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A Family Based Association Study of DRD4, DAT1, and 5HTT and Continuous Traits of Attention-Deficit Hyperactivity Disorder

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Abstract

Despite its high heritability, genetic association studies of attention deficit-hyperactivity disorder (ADHD) have often resulted in somewhat small, inconsistent effects. Refining the ADHD phenotype beyond a dichotomous diagnosis and testing associations with continuous information from the underlying symptom dimensions may result in more consistent genetic findings. This study further examined the association between ADHD and the DRD4, DAT1, and 5HTT genes by testing their association with multivariate phenotypes derived from continuous measures of ADHD symptom severity. DNA was collected in 202 families consisting of at least one ADHD proband and at least one parent or sibling. VNTR polymorphisms of the DRD4 and DAT1 genes were significantly associated with the continuous ADHD phenotype. The association with DRD4 was driven by both inattentive and hyperactive symptoms, while the association with DAT1 was driven primarily by inattentive symptoms. These results use novel methods to build upon important connections between dopamine genes and their final behavioral manifestation as symptoms of ADHD.

Keywords

ADHD; dopamine; FBAT; genetic; serotonin

Introduction

Overview

Attention deficit-hyperactivity disorder (ADHD) is a heterogeneous disorder with a complex, multifactorial etiology (Faraone & Doyle, 2001; Willcutt & Carlson, 2005). Twin studies indicate that ADHD is highly heritable, and molecular genetic studies have identified 7–9 candidate genes that may increase susceptibility to ADHD (Faraone & Khan, 2006; Gizer et al., 2009). However, each of these genes accounts for a relatively small proportion of the total variance in ADHD and the majority of the genetic variance in ADHD remains unexplained.

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Clarifying the multifactorial etiology of a heterogenous disorder such as ADHD has been difficult. One explanation for inconsistent results is the use of a categorical ADHD diagnosis. Most molecular genetic studies have largely focused on the association with a diagnosis of ADHD. These studies have yielded significant associations, albeit with mixed results, with both successful and failed replications for many of the candidate genes of interest. Such mixed findings have called into question whether categorical diagnoses are the most useful phenotypes for genetic analyses of ADHD. Examining genetic associations with behaviors other than a categorical diagnosis of ADHD, including information about underlying symptom dimensions, may result in more consistent molecular genetic findings.

Refining the ADHD phenotype

Categorical vs. continuous symptom measures

Given that the diagnostic cutoff for most psychopathology is likely to represent a clinically imposed threshold on an underlying symptom dimension, many researchers have advocated for the analysis of continuous measures of psychopathology rather than categorical diagnoses (e.g. Plomin et al., 2008). In addition, there is strong evidence to suggest that ADHD may exist on the extreme end of a continuum of behavior and therefore developing quantitative phenotypes using the underlying symptom dimensions may more accurately reflect the heterogeneity in ADHD and is likely to improve power to detect etiological effects (Levy et al., 1997).

Symptom dimensions

Another reason to use continuous instead of categorical measures of ADHD is because it is important to distinguish between the inattentive and hyperactive-impulsive symptom dimensions. Both exploratory and confirmatory factor analyses consistently support the distinction between inattention and hyperactivity-impulsivity symptoms (e.g., DuPaul et al., 1997), and both symptom dimensions are associated with multiple aspects of global, academic, and social impairment (e.g., Lahey et al., 1994). The discriminant validity of the symptom dimensions was demonstrated by multiple regression analyses showing that inattention symptoms were more strongly associated with internalizing symptoms, academic difficulties, and neurocognitive weaknesses, whereas hyperactive-impulsive symptoms more strongly predicted comorbid externalizing behaviors, peer rejection, and accidental physical injuries (Lahey & Willcutt, 2002).

Genetic association and dimensional phenotypes

Based on evidence pointing to the importance of dimensional symptom information, some molecular genetic studies of ADHD have begun to use definitions derived quantitatively from continuous symptom measures (e.g. Park et al., 2005; Waldman et al., 2006; Lasky-Su et al., 2008a). Using a statistically derived and maximally heritable ADHD phenotype that allowed each of the nine inattentive and nine hyperactive symptoms to load separately, Lasky-Su et al. (2008a) reported the genetic association between ADHD and DRD4 variants found in their sample was primarily attributable to inattentive symptoms. However, two hyperactive symptoms (restlessness and excessive fidgeting) were also strongly correlated with DRD4 variants. In a study examining the association between a statistically derived ADHD phenotype and the ADRA2A gene, both inattentive and hyperactive symptoms were significantly associated with the gene, albeit the effects of ADRA2A were stronger for the hyperactive than the inattentive symptom dimensions (Waldman et al., 2006). The current study expands upon this small, but growing body of literature by using Family-Based Association Tests (FBAT) methodology to conduct tests of association using novel phenotypes with 3 important candidate genes in ADHD.

