Polymorphisms in Dopamine System Genes DAT1 and DRD4 are Associated with Disinhibitory Psychopathology in Adolescence

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Abstract

The specificity of genetic influence upon childhood disorders of behavioral disinhibition is uncertain. Polymorphisms in dopamine system genes have been implicated in the contribution of risk for attention-deficit/hyperactivity disorder (ADHD), and other externalizing behavior disorders, but the range of behaviors affected by variation in these genes is ambiguous. To address this problem, we examined the relationship between polymorphisms in the dopamine receptor D4 (DRD4) and dopamine transporter (DAT1) genes and the symptoms and diagnoses of ADHD, ADHD subtypes, oppositional defiant disorder (ODD), and conduct disorder (CD) in 2902 individuals from a population-based twin sample. We observed an association between risk alleles in both DAT1 and DRD4 polymorphisms and increased risk for ADHD, but no main effects of variation in these genes upon risk for ODD or CD. Furthermore, the risk contributed by DAT1 and DRD4 to ADHD was not general; risk alleles in each gene were associated with specific patterns of changes in ADHD subtypes and symptom dimensions.

Polymorphisms in dopamine system genes SLC6A4 and DRD4 are associated with disinhibited psychopathology in adolescence

Introduction

Attention-deficit/hyperactivity disorder (ADHD), oppositional defiant disorder (ODD), and conduct disorder (CD) are common psychiatric disorders that typically have onset during childhood or adolescence, are each characterized by symptoms of behavioral disinhibition or externalization, and frequently co-occur (Angold, Costello, & Erkanli, 1999). In addition to their comorbidity with each other, childhood-onset disorders of disinhibited behavior are related to later externalizing problems, such as early onset of substance use (King, Iacono, & McGue, 2004), higher rates of substance abuse and antisocial personality disorder (ASPD) in adulthood (Mannuzza, Klein, Bessler, Malloy, & LaPadula, 1998), and adult criminal activity (Babinski, Hartsough, & Lambert, 1999; Fergusson, Horwood, & Ridder, 2005).

ADHD, ODD, and CD have each been individually shown to be substantially influenced by genetic factors (Faraone et al., 2005; Eaves et al., 1997), but the nature and origin of the comorbidity among them is still not certain. The simultaneous exhibition of ADHD and either CD or ODD may be related to a more severe overall syndrome, with poorer social and life outcomes (Steinhausen et al., 2006) and a psychophysiological presentation distinct from that displayed by individuals with a single diagnosis (Albrecht, Banaschewski, Brandeis, Heinrich, & Rothenberger, 2005). Furthermore, studies examining patterns of comorbidity in the family members of clinically-assessed probands support the idea that individuals with both ADHD and CD might be properly considered to be affected by a disorder nosologically distinct from ADHD alone (Faraone, Biederman, Jetton, & Tsuang, 1997). Although such studies implicate familial factors in

the etiology of the putatively distinct combination of ADHD and CD, due to methodological limitations they cannot explicitly show that genetic factors are involved (Faraone et al., 1997).

Contrary to the implications of family-of-proband studies, multiple studies using population-based twin study methods indicate that common genetic influences are largely responsible for the comorbidity in externalizing psychopathology, with extant but smaller genetic influences unique to different categories of disinhibitory psychopathology (Silberg et al., 1996; Dick, Viken, Kaprio, Pulkkinen, & Rose, 2005). Other studies of twins suggest that shared environmental factors are the most important source of the covariation between externalizing disorders (Burt, McGue, Iacono, Comings, & MacMurray, 2001). A recent twin study explicitly comparing several possible models for explaining the comorbidity between ADHD and CD (Rhee, Willcutt, Hartman, Pennington, & DeFries, 2008) found that the model corresponding to the idea that ADHD with comorbid CD represents an etiologically distinct subtype from ADHD or CD alone did not fit the data as well as alternative models suggesting both shared genetic and shared environmental risk factors between the two disorders.

Uncertainty regarding the etiology of the co-occurrence of childhood disinhibitory psychopathology is also apparent in attempts to account for the behavioral variation observed *within* ADHD. The DSM-IV (DSM-IV-TR; American Psychiatric Association, 2000) provides for the differential diagnosis of ADHD subtypes, made distinct by different patterns of symptomology. A particular diagnosis of ADHD may be of the Inattentive type (IA), the Hyperactive-impulsive (HI) type, or the Combined (C) type, which involves several symptoms of both inattention and hyperactivity-impulsivity.

ADHD subtypes have different prevalence rates, different rates of affectedness by sex, and different developmental courses (Baeyens, Roeyers, & Walle, 2006). ADHD subtypes may also have different rates of co-occurrence with other externalizing disorders, so that subtypes involving a greater overall number of ADHD symptoms and/or a preponderance of hyperactive-impulsive symptoms are particularly frequently concurrent with CD or ODD (Volk, Neuman, & Todd, 2005; Willcutt, Pennington, Chhabildas, Friedman, & Alexander, 1999; LaLonde, Turgay, & Hudson, 1998), and are related to the development of aggressiveness and conduct problems later in life (Thapar, Bree, Fowler, Langley, & Whittinger, 2006). Twin studies implicate both shared and unique genetic influences upon the hyperactive-impulsive and inattentive dimensions of ADHD (Sherman, Iacono, & McGue, 1997; Larsson, Lichtenstein, & Larsson, 2006), but also suggest that shared genetic influences between the two are likely more substantial than unique genetic influences (McLoughlin, Ronald, Kuntsi, Asherson, & Plomin, 2007).

The role of dopamine system genes in disinhibitory psychopathology

Because of dopamine's role in the neurological reward, motor activity, and attention apparatus, variation in dopamine system genes is of particular interest with respect to behavioral pathology related to attentional deficit, impulsivity, addiction, and other manifestations of behavioral disinhibition. Two dopamine genes genes, DAT1 and DRD4, have been the focus of the majority of genetic association studies of ADHD, as well as many studies of related externalizing phenotypes.

The dopamine transporter gene (DAT1)

The dopamine transporter gene (DAT1, locus symbol: SlC6A3) codes for a protein that mediates synaptic dopamine reuptake, regulating synaptic dopamine concentration (Giros & Caron, 1993). A variable number of tandem repeats (VNTR) polymorphism exists in the 3' untranslated region of DAT1, consisting of 3-13 copies of a 40 base-pair sequence. (Vandenbergh et al., 1992; Yang, Chan, Jing, Sham, & Chen 2007). The relationship between DAT1 VNTR variation and dopamine transporter expression is still unclear. Some studies indicate that possession of the 10-repeat (10R) allele is associated with greater DAT1 expression, while others show increased expression in association with the 9-repeat (9R) allele, or no relationship at all between DAT1 VNTR genotype and dopamine transporter expression (Purper-Ouakil et al., 2005).

The rationale for suspecting the involvement of DAT1 variation in ADHD rests largely upon the fact that the dopamine transporter is the target of stimulant drugs like methylphenidate, that are used to treat ADHD (Spencer, Biederman, Wilens, Harding, & O'Donnell). Such drugs may vary in efficacy depending upon DAT1 VNTR genotype (Kooij et al., 2008). Individuals with ADHD also display increased dopamine transporter density compared to those without ADHD (Dougherty et al., 1999).

The results of studies testing for association between the 10 repeat allele of the DAT1 VNTR and ADHD have been mixed, so that even meta-analytic analyses are inconclusive. Two meta-analyses showed no relationship between DAT1 VNTR variation and ADHD, although both demonstrated the existence of heterogeneity between the various samples included in their analyses (Li, Sham, Owen, & He, 2006; Purper-Ouakil et al., 2005). A third meta-analysis revealed a significant, albeit small, relationship

between the DAT1 10R allele and ADHD in transmission disequilibrium test (TDT) based studies, but not in studies that used haplotype-based haplotype relative risk (HHRR), nor case-control designs (Yang et al., 2007).

Some studies have found that the putative connection between DAT1 and ADHD varies between subtypes or across different symptom dimensions related to the disorder. Waldman et al., (1998) observed an association between DAT1 VNTR 10R alleles and more severe hyperactive-impulsive, but not inattentive, symptoms, in between-family analyses; and within-family analyses conducted in the same study showed association between the 10R allele and the combined ADHD subtype, but not the inattentive subtype. Another study found no relationship between the DAT1 VNTR and DSM-IV defined ADHD subtypes, but that the 9R allele, not the 10R allele, was related to a severe combined subtype of ADHD as defined by population-based latent class analysis (Todd et al., 2005).

Possession of the 10R allele of the DAT1 VNTR may also be associated with other measures of externalizing behavior, such as pathological violence and delinquency (Chen et al., 2007; Guo, Roettger, & Shih, 2007), although some research indicates that the relationship between the 10R allele and antisocial behavior in adolescents may be limited to non-aggressive, rule-breaking behaviors (Burt & Mikolajewski, 2008), or may be restricted to individuals who both possess the 10R allele and were raised in a high-risk familial environment (Beaver, Wright, & DeLisi, 2008). Other studies, however, instead implicate the 9R allele in risk for externalizing behaviors in children (Young et al., 2002).

The dopamine D4 receptor gene (DRD4)

The dopamine D4 receptor gene (DRD4), a D2-like receptor located at chromosome 11p15.5 (Oak, Oldenhof, &Van Tol, 2000) notable for its high affinity with the antipsychotic clozapine (Van Tol et al., 1991), has been the subject of many psychiatric association studies. Among brain structures, the D4 receptor is found most abundantly in the prefrontal cortex (Noain et al., 2006), which makes this gene of interest for disorders like ADHD that involve dysregulation in frontal cortical areas (Bush, Valera, & Seidman, 2005).

The dopamine receptor D4 gene (DRD4) contains a highly variable 48-bp variable number tandem repeat (VNTR) in the third exon of the gene, which codes for the putative third cytoplasmic loop (Van Tol et al., 1992) of the D4 receptor protein. The VNTR may be between 2 and 11 repeats in length, but the 4 and 7 repeat (7R) alleles are most common. In vitro research suggests that, compared to the 2 and 4 repeat alleles, the 7 repeat allele is related to reduced inhibition of adenylate cyclase, and, in turn, reduced inhibition of cyclic AMP formation (Asghari, et al., 1995; Jackson & Westlind-Danielsson, 1994).

Many studies have been conducted to explore the potential relationship between 48-bp DRD4 VNTR variation and ADHD, with mixed results. A recent meta-analysis, using data from 33 family-based and case-control studies, found substantiative evidence for increased risk for ADHD associated with the 7R allele of the VNTR (Li, Sham, Owen, & He, 2006), supporting similar findings in previous meta-analyses (Faraone, Doyle, Mick, & Biederman, 2001; Maher, Marazita, Ferrell, & Vankyukov, 2002). DRD4 VNTR genotype has also been observed to be related to structural differences in ADHD-

related cortical areas, although the nature of this relationship is still uncertain (Durston et al., 2005; Shaw et al., 2007).

DRD4 48-bp VNTR variation may also be related to the different subtypes and symptom dimensions of ADHD in a non-uniform manner. Symptoms of inattention have been shown to be more strongly associated with the 7R allele than were hyperactive-impulsive symptoms, in both children with ADHD (Rowe et al., 1998) and in the retrospective self-report of the fathers of children with ADHD (Rowe et al., 2001). Other studies showed no relationship between ADHD subtype symptom dimensions and variation in the DRD4 48-bp VNTR (Todd et al., 2001; Kirley et al., 2004).

Some evidence suggests that DRD4 might be particularly associated with specific patterns of externalizing symptoms, syndromes, or combinations of comorbid externalizing disorders. Holmes et al., (2002) found an association between DRD4 and ADHD with comorbid conduct problems (ODD with at least one CD symptom) in a sample that had previously not shown an association between DRD4 and ADHD alone. Likewise, Kirley et al., (2004) observed a relationship between the DRD4 3' VNTR and ADHD only in conjunction with ODD, not alone. The association between the DRD4 7-repeat allele and ADHD comorbid with other externalizing disorders could either implicate the polymorphism in the etiology of a single broad externalizing factor underlying all such behavior, or it could instead imply that DRD4 is related to greater severity of ADHD (Kirley et al., 2004).

Although substantial evidence now exists implicating DRD4 48-bp VNTR variation in ADHD, either alone or in the presence of additional behavior problems, the independent relationship between this polymorphism and other manifestations of

disinhibited or externalizing behavior is less well-established. Several early studies of the DRD4 48-bp VNTR linked possession of the 7R allele to increased levels of the novelty seeking (NS) personality trait, but meta-analyses of this body of research suggest that no genuine link exists (Kluger, Siegfried, & Ebstein, 2002; Schinka, Letsch, & Crawford, 2002; Munafò, Yalcin, Willis-Owen, & Flint, 2008). Because of the relative paucity of substantiative links between DRD4 and externalizing traits or psychopathology in the absence of ADHD, it seems possible that liability associated with DRD4 48-bp VNTR variation is specific to syndromes that include ADHD (Mick & Faraone, 2008).

Another, less often studied polymorphism near the DRD4 gene may also be associated with variation in externalizing behaviors. A 120-bp tandem duplication polymorphism exists 1.2kb upstream of the DRD4 gene initiation codon (Seaman, Fisher, Chang, & Kidd, 1999). The duplicated, longer allele of this polymorphism results in reduced transcription of the DRD4 gene, relative to the shorter allelic variant (D'Souza et al., 2004).

Variation in the 120-bp repeat DRD4 polymorphism has been inconsistently related to ADHD. Some studies have found an association between the long allele and ADHD (McCracken et al., 2000; Kustanovich et al., 2004), and that the long allele may be related to the inattentive subtype, but not the hyperactive-impulsive or combined subtypes (McCracken et al., 2000). Others have failed to detect any relationship between variation in this polymorphism and ADHD (Todd et al., 2001; Barr et al., 2001; Bhaduri et al., 2006), or have found the short allele, rather than the long allele, to be associated with ADHD (Kereszturi et al., 2007). Investigations examining this polymorphism in relation to externalizing outcomes other than ADHD are rare, but one such study found

no association between the long allele and ADHD, ADHD subtypes, nor with ADHD comorbid with CD or ODD (Kirley et al., 2004).

The present study

Past research has been equivocal about both the nature of the etiology of the covariation between childhood disinhibitory disorders, and the nature of the relationship between polymorphisms in dopamine system genes and externalizing behaviors and psychopathology. The present study is an investigation of, first, whether the possession of putative risk alleles in DRD4 and DAT1 polymorphisms contributes to risk for experiencing the symptoms of, or to being diagnosed as having, individual childhood externalizing disorders; and second, whether this influence contributes to risk for childhood disinhibition in general, or is specific to particular disorders or subtypes.

Method

Sample

The Minnesota Twin Family Study (MTFS) is a population-based longitudinal study attempting to assess all twins, and their parents, born in Minnesota during specified target years, using a broad array of behavioral, demographic, and psychophysiological measures. Our participants were twins who had participated in MTFS assessment. More detailed descriptions of the general aims and methodologies of the MTFS have been provided in previous publications (Iacono, McGue, & Krueger, 2006). The MTFS drew participants from two separate cohorts: one consisting of twins born 1971-1985 and assessed for the first time during the year they turned 17 (the older cohort), and one consisting of twins born 1988-1994 and assessed for the first time during the year they turned 11 (the younger cohort). Adolescent twin participants returned to the MCTFR

every three years for further assessment. The present study makes use of data collected from the first assessment of participants in the older twin cohort, and the first and second assessments of participants in the younger twin cohort. We included data collected from 2902 twin MTFS participants, of which 1852 were monozygotic (MZ) twins, and 1050 were dizygotic (DZ) twins. 1859 participants from the younger sample were included in our analyses (51% female; mean age at intake (SD): 11.79 (.44); mean age at follow-up 1 (SD): 14.89 (.54)). From the older cohort, 1043 participants were included in our analyses (55% female; mean age at intake (SD): 17.48 (.45)). An additional 271 twin participants were recruited by the MTFS following a screening procedure in order to insure that they exhibited elevated levels of externalizing symptoms (Elkins et al., 2009).

Twin zygosity was determined using three separate estimates in combination: parental report on a standard zygosity questionnaire, evaluation of between-twin physical similarity by MTFS staff, and an algorithm that uses ponderal and cephalic indices and fingerprint ridge count. When these three estimates did not concur, serological analysis was performed to confirm accurate zygosity status. All participants were white. Parents of participants below age 18 granted informed consent to their children's participation, while the participants themselves gave written informed assent to participate. Those participants who were 18 or older during assessment also granted their informed consent to participate. Participants included all MCTFR-participating twins from the older and younger cohorts who granted a biological sample for DNA extraction and for whom we were able to successfully determine genotype for at least one of the three dopamine system polymorphisms that we examined.

Measures

Trained interviewers with either a bachelor's or master's degree in psychology administered a series of structured interviews separately and simultaneously to individual twins and their mothers in order to assess the occurrence of DSM-III-R defined mental disorders (3rd edition, revised; DSM-III-R; American Psychiatric Association, 1987). Participants in the older cohort were assessed for DSM-III-R symptoms of ADHD and ODD using the Diagnostic Interview for Children and Adolescents – Revised (DICA-R) (Reich, & Welner, 1988), and for CD using an interview created by the MTFS in order to measure the symptoms of CD and other antisocial behaviors. Participants in the younger cohort were assessed for DSM-III-R symptoms of ADHD, ODD, and CD using the DICA-R. A variant of the DICA-R was also used to obtain maternal reports of ADHD, ODD, and CD in both younger and older cohort twins. In the younger cohort, the presence of a diagnosis at either the intake or the follow-up 1 assessments was taken as positive evidence that an individual had experienced the disorder in question. Likewise, in the younger cohort, we took for analysis the highest number of symptoms for each disorder displayed at a single assessment.

Symptoms were assigned using a combination of mother and child reports, so that the endorsement of a symptom by either informant was taken as evidence of the presence of that symptom (See Iacono, Carlson, Taylor, Elkins, & McGue, 1999 and Burt, McGue, Iacono, Comings, & MacMurray, 2001 for detailed defense of this method). Individuals were classified as having a diagnosis if they had ever probably (defined as one symptom short of meeting full criteria) or definitely met the criteria for a disorder. For diagnoses made using this method, kappa reliabilities were: ADHD (.77), ODD (.71), and CD (.81) (Iacono et al., 1999).

Although participants were assessed using DSM-III-R criteria, we were able to calculate DSM-IV-equivalent dimensional symptom counts and categorical diagnoses of the Inattentive, Hyperactive-Impulsive, and Combined subtypes of ADHD, using items from the DSM-III-R assessment (Elkins, McGue, & Iacono, 2007). Because DSM-IV ADHD criteria requiring onset of disorder-related impairment before the age of seven have been shown to under-indentify genuinely affected individuals (Todd, Huang, & Henderson, 2008), we did not require onset before age seven in order to assign diagnoses of DSM-IV ADHD subtypes.

We also computed composite externalizing diagnosis and symptom count summary variables (EXT DX and EXT SX). EXT DX is a binary-coded variable indicating the diagnosis of at least one of the DSM-III-R disorders we examined (ADHD, CD, ODD), while EXT SX reflects the sum total of the number of symptoms of those three disorders.

Genetic analysis

Samples of either peripheral blood or buccal swabs were taken from participants during the assessment session. We used PCR (Anchordoquy, McGeary, Liu, Krauter, & Smolen, 2003) followed by optical genotyping with fluorescent probes on an Applied Biosystems 3130 genetic analyzer to discriminate between allele variants. We conducted genotyping of the DRD4 48-bp Exon-III VNTR (forward primer sequence 5'-AGGACCCTCATGGCCTTG-3', reverse sequence 5'-GCGACTACGTGGTCTACTCG-3') and DAT1 40-bp VNTR (forward primer sequence 5'-TGTGGTGTAGGGAACGGCCTGAG-3', reverse sequence 5'-CTTCCTGGAGGTCACGCTCAAGG-3') polymorphisms as described by Anchordoquy

et al., (2003). We genotyped for the DRD4 120-bp polymorphism (forward sequence 5'-GTTGTCTGTCTTTTCTCATTGTTTCCATTG-3', reverse sequence 5'-GAAGGAGCAGGCACCGTGAGC-3') using the method of Seaman, Fisher, Chang, and Kidd, (1999). Because of PCR-related technical difficulties, the number of individuals for whom genotypes were available was fewer for the 48-bp DRD4 VNTR than for the DRD4 120-bp polymorphism and the DAT1 VNTR. The genotypic frequencies of the three polymorphsisms did not differ between cohorts, nor between sexes (in all cases, $p > .05$).

We detected a small number of cases in which the called genotypes for a particular marker either differed between the members of a monozygotic twin pair, or between two different DNA samples thought to have been drawn from the same individual. It is likely that most of these cases represent errors in genotyping, and they may be used to estimate the rate of genotyping error for particular genetic markers within the overall group of genotyped individuals in our sample. We estimated the genotyping error rate as half the observed proportion of genotype disagreement among all genotyped MZ twin pairs and duplicate DNA samples. For the DRD4 48-bp VNTR, among all genotyped individuals, genotypes were identical for 221 of 224 MZ pairs, with no duplicate DNA samples having been genotyped, resulting in an estimated rate of genotyping error for this marker of .7%. For the DRD4 120-bp polymorphism, there was a genotype mismatch in 1 out of 41 MZ pairs, and in none of the 87 duplicated DNA samples, resulting in an estimated genotyping error rate of .4%. For the DAT1 VNTR, genotypes mismatched in 3 of 39 MZ pairs, and duplicate DNA sample genotypes mismatched 3 times out of 85, resulting in an estimated genotyping error rate of 2.4%.

Statistical analysis

We used the generalized estimating equations (GEE) - a method capable of accounting for the non-independent observations within twin pairs – as implemented in SPSS version 15 (SPSS, Inc., Chicago, IL) to test for association between DRD4 & DAT1 genotypes, and the diagnoses and symptom counts of ADHD, ODD, CD, and ADHD subtypes, as well as the EXT DX & EXT SX summary variables. For analysis, all symptom count variables, whether of individual disorders or summarizing the symptoms of multiple disorders, were log-transformed to minimize the positive skew of their distributions. Sex, cohort, and genotype were entered as predictors. All genotypes were entered as additive genetic predictors, with values indicating number of risk alleles (0, 1, or 2). Each analysis was run both with and without gene-by-sex interaction terms, and also with and without terms reflecