internalizing disorders would moderate the magnitude of associations between ADHD and *DRD4* and *5-HTT*.

In the current study, the overall associations between ADHD and *DRD4* and *5-HTT* were tested using ordinal logistic regression analyses, but revealed no evidence of

significant association between the categorical ADHD diagnosis and the putative high-risk alleles of *DRD4* (the 7-repeat allele) or *5-HTT* (the long allele). As predicted, tests of interactions showed that co-occurring symptoms of *ODD/CD* significantly moderated the relation between *DRD4* and ADHD, wherein the association was strengthened among children with ADHD who also exhibited elevated symptoms of ODD and CD. Also consistent with our hypotheses, tests of the moderating effects of co-occurring internalizing symptoms (i.e., *Distress* and *Fear*) on the *DRD4*-ADHD association were nonsignificant.

This study replicates similar findings that have shown significant association between the *DRD4* 7-repeat allele and ADHD with comorbid ODD/CD (Holmes et al., 2002; Kirley et al., 2004). Consistent with Holmes et al.'s (2002) results, the current study found evidence for a significant relation between *DRD4* and children with ADHD and concurrent conduct problems, whereas no association was detected between *DRD4* and the overall ADHD diagnosis. In the study by Kirley et al. (2004), the association analyses were conducted separately for ADHD children with comorbid ODD and comorbid CD. Their analyses revealed that the 7-repeat allele of *DRD4* was significantly associated with ADHD children with comorbid ODD, but not with comorbid CD. As the authors stated, the lack of association between *DRD4* and ADHD with comorbid CD may have been due to the low number of informative transmissions in the CD group (N=16), thus limiting the generalizability of their results.

Further ordinal logistic regression analyses were conducted in the present investigation to examine possible moderating influences of co-occurring externalizing and internalizing disorders on the association between ADHD and the *5-HTTLPR*. Consistent

with our hypotheses, tests of moderation revealed a statistical trend whereby the association between *5-HTT* and ADHD was enhanced for ADHD children with higher symptoms of *Fear* (i.e., SAD, specific phobias, and OCD). It is noteworthy that the ADHD children with concurrent elevated symptoms of *Fear* were more likely to possess 1 or 2 copies of the *short* variant of the *5-HTTLPR*, rather than the putative high risk variant for ADHD (i.e., the *long* variant). This finding provides additional support for Jensen et al.'s (1997) recommendation of the addition of an "ADHD, anxious subtype," as it appears that ADHD with co-occurring symptoms of anxiety may reflect a somewhat etiologically distinct subgroup of ADHD children as compared with those with predominant co-occurring symptoms of disruptive behavior disorders (e.g., ODD, CD).

As predicted, the association between ADHD and *5-HTT* was virtually unchanged when moderating levels of *Distress* were included in the ordinal logistic regression model. The extant literature suggests that ADHD is preferentially associated with the long allele of the *5-HTTLPR* (Faraone et al., 2005), whereas MDD and GAD are more strongly associated with the short allele (Lotrich & Pollock et al., 2004; You et al., 2005). The current study provides additional evidence that different variants of the *5-HTTLPR* appear to confer risk for ADHD (i.e., long allele) as opposed to MDD/GAD (i.e., short allele), thus suggesting that *5-HTT* may have unique and specific genetic effects on these conditions. Nonetheless, considering that roughly 10 – 20% of children with ADHD also have comorbid depressive disorders (Acosta et al., 2004; Biederman et al., 1991), further research is needed to help identify shared vulnerability genes underlying the phenotypic overlap between ADHD and MDD/GAD. Towards that end, Kim et al. (2007) recently demonstrated evidence of enhanced association for the gene coding for Synaptosomal-

associated protein of 25 kDa (*SNAP-25)* in a subset of ADHD patients with comorbid MDD.

Based on Seeger et al.'s (2001) finding that children diagnosed with ICD-10 hyperkinetic disorder (HD) with comorbid CD showed a significant over-representation of the long allele of *5-HTT* as compared with controls, we hypothesized that the magnitude of the association between ADHD and *5-HTT* would be strengthened with the inclusion of moderating levels of *ODD/CD*. This prediction was not supported in the current study, however. Possible reasons for the nonreplication of Seeger et al.'s findings in this study include differences in ascertainment methods, diagnostic assessment (due to use of ICD-10 versus *DSM-IV* diagnostic criteria for HD/ADHD and/or categorical versus dimensional definitions of CD), and genetic heterogeneity across samples.

The proposed ADHD comorbid conditions were next compared directly to the *DSM-IV* ADHD diagnostic subtypes in order to evaluate which system better captures the heterogeneity within the overall ADHD diagnosis. Ordinal logistic regression analyses using *a priori* contrasts yielded significant differences among the *DSM-IV* ADHD subtypes with respect to their associations with *DRD4* and *5-HTT*. For *DRD4*, the subtype analyses revealed significantly greater association with CT and IT (to a lesser extent) than HT, confirming findings from several previous studies suggesting a stronger relation between *DRD4* and inattentive (rather than hyperactive-impulsive) symptoms of ADHD (Lasky-Su et al., 2008; McCracken et al., 2000; Rowe et al., 1998). In comparison, the moderation analyses yielded marginally significant evidence for a moderating influence of *ODD/CD* on the association between *DRD4* and ADHD,

whereas there was no evidence for moderating effects of *Distress* or *Fear* on the *DRD4*-ADHD relation.

For *5-HTT*, the subtype analyses showed a significantly stronger association between the *5-HTTLPR* and IT as compared with the other two subtypes (CT and HT). This finding conflicts with two previous studies that demonstrated a stronger relation between *5-HTT* and CT (Manor et al., 2001; Seeger et al., 2001). In comparison, tests of moderation showed a significant moderating influence of *Fear* on the association between the *5-HTTLPR* and ADHD, but no moderating effects were demonstrated for *ODD/CD* or *Distress*.

Although a nascent literature examining the role of the *5-HTTLPR* in regulating fear-related traits in childhood has been mixed, several studies have shown that the short allele is associated with higher levels of fearfulness and inhibition (Battaglia et al., 2005; Fox et al., 2005; Hayden et al., 2007). One possible interpretation of the findings in the current sample is that a subset of ADHD children may present with predominant co-occurring symptoms of anxiety (e.g., SAD, specific phobias, and OCD) that are linked by the common feature of *fear of physical harm* (Lahey et al., 2004). As suggested in a recent review of ADHD with comorbid anxiety by Schatz and Rostain (2006), we would expect that this anxious subtype of ADHD would exhibit lower levels of impulsivity and disinhibition, combined with relatively higher levels of inattention and distractibility. Further support for a separate anxious subtype of ADHD stems from several pharmacological studies demonstrating that children diagnosed with ADHD accompanied by anxiety tend to show significantly poorer responses to stimulant medications than other children with ADHD (reviewed in Jensen et al., 1997 and Newcorn et al., 2001).

Finally, the proposed comorbid conditions (ADHD X *ODD/CD*, ADHD X *Distress*, ADHD X *Fear*) were evaluated on two additional external validity indicators (viz., gender composition and mean age). Logistic regression analyses and planned comparisons in ANOVA did not yield any significant moderating effects of the comorbid conditions on the relation of ADHD with gender or age. Therefore, the associations between ADHD and gender and age do not appear to be dependent on levels of concurrent externalizing (ODD, CD) or internalizing (depression, anxiety, fear) symptoms. Given the current finding that children diagnosed with ADHD were on average more likely to be male and younger than children without ADHD ($p = .069$ and $p = .014$ for gender and age, respectively), one might expect to find only a slight incremental relation when symptoms of co-occurring externalizing and internalizing disorders are added to the model.

At first glance, the results presented in Tables 4 and 5 may appear to suggest that the *DSM-IV* ADHD subtype classification is superior to the proposed alternative comorbid conditions with respect to its ability to segregate more genetically homogeneous subgroups of children within the ADHD diagnosis. There are several key considerations that should be weighed when comparing the relative success of these two subtyping systems. Due to the fact that the diagnostic subtypes are by definition nonoverlapping entities, it seems logical that the *DSM-IV* ADHD subtypes would more exhaustively capture the variance within the categorical diagnosis as compared with an alternative ADHD phenotype defined by co-occurring conditions. In contrast, the most severe ADHD cases in this clinic-referred sample are likely to have multiple concurrent disorders, spanning both externalizing and internalizing dimensions (due in part to

referral bias; Berkson, 1946), thus, the proposed comorbid conditions examined in this study clearly are not mutually exclusive. More importantly, the inclusion of co-occurring externalizing and internalizing symptoms in the ADHD phenotype also proved to be quite effective in identifying children for whom *DRD4* and *5-HTT* conferred stronger genetic susceptibility. Specifically, *ODD/CD* moderated the association between ADHD and *DRD4* and *Fear* moderated the association between ADHD and *5-HTT*. This suggests that considering co-occurring symptoms of conduct problems and anxiety, in addition to ADHD symptoms, provides incremental validity with respect to detecting genetic associations with *DRD4* and *5-HTT*.

Taken together, the findings from the current study suggest that there are merits to both approaches with respect to their ability to distinguish more phenotypically homogeneous clusters of children within the ADHD diagnosis. The results further suggest that when conducting genetic association analyses, the addition of information on diagnostic subtypes or comorbid ADHD conditions provides increased external validity of the ADHD phenotype as compared with only analyzing the categorical ADHD diagnosis. In contrast, there was no evidence of significant moderating influences of the co-occurring externalizing and internalizing composite variables on the association between *DSM-IV* ADHD subtypes and *DRD4* and *5-HTT*. This finding suggests that there is no additional benefit gained when these two approaches are used simultaneously, likely owing to the fact that the majority of variance has been already accounted for by parsing the overall ADHD diagnosis into subtypes. Overall, the results provide modest support for the external validity of the *DSM-IV* classification of ADHD diagnostic subtypes, as well as the proposed comorbid conditions.

Several key findings from the current investigation have important implications for future research as well as clinical practice. First, the results from this study raise the possibility that some of the negative findings reported for the associations between ADHD and *DRD4* and *5-HTT* may have been due to sample differences such as diagnostic or genetic heterogeneity that resulted in reduced power to detect true associations. Importantly, this study demonstrates that undertaking subgroup analyses (e.g., examining diagnostic subtypes or comorbid subgroups) or incorporating continuous measures of comorbid psychopathology as moderators can be a useful strategy for enhancing our ability to detect genetic associations that might be otherwise masked when testing a phenotypically heterogeneous condition such as ADHD (e.g., Kim et al., 2007; Kirley et al., 2004). One important caveat deserves mention when considering whether to conduct subtype analyses. Ideally, such phenotypic refinements should proceed from *a priori* hypotheses that are well grounded in the extant empirical literature to limit problems associated with multiple testing (Thapar et al., 2006) and to counterbalance the loss of power inherent in dividing a diagnostic category into smaller subcomponents, each comprised of fewer subjects.

Second, our results provide further validational support for the hypothesis that ADHD with comorbid conduct problems represents a phenotype characterized by increased genetic loading (e.g., Thapar et al., 2001). Despite substantial evidence from family and twin studies suggesting that ADHD, ODD, and CD share a common genetic etiology (e.g., Coolidge et al., 2000; Dick et al., 2005; Faraone et al.., 1998, 2000; Nadder et al., 1998; Silberg et al., 1996; Thapar et al., 2001; Waldman et al., 2001), few studies have investigated specific genes that contribute to the shared genetic susceptibility among these

disorders. Thus, further research is clearly needed to help elucidate whether ADHD and CD are generally influenced by the same genes or if their co-occurrence has unique genetic influences that are distinct from those acting on ADHD and CD in isolation (Stevenson et al., 2005). This line of research should also make important contributions in determining whether a separate diagnostic category for ADHD with comorbid CD is warranted in *DSM-V*, analogous to "hyperkinetic conduct disorder" as defined in the ICD-10 classification system (World Health Organization, 1992).

Third, the current results also lend validational support for an "ADHD, anxious subtype" as proposed by Jensen et al. (1997), given initial evidence that ADHD with co-occurring symptoms of anxiety appears to reflect a somewhat etiologically distinct subgroup of ADHD children. The putative anxious subtype of ADHD warrants further research to examine its external validity, as well as its potential clinical utility. Future studies should test whether the ADHD, anxious subtype can be distinguished from other ADHD subgroups on the basis of external characteristics, such as family history of psychopathology, genetic and environmental influences, developmental course, and differential treatment response.

Study Limitations

Finally, several potential limitations should be considered when interpreting the results of the current study. First, ADHD diagnoses were based on symptom ratings by parents alone. The lack of complementary teacher ratings imposes limits on our ability to determine the extent to which functional impairment was present in two or more settings. Furthermore, there is evidence suggesting that using multiple raters to establish an ADHD diagnosis results in decreased measurement error and higher reliability (reviewed

in Thapar et al., 2006). Alternatively, there is not consensus with respect to how best to combine data from parent and teacher reports to yield categorical clinical diagnoses, and thus, different methods of integrating data from multiple informants may also act as another source of error variance across studies (cf. Gizer et al., in press).

Second, a dimensional approach was used to quantify symptoms of co-occurring externalizing and internalizing disorders in this study given the low base rate of categorical internalizing disorders, as well as the loss of power for detecting genetic associations that would result from parsing ADHD into subtypes based on comorbid clinical diagnoses. Importantly, there is specific evidence demonstrating comparably high heritability estimates regardless of whether a categorical (diagnostic) or dimensional (trait) approach is used to characterize ADHD (e.g., Levy, Hay, McStephen, Wood, & Waldman, 1997). Although genetic and clinical evidence exists to support the validity of using both categorical and dimensional approaches in molecular genetic studies, it is not clear that the same susceptibility genes influence clinical diagnoses and dimensional traits in the same manner (reviewed in Thapar et al., 2006). Therefore, caution should be used in generalizing the results from the current study beyond a dimensional representation of ADHD comorbidity. In addition, though the dimensional composite variables composed of co-occurring symptoms of externalizing and internalizing disorders (i.e., *ODD/CD*, *Distress*, *Fear*) were created based on findings from empirical studies of the structure of psychopathology (Krueger, 1999; Lahey et al., 2004) and corresponding behavior genetic studies (Kendler et al., 2003), further external validation studies of these composite variables are necessary.

Lastly, it was beyond the scope of the current study to examine more than single markers in the two genes of interest, *DRD4* and *5-HTT*. Ideally, future research focused on the examination of the shared genetic susceptibility of ADHD and comorbid disorders will examine multiple markers in both selected candidate genes. A multi-marker approach will yield increased confidence that a failed association for multiple markers truly represents a lack of association with the candidate gene, as well as provide additional power to detect genes that underlie ADHD.

References

Acosta, M.T., Arcos-Burgos, M., Muenke, M., (2004). Attention deficit/hyperactivity disorders (ADHD): Complex phenotype, simple genotype? *Genetics in Medicine, 6*(1), 1-15.

American Psychiatric Association. (1987). *Diagnostic and Statistical Manual of Mental Disorders* (3rd ed., revised). Washington, D.C.: American Psychiatric Association.

American Psychiatric Association. (1994). *Diagnostic and Statistical Manual of Mental Disorders* (4th ed.). Washington, D.C.: American Psychiatric Association.

Arbelle, S., Benjamin, J., Golin, M., Kremer, I, Belmaker, R.H., & Ebstein, R.P. (2003). Relation of shyness in grade school children to the genotype for the long form of the serotonin transporter promoter region polymorphism. *American Journal of Psychiatry, 160*, 671-676.

Auerbach, J.G., Faroy, M., Ebstein, R., Kahana, M., & Levine, J. (2001). The association of the dopamine D4 receptor gene (DRD4) and the serotonin transporter promoter gene (5-HTTLPR) with temperament in 12-month-old infants. *Journal of Child Psychology & Psychiatry, 42*, 777-783.

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

Battaglia, M., Ogliari, A., Zanoni, A., Citterio, A., Pozzoli, U., Giorda, R., et al. (2005). Influence of the serotonin transporter promoter gene and shyness on children's cerebral responses to facial expressions. *Archives of General Psychiatry, 62*, 85-

94.

Bauermeister, J.J., Shrout, P.E., Ramirez, R., Bravo, M., Alegria, M., Martinez-Taboas, A., et al. (2007). ADHD correlates, comorbidity, and impairment in community and treated samples of children and adolescents. *Journal of Abnormal Child Psychology, 35*, 883-898.

Beitchman, J.H., Davidge, K.M., Kennedy, J.L., Atkinson, L., Lee, V., Shapiro, S., et al. (2003). The serotonin transporter gene in aggressive children with and without ADHD and nonaggressive matched controls. *Annals of the New York Academy of Sciences, 1008*, 248-251.

Benjamin, J., Li, L., Patterson, C., Greenberg, B., Murphy, D., & Hamer, D. (1996). Population and familial association between the D4 dopamine receptor gene and measures of Novelty Seeking. *Nature Genetics, 12*, 81-84.

Berkson, J. (1946). Limitations of the application of fourfold tables to hospital data. *Biometrics Bulletin, 2*(3), 47-53.

Biederman, J., Faraone, S.V., Keenan, K., Benjamin, J., Krifcher, B., Moore, C., et al. (1992). Further evidence for family-genetic risk factors in attention deficit hyperactivity disorder: Patterns of comorbidity in probands and relatives in psychiatrically and pediatrically referred samples. *Archives of General Psychiatry, 49*, 728-738.

Biederman, J., Faraone, S.V., Keenan, K., Steingard, R., & Tsuang, M. (1991). Familial association between attention deficit disorder and anxiety disorders. *American Journal of Psychiatry, 148*, 251-256.

Biederman, J., Faraone, S.V., Keenan, K., & Tsuang, M.T. (1991). Evidence of familial

association between attention deficit disorder and major affective disorders. *Archives of General Psychiatry, 48*, 633-642.

Biederman, J., Newcorn, J., & Sprich, S. (1991). Comorbidity of attention deficit hyperactivity disorder with conduct, depressive, anxiety, and other disorders. *American Journal of Psychiatry, 148*, 564-577.

Burns, G.L., Walsh, J.A., Patterson, D.R., Holte, C.S., Sommers-Flannagan, R., & Parker, C.M. (1997). Internal validity of disruptive behavior disorder symptoms: Implications from parent ratings for a dimensional approach to symptom validity. *Journal of Abnormal Child Psychology, 25*, 307-319.

Cadoret, R.J., Langbehn, D., Caspers, K., Troughton, E.P., Yucuis, R., Sandhu, H.K., & Philibert, R. (2003). Associations of the serotonin transporter promoter polymorphism with aggressivity, attention deficit, and conduct disorder in an adoptee population. *Comprehensive Psychiatry, 44*(2), 88-101.

Caspi, A., Langley, K., Milne, B., Moffitt, T.E., O'Donovan, M., Owen, M.J., et al. (2008). A replicated molecular genetic basis for subtyping antisocial behavior in children with attention-deficit/hyperactivity disorder. *Archives of General Psychiatry, 65*(2), 203-210.

Caspi, A., Sugden, K., Moffitt, T.E., Taylor, A., Craig, I.W., Harrington, H., et al. (2003). Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science, 301*, 386-389.

Coffman, J.K, Wright, G., & Gottfried, A.W. (2006). Reliability and validity of the parent-child relationship inventory (PCRI): Evidence from a longitudinal cross-informant investigation. *Psychological Assessment, 18*(2), 209-214.

Coolidge, F.L., Thede, L.L., & Young, S.E. (2000). Attention deficit hyperactivity disorder with behavioral disorders and executive function deficits: A preliminary investigation. *Developmental Neuropsychology, 17*(3), 273-287.

Cohen, J., Cohen, P., West, S.G., & Aiken, L.S. (Eds.). (2003). *Applied multiple regression / correlation analysis for the behavioral sciences* (3rd ed.). Mahwah, N.J.: Lawrence Erlbaum Associates, Publishers.

Crick, N.R., & Zahn-Waxler, C. (2003). The development of psychopathology in females and males: Current progress and future challenges. *Development and Psychopathology, 15*, 719-742.

Dick, D.M., Viken, R.J., Kaprio, J., Pulkkinen, L., & Rose, R.J. (2005). Understanding the covariation among childhood externalizing symptoms: Genetic and environmental influences on conduct disorder, attention deficit hyperactivity disorder, and oppositional defiant disorder symptoms. *Journal of Abnormal Child Psychology, 33*(2), 219-229.

Eaves, L.J., Rutter, M., Silberg, J.L., Shillady, L., Maes, H.H., & Pickles, A. (2000). Genetic and environmental causes of covariation in interview assessments of disruptive behavior in child and adolescent twins. *Behavior Genetics, 30*, 321-334.

Ebstein, R., Novick, O., Umansky, R., Priel, B., Osher, Y., Blaine, D., et al. (1996). Dopamine D4 receptor (DRD4) exon III polymorphism associated with the human personality trait of Novelty Seeking. *Nature Genetics, 12*, 78-80.

Faraone, S.V. & Biederman, J. (1998). Neurobiology of attention-deficit / hyperactivity disorder. *Biological Psychiatry, 44*, 951-958.

Faraone, S.V., Biederman, J., Chen, W.J., Milberger, S., Warburton, R., & Tsuang, M. (1995). Genetic heterogeneity in attention-deficit hyperactivity disorder (ADHD): Gender, psychiatry comorbidity, and maternal ADHD. *Journal of Abnormal Psychology, 104*(2), 334-345.

Faraone, S.V., Biederman, J., & Friedman, D. (2000). Validity of DSM-IV subtypes of Attention-deficit/hyperactivity disorder: A family study perspective. *Journal of the American Academy of Child & Adolescent Psychiatry, 39*(3), 300-307.

Faraone, S.V., Biederman, J., Jetton, J.G., & Tsuang, M.T. (1997). Attention deficit disorder and conduct disorder: Longitudinal evidence for a familial subtype. *Psychological Medicine, 27*, 291-300.

Faraone, S.V., Biederman, J., Weber, W., & Russell, R.L. (1998). Psychiatric, neuropsychological, and psychosocial features of DSM-IV subtypes of Attention-deficit/hyperactivity disorder: Results from a clinically referred sample. *Journal of the American Academy of Child & Adolescent Psychiatry, 37*(2), 185-193.

Faraone, S.V., Biederman, J., Weiffenbach, B., Keith, T., Chu, M.P.,Weaver, A., Spencer, T.J., Wilens, T.E., Frazier, J., Cleves, M., & Sakai, J. (1999). Dopamine D4 Gene 7-Repeat Allele and Attention Deficit Hyperactivity Disorder. *The American Journal of Psychiatry, 156*(5), 768-770.

Faraone, S. V., Doyle, A. E., Mick, E., & Biederman, J. (2001). Meta-analysis of the association between the 7-repeat allele of the dopamine D(4) receptor gene and attention deficit hyperactivity disorder. *American Journal of Psychiatry, 158,* 1052-1057.

Faraone, S., Perlis, R., Doyle, A., Smoller, J., Goralnick, J., Holmgren, M., et al. (2005). Molecular genetics of Attention-Deficit/Hyperactivity Disorder. *Biological Psychiatry, 57*, 1313-1323.

Faraone, S.V. & Khan, S.A. (2006). Candidate gene studies of attention-deficit / hyperactivity disorder. *Journal of Clinical Psychiatry, 67*(Suppl 8), 13-20.

Fox, N.A., Nichols, K.E., Henderson, H.A., Rubin, K., Schmidt, L., Hamer, D., et al. (2005). Evidence for a gene-environment interaction in predicting behavioral inhibition in middle childhood. *Psychological Science, 16*, 921-926.

Frisch, A., Postilnick, D., Rockah, R., Michaelovsky, E., Postilnick, S., Birman, E., et al. (1999). Association of unipolar major depressive disorder with genes of serotonergic and dopaminergic pathways. *Molecular Psychiatry, 4*, 389-392.

Gaub, M., & Carlson, C. (1997). Behavioral characteristics of DSM-IV ADHD subtypes in a school-based population. *Journal of Clinical Child Psychology, 21*, 103-111.

Gerra, G., Garofano, L., Castaldini, L., Rovetto, F., Zaimovic, A., Moi, G., et al. (2005). Serotonin transporter promoter polymorphism genotype is associated with temperament, personality traits, and illegal drug use among adolescents. *Journal of Neural Transmission, 112*, 1397-1410.

Gilliom, M. & Shaw, D.S. (2004). Codevelopment of externalizing and internalizing problems in early childhood. *Development and Psychopathology, 16*, 313-333.

Gizer, I., Waldman, I.D., Abramowitz, A., Barr, C.L., Feng, Y., Wigg, K., Misener, V., & Rowe, D.C. (in press). Relations between multi-informant assessments of ADHD symptoms and DAT1 and DRD4. *Journal of Abnormal Psychology*.

Haberstick, B.C., Smolen, A., & Hewitt, J.K. (2006). Family-based association test of the

5HTTLPR and aggressive behavior in a general population sample of children. *Biological Psychiatry, 59*, 836-843.

Halperin, J. M., Newcorn, J. H., Schwartz, S. T., Sharma, V., Siever, L. J., Koda, V. H., et al. (1997). Age-related changes in the association between serotonergic function and aggression in boys with ADHD. *Biological Psychiatry, 41*, 682-689.

Hamilton, S.P., Haghighi, F., Heiman, G.A., Klein, D.F., Hodge, S.E., Fyer, A.J. et al. (2000). Investigation of dopamine receptor (DRD4) and dopamine transporter (DAT1) polymorphisms for genetic linkage or association to panic disorder. *American Journal Medical Genetics, 96*(3), 324-330.

Hayden, E.P., Dougherty, L.R., Maloney, B., Durbin, C.E., Olino, T.M., Nurnberger, J.I., et al. (2007). Temperamental fearfulness in childhood and the serotonin transporter promoter region polymorphism: A multimethod association study. *Psychiatric Genetics, 17*, 135-142.

Hechtman, L. (1999). Predictors of long-term outcome in children with attention-deficit/ hyperactivity disorder. *Pediatric Clinics of North America, 46*(5), 1039-1052.

Hinshaw, S.P. (1987). On the distinction between attentional deficits/hyperactivity and conduct problems/aggression in child psychopathology. *Psychological Bulletin, 101*, 443-463.

Holmes, J., Payton, A., Barrett, J., Harrington, R., McGuffin, P., Owen, M., et al. (2002). Association of DRD4 in children with ADHD and comorbid conduct problems *American Journal of Metical Genetics Part B (Neuropsychiatric Genetics), 114*, 150-153.

Hutchison, K.E., Stallings, M., McGeary, J., & Bryan, A. (2004). Population stratification

in the candidate gene study: fatal threat or red herring? *Psychological Bulletin, 130*(1), 66-79.

Jensen, P.S., Hinshaw, S.P., Kraemer, H.C., Lenora, N., Newcorn, J.H., Abikoff, H.B., et al. (2001). ADHD comorbidity findings from the MTA study: Comparing comorbid subgroups. *Journal of the American Academy of Child & Adolescent Psychiatry, 40*(2), 147-158.

Jensen, P.S., Martin, D., & Cantwell, D.P. (1997). Comorbidity in ADHD: Implications for research, practice, and *DSM-IV*. *Journal of the American Academy of Child & Adolescent Psychiatry, 36*(8), 1065-1079.

Jorm, A.F., Prior, M., Sanson, A., Smart, D., Zhang, Y., & Easteal, S. (2000). Association of a functional polymorphism of the serotonin transporter gene with anxiety-related temperament and behavior problems in children: a longitudinal study from infancy to the mid-teens. *Molecular Psychiatry, 5*, 542-547.

Kaufman, J., Yang, B.Z., Douglas-Palumberi, H., Houshyar, S., Lipschitz, D., Krystal, J.H., & Gelernter, J. (2004). Social supports and serotonin transporter genes moderate depression in maltreated children. *Proceedings of the National Academy of Sciences, 101*, 17316-17321.

Kendler, K.S., Kuhn, J.W., Vittum, J., Prescott, C.A., & Riley, B. (2005). The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression. *Archives of General Psychiatry, 62*, 529-535.

Kendler, K.S., Prescott, C.A., Myers, J., & Neale, M.C. (2003). The structure of genetic and environmental risk factors for common psychiatric and substance use disorders in men and women. *Archives of General Psychiatry, 60*, 929-937.

Kennedy, J.L., Neves-Pereira, M., King, N., Lizak, M.V., Basile, V.S., Chartier, M.J., & Stein, M.B. (2001). Dopamine system genes not linked to social phobia. *Psychiatric Genetics, 11*, 213-217.

Kim, J.W., Biederman, J., Arbeitman, L., Fagerness, J., Doyle, A.E., Petty, C., et al. (2007). Investigation of variation in SNAP-25 and ADHD and relationship to comorbid major depressive disorder. *American Journal of Medical Genetics (Neuropsychiatric Genetics), 144B*, 781-790.

Kirley, A., Lowe, N., Mullins, C., McCarron, M., Daly, G., Waldman, I., et al. (2004). Phenotype studies of the *DRD4* gene polymorphisms in ADHD: association with oppositional defiant disorder and positive family history. *American Journal of Medical Genetics Part B (Neuropsychiatric Genetics), 131B*, 38-42.

Krueger, R.F. (1999). The structure of common mental disorders. *Archives of General Psychiatry, 56*, 921-926.

Krueger, R.F. & Markon, K.E. (2006). Reinterpreting comorbidity: A model-based approach to understanding and classifying psychopathology. *Annual Reviews in Clinical Psychology, 2*, 111-133.

Kuhne, M., Schachar, R., & Tannock, R. (1997). Impact of comorbid oppositional or conduct problems on attention-deficit hyperactivity disorder. *Journal of the American Academy of Child & Adolescent Psychiatry, 36*(12), 1715-1725.

Lahey, B.B., Waldman, I.D., Hankin, B.L., Applegate, B., Loft, J.D., & Rick, J. (2004). The structure of child and adolescent psychopathology: Generating new hypotheses. *Journal of Abnormal Psychology, 113*(3), 358-385.

Lasky-Su, J., Lange, C., Biederman, J., Tsuang, M., Doyle, A.E., Smoller, J.W., et al. (2008). Family-based association analysis of a statistically derived quantitative trait for ADHD reveals an association in DRD4 with inattentive symptoms in ADHD individuals. *American Journal of Medical Genetics Part B (Neuropsychiatric Genetics), 147B*, 100-106.

LeMarquand, D.G., Pihl, R.O., Young, S.N., Tremblay, R.E., Seguin, J.R., Palmour, R.M., & Benkelfat, C. (1998). Tryptophan depletion, executive functions, and disinhibition in aggressive, adolescent males. *Neuropsychopharmacology, 19*, 333-341.

Liao, D.L., Hong, C.L., Shih, H.L., & Tsai, S.J. (2004). Possible association between serotonin transporter promoter region polymorphism and extremely violent crime in Chinese males. *Biological Psychiatry, 50*, 284-287.

Loeber, R., Lahey, B.B., & Thomas, C. (1991). Diagnostic conundrum of oppositional defiant disorder and conduct disorder. *Journal of Abnormal Psychology, 100*, 379-390.

López León, S.L., Croes, E.A., Sayed-Tabatabaei, F.A., Claes, S., Van Broeckhoven, C., & van Duijn, C.M. (2005). The dopamine D4 receptor gene 48-base-pair-repeat polymorphism and mood disorders: a meta-analysis. *Biological Psychiatry, 57*, 999-1003.

Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science, 274,* 1527-1531.

Levy, F., Hay, D.A., 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 American Academy of Child & Adolescent Psychiatry, 36,* 737-744.

Li, D., Sham, P.C., Owen, M.J., & He, L. (2006). Meta-analysis shows significant Association between dopamine system genes and attention deficit hyperactivity disorder (ADHD). *Human Molecular Genetics, 15*(14), 2276-2284.

Lotrich, F.E., & Pollock, B.G. (2004). Meta-analysis of serotonin transporter polymorphisms and affective disorders. *Psychiatric Genetics, 14,* 121-129.

Lucki, I. (1998). The spectrum of behaviors influenced by serotonin. *Biological Psychiatry, 44,* 151-162.

Maher, B.S., Marazita, M.L., Ferrell, R.E., Vanyukov, M.M. (2002). Dopamine system genes and attention deficit hyperactivity disorder: A meta-analysis. *Psychiatric Genetics, 12,* 207-215.

Manor, I., Eisenberg, J., Tyano, S., Sever, Y., Cohen, H., Ebstein, R. P., et al. (2001). Family-based association study of the serotonin transporter promoter region polymorphism (5-HTTLPR) in attention deficit hyperactivity disorder. *American Journal of Medical Genetics, 105,* 91-95.

McCracken, J.T., Smalley, S.L., McGough, J.J., Crawford, L., Del'Homme, M., Cantor, R.M., et al. (2000). Evidence for linkage of a tandem duplication polymorphism upstream of the dopamine D4 receptor gene (DRD4) with attention deficit hyperactivity disorder (ADHD). *Molecular Psychiatry,*5(5), 531-536.

McDougle, C.J., Epperson, C.N., Price, L.H., & Gelernter, J. (1998). Evidence for linkage disequilibrium between serotonin transporter protein gene (SLC6A4) and obsessive compulsive disorder. *Molecular Psychiatry, 3*, 270-273.

Nadder, T.S., Rutter, M., Silberg, J.L., Maes, H.H., & Eaves, L.J. (2002). Genetic effects on the variation and covariation of attention-deficit/hyperactivity disorder (ADHD) and oppositional defiant disorder/conduct disorder (ODD/CD) symptomatologies across informant and occasion of measurement. *Psychological Medicine, 32*, 39-53.

Nadder, T.S., Silberg, J.L., Eaves, L.J., Maes, H.H., & Meyer, J.M. (1998). Genetic effects on ADHD symtomatology in 7- to 13-year-old twins: Results from a telephone survey. *Behavior Genetics, 28*, 83-99.

Neuman, R.J., Heath, A., Reich, W., Bucholz, K.K., Madden, P.A.F., Sun, L., et al. (2001). Latent class analysis of ADHD and comorbid symptoms in a population sample of adolescent female twins. *Journal of Child Psychology & Psychiatry, 42*(7), 933-942.

Neumeister, A., Young, T., & Stastny, J. (2004). Implications of genetic research on the role of serotonin in depression: emphasis on the serotonin type 1_A receptor and the serotonin transporter. *Psychopharmocology, 174*, 512-524.

Newcorn, J.H., Halperin, J.M., Jensen, P.S., Abikoff, H.B., Arnold, E., Cantwell, D.P., et al. (2001). Symptom profiles in children with ADHD: Effects of comorbidity and gender. *Journal of the American Academy of Child & Adolescent Psychiatry, 40*(2), 137-146.

Paterson, A.D., Sunohara, G.A., & Kennedy, J.L. (1999). Dopamine receptor gene:

Novelty or nonsense? *Neuropsychopharmacology, 21,* 3-16.

Perrin, S. & Last, C.G. (1998). Relationship between ADHD and anxiety in boys: Results from a family study. *Journal of the American Academy of Child & Adolescent Psychiatry,35*(8), 988-996.

Pliszka, S.R., Carlson, C., & Swanson, J.M., (1999). *ADHD with comorbid disorders: Clinical assessment and management.* New York: Guilford.

Retz, W., Freitage, C.M., Retz-Junginger, P., Wenzler, D., Schneider, M., Kissling, C., et al. (2008). A functional serotonin transporter promoter gene polymorphism increased ADHD symptoms in delinquents: Interaction with adverse childhood environment. *Psychiatry Research, 158,* 123-131.

Retz, W., Retz-Junginger, P., Supprian, T., Thome, J., & Rosler, M. (2004). Association of serotonin transporter promoter gene polymorphism with violence: Relation with personality disorders, impulsivity, and childhood ADHD psychopathology. *Behavioral Science and the Law, 22,* 415-425.

Rowe, D.C., Stever, C., Giedinghagen, L.N., Gard, J.M., Cleveland, H.H., Terris, S.T., et al. (1998). Dopamine DRD4 receptor polymorphism and attention deficit hyperactivity disorder. *Molecular Psychiatry, 3,* 419-426.

Sakai, J.T., Lessem, J.M., Haberstick, B.C., Hopfer, C.J., Smolen, A., Ehringer, M.A., et al. (2007). Case-control and within-family tests for association between 5HTTLPR and conduct problems in a longitudinal adolescent sample. *Psychiatric Genetics, 17,* 207-214.

Sakai, J.T., Young, S.E., Stallings, M.C., Timberlake, D., Smolen, A., Stetler, G.L., et al. (2006). Case-control and within-family tests for an association between conduct

disorder and 5HTTLPR. *American Journal of Medical Genetics, Part B (Neuropsychiatric Genetics), 141*(8), 825-832.

Schatz, D.B., & Rostain, A.L. (2006). ADHD with comorbid anxiety: a review of the current literature. *Journal of Attention Disorders, 10*(2), 141-149.

Schinka, J.A., Letsch, E.A., & Crawford, F.C. (2002). DRD4 and novelty seeking: Results of meta-analyses. *American Journal of Medical Genetics, Part B (Neuropsychiatric Genetics), 114*(6), 643-648.

Seeger, G., Schloss, P., & Schmidt, M. H. (2001). Functional polymorphism within the promotor of the serotonin transporter gene is associated with severe hyperkinetic disorders. *Molecular Psychiatry, 6,* 235−238.

Serretti, A., Cristina, S., Lilli, R., Cusin, C., Lattuada, E., Lorenzi, C., et al. (2002). Family-based association study of 5-HTTLPR, TPH, MAO-A, and DRD4 polymorphisms in mood disorders. *American Journal of Medical Genetics, 114,* 361-369.

Silberg, J.L., Rutter, M., Meyer, J.M., Maes, H.H., Hewitt, J.K., Simonoff, E., et al., (1996). Genetic and environmental influences n the covariation between hyperactivity and conduct disturbance in juvenile twins. *Journal of Child Psychology & Psychiatry, 37,* 803-816.

Smalley, S.L., McGough, J.J., Del'Homme, M., NewDelman, J., Gordon, E., Kim, T., et al. (2000). Familial clustering of symptoms and disruptive behaviors in multiplex families with attention-deficit/hyperactivity disorder. *Journal of the American Academy of Child & Adolescent Psychiatry, 39*(9), 1135-1143.

Spivak, B., Vered, Y., Yoran-Hegesh, R., Averbuch, E., Mester, R., Graf, E., et al.

(1999). Circulatory levels of catecholamines, serotonin and lipids in attention deficit hyperactivity disorder. *Acta Psychiatrica Scandinavica, 99,* 300-304.

Stevenson, J., Asherson, P., Hay, D., Levy, F., Swanson, J., Thapar, A., & Willcutt, E. (2005). Characterizing the ADHD phenotype for genetic studies. *Developmental Science, 8*(2), 115-121.

Thapar, A., Harrington, R., & McGuffin, P. (2001). Examining the comorbidity of ADHD-related behaviors and conduct problems using a twin study design. *British Journal of Psychiatry, 179*, 224-229.

Thapar, A., Holmes, J., Poulton, K., & Harrington, R. (1999). Genetic basis of attention deficit and hyperactivity. *British Journal of Psychiatry, 174,* 105-111.

Thapar, A., Langley, K., O'Donovan, M., & Owen, M. (2006). Refining the attention deficit hyperactivity disorder phenotype for molecular genetic studies. *Molecular Psychiatry, 11*, 714-720.

Waldman, I.D., & Gizer, I.R. (2006). The genetics of attention deficit hyperactivity disorder. *Clinical Psychology Review, 26,* 396-432.

Waldman, I. D., & Rhee, S. H. (2002). Behavioral and molecular genetic studies. In S. Sandberg (Ed.), *Hyperactivity and attention disorders of childhood, 2nd Ed.* (pp. 290-335). New York: Cambridge University Press.

Waldman, I.D., Rhee, S.H., Levy, F., & Hay, D.A. (2001). Causes of the overlap among symptoms of ADHD, oppositional defiant disorder, and conduct disorder. In F. Levy & D.A. Hay (Eds.), *Attention, genes, and ADHD* (pp. 115-138). New York: Brunner-Routledge.

World Health Organization (1992). *International Classification of Diseases and Related*

Disorders (ICD-10). Geneva: WHO.

Wigginton, J.E. & Abecasis, G.R. (2005). PEDSTATS: descriptive statistics, graphics and quality assessment for gene mapping data. *Bioinformatics, 21*(16), 3445-3447.

Wittke-Thompson, J.K., Pluzhnikov, A., & Cox, N.J. (2005). Rational inferences about departures from Hardy-Weinberg equilibrium. *American Journal of Human Genetics, 76*(6), 967-986.

You, J-S, Hu, S-Y, Chen, B., & Zhang, H-G (2005). Serotonin transporter and tryptophan hydroxylase gene polymorphisms in Chinese patients with generalized anxiety disorder. *Psychiatric Genetics, 15*, 7-11.

Zalsman, G., Huang, Y.Y., Oquendo, M.A., Burke, A.K., Hu, X.Z., & Brent, D.A. (2006). Association of a triallelic serotonin transporter gene promoter region (5-HTTLPR) polymorphism with stressful life events and severity of depression. *American Journal of Psychiatry, 163*, 1588-1593.

Table 1. *Demographic Characteristics and Mean Symptom Levels for ADHD Diagnostic Subtypes and a Nondisordered Sample*

	No ADHD diagnosis	Overall ADHD	ADHD/IT	ADHD/CT	ADHD/HT
Participants, N	135	237	82	132	23
ADHD subtype diagnoses (%)	--	--	34%	56%	10%
Gender, N (%) male	82 (61%)	172 (73%)	55 (67%)	103 (78%)	14 (61%)
Mean age at assessment (SD)	10.8 (3.5)	10.3 (3.0)	11.5 (3.0)	10.0 (2.8)	8.4 (2.5)
Mean inattention sx (SD)	.67 (.55)	2.81 (.76)	2.81 (.62)	3.10 (.56)	1.40 (.66)
Mean hyp-imp. sx (SD)	.47 (.47)	2.18 (1.13)	.95 (.68)	2.92 (.63)	2.77 (.58)
Mean ODD sx (SD)	.80 (.74)	1.96 (1.14)	1.36 (1.00)	2.31 (1.04)	2.31 (1.11)
Mean CD sx (SD)	.15 (.26)	.41 (.42)	.27 (.31)	.51 (.45)	.41 (.37)
Mean depression sx (SD)	.20 (.39)	.58 (.57)	.45 (.49)	.73 (.59)	.36 (.41)
Mean GAD sx (SD)	.37 (.56)	.67 (.64)	.52 (.53)	.84 (.70)	.55 (.56)
Mean social phobia sx (SD)	.40 (.57)	.55 (.69)	.56 (.66)	.59 (.77)	.60 (.83)
Mean SAD Sx (SD)	.15 (.34)	.28 (.48)	.18 (.40)	.39 (.57)	.27 (.39)
Mean specific phobia sx (SD)	.24 (.31)	.39 (.40)	.28 (.33)	.41 (.43)	.31 (.37)
Mean obsessive sx (SD)	.12 (.35)	.27 (.48)	.14 (.29)	.37 (.59)	.26 (.31)
Mean compulsive sx (SD)	.04 (.15)	.14 (.34)	.08 (.27)	.19 (.40)	.20 (.35)

Note. ADHD/IT = Attention-Deficit/Hyperactivity Disorder, Inattentive Type; ADHD/CT = Combined Type; ADHD/HT = Hyperactive-Impulsive Type; sx = symptoms; hyp-imp. = hyperactive-impulsive; ODD = Oppositional Defiant Disorder; CD = Conduct Disorder; GAD = Generalized Anxiety Disorder; SAD = Separation Anxiety Disorder.

Table 2. *A Priori Orthogonal Contrasts for Comparing DSM-IV ADHD Subtypes*

Contrast set 1	Contrast 1	Contrast 2	Contrast 3
ADHD diagnostic subtype			
No ADHD	-3	0	0
Inattentive	1	1	1
Hyperactive-impulsive	1	-2	0
Combined	1	1	-1
Contrast set 2	**Contrast 1a**	**Contrast 2a**	**Contrast 3a**
ADHD diagnostic subtype			
No ADHD	-3	0	0
Inattentive	1	2	0
Hyperactive-impulsive	1	-1	-1
Combined	1	-1	1

Table 3. *Correlations among Symptom Dimensions Comprising the ODD/CD, Distress, and Fear Composite Variables*

	ODD	CD	Depress.	GAD	Social Phobia	SAD	Specific Phobias	Obsess.	Comp.
ODD	—								
CD	.71**	—							
Depression	.51**	.47**	—						
GAD	.43**	.33**	.68**	—					
Social Phobia	.19**	.17*	.34**	.52**	—				
SAD	.25**	.27**	.39**	.55**	.38**	—			
Specific Phobias	.20**	.18*	.28**	.34**	.22**	.31**	—		
Obsessions	.25**	.32**	.40**	.50**	.35**	.52**	.27**	—	
Compulsions	.25**	.20**	.30**	.42**	.31**	.39**	.22**	.38**	—

Note. ODD = Oppositional Defiant Disorder; CD = Conduct Disorder; GAD = Generalized Anxiety Disorder; SAD = Separation Anxiety Disorder; Obsess. = Obsessions; Comp. = Compulsions. Light grey shading highlights the correlation between *ODD* and *CD* symptom dimensions; medium grey shading highlights the correlations among symptom dimensions comprising the *Distress* composite (Depression, GAD, Social Phobia); dark grey shading highlights the correlations among symptom dimensions comprising the *Fear* composite (SAD, Specific Phobias, Obsessions, Compulsions).

*$p < .01$; **$p < .001$.

Table 4. *Moderating Effects of Co-occurring Conditions on the Association between ADHD and DRD4 and 5-HTTLPR*

	DRD4 (7-repeat allele)			*5-HTTLPR* (long allele)		
Phenotype	*p*	*OR*	R^2	*p*	*OR*	R^2
Categorical ADHD diagnosis	.398	1.07	.01	.219	1.18	.02
ADHD X *ODD/CD*	.036**	2.90	.02	.470	1.03	.02
ADHD X *Distress*	.366	0.83	.02	.252	0.72	.02
ADHD X *Fear*	.487	1.03	.01	.081*	0.31	.03

Note. "X" indicates statistical interaction.

*$p < .10$; **$p < .05$.

Table 5. *Comparison of DSM-IV ADHD Subtypes versus ADHD Comorbid Conditions in their Association with DRD4*

Moderator	*p*	OR	R^2	*p*	OR	R^2	*p*	OR	R^2	Significant planned comparisons
Contrast set 1	No ADHD vs. ADHD/Any Dx			ADHD/IT + CT vs. ADHD/HT			ADHD/IT vs. ADHD/CT			
No moderator	.073*	1.16	.05	.023**	2.01	.05	.022**	.71	.05	ADHD > No Dx; IT+CT > HT; CT > IT
ODD/CD	.059*	1.52	.07	.178	.44	.07	.331	.89	.07	ADHD X *ODD/CD*
Distress	.252	.82	.06	.334	1.56	.06	.351	.88	.06	
Fear	.271	.73	.06	.374	1.82	.06	.217	.51	.06	
Contrast set 2	No ADHD vs. ADHD/Any Dx			ADHD/IT vs. ADHD/HT + CT			ADHD/CT vs. ADHD/HT			
No moderator	.073*	1.16	.05	.187	1.19	.05	.010**	3.40	.05	ADHD > No Dx; CT > HT
ODD/CD	.059*	1.52	.07	.160	.62	.07	.189	.30	.07	ADHD X *ODD/CD*
Distress	.252	.82	.06	.389	1.17	.06	.319[a]	2.07	.06	
Fear	.271	.73	.06	.487	.97	.06	.329	3.42	.06	

Note. "X" indicates statistical interaction; [a] indicates that the test of parallel lines was violated.

*$p < .10$; **$p < .05$.

Table 6. *Comparison of DSM-IV ADHD Subtypes versus ADHD Comorbid Conditions in their Association with 5-HTTLPR*

Moderator	*p*	OR	R^2	*p*	OR	R^2	*p*	OR	R^2	Significant planned comparisons
Contrast set 1	No ADHD vs. ADHD/Any Dx			ADHD/IT + CT vs. ADHD/HT			ADHD/IT vs. ADHD/CT			
No moderator	.478	1.00	.03	.045**	1.30	.03	.098*	1.20	.03	IT+CT > HT; IT > CT
ODD/CD	.497	1.00	.03	.260	1.16	.03	.336	.91	.03	
Distress	.195	.89	.04	.300	1.18	.04	.295	.85	.04	
Fear	.044**	.65	.05	.304	1.35	.05	.167	.56	.05	ADHD X *Fear*
Contrast set 2	No ADHD vs. ADHD/Any Dx			ADHD/IT vs. ADHD/HT + CT			ADHD/CT vs. ADHD/HT			
No moderator	.478	1.00	.03	.019**	1.25	.03	.103*	1.36	.03	IT > HT+CT; CT > HT
ODD/CD	.497	1.00	.03	.433	1.03	.03	.223	1.31	.03	
Distress	.195	.89	.04	.493	1.00	.04	.249	1.38	.04	
Fear	.044**	.65	.05	.381	.87	.05	.196	2.10	.05	ADHD X *Fear*

Note. "X" indicates statistical interaction.

*$p \leq .10$; **$p < .05$.

Table 7. *Differences in Gender Composition and Mean Age for ADHD Comorbid Conditions*

	Gender composition			*Mean age*	
Phenotype	*p*	*OR (95% CI)*	R^2	*p*	h^2
Categorical ADHD diagnosis	.069*	1.10 (.97 – 1.23)	.04	.014**	.01
ADHD X *ODD/CD*	.277	1.07 (.85 – 1.33)	.04	.290	.00
ADHD X *Distress*	.288	1.07 (.83 – 1.39)	.04	.456	.00
ADHD X *Fear*	.234	1.18 (.75 – 1.89)	.05	.343	.00

Note. "X" indicates statistical interaction.

*$p < .10$; **$p < .05$.

General Discussion

The overarching objective of this dissertation was to help address two major challenges that clinicians and researchers face concerning the classification, treatment, and etiology of ADHD. First, researchers lack consensus with respect to the best way to classify and diagnose ADHD, despite intense taxonomic study of this prevalent childhood disorder. Second, the search for specific susceptibility genes for ADHD is complicated by the fact that this condition is a genetically complex disorder and by clinical heterogeneity within the ADHD diagnosis. In order for quantitative and molecular genetic methods to be maximally utilized to uncover the genetic and environmental risk factors underlying ADHD, accurate and valid characterization of the ADHD phenotype is of utmost importance (Lahey et al., 2004). With these challenges in mind, the current dissertation 1) examined the external validity and distinctiveness of the *DSM-IV* ADHD diagnostic subtypes and 2) evaluated whether an alternative comorbid ADHD phenotype enhances the external validity of ADHD, as a function of including co-occurring symptom dimensions, over and above the extant *DSM-IV* diagnostic subtypes.

The results of the first paper of this thesis demonstrated that the *DSM-IV* ADHD diagnostic subtypes were distinguishable with respect to the proportion of males, mean age at assessment, and symptom levels of co-occurring externalizing and internalizing disorders. On average, children diagnosed with the Combined Type (CT) relative to the Inattentive Type (IT) were 1.5 years older, 30% more likely to be male, and more likely to show elevated levels of ODD, CD, depression, and a variety of anxiety symptoms. The Hyperactive-Impulsive Type (HT) generally appeared more similar to the CT than the IT, showing comparable levels of ODD and CD, as well as a range of internalizing symptoms, with the

exception of depression and GAD. In addition, the molecular genetic analyses suggested that the extant *DSM-IV* diagnostic subtypes can be differentiated with respect to their strength of associations with the candidate genes, *DRD4* and *5-HTT*. That is, the CT showed the strongest association with the 7-repeat allele of *DRD4*, whereas the IT showed the strongest relation with the long allele of the *5-HTTLPR*.

Taken together, this set of results from a clinically-referred sample provides only modest support for the external validity and distinctiveness of the *DSM-IV*–defined ADHD diagnostic subtypes. Although the findings from the current study suggest that the ADHD subtypes are somewhat distinguishable on several external correlates, it is important to highlight that there are greater similarities than differences across the subtypes as compared with a group of non-ADHD children. Thus, the current study does not provide robust evidence for Milich et al.'s (2001) assertion that the IT and the CT may be best characterized as "distinct and unrelated disorders."

It is a well documented finding that children diagnosed with CT relative to IT are generally rated higher by parents and teachers on other externalizing symptoms (Milich et al., 2001). However, a distinct pattern of ADHD subtype differences in internalizing symptoms has not previously been reported (Power, Costigan, Eiraldi, & Leff, 2004). The current analyses revealed that the CT is characterized by significantly higher levels of internalizing symptoms (depression, anxiety) as well as externalizing symptoms, however, and thus represents a novel finding of this study. Notably, a recent meta-analysis of ADHD subtype differences in co-occurring externalizing and internalizing disorders provides additional evidence that children with CT tend to be at higher risk for developing both concurrent externalizing and internalizing symptoms than children with IT (Harrington

& Waldman, 2008). Overall, these findings suggest that within the heterogeneous diagnostic category of ADHD, the CT represents a more severely affected group than the IT. Specifically, there is increasing evidence that CT is associated with greater severity of psychopathology, including higher rates of comorbidity as well as elevated essential symptoms (i.e., inattention and hyperactivity-impulsivity) relative to IT. It is noteworthy that a recent study by Gadow et al. (2004) demonstrated that the CT still showed significantly higher levels of anxiety and depression than the IT and HT groups after controlling statistically for the severity of the essential ADHD symptoms. Thus, the higher rate of internalizing symptoms exhibited by the CT cannot be solely attributed to the fact that the CT possesses more severe inattentive and hyperactive-impulsive symptoms.

As previously stated, there continues to be controversy among researchers about whether ADHD is best conceptualized as a) a unitary disorder, b) a unitary disorder with subtypes, or c) multiple distinct disorders. The findings from the current investigation are most consistent with the representation of ADHD as a unitary disorder with subtypes, given that the ADHD subtypes were found to differ significantly on several external correlates, while their overall symptom profiles appear more similar than different when compared with non-ADHD children. Nevertheless, one important question that cannot be adequately resolved in the current study relates to whether these subtypes differ sufficiently to justify partitioning the overall ADHD diagnosis into qualitatively distinct subcategories. That is, do the observed ADHD subtype differences more accurately reflect *quantitative* variation in severity (e.g., opposite ends of a continuum or continua) or *qualitatively* discrete categories? Proper identification of the latent structure of ADHD clearly has implications for the taxonomy of ADHD and thus merits exploration in future validation studies. For example,

accurate identification of the latent structure of ADHD would allow researchers to focus on the most probable etiological pathways for this highly heritable condition (Frazier, Youngstrom, & Naugle, 2007). More specifically, Haslam (1997) suggested that taxonicity is indicative of all-or-none causes, whereas dimensionality is more consistent with multiple additive or graded etiologies.

Psychopathology researchers have used several approaches to examine the latent structure of ADHD, including factor analysis, latent class analysis, and taxometric methods[1] (e.g., Frazier et al., 2007; Gomez et al., 2005; Hartman et al., 2001; Haslam et al., 2006; Hudziak et al., 1998; Neuman et al., 1999; Rasmussen et al., 2002; Rohde et al., 2001). A superior fit of latent class models or taxometric models would provide evidence in support of an underlying categorical structure comprised of distinct subtypes (i.e., classes or taxa), whereas a superior fit of factor models would be consistent with a continuous underlying trait (i.e., one or more dimensional factors). Nonetheless, it is necessary to be able to directly compare the fit of such models in order to make such inferences, which neither factor analysis nor latent class analysis permit.

Frazier et al. (2007) recently conducted a series of taxometric analyses and the results suggested that the core symptoms of ADHD may be best represented by a dimensional (not categorical) latent structure. Furthermore, Frazier and colleagues did not find evidence for a categorical distinction among the ADHD subtypes. They concluded that this finding may suggest that ADHD subtypes simply represent alternative ways of parsing the inattention and hyperactivity-impulsivity symptom dimensions. Nonetheless, the authors warn that "the

[1] Briefly, "taxometrics" represent one method of latent variable techniques developed by Paul Meehl and his colleagues. Taxometric methods are useful in identifying whether or not a set of indicators relate to one another in a manner that is consistent with the presence of a meaningfully identified type or category (i.e., taxon) (Widiger, 2001).

absence of taxonic findings in a taxometric analysis should not be mistaken for strong evidence of dimensionality," as the results may be better explained by model misspecification or poor validity indicators (Frazier et al., 2007, p. 57). Similarly, Waldman and Lilienfeld (2001) argue that the latent taxa uncovered by taxometric methods initially possess a provisional status and should be subjected to an iterative process of external validation in order to firmly establish the validity of such constructs.

Lubke et al. (2007) acknowledged that these previous approaches have suffered from a serious limitation, namely the lack of a statistical framework that is capable of directly testing alternative latent variable models. Hence, the use of a single approach will be limited to the assumptions intrinsic to that specific model and therefore would preclude the possibility of drawing inferences about which model best represents the actual latent structure of ADHD. To address this limitation, Lubke et al. (2007) used a novel statistical approach involving factor mixture modeling (FMM), which simultaneously incorporates aspects of both factor analysis and latent class analysis. The FMM approach represents a promising direction for future research on the validation of ADHD classification, whereby alternative conceptualizations of the ADHD phenotype can be tested concurrently and contrasted against one another. That is, FMM can be utilized to help further tease apart whether symptoms of inattention, hyperactivity, and impulsivity reflect 1) qualitatively distinct subtypes of ADHD, 2) variants along a single continuum of severity, or 3) severity differences within subtypes. Interestingly, the initial results from Lubke and colleagues' FMM analysis of an ADHD rating scale suggested that ADHD is best characterized by two moderately correlated continuous dimensions (inattention and hyperactivity-impulsivity) that vary in severity, rather than clusters of qualitatively distinct subtypes.

There is support for the clinical utility of continuous ratings of inattentive and hyperactive-impulsive symptoms, over and above nominal diagnostic categories, based on recent findings from two longitudinal studies that demonstrated evidence of considerable instability of the *DSM-IV* ADHD subtypes across development (Lahey et al., 2005; Todd et al., 2008). Furthermore, important information about the phenotype may be lost as a consequence of regarding ADHD as an "all-or-nothing" trait, rather than taking into account the severity level of ADHD symptoms (Neuman et al., 2001). Less emphasis on categorical diagnoses in favor of continuous symptom ratings would seem particularly salient for two subgroups of ADHD children: 1) those diagnosed initially with HT, who often shift to CT in later years as academic tasks demand greater attentional resources (Lahey et al., 2005) and 2) those initially diagnosed with CT, who later shift to IT as hyperactive-impulsive symptoms decline with normative developmental maturity (Hart et al., 1995). Importantly, another recently published longitudinal study by Larsson, Lichtenstein, and Larsson (2006) found initial evidence of both persistent cross-subtype and subtype-specific genetic influences, suggesting that a substantial part of the genetic susceptibility underlying ADHD persists over time, despite developmental changes in the overall manifest symptom profile.

As previously mentioned, a second major challenge for researchers stems from the difficulty in identifying specific susceptibility genes for ADHD given that ADHD, like all psychiatric conditions, is a genetically complex disorder (Faraone & Biederman, 1998; Kendler, 2005; Waldman & Gizer, 2006). Additionally, diagnostic or phenotypic heterogeneity (e.g., due to variation in presenting core symptoms, co-occurring disorders, assessment methods) appears to represent a key source of discrepancy across candidate gene studies of ADHD that serves to further complicate molecular genetic research. It is

noteworthy that the majority of molecular genetic studies have only investigated the relation between a gene of interest and the categorical ADHD diagnosis (Kirley et al., 2004). Therefore, the current investigation undertook both subtype analyses and moderation analyses based on the hypothesis that using only a single categorical diagnosis of ADHD when conducting tests of association potentially may mask a significant effect if the candidate gene is associated only with certain aspects of the ADHD phenotype (e.g., a particular diagnostic subtype or comorbid condition).

As predicted, the current findings suggested specificity in the relations of candidate genes with a particular diagnostic subtype or symptom dimension of ADHD. On the one hand, tests of association between ADHD and *DRD4* and *5-HTT* revealed no evidence of significant association between the overall categorical ADHD diagnosis and the putative high-risk alleles of *DRD4* (the 7-repeat allele) or *5-HTT* (the long allele). On the other hand, evidence for significant associations between specific aspects of the ADHD phenotype and *DRD4* and *5-HTT* emerged in the subgroup analyses. For *DRD4*, the subtype analyses revealed significantly greater association with CT and IT (to a lesser extent) than HT, confirming findings from several previous studies suggesting a stronger relation between *DRD4* and the inattentive (rather than hyperactive-impulsive) symptoms of ADHD (Lasky-Su et al., 2008; McCracken et al., 2000; Rowe et al., 1998). For *5-HTT*, the subtype analyses suggested that the long allele of the *5-HTTLPR* is preferentially related to inattentive symptoms, given that the IT showed stronger evidence for association than the CT and HT groups combined. This finding conflicts with earlier studies that reported a specific association between the long allele of *5-HTT* and the CT, as well as hyperactive-impulsive symptoms (e.g., Manor et al., 2001; Seeger et al., 2001).

The major premise of the second paper of this thesis rested on the question of whether there are viable alternative representations of the ADHD phenotype that may serve to identify more genetically homogeneous clusters of children with ADHD in a more effective manner than the current *DSM-IV*-defined diagnostic subtypes. It was hypothesized that methods aimed at reducing heterogeneity in the ADHD phenotype by discriminating among common comorbid conditions would enhance the power to detect associations between genes and ADHD. Based on initial evidence suggesting that some true associations may be obscured if a given candidate gene is associated only with a particular comorbid subgroup (e.g., Caspi et al., 2008; Kim et al., 2008; Kirley et al., 2004), it was further hypothesized that symptoms of co-occurring externalizing and internalizing disorders would moderate the magnitude of associations between ADHD and *DRD4* and *5-HTT*. As predicted, tests of moderation showed that co-occurring symptoms of *ODD/CD* significantly increased the association between *DRD4* and ADHD. This study replicates similar findings that have shown significant association between the *DRD4* 7-repeat allele and ADHD with comorbid ODD/CD, whereas no association was detected between *DRD4* and the overall ADHD sample (Holmes et al., 2002; Kirley et al., 2004).

This replicated finding seems to raise the question of whether this subgroup with both ADHD and comorbid conduct disorder (ADHD + CD) represents a quantitative, continuous co-occurrence or an etiologically distinct subtype (e.g., Faraone et al., 1997). Interestingly, the ICD-10 (World Health Organization, 1992) already classifies children in a separate diagnostic category (i.e., “hyperkinetic conduct disorder”) if they fulfill diagnostic criteria for both hyperkinetic disorder and conduct disorder. There is some clinical support for this

distinction, based on evidence suggesting that ADHD + CD is a more severe condition with a worse outcome than either disorder alone (e.g., Barkley et al., 1990; Jensen et al., 1997; Kuhne et al., 1997).

Family prevalence studies have also suggested ADHD + CD represents a distinct familial type of ADHD (e.g., Faraone et al., 1997, 1991), though a recent study (Rhee, Hewitt, Corley, & Stallings, 2003) demonstrated that such family prevalence analyses are very limited in their ability to discriminate the correct comorbidity model. Specifically, Rhee et al. used simulated datasets to test the validity of three alternative comorbidity models that are most often examined in the literature (i.e., the alternate forms model, the correlated liabilities model, and the three independent disorders model).[2] The results revealed that none of the family prevalence analyses of ADHD and CD published before 2003 proved to be a valid test of the *three independent disorders model*, despite the fact that several studies concluded that this model (i.e., ADHD + CD is etiologically distinct from ADHD or CD occurring alone) is the correct explanation for the comorbidity between ADHD and CD (Faraone et al., 1997, 1991). Thus, Rhee et al.'s results call into question previous investigators' conclusions from family prevalence analyses regarding the etiology of the comorbidity between ADHD and CD.

In contrast to the family prevalence method, Thapar, Harrington, and McGuffin (2001) conducted a population-based twin study using a liability threshold model approach to examine how the ADHD + CD group is genetically related to ADHD. Their results provided support for the notion that ADHD with comorbid CD is a quantitative variant of ADHD that

[2] The *alternate forms model* hypothesizes that comorbidity between two disorders exists because the two disorders are alternative manifestations of the same underlying liability distributions, the *correlated liabilities model* hypothesizes that the liabilities for the two disorders are significantly correlated, and the *three independent disorders model* hypothesizes that the comorbid disorder is a third disorder etiologically separate from either disorder occurring alone (Rhee et al., 2003).

is associated with higher genetic loading and higher clinical severity, rather than a genetically distinct subtype. Considering Thapar et al.'s results along with the current study's finding that the association between *DRD4* and ADHD was strengthened among children with a concurrent elevation in ODD/CD symptoms, it appears that the identification of individuals with higher clinical severity (i.e., the CT or ADHD + ODD/CD) may have enhanced the statistical power to detect a significant association between ADHD and *DRD4*. Thus, the current findings seem consistent with Thapar et al.'s assertion that ADHD with comorbid CD represents a more severe quantitative variant of ADHD with a higher genetic loading.

As hypothesized in the second paper of this thesis, moderation analyses also revealed a significant moderating influence of *Fear* on the association between the *5-HTTLPR* and ADHD such that this association was enhanced for ADHD children with higher symptoms of SAD, specific phobias, and OCD. It is noteworthy, however, that ADHD children with concurrent elevated symptoms of *Fear* were more likely to possess 1 or 2 copies of the *short* variant of the *5-HTTLPR* rather than the putative high risk variant for ADHD (i.e., the *long* variant). Although replication of this finding is needed before drawing firm conclusions, it is possible that ADHD with co-occurring symptoms of anxiety may reflect a somewhat etiologically distinct subgroup of ADHD children as compared with those with predominant co-occurring symptoms of disruptive behavior disorders (e.g., ODD, CD). Additionally, the symptom presentation of this subset of ADHD children with elevated co-occurring symptoms of anxiety (i.e., SAD, specific phobias, and OCD) seems to be linked by the common feature of *fear of physical harm* (Lahey et al., 2004). Therefore, we might expect that this "ADHD, anxious subtype"

would be characterized by a unique clinical profile that includes *lower* levels of impulsivity and disinhibition, combined with relatively higher levels of inattention and distractibility (cf. Jensen et al., 1997; Schatz & Rostain, 2006). Further support for a separate anxious subtype of ADHD stems from several pharmacological studies demonstrating that children diagnosed with ADHD accompanied by anxiety tend to show significantly poorer responses to stimulant medications than other children with ADHD (reviewed in Jensen et al., 1997 and Newcorn et al., 2001).

Alternatively, it is possible that the association between *Fear* and the short allele of the *5-HTTLPR* may be operating independently of children's ADHD diagnostic status. For example, an association between the short allele of the *5-HTTLPR* and fear-related traits may be directly driving this association, without depending on the child's ADHD diagnosis *per se*. In support of this alternative hypothesis, a significant main effect was found for the *Fear* composite, such that the short allele of the *5-HTTLPR* was associated with higher levels of SAD, specific phobias, obsessions, and compulsions. Further support for this hypothesis is provided by several studies that have demonstrated a significant association between the short allele of the *5-HTTLPR* and higher levels of fearfulness and inhibition in children (Battaglia et al., 2005; Fox et al., 2005; Hayden et al., 2007). Furthermore, this alternative interpretation is consistent with the findings from family genetic studies suggesting that ADHD and anxiety disorders are independently transmitted within families (e.g., Biederman et al., 1992; Perrin & Last, 1996).

A comparison of the proposed alternative comorbid conditions versus the *DSM-IV* ADHD diagnostic subtypes revealed that there are merits to both approaches with respect to their ability to distinguish more homogeneous phenotypes within the ADHD diagnosis.

Overall, the results provide modest support for the external validity of the *DSM-IV* ADHD diagnostic subtypes, as well as the proposed comorbid ADHD conditions. The current findings also suggest that no additional benefit is gained when these two approaches are used simultaneously, likely owing to the fact that the majority of variance has been already accounted for by parsing the overall ADHD diagnosis into subtypes.

Given the high rates of comorbidity among children diagnosed with ADHD in both clinic-referred and community-based samples, a better understanding of the role and significance of comorbidity in the taxonomy of ADHD is necessary (Waschbusch, 2002). A number of alternative models have been proposed by several researchers to explain the etiology of comorbidity between two disorders (e.g., Klein & Riso, 1993; Neale & Kendler, 1995; Rutter, 1997). Notably, it is beyond the scope of the current study to either test alternative models of the etiology of the co-occurrence of ADHD and other disorders, or to even exhaustively consider each of the possible causal models. Nevertheless, it is important to acknowledge that this line of research has significant implications for furthering our understanding of the classification, treatment, and etiology of childhood disorders.

First, the ability to discriminate the correct comorbidity model is important for improving the classification of psychiatric disorders, as Rhee, Hewitt, Corley, and Stallings (2003) elegantly demonstrate. There is reason to be concerned that artificial comorbidity may result from the lack of well-validated diagnostic criteria for the disorders (e.g., observed comorbidity between two disorders actually may be the product of an artificial subdivision of one disorder into two disorders). Second, Achenbach (1995) suggests that increased understanding of the causes of comorbidity between two disorders may be key for improving treatment research (e.g., a single treatment may be appropriate if two comorbid disorders are

truly manifestations of the same underlying liability, whereas different treatments may be optimally effective if the etiology of the co-occurrence is distinct from either disorder occurring alone). Third, increased knowledge of the etiological nature of comorbidity between two disorders will help inform the search for specific genetic and environmental factors underlying the two disorders. For example, if a majority of studies reveal that etiological heterogeneity is operating such that the "comorbid disorder" is etiologically distinct from either disorder occurring alone, molecular genetic studies would have increased power for detecting susceptibility genes by examining these groups separately rather than together (Rhee et al., 2003).

Implications, Limitations, and Directions for Future Research

There are several key findings from the current dissertation that have important implications for future research as well as clinical practice. First, the results suggest that within the heterogeneous diagnostic category of ADHD, the CT represents a more severely affected group of children than the IT or the HT, characterized by greater severity of psychopathology including higher rates of comorbidity as well as elevated essential symptoms. Given that children diagnosed with the CT are at increased risk for developing co-occurring externalizing and internalizing disorders, clinicians should be particularly careful when assessing this subgroup of children to ensure that concurrent problems are identified early and treated appropriately. From a research perspective, this finding provides initial evidence suggesting that the identification of a subgroup with greater clinical severity, such as the CT, may increase the statistical power to detect a significant association between ADHD and certain susceptibility genes.

Second, the current findings raise the possibility that some of the negative findings reported for the associations between ADHD and *DRD4* and *5-HTT* may have been due to sample differences such as diagnostic or genetic heterogeneity that resulted in reduced power to detect true associations. Moreover, this thesis demonstrated that when testing a phenotypically heterogeneous condition such as ADHD, undertaking subgroup analyses (e.g., examining diagnostic subtypes) or moderation analyses (e.g., examining the interaction between ADHD and comorbid conditions) can be a useful strategy for enhancing our ability to detect genetic associations that might be otherwise obscured. Although the current results suggest that examining association with more narrowly defined phenotypes can be a productive approach for psychiatric genetic studies (Cadoret et al., 2003), other investigators have advocated the utility of studying the genetics of broad diagnostic constructs that span several *DSM-IV* diagnoses such as externalizing symptom dimensions (e.g., Krueger et al., 2002; Young et al., 2002). Given the rudimentary stage of our knowledge of the association between specific genes and disorders, as well as the likelihood that some genes will be risk factors for several related disorders whereas others will only confer risk on narrower disorder phenotypes, it is ideal to pursue both of these promising approaches simultaneously (Waldman & Gizer, 2006).

Third, the findings from the current investigation appear most consistent with the representation of ADHD as a unitary disorder with subtypes, though the current study cannot conclusively resolve the question of whether the observed ADHD subtype differences more accurately reflect *quantitative* variation in severity or *qualitatively* discrete categories. More specifically, while the current results suggest that CT is the most severely affected group of children included with the overall ADHD diagnosis, this finding nevertheless could be

explained equally well by a qualitative difference model (i.e., subtypes are distinct diagnostic entities) as by a dimensional model in which the ADHD subtypes are expressions of the same underlying symptom dimensions but differ in severity (Waldman & Lilienfeld, 2001). Therefore, additional studies are sorely needed to identify the true latent structure of ADHD, specifically by simultaneously testing alternative conceptualizations of the ADHD phenotype (e.g., Factor Mixture Modeling, Lubke et al., 2007).

Fourth, the current results provide further validational support for the hypothesis that ADHD with comorbid conduct problems represents a more severe quantitative variant of ADHD with a higher genetic loading, rather than a genetically distinct subtype (e.g., Thapar et al., 2001). This study also lends initial validational support for an "ADHD, anxious subtype" as proposed by Jensen et al. (1997), given our finding that ADHD with co-occurring symptoms of anxiety appears to reflect a somewhat etiologically distinct phenotype (i.e., ADHD accompanied by fear/anxiety showed stronger association with the *5-HTTLPR short* allele, rather than the *long* allele that is typically found to confer risk for ADHD). These putative comorbid conditions of ADHD (ADHD + CD and ADHD + Anxiety) warrant further research to examine their external validity, as well as potential clinical utility for inclusion in the *DSM-V*. Future studies should test whether children with these putative comorbid conditions can be distinguished from other children with ADHD on the basis of external characteristics, such as family history of psychopathology, genetic and environmental influences, developmental course, and differential treatment response.

Several limitations should be considered when interpreting the findings reported herein. The first major limitation concerns the generalizability of the results from the

current study to other populations, due to variation across studies in phenotypic measurement (e.g., diagnoses based on parent ratings, teacher ratings, clinical interviews, multiple raters, or multiple assessment instruments), as well as sample type or referral source (i.e., community-based vs. clinic-referred samples). Considering that ADHD diagnoses were based on symptom ratings by parents alone in the current study, the lack of complementary teacher ratings imposes limits on our ability to determine the extent to which functional impairment was present in two or more settings, as per the official *DSM-IV* criteria. Thus, caution should be used in generalizing the results from the current study beyond the methodology described herein (i.e., clinic-referred sample, clinical diagnosis of ADHD based on parents' symptom ratings).

A second important limitation relates to the relatively modest sample size for undertaking analyses of diagnostic subtypes of ADHD. Dividing a diagnostic category (i.e., ADHD) into its constituent subtypes reduces the sample size per group, which likely led to decreased power in detecting differences among the subgroups of ADHD, particularly for the genetic analyses. A third and related limitation pertains to the fact that a dimensional approach was used to quantify symptoms of co-occurring externalizing and internalizing disorders in the second paper, given the low base rate of categorical internalizing disorders, as well as the loss of power for detecting genetic associations that would result from parsing ADHD into subtypes based on comorbid clinical diagnoses. Although genetic and clinical evidence exists to support the validity of using both categorical and dimensional approaches in molecular genetic studies (e.g., Levy, Hay, McStephen, Wood, & Waldman, 1997), it is not clear that the same susceptibility genes influence clinical diagnoses and dimensional traits in the same manner (reviewed in

Thapar et al., 2006). Therefore, caution should be used in generalizing the results from the current study beyond a dimensional representation of ADHD comorbidity. Furthermore, though the dimensional composite variables composed of co-occurring symptoms of externalizing and internalizing disorders (i.e., *ODD/CD*, *Distress*, *Fears*) were created based on findings from empirical studies of the structure of psychopathology (Krueger, 1999; Lahey et al., 2004) and corresponding behavior genetic studies (Kendler et al., 2003), further external validation studies of these composite variables are necessary.

The fourth and final limitation involves the use of only single markers in the two genes of interest, *DRD4* and *5-HTT*. Although there is considerable evidence suggesting associations between these markers and ADHD, single markers nevertheless represent only a small proportion of the known and novel genetic variation in *DRD4* and *5-HTT*. Ideally, future research focused on the examination of the shared genetic susceptibility of ADHD and comorbid disorders will examine multiple markers in both selected candidate genes. A multi-marker approach offers the advantage of effectively capturing the majority of the common genetic variation in a candidate gene and thus has the capacity to effectively yield increased confidence that a failed association for multiple markers truly represents a lack of association with the candidate gene, as well as provide additional power to detect genes that underlie ADHD.

References

Achenbach, T.M. (1995). Diagnosis, assessment, and comorbidity in psychosocial treatment research. *Journal of Abnormal Child Psychology, 23,* 45-65.

Acosta, M.T., Arcos-Burgos, M., Muenke, M., (2004). Attention deficit/hyperactivity disorders (ADHD): Complex phenotype, simple genotype? *Genetics in Medicine, 6*(1), 1-15.

Alsobrook, J. P., 2nd, & Pauls, D. L. (1998). Molecular approaches to child psychopathology. *Human Biology, 70,* 413-432.

American Psychiatric Association. (1968). *Diagnostic and Statistical Manual of Mental Disorders* (2nd ed.). Washington D.C.: American Psychiatric Association.

American Psychiatric Association. (1980). *Diagnostic and Statistical Manual of Mental Disorders* (3rd ed.). Washington D.C.: American Psychiatric Association.

American Psychiatric Association. (1987). *Diagnostic and Statistical Manual of Mental Disorders* (3rd ed., revised). Washington, D.C.: American Psychiatric Association.

American Psychiatric Association. (1994). *Diagnostic and Statistical Manual of Mental Disorders* (4th ed.). Washington, D.C.: American Psychiatric Association.

American Psychiatric Association. (2000). *Diagnostic and Statistical Manual of Mental Disorders* (4th ed., text revision). Washington, D.C.: American Psychiatric Association.

Baeyens, D. Roeyers, H., & Walle, J.V. (2006). Subtypes of attention-deficit / hyperactivity disorder: Distinct or related disorders across measurement levels? *Child Psychology & Human Development, 36,* 403-417.

Barkley, R.A., Fischer, M., Edelbrock, C.S., & Smallish, L. (1990). The adolescent

outcome of hyperactive children diagnosed by research criteria: I. An 8-year prospective follow-up study. *Journal of the American Academy of Child & Adolescent Psychiatry, 29*, 546-557.

Barkley, R., Murphy, K., & Kwasnik, D. (1996). Motor vehicle driving competencies and risks in teens and young adults with attention deficit hyperactivity disorder. *Pediatrics, 98*, 1089-1095.

Battaglia, M., Ogliari, A., Zanoni, A., Citterio, A., Pozzoli, U., Giorda, R., et al. (2005). Influence of the serotonin transporter promoter gene and shyness on children's cerebral responses to facial expressions. *Archives of General Psychiatry, 62*, 85-94.

Baughman, F.A., Jr. (2001). Questioning the treatment for ADHD. *Science, 291*, 595.

Biederman, J., Faraone, S.V., Keenan, K., Benjamin, J., Krifcher, B., Moore, C., et al. (1992). Further evidence for family-genetic risk factors in attention deficit hyperactivity disorder: Patterns of comorbidity in probands and relatives in psychiatrically and pediatrically referred samples. *Archives of General Psychiatry, 49*, 728-738.

Biederman, J., Newcorn, J., & Sprich, S. (1991). Comorbidity of attention deficit hyperactivity disorder with conduct, depressive, anxiety, and other disorders. *American Journal of Psychiatry, 148*, 564-577.

Biederman, J., Wilens, T., Mick, E., Faraone, S.V., Weber, W., Curtis, S., et al. (2003). Is ADHD a risk factor for psychoactive substance use disorders? Findings from a four-year prospective follow-up study. *Focus, 1*, 196-204.

Burns, G.L., Walsh, J.A., Patterson, D.R., Holte, C.S., Sommers-Flannagan, R., &

Parker, C.M. (1997). Internal validity of disruptive behavior disorder symptoms: Implications from parent ratings for a dimensional approach to symptom validity. *Journal of Abnormal Child Psychology, 25*, 307-319.

Cadoret, R.J., Langbehn, D., Caspers, K., Troughton, E.P., Yucuis, R., Sandhu, H.K., et al. (2003). Associations of the serotonin transporter promoter polymorphism with aggressivity, attention deficit, and conduct disorder in an adoptee population. *Comprehensive Psychiatry, 44*(2), 88-101.

Carlson, C.L., & Mann, M. (2002). Sluggish cognitive tempo predicts a different pattern of impairment in the Attention Deficit Hyperactivity Disorder, predominantly inattentive type. *Journal of Clinical Child & Adolescent Psychology, 31*(1), 123-129.

Caspi, A., Langley, K., Milne, B., Moffitt, T.E., O'Donovan, M., Owen, M.J., et al. (2008). A replicated molecular genetic basis for subtyping antisocial behavior in children with attention-deficit/hyperactivity disorder. *Archives of General Psychiatry, 65*(2), 203-210.

Coolidge, F.L., Thede, L.L., & Young, S.E. (2000). Attention deficit hyperactivity disorder with behavioral disorders and executive function deficits: A preliminary investigation. *Developmental Neuropsychology, 17*(3), 273-287.

Dick, D.M., Viken, R.J., Kaprio, J., Pulkkinen, L., & Rose, R.J. (2005). Understanding the covariation among childhood externalizing symptoms: Genetic and environmental influences on conduct disorder, attention deficit hyperactivity disorder, and oppositional defiant disorder symptoms. *Journal of Abnormal Child Psychology, 33*(2), 219-229.

DiScala, C., Lescohier, I., Barthel, M., & Li, G. (1998). Injuries to children with attention deficit hyperactivity disorder. *Pediatrics, 102*, 1415-1421.

Eaves, L.J., Rutter, M., Silberg, J.L., Shillady, L., Maes, H.H., & Pickles, A. (2000). Genetic and environmental causes of covariation in interview assessments of disruptive behavior in child and adolescent twins. *Behavior Genetics, 30*, 321-334.

Faraone, S.V. (2005). The scientific foundation for understanding attention-deficit / hyperactivity disorder as a valid psychiatric disorder. *European Child & Adolescent Psychiatry, 14*, 1-10.

Faraone, S.V. & Biederman, J. (1998). Neurobiology of attention-deficit / hyperactivity disorder. *Biological Psychiatry, 44*, 951-958.

Faraone, S.V., Biederman, J., Chen, W.J., Milberger, S., Warburton, R., & Tsuang, M. (1995). Genetic heterogeneity in attention-deficit hyperactivity disorder (ADHD): Gender, psychiatry comorbidity, and maternal ADHD. *Journal of Abnormal Psychology, 104*(2), 334-345.

Faraone, S.V., Biederman, J., Jetton, J.G., & Tsuang, M.T. (1997). Attention deficit disorder and conduct disorder: Longitudinal evidence for a familial subtype. *Psychological Medicine, 27*, 291-300.

Faraone, S.V., Biederman, J., Keenan, K., & Tsuang, M. (1991). Separation of DSM-III attention deficit disorder and conduct disorder: Evidence from a family-genetic study of American child psychiatric patients. *Psychological Medicine, 21*, 109-121.

Faraone, S.V., Biederman, J., Mennin, D., Gershon, J., & Tsuang, M.T. (1996). A

prospective four-year follow-up study of children at risk for ADHD: Psychiatric, neuropsychological, and psychosocial outcome. *Journal of the American Academy of Child & Adolescent Psychiatry, 35*, 1449-1459.

Faraone, S. V., Doyle, A. E., Mick, E., & Biederman, J. (2001). Meta-analysis of the association between the 7-repeat allele of the dopamine D(4) receptor gene and attention deficit hyperactivity disorder. *American Journal of Psychiatry, 158,* 1052-1057.

Faraone, S.V. & Khan, S.A. (2006). Candidate gene studies of attention-deficit/hyperactivity disorder. *Journal of Clinical Psychiatry, 67*(Suppl 8), 13-20.

Faraone, S., Perlis, R., Doyle, A., Smoller, J., Goralnick, J., Holmgren, M., et al. (2005). Molecular genetics of Attention-Deficit/Hyperactivity Disorder. *Biological Psychiatry, 57,* 1313-1323.

Faraone, S.V., Sergeant, J., Gillberg, C., & Biederman, J. (2003). The worldwide prevalence of ADHD: Is it an American condition? *World Psychiatry, 2*, 104-113.

Foley, H.A., Carlton, C.O., & Howell, R.J. (1996). The relationship of attention deficit hyperactivity disorder and conduct disorder to juvenile delinquency: Legal implications. *Bulletin of the American Academy of Psychiatry & the Law, 24*, 333-345.

Fox, N.A., Nichols, K.E., Henderson, H.A., Rubin, K., Schmidt, L., Hamer, D., et al. (2005). Evidence for a gene-environment interaction in predicting behavioral inhibition in middle childhood. *Psychological Science, 16*, 921-926.

Frazier, T.W., Youngstrom, E.A., & Naugle, R.I. (2007). The structure of attention-deficit / hyperactivity disorder in a clinic-referred sample. *Neuropsychology,*

21(1), 45-64.

Gadow, K.D., Drabick, D.A.G., Loney, J., Sprafkin, J., Salisbury, H., Azizian, A., et al. (2004). Comparison of ADHD symptom subtypes as source-specific syndromes. *Journal of Child Psychology & Psychiatry, 45*(6), 1135-1149.

Gomez, R., Burns, G.L., Walsh, J.A., & Hafetz, N. (2005). A multitrait-multisource confirmatory factor analytic approach to the construct validity of ADHD and ODD rating scales with Malaysian children. *Journal of Abnormal Child Psychology, 33*, 241-254.

Goodman, R., & Stevenson, J. (1989). A twin study of hyperactivity, II: The aetiological role of genes, family relationships and perinatal adversity. *Journal of Child Psychology & Psychiatry, 30,* 691-709.

Greene, R.W., Biederman, J., Faraone, S.V., Monuteaux, M.C., Mick, E., DuPre, E.P., et al. (2001). Social impairment in girls with ADHD: Patterns, gender comparisons, and correlates. *Journal of the American Academy of Child & Adolescent Psychiatry, 40*, 704-710.

Harrington, K.M., & Waldman, I.D. (2008). Differences among the attention-deficit / hyperactivity disorder subtypes in their overlap with internalizing and externalizing disorders: A meta-analytic review. Manuscript in preparation.

Hart, E.L., Lahey, B.B., Loeber, R., Applegate, B., & Frick, P.J. (1995). Developmental change in Attention-Deficit Hyperactivity Disorder in boys: A four-year longitudinal study. *Journal of Abnormal & Child Psychology, 23*, 729-749.

Hartman, C.A., Hox, J., Mellenbergh, G.J., Boyle, M.H., Offord, D.R., Racine, Y., et al. (2001). DSM-IV internal construct validity: When a taxonomy meets data. *Journal of Child*

Psychology & Psychiatry, 42(6), 817-836.

Haslam, N. (1997). Evidence that male sexual orientation is a matter of degree. *Journal of Personality and Social Psychology, 73*, 862-870.

Haslam, N., Williams, B., Prior, M., Haslam, R. Graetz, B., & Sawyer, M. (2006). The latent structure of attention-deficit /hyperactivity disorder: A taxometric analysis. *Australian & New Zealand Journal of Psychiatry, 40*, 639-647.

Hayden, E.P., Dougherty, L.R., Maloney, B., Durbin, C.E., Olino, T.M., Nurnberger, J.I., et al. (2007). Temperamental fearfulness in childhood and the serotonin transporter promoter region polymorphism: A multimethod association study. *Psychiatric Genetics, 17*, 135-142.

Hechtman, L. (1999). Predictors of long-term outcome in children with attention-deficit/ hyperactivity disorder. *Pediatric Clinics of North America, 46*(5), 1039-1052.

Heils, A., Teufel, A., Petri, S., Stober, G., Riederer, P., Bengel, D., et al (1996). Allelic variation of human serotonin transporter gene expression. *Journal of Neurochemistry, 66*, 2621-2624.

Hinshaw, S.P. (2001). Is the inattentive type of ADHD a separate disorder? *Clinical Psychology-Science & Practice, 8*(4), 498-501.

Holmes, J., Payton, A., Barrett, J., Harrington, R., McGuffin, P., Owen, M., et al. (2002). Association of DRD4 in children with ADHD and comorbid conduct problems *American Journal of Metical Genetics Part B (Neuropsychiatric Genetics), 114*, 150-153.

Hudziak, J., Heath, A.C., Madden, P., Reich, W., Bucholz, K., Slutske, W., et al. (1998). The latent class and factor analysis of DSM-IV ADHD in girls. *Journal of the*

American Academy of Child & Adolescent Psychiatry, 37, 848-857.

Ioannidis, J. P., Ntzani, E. E., Trikalinos, T. A., & Contopoulos-Ioannidis, D. G. (2001). Replication validity of genetic association studies. *Nature Genetics., 29*, 306-309.

Jensen, P.S., Hinshaw, S.P., Kraemer, H.C., Lenora, N., Newcorn, J.H., Abikoff, H.B., et al. (2001). ADHD comorbidity findings from the MTA study: Comparing comorbid subgroups. *Journal of the American Academy of Child & Adolescent Psychiatry, 40*(2), 147-158.

Jensen, P.S., Martin, D., & Cantwell, D.P. (1997). Comorbidity in ADHD: Implications for research, practice, and *DSM-IV*. *Journal of the American Academy of Child & Adolescent Psychiatry, 36*(8), 1065-1079.

Kendler, K. S. (2005). "A gene for...": the nature of gene action in psychiatric disorders. *American Journal of Psychiatry, 162*, 1243-1252.

Kendler, K.S., Prescott, C.A., Myers, J., & Neale, M.C. (2003). The structure of genetic and environmental risk factors for common psychiatric and substance use disorders in men and women. *Archives of General Psychiatry, 60*, 929-937.

Kim, J.W., Biederman, J., Arbeitman, L., Fagerness, J., Doyle, A.E., Petty, C., et al. (2007). Investigation of variation in SNAP-25 and ADHD and relationship to comorbid major depressive disorder. *American Journal of Medical Genetics (Neuropsychiatric Genetics), 144B*, 781-790.

Kirley, A., Lowe, N., Mullins, C., McCarron, M., Daly, G., Waldman, I., et al. (2004). Phenotype studies of the *DRD4* gene polymorphisms in ADHD: association with oppositional defiant disorder and positive family history. *American Journal of Medical Genetics Part B (Neuropsychiatric Genetics), 131B*, 38-42.

Klein, D.N. & Riso, LP. (1993). Psychiatric disorders: Problems of boundaries and comorbidity. In C.G. Costello (Ed.), *Basic issues in psychopathology* (pp. 19-66). New York: The Guilford Press.

Krueger, R.F. (1999). The structure of common mental disorders. *Archives of General Psychiatry, 56,* 921-926.

Krueger, R.F., Hicks, B.M., Patrick, C.J., Carlson, S.R., Iacono, W.G., & McGue, M. (2002). Etiologic connections among substance dependence, antisocial behavior, and personality: Modeling the externalizing spectrum. *Journal of Abnormal Psychology, 111,* 411-424.

Kuhne, M., Schachar, R., & Tannock, R. (1997). Impact of comorbid oppositional or conduct problems on attention-deficit hyperactivity disorder. *Journal of the American Academy of Child & Adolescent Psychiatry, 36*(12), 1715-1725.

Lahey, B.B., & Carlson, C.L. (1991). Validity of the diagnostic category of attention deficit disorder without hyperactivity: A review of the literature. *Journal of Learning Disabilities, 24*(2), 110-120.

Lahey, B.B., Carlson, C.L., & Frick, P.J. (1997). Attention-deficit disorder without hyperactivity. In Widiger, T.A., Frances, A.J., Pincus, H.A, et al. (Eds): *DSM-IV source book* (Vol. 3, pp.163-188). Washington, D.C.: American Psychiatric Association.

Lahey, B.B., Pelham, W.E., Loney, J., Lee, S.S., & Willcutt, E. (2005). Instability of the *DSM-IV* subtypes of ADHD from preschool through elementary school. *Archives of General Psychiatry, 62,* 896-902.

Lahey, B.B., Pelham, W.E., Schaughency, E.A., Atkins, M.S., Murphy, H.A., Hynd,

G.W., et al. (1988). Dimensions and types of attention deficit disorder. *Journal of the American Academy of Child and Adolescent Psychiatry, 27*(3), 330-335.

Lahey, B.B., Rathouz, P.J., Van Hulle, C., Urbano, R.C., Krueger, R.F., Applegate, B., et al. (2008). Testing the structural models of DSM-IV symptoms of common forms of child and adolescent psychopathology. *Journal of Abnormal Child Psychology, 36*, 187-206.

Lahey, B.B., Waldman, I.D., Hankin, B.L., Applegate, B., Loft, J.D., & Rick, J. (2004). The structure of child and adolescent psychopathology: Generating new hypotheses. *Journal of Abnormal Psychology, 113*(3), 358-385.

Lahey, B.B. & Willcutt, E. (2002). The validity of attention-deficit/hyperactivity disorder among children and adolescents. In P.S. Jensen & J.R. Cooper (Eds.), *Diagnosis and treatment of attention deficit hyperactivity disorder: An evidence-based approach.* New York: Civic Research Institute, pp. 1-23.

Larsson, H., Lichtenstein, P., & Larsson, J.O. (2006). Genetic contributions to the development of ADHD subtypes from childhood to adolescence. *Journal of the American Academy of Child & Adolescent Psychiatry, 45*(8), 973-981.

Lasky-Su, J., Lange, C., Biederman, J., Tsuang, M., Doyle, A.E., Smoller, J.W., et al. (2008). Family-based association analysis of a statistically derived quantitative trait for ADHD reveals an association in DRD4 with inattentive symptoms in ADHD individuals. *American Journal of Medical Genetics Part B (Neuropsychiatric Genetics), 147B*, 100-106.

Levy, F., Hay, D.A., 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 American Academy of Child & Adolescent Psychiatry, 36,* 737-744.

Loeber, R., Lahey, B.B., & Thomas, C. (1991). Diagnostic conundrum of oppositional defiant disorder and conduct disorder. *Journal of Abnormal Psychology, 100,* 379-390.

Maher, B.S., Marazita, M.L., Ferrell, R.E., Vanyukov, M.M. (2002). Dopamine system genes and attention deficit hyperactivity disorder: A meta-analysis. *Psychiatric Genetics, 12*, 207-215.

Mannuzza, S., Klein, R., Bessler, A., Malloy, P., & LaPadula, M. (1993). Adult outcome of hyperactive boys: Educational achievement, occupational rank, and psychiatric status. *Archives of General Psychiatry, 50*, 565-576.

Manor, I., Eisenberg, J., Tyano, S., Sever, Y., Cohen, H., Ebstein, R. P., et al. (2001). Family-based association study of the serotonin transporter promoter region polymorphism (5-HTTLPR) in attention deficit hyperactivity disorder. *American Journal of Medical Genetics, 105,* 91-95.

McCracken, J.T., Smalley, S.L., McGough, J.J., Crawford, L., Del'Homme, M., Cantor, R.M., et al. (2000). Evidence for linkage of a tandem duplication polymorphism upstream of the dopamine D4 receptor gene (DRD4) with attention deficit hyperactivity disorder (ADHD). *Molecular Psychiatry,*5(5), 531-536.

McCubbin, M. & Cohen, D. (1997). Empirical, ethical, and political perspectives on the use of methylphenidate. *Ethical Human Sciences and Services, 1*, 81-101.

Mick, E. & Faraone, S.V. (2008). Genetics of attention deficit hyperactivity disorder.

Child & Adolescent Psychiatric Clinics of North America, 17, 261-284.

Milich, R., Balentine, A.C., & Lynam, D.R. (2001). ADHD combined type and ADHD predominantly inattentive type are distinct and unrelated disorders. *Clinical Psychology: Science & Practice, 8*(4), 463-506.

Munafo, M.R. & Flint, J. (2004). Meta-analysis of genetic association studies. *Trends in Genetics, 20*, 439-444.

Nada-Raja, S., Langley, J., McGee, R., Williams, S., Begg, D., & Reeder, A. (1997). Inattentive and hyperactive behaviors and driving offenses in adolescence. *Journal of the American Academy of Child & Adolescent Psychiatry, 36*, 515-522.

Nadder, T.S., Rutter, M., Silberg, J.L., Maes, H.H., & Eaves, L.J. (2002). Genetic effects on the variation and covariation of attention-deficit/hyperactivity disorder (ADHD) and oppositional defiant disorder/conduct disorder (ODD/CD) symptomatologies across informant and occasion of measurement. *Psychological Medicine, 32,* 39-53.

Nadder, T.S., Silberg, J.L., Eaves, L.J., Maes, H.H., & Meyer, J.M. (1998). Genetic effects on ADHD symtomatology in 7- to 13-year-old twins: Results from a telephone survey. *Behavior Genetics, 28,* 83-99.

Neale, M.C. & Kendler, K.S. (1995). Models of comorbidity for multifactorial disorders. *American Journal of Human Genetics, 57,* 935-953.

Neuman, R.J., Heath, A., Reich, W., Bucholz, K.K., Madden, P.A.F., Sun, L., et al. (2001). Latent class analysis of ADHD and comorbid symptoms in a population sample of adolescent female twins. *Journal of Child Psychology & Psychiatry, 42*(7), 933-942.

Neuman, R.J., Todd, R.D., Heath, A.C., Reich, W., Hudziak, J.J., Bucholz, K.K., et al., (1999). The evaluation of the ADHD typology in three contrasting samples: A latent class approach. *Journal of the American Academy of Child & Adolescent Psychiatry, 38*, 25-33.

Newcorn, J.H., Halperin, J.M., Jensen, P.S., Abikoff, H.B., Arnold, E., Cantwell, D.P., et al. (2001). Symptom profiles in children with ADHD: Effects of comorbidity and gender. *Journal of the American Academy of Child & Adolescent Psychiatry, 40*(2), 137-146.

Perrin, S. & Last, C.G. (1998). Relationship between ADHD and anxiety in boys: Results from a family study. *Journal of the American Academy of Child & Adolescent Psychiatry,35*(8), 988-996.

Plomin, R., & Crabbe, J. (2000). DNA. *Psychological Bulletin, 126*, 806-828.

Power, T.J., Costigan, T.E., Eiraldi, R.B., & Leff, S.S. (2004). Variations in anxiety and depression as a function of ADHD subtypes defines by DSM-V: Do subtype differences exist or not? *Journal of Abnormal Child Psychology, 32*(1), 27-37.

Rasmussen, E.R., Todd, R.D., Neuman, R.J., Heath, A.C., Reich, W., & Rohde, L.A. (2002). Comparison of male adolescent-report of attention-deficit /hyperactivity disorder (ADHD) symptoms using latent class and principal components analysis. *Journal of Child Psychology & Psychiatry, 43*, 797-805.

Rhee, S.H., Hewitt, J.K., Corley, R.P., & Stallings, M.C. (2003). The validity of analyses testing the etiology of comorbidity between two disorders: A review of family studies. *Journal of Child Psychology & Psychiatry, 44*(4), 612-636.

Robins, E. & Guze, S.B. (1970). Establishment of diagnostic validity in psychiatric

illness: it's application to schizophrenia. *American Journal of Psychiatry, 126,* 983-987.

Rohde, L.A. (2008). Is there a need to reformulate attention deficit hyperactivity disorder in future nosologic classifications? *Child & Adolescent Psychiatric Clinics of North America, 17,* 405-420.

Rohde, L.A., Barbosa, G., Polanczyk, G., Guilherme, E.M., Rasmussen, E.R., Neuman, R.J., et al. (2001). Factor and latent class analysis of DSM-IV ADHD symptoms in a school sample of Brazilian adolescents. *Journal of the American Academy of Child & Adolescent Psychiatry, 40*(6), 711-718.

Rowe, D.C., Stever, C., Giedinghagen, L.N., Gard, J.M., Cleveland, H.H., Terris, S.T., et al. (1998). Dopamine DRD4 receptor polymorphism and attention deficit hyperactivity disorder. *Molecular Psychiatry, 3,* 419-426.

Rutter, M. (1997). Comorbidity: Concepts, claims and choices. *Criminal Behaviour & Mental Health, 7,* 265-285.

Schatz, D.B., & Rostain, A.L. (2006). ADHD with comorbid anxiety: a review of the current literature. *Journal of Attention Disorders, 10*(2), 141-149.

Seeger, G., Schloss, P., & Schmidt, M. H. (2001). Functional polymorphism within the promotor of the serotonin transporter gene is associated with severe hyperkinetic disorders. *Molecular Psychiatry, 6,* 235−238.

Silberg, J.L., Rutter, M., Meyer, J.M., Maes, H.H., Hewitt, J.K., Simonoff, E., et al., (1996). Genetic and environmental influences n the covariation between hyperactivity and conduct disturbance in juvenile twins. *Journal of Child Psychology & Psychiatry, 37,* 803-816.

Smalley, S.L., McGough, J.J., Del'Homme, M., NewDelman, J., Gordon, E., Kim, T., et al. (2000). Familial clustering of symptoms and disruptive behaviors in multiplex families with attention-deficit/hyperactivity disorder. *Journal of the American Academy of Child & Adolescent Psychiatry, 39*(9), 1135-1143.

Speer, M.C. (1998). Basic concepts in genetics. In J.L. Haines & M.A. Pericak-Vance (Eds.), *Approaches to gene mapping in complex human diseases* (pp. 309-330). New York: Wiley-Liss.

Thapar, A., Harrington, R., & McGuffin, P. (2001). Examining the comorbidity of ADHD-related behaviors and conduct problems using a twin study design. *British Journal of Psychiatry, 179*, 224-229.

Thapar, A., Langley, K., O'Donovan, M., & Owen, M. (2006). Refining the attention deficit hyperactivity disorder for molecular genetic studies. *Molecular Psychiatry, 11*, 714-720.

Todd, R.D. (2000). Genetics of attention deficit / hyperactivity disorder: Are we ready for molecular genetic studies? *American Journal of Medical Genetics, 96*, 241-243.

Todd, R.D., Huang, H., Todorov, A.A., Neuman, R.J., Reiersen, A.M., Henderson, C.A., et al. (2008). Predictors of stability of attention-deficit/hyperactivity disorder subtypes from childhood to young adulthood. *Journal of the American Academy of Child & Adolescent Psychiatry, 47*(1), 76-85.

Waldman, I. D., & Gizer, I. R. (2006). The genetics of Attention Deficit Hyperactivity Disorder. *Clinical Psychology Review, 26*, 396-432.

Waldman, I.D., & Lilienfeld, S.O. (2001). Applications of taxometric methods to problems of comorbidity: Perspectives and challenges. *Clinical Psychology:*

Science & Practice, 8, 520-527.

Waldman, I. D., & Rhee, S. H. (2002). Behavioral and molecular genetic studies. In S. Sandberg (Ed.), *Hyperactivity and attention disorders of childhood, 2nd Ed.* (pp. 290-335). New York: Cambridge University Press.

Waldman, I.D., Rhee, S.H., Levy, F., & Hay, D.A. (2001). Causes of the overlap among symptoms of ADHD, oppositional defiant disorder, and conduct disorder. In F. Levy & D.A. Hay (Eds.), *Attention, genes, and ADHD* (pp. 115-138). New York: Brunner-Routledge.

Waschbusch, D.A. (2002). A meta-analytic examination of comorbid hyperactive-impulsive-attention problems and conduct problems. *Psychological Bulletin, 128*(1), 118-150.

Widiger, T.A. (2001). What can be learned from taxometric analyses? *Clinical Psychology: Science & Practice, 8*, 528-533.

Willcutt, E.G., Pennington, B.F., Chhabildas, N.A., Friedman, M.C., & Alexander, J. (1999). Psychiatry comorbidity associated with DSM-IV ADHD in a nonreferred sample of twins. *Journal of the American Academy of Child & Adolescent Psychiatry, 38*(11), 1355-1362.

Woo, S.C., & Rey, J.M. (2005). The validity of the DSM-IV subtypes of attention-deficit/ hyperactivity disorder. *Australian & New Zealand Journal of Psychiatry, 39*, 344-353.

World Health Organization (1992). *International Classification of Diseases and Related Disorders* (ICD-10). Geneva: WHO.

Young, S.E., Smolen, A., Corley, R.P., Krauter, K.S., DeFries, J.C., Crowley, T.J., et al.

(2002). Dopamine transporter polymorphism associated with externalizing behavior problems in children. *American Journal of Medical Genetics, 114*, 144-149.

Appendix

Alternate Genotyping Protocol for *DRD4* and *5-HTT*

Genotyping of the 5-HTT (aka SLC6A4) promoter polymorphism and the DRD4 VNTR was performed by Jill Platko, Ph.D., using the following protocol at the Psychiatric and Neurodevelopmental Genetics Unit (PNGU) in the Center for Human Genetic Research at Massachusetts General Hospital (MGH) in Boston, MA. Genomic DNA (5 ng) was amplified in a 7-µl reaction using the marker specific primers (0.2 µM), KlenTaq DNA Polymerase (0.2 U), the proprietary KlenTaq Buffer (1X), dNTPs (200 µM each), glycerol (5% for 5-HTT and 10% for DRD4), and Betaine (1 M). The DRD4 VNTR primers were ordered from Applied Biosystems (ABI) and were as follows: DRD4-EX03B-F VIC-GACCGCGACTACGTGGTCTACTC, DRD4-EX03B-R CTCAGGACAGGAACCCACCGAC. The DRD4-EX03B-R primer also contained a proprietary tail that helps stabilize the amplified product. The SLC6A4 promoter primers were ordered from Integrated DNA Technologies (IDT) and were as follows: SLC6A4_PRO-F 6FAM-ATGCCAGCACCTAACCCCTAATGT, SLC6A4_PRO-R GGACCGCAAGGTGGGCGGGA. Amplification was performed with the following protocol: 13 cycles of (1) denaturation for 30 seconds at 93ºC, (2) annealing for 30 seconds beginning at 61.5ºC for the SLC6A4 marker and 69.5ºC for the DRD4 marker and dropped 0.5º C every cycle and (3) primer extension at 72ºC for 30 seconds, followed by 37 cycles of (1) denaturation for 30 seconds at 93ºC, (2) annealing for 30 seconds at 55º C for the SLC6A4 marker and 63ºC for the DRD4 marker and (3) primer extension at 72ºC for 30 seconds, and finally 72ºC for 1 hour.

Amplified products were pooled and combined with size standard (LIZ-250) before being analyzed on an ABI-3730 (Applied Biosystems). GeneMapper v3.5 software (Applied Biosystems) was used to analyze the raw results from the ABI-3730. A genotype was not considered final until two PNGU personnel had independently checked (and if necessary, corrected) the GeneMapper results and both individuals were in agreement.