Candidate genes for the proposed study

The candidate polymorphisms examined here (i.e. DAT1, DRD4, and 5HTT) are among the most commonly studied in ADHD and have shown a replicable association with ADHD across studies (Gizer et al, 2009). Table I lists the candidate genes, their putative risk alleles, and the pooled odds ratios of previous association studies of ADHD. Further, the results of a recent meta-analysis suggest that while each of these polymorphisms is significantly associated with ADHD, there also exists significant heterogeneity in the effects found across the different studies, suggesting there are likely to be important moderators influencing the associations (Gizer et al., 2009). This study aims to examine the associations more closely, using methods that allow continuous rather than dichotomous ADHD phenotypes and measure the relative input of the inattentive and hyperactive/impulsive symptoms and symptom dimensions. Below we briefly review the evidence for the candidate genes tested in the current study.

Dopamine DR receptor (DRD4)

The DRD4 gene, located on chromosome 11p15, is particularly relevant to ADHD because D4 receptors are expressed in brain regions that are known to be important for attention (Posner and Peterson, 1990) and inhibition (Rubia et al., 1999), such as the anterior cingulate. Many association studies have evaluated a 48 base-pair VNTR polymorphism in exon III, which codes for the third putative cytoplasmic loop of the receptor (Van Tol et al 1992). The most common allele contains 4-repeats, while two less common alleles contain 2-repeat and 7-repeat alleles. The 7-repeat allele of DRD4 has been shown to be significantly associated with ADHD (see Gizer et al., 2009 for a meta-analysis). As such, much effort has been put into identifying functional differences in the pharmacologic properties of DRD4 polymorphic repeat sequences (e.g. Asghari et al 1994; Jovanovic et al 1999; Van Tol et al 1992); However results have not consistently shown a direct relationship between length of the polymorphism at exon III and changes at the neuropharmacological level. In one study, the variant forms (i.e. the 2-, and 7-repeat alleles) were associated with lower transcription and receptor binding relative to the 4-repeat allele (Paterson et al. 1999). Other studies have demonstrated that all the 2-, 4-, and 7- repeat alleles displayed similar binding profiles (Asghari et al., 1994). Further, no evidence of any quantitative difference in G protein coupling related to the number of DRD4 repeats was observed (Kazmi et al 2000).

Dopamine Transporter (DAT1)

The dopamine transporter (DAT1) gene, located on chromosome 5p15.3, is heavily expressed in the human striatum, where it acts as the primary means of dopamine re-uptake (Sesack et al., 1998). The dopamine transporter is the principal site of action of methylphenidate (MPH), a common stimulant treatment for ADHD. MPH inhibits the transporter, thereby increasing the level of extracellular dopamine (Volkow et al.,1998). The most well-studied polymorphism is a variable nucleotide tandem repeat (VNTR) in the 3' untranslated region (UTR) of the DAT1 gene (Mitchell et al., 2000). Alleles from 3–13 repeats have been described, but alleles with 9 and 10 repeats are the most frequently reported (Mitchell et al., 2000). Since this VNTR is not in the coding region of the gene, it does not affect the protein sequence of the dopamine transporter. However, it is thought to have functional significance with *in vitro* studies indicating altered expression of the transporter as a function of VNTR alleles (Fuke et al., 2001; Miller & Madras, 2002), such that carrying 1 or 2 copies of the 10-repeat allele is associated with increased DAT1 expression. In addition, normal adults who are 10/10 homozygotes have significantly reduced dopamine transporter binding in the striatum relative to those having at least one 9-repeat allele (Jacobsen et al., 2000). In this way, the DAT1 genotype is thought to affect

dopamine levels indirectly by altering translational efficiency and the amount of protein expressed.

Serotonin Transporter (5HTT)

Although the role of DAT1 and DRD4 genes in ADHD has been studied extensively, the role of serotonergic genes is less known. However, there is significant evidence to suggest that genes that affect serotonergic pathways should also be considered as potential candidates in genetic studies in ADHD. Animal studies indicate that frontal cortex dopamine and serotonin play an important role in the modulation of attention and response control (Ruotsalainen et al., 1997; Puumala and Sirvio, 1998). Disruption of the serotonin transporter in mice results in a phenotype characterized by marked hyperactivity (Giros et al., 1996). The effect of psychostimulants on mice with this disruption depends on serotonergic neurotransmission (Gainetdinov et al., 1999; Tanaka et al., 2006). The most studied serotonin transporter polymorphism in ADHD is a 44-bp deletion in the gene's promoter region (5-HTTLPR). The short version of this allele is associated with less transcription and reduced levels of the transporter (Heils et al., 1996; Lesch et al., 1996). Meta-analyses that combined the results from case-control and family-based studies of ADHD result in a significant pooled odds ratio for the long allele (Gizer et al., 2009).

The current study

The current study used a well-characterized sample with rich phenotypic data to test VNTR polymorphisms in three candidate genes (DRD4, DAT1, and 5HTT) for association with novel phenotypes developed from continuous measures of ADHD symptom dimensions. FBAT-GEE, an extension of the traditional transmission disequilibrium test for association (TDT) that allows for multivariate continuous phenotypes, was used to test association with three continuous phenotypes, including total ADHD, inattentive, and hyperactive-impulsive symptoms.

Method

Participants

Sample—Participants completed the procedures described below as part of the Colorado Learning Disabilities Research Center (CLDRC) twin study, an ongoing study of the etiology of learning disabilities, ADHD, and other related disorders (e.g., DeFries et al., 1997; Willcutt et al., 2003). Recruitment and testing procedures have been described previously (i.e. Willcutt et al., 2003; Bidwell et al., 2007). The current sample includes 202 families consisting of at least one ADHD proband and at least one parent, dizygotic twin, or sibling.

Exclusionary criteria—Because the focus of the overall project is on the etiology and correlates of familial ADHD, potential participants with a documented brain injury, significant hearing or visual impairment, or other rare genetic or environmental etiology (e.g., Down syndrome, Fragile X syndrome, or other sex chromosome anomalies) were excluded from the sample. In addition, participants were excluded if their Full Scale IQ score was below 75 on the Wechsler Intelligence Scale for Children, Revised (Wechsler, 1974).

Diagnostic measures and operational definition of ADHD

Assessment of ADHD—The Disruptive Behavior Rating Scale (DBRS; Barkley & Murphy, 1998) was used to obtain parent and teacher ratings of the 18 symptoms of *DSM-IV* ADHD. Each symptom on the DBRS was rated on a four point scale (*never or rarely*,

sometimes, *often*, and *very often*). Items rated as *often* or *very often* were scored as positive symptoms and items rated as *never or rarely* or *sometimes* were scored as negative symptoms, consistent with the procedure used in previous studies of similar rating scales (e.g., Pelham et al. 1992). Results from this sample and others indicate that parent and teacher ratings on the DBRS and other similar scales are internally consistent (α = .92 – .96) and have adequate to high test-retest reliability (r = .59 – .89; e.g., DuPaul et al. 1998; Willcutt et al. 2001). The parent and teacher ratings of each of the 18 ADHD symptoms were coded (*never* or *rarely* = 0, *sometimes* = 1, *often* = 2, *very often* = 3) and combined using a modified "or rule" algorithm, similar to that used in the *DSM-IV* field trials for disruptive behavior disorders (Lahey et al., 1994), by taking the higher of the two ratings. The "or rule" is a highly reliable and frequently used approach for assessing ADHD symptoms and dimensions (e.g. Lahey & Willcutt, 2002).

Procedures

Genetic data were collected as part of a larger study in which participants were administered a variety of cognitive, neuropsychological, and achievement measures across four testing sessions. Full Scale IQ was assessed with the Wechsler Intelligence Scale for Children, Revised (for twins 16 years old and younger; WISC-R; Wechsler, 1974) or the Wechsler Adult Intelligence Scale (twins 17 and 18 years old; WAIS; Wechsler, 1981). Reading achievement was assessed with the Peabody Individual Achievement Test (PIAT; Dunn & Markwardt, 1970). The mean age, socioeconomic status (SES; Hollingshead, 1975), IQ scores, ADHD symptoms, and comorbidity rates of the groups of probands with ADHD and their siblings are reported in Table II.

Genetic Data Acquisition and Genotyping

DNA was obtained from blood samples, buccal swabs, or saliva (Oragene; DNAGenotek, Kanata, Ontario, Canada), and extraction was done using the appropriate Gentra PureGene kit (Qiagen, Valencia, CA) for the blood and buccal samples and with the Oragene procedure for saliva samples.

Genotyping for the DRD4 and 5HTTLPR polymorphisms was done using a touchdown polymerase chain reaction (PCR) procedure. For the 5HTTLPR, the forward primer sequence was 5' - GGC GTT GCC GCT CTG AAT GC - 3' and the reverse primer was 5' – GAG GGA CTG AGC TGG ACA ACC AC – 3', For DRD4, the primers were 5' - AGG ACC CTC ATG GCC TTG - 3' forward and 5' - GCG ACT ACG TGG TCT ACT CG - 3' reverse. All primers were obtained from IDT (Integrated DNA Technologies, Coralville, IA). The reactions contained 2.00 μl of genomic DNA at a concentration of 50 ng/μl along with 0.20 μl of AmpliTaq Gold DNA polymerase at 5U/μl (Applied Biosystems, Foster City, CA), 2 μl each of 10X buffer and DMSO, 1.44 μl of 25 mM MgCl2, 0.36 μl each of 10 mM dATP, dCTP, and dTTP, 0.18 μl each of 10 mM dGTP and 7-deaza-dGTP, 1 μl of each of the primers at 1 μM concentration, and 8.92 μl water. Amplification was done with a 10 minute denaturation at 95oC, followed by 2 cycles of 30 seconds denaturation at 95°, 30 seconds annealing at 65°, and 60 seconds extension at 72°. The first annealing temperature was gradually decreased to 55o by 2 degree intervals for 2 cycles each, ending with 30 cycles at 95° for 30 seconds, 55° for 30 seconds, and 60 seconds at 72°. This was followed by a final extension at 72° for 30 minutes and storage at 4°.

Genotyping for the DAT1 30 bp polymorphism was done by standard PCR using 5'- GCA CAA ATG AGT GTT CGT GCA TGT G - 3' as the forward primer sequence and 5' - AGC AGG AGG GGC TTC CAG GC - 3' for the reverse primer. The reagents and amounts were the same as above except that 0.36 μl of dGTP was used rather than the dGTP/ 7-deaza-dGTP combination and 10.92μl of water was added. The PCR reaction started at 95° for 7

minutes followed by 40 cycles at 94° for 1 minute, 65.6° for 1 minute, and 72° for 1 minute. This was followed by extension at 72° for 30 minutes and storage at 4°.

The alleles were separated by electrophoresis on a 2.5% NuSieve (Lonza Rockland Inc., Rockland, MD) agarose gel with a 100 bp size standard ladder. Bands were visualized by staining with ethidium bromide and imaged with a Fotodyne transillumination and imaging system (Fotodyne Inc., Hartland, WI).

Genotypes were examined for departures from Hardy-Weinberg equilibrium and for any inconsistencies with Mendelian inheritance using the routines incorporated in Pedstats, included in the Merlin distribution, and the GAS program (Young, 1995). The MERLIN error program was also run to detect unusual recombination between closely linked markers (Abecasis et al., 2002). Genotypes that were potentially erroneous were not included in the analyses.

Data Analyses

Allele and Genotype Frequencies

Allele and genotype frequencies were calculated and reported. Table III shows the allele frequencies for polymorphisms of interest in the parents and children. Allele frequencies are consistent with allele frequencies for European and multinational North American populations (Chang et al., 1996, Steffens et al., 2002, Vandenbergh et al., 1992).

Power analysis

Using PBAT software (version 3.61), we conducted power analysis for each candidate polymorphisms using the family configurations in the current sample, .20 as an estimate of minor allele frequency, and .01 as an estimate of heritability. These calculations reflected that the current sample is adequately power to detect effects for DRD4 and DAT (Power = . 89 and .82, respectively) and slightly under powered to detect effects for 5HTTLPR (Power = .72).

Statistical Tests for Genetic Association

Family Based Association Tests (FBAT) is a non-parametric extension of the transmission equilibrium test (TDT), which is robust to population stratification. FBAT used family data to test for an association between a genetic polymorphism and a disease or a quantitative trait. FBAT-GEE is an FBAT test that allows for continuous multivariate phenotypic data and tests multiple phenotypes simultaneously, and thus allows for testing of continuous phenotypes and avoids multiple testing. FBAT-GEE is robust to missing parental genotypes and assumptions about the distribution of the phenotype (Laird & Lange, 2006).

Phenotypes tested in the current study

In order to take advantage of the available multivariate symptom data, the FBAT-GEE test (implemented in PBAT Version 3.5) was used to evaluate association with each candidate gene and the continuous phenotypes based on the DSM-IV ADHD symptoms. All genotypes that included a minimum of 20 families were tested. Our Total ADHD phenotype consisted of a summary score of the 18 individual severity ratings for each of the DSM-IV ADHD symptoms (*never or rarely*=0, *sometimes*= 1, *often*=2, and *very often*=3). The nine inattentive symptoms include: (1) inability to pay attention to details; (2) difficulty with sustained attention in tasks or play activities; (3) listening problems; (4) difficulty following instructions; (5) problems organizing tasks and activities; (6) avoidance or dislike of tasks that require mental effort; (7) tendency to lose things like toys, notebooks, or homework; (8) distractibility; and (9) forgetfulness in daily activities. Hyperactive-impulsivity symptoms

include: (1) fidgeting or squirming; (2) difficulty remaining seated; (3) restlessness; (4) difficulty playing quietly; (5) always seeming to be ‘‘on the go;’’ (6) excessive talking; (7) blurting out answers before hearing the full question; (8) difficulty waiting for a turn or in line; and (9) problems with interrupting or intruding.

In addition, since both theory and factor analysis suggest that the hyperactive-impulsive and inattentive symptom dimensions of ADHD are distinguishable but overlapping constructs (Lahey & Willcutt, 2002), we also examined phenotypic information from the symptom dimensions separately. If a significant association was found with the candidate polymorphism and the Total ADHD symptom phenotype, the FBAT-GEE statistic was evaluated for the 9 inattentive and 9 hyperactive-impulsive symptoms separately. The Inattention phenotype included a summary score of the severity ratings from each of the 9 inattentive symptoms and the Hyperactive-Impulsive phenotype included a summary score of the severity scores from each of the 9 Hyperactive-Impulsive symptoms.

Results

Table IV shows the results for each of the phenotypes tested. In addition to the empirical p values for the tests, this table shows the number of informative families, the range of the variance explained by the individual variables in the phenotype, and the significance of the aggregated multivariate phenotype.

FBAT-GEE Analyses

1. Association with the common ADHD phenotype

   When testing the Total ADHD phenotype, there was a statistically significant association with the DRD4 gene, with the 4-repeat allele significantly associated (p=.007) with higher ADHD symptom scores. A significant association was also found with the DAT1 gene with the 10-repeat allele significantly associated (p=.02) with higher symptoms. When testing for association with 5HTT, no significant associations were found.

2. Association with symptom dimensions

   When a significant association was found for the Total ADHD phenotype, the hyperactive-impulsive and inattentive symptom dimensions were evaluated by rerunning the FBAT-GEE analyses for inattentive and hyperactive-impulsive symptoms separately. Results for these tests are also listed in Table IV. Significant associations were found with the 4-repeat allele of the DRD4 gene and both the inattentive and hyperactive-impulsive symptom dimensions (p= .007 and .003, respectively). With regard to DAT1, the 10-repeat (p= .005) allele showed significant association for the inattentive symptoms, but not for the hyperactive-impulsive symptoms.

Finally, because the inattention and hyperactive-impulsive symptoms are separable but related, we followed up the above findings with a post-hoc test examining whether significant associations remained when testing association with the independent variance accounted for by given a symptom dimension. Due to the high correlation between inattentive and hyperactive-impulsive symptoms (r=.69 in this sample), this is a very stringent test for which the current sample is likely underpowered. However, examination of association with the unique variance in the symptom dimensions may further clarify any potential specificity of association between the symptom dimensions and the candidate genes. We used SPSS (Version 17.0) to calculate the unique variance accounted for by the inattentive and hyperactive-impulsive symptom dimensions individually. In separate tests, we then tested the association in FBAT with: DRD4 and the unique variance associated with

1) hyperactivity-impulsivity and 2) inattention; and 3) DAT1 and the unique variance associated with inattention. None of these tests were significant.

Discussion

Using novel analytic methods, our results add to an extensive body of literature suggesting an association between continuous ADHD symptoms ratings and two theoretically plausible candidate genes. Using an extension of the traditional TDT test, two polymorphic regions of DRD4 and DAT1 were found to be significantly associated with a continuous phenotype combining information from each of the 18 ADHD symptoms. The DRD4 exon 3 VNTR polymorphism was significantly associated with ADHD and this association was driven by both hyperactive-impulsive symptoms and inattentive symptoms. The association with ADHD and the VNTR polymorphism at the 3' untranslated region of the DAT1 gene was also significant and was primarily explained by the inattentive symptom dimension. A significant association was not found in the current study when examining the 44-bp deletion polymorphism region of the 5HTT gene.

DRD4

In the current study, the DRD4 VNTR 4-repeat allele was associated with increased ADHD symptomatology. While previous studies have shown an association with the 4-repeat allele and less efficient executive attention in healthy populations (Fossella et al., 2002), it is the 7-repeat allele that has consistently shown association with ADHD (Gizer et al., 2009). It is the case that evidence remains mixed with regard to whether the 7-repeat allele is the true causal variant with functional characteristics (e.g. McCracken, et. al., 2004; D' Souza et al. 2004). However, a more parsimonious explanation for this unexpected result may be that normal sampling variability in the current study resulted in a significant effect in the opposite direction. Thus, our result may be considered a non-replication of previous findings.

DAT1

The current analysis replicated previously found associations with ADHD and 3' untranslated region of the DAT1 gene (Gizer et al., 2009), with the 10-repeat allele conferring greater risk for ADHD symptoms. For DAT1, the results of analysis examining inattentive and hyperactive-impulsive symptom dimensions separately indicate that this association may be stronger for symptoms of inattention than symptoms of hyperactivity-impulsivity.

A previous report examining the association with DRD4 and DAT1 polymorphic regions and phenotypes statistically derived from ADHD symptoms found no association with DAT1 and a significant association with DRD4 that was primarily attributable to symptoms of inattention (Lasky-Su et al., 2008a). While this is not entirely inconsistent with the results reported here, it is worth noting that there are substantial differences between the sample reported on previously by Lasky-Su et al. and the current sample. The sample reported on previously was a highly comorbid, clinical sample that included a mixture of children, adolescents, and adults with ADHD. Age and clinical factors within a given sample are thought to be important moderating factors in genetic association (Gizer et al., 2009; Lasky-Su et al., 2008b) and these important differences between the two samples may be one explanation for the discrepant findings.

5HTT

The current study did not replicate previous associations with ADHD and the 5HTT gene. Serotonergic brain pathways have been linked to ADHD primarily though aggression and

conduct disorder (Halperin et al., 1997; Halperin et al., 2006). While conduct disorder and oppositional defiant disorder are common comorbid conditions associated with ADHD, the role of serotonin systems in ADHD symptoms is not well understood. Future studies that examine its association with ADHD in relation to comorbid oppositional symptoms and conduct problems may be warranted.

Limitations and future directions

Twins with reading disability were also recruited as part of the overall study, but twin pairs with RD alone were not included in the analyses described here. Because probands with ADHD and probands with RD were selected independently (i.e., probands with ADHD were selected without regard to their reading status), this recruitment method does not over-select probands with both ADHD and RD. Nonetheless, it is possible that parents of children with both RD and ADHD might be more likely to agree to participate because the study focuses on both disorders. Therefore, our results warrant replication in other studies that selected a sample for ADHD only, with RD free to vary alone.

Another limitation is the relatively small sample size (202 families). Power analysis indicated that our study may have been underpowered to detect associations with 5HTTLPR. In addition, some studies have suggested that an A->G substitution on the 5HTTLPR gene may alter functionality of the long allele (Hu et al., 2006). The lack of genotype data for this substitution in the current analysis may further have contributed to the lack of significant association with 5HTT. However, our results are not inconsistent with some previous studies that have failed to find a significant association with the 5HTTLPR polymorphism and ADHD (Hieser, et al., 2007; Li et al., 2005; Wigg et al., 2006; Xu et al., 2005). Further, significant heterogeneity has been observed across the effect sizes of associations with this polymorphism and ADHD, indicating that future studies should explore potential moderators (e.g. gender, comorbidities, environmental risk factors) of the association (Gizer et al., 2009).

Associations with dopaminergic genes have also been found in other disorders which are frequently comorbid with ADHD, such as learning disabilities (Hsiung et al., 2004), antisocial behavior (Dolan & Park, 2002) and autism (Anderson et al., 2008). Because ADHD and most other childhood disorders have polygenic, multifactorial etiologies, it is perhaps not surprising that some genetic risk factors would be associated with multiple disorders. Future studies are needed to clarify which genetic risk factors are specific to ADHD, and which are more general risk factors that increase susceptibility to ADHD and other disorders.

Conclusion

Our results add to an extensive body of literature indicating association of ADHD with two polymorphisms in the DAT1 and DRD4 genes. Very few studies have examined the influence of these genetic variants on continuous ADHD symptomatology. These results use novel methods to build upon important connections between dopamine genes and their final behavioral manifestation as symptoms of ADHD. By clarifying the nature of these small effects, candidate gene studies such as this one continue to be important in the process of phenotype refinement and increasing the understanding of the complex etiology of ADHD.

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Table 1

Pooled Odds Ratios in Studies of ADHD for Gene Variants Examined in the Current Study.

Gene	Risk Allele	Number of Studies	Pooled Odds Ratio[1]
Dopamine D4 Receptor (DRD4)	exon III VNTR, 7-repeat	34	1.33
Dopamine Transporter (DAT1)	3' UTR VNTR, 10-repeat	25	1.12
Serotonin Transporter (5HTT)	5-HTTLPR long	19	1.17

[1] Odds Ratio (OR) as calculated in a meta-analysis by Gizer et al., 2009. An OR represents the magnitude of the association between ADHD and the putative risk alleles. An OR of 1.0 indicates no association, those greater than 1.0 indicate that the allele increased risk for ADHD, and those less than 1.0 indicate that allele decreases risk for ADHD.

Table II

Descriptive Characteristics of Participants

	ADHD probands M (SD)	siblings of ADHD probands M (SD)
Demographic Variables		
N	202	93
Sex	168 M, 34 F	34 M, 59 F
Age	10.7 (2.5)	10.9 (2.6)
Socioeconomic status	2.9 (1.2)	3.0 (1.2)
WISC-R		
Full Scale IQ *	103.2 (12.0)	107.8 (13.6)
Verbal IQ *	104.2 (15.4)	108.7 (14.2)
Performance IQ *	101.1 (11.0)	105.1 (12.2)
ADHD symptoms		
Inattention *	7.3 (1.8)	1.2 (1.6)
Hyperactivity – impulsivity *	4.0 (2.8)	.9 (1.6)
Total symptoms *	11.2 (3.2)	2.2 (2.6)
Rate of Comorbidity		
Oppositional Defiant Disorder	17%	--
Conduct Disorder	12%	--
Social Anxiety Disorder	10%	--
Generalized Anxiety Disorder	17%	--
Major Depressive Disorder	5%	--

* Indicates means are significantly different at the $p < .01$ level.

Note. WISC-R = Wechsler Intelligence Scale for Children (Wechsler, 1974).

Table III

Allele Frequencies

Polymorphism	Children	Parents
Dopamine D4 Receptor (DRD4) exon III VNTR		
2-repeat	.07	.07
3-repeat	.05	.05
4-repeat	.67	.65
7-repeat	.20	.21
Dopamine Transporter (DAT1) 3' UTR VNTR		
9-repeat	.24	.24
10-repeat	.75	.76
Serotonin Transporter (5HTT) LPR		
Short	.44	.43
Long	.56	.57

Table IV

Results of FBAT statistics for each candidate polymorphism and the total ADHD and dimensional symptom phenotypes

Polymorphism	Informative families	FBAT-GEE					
		Total ADHD		Inattentive		Hyperactive/Impulsive	
		P-value	Variance Explained	P-value	Variance Explained	P-value	Variance Explained
DRD4 exon III VNTR							
4-repeat	160	**.007**$^{+}$	**.01**	**.007**$^{+}$	**.01**	**.003**$^{+}$	**.01**
7-repeat	122	**.05**$^{-}$	**.01**	.21	.01	.08	.01
DAT1 3' UTR VNTR							
9-repeat	139	**.03**$^{-}$	**.00**	**.009** $^{-}$	**.01**	.57	.00
10-repeat	141	**.02**$^{+}$	**.01**	**.005**$^{+}$	**.02**	.47	.00
5HTT LPR							
Short	110	.23	.00	--	--	--	--
Long	110	.23	.00	--	--	--	--

$^{+}$Indicates allele is positively associated with phenotype

$^{-}$Indicates allele is negatively associated with phenotype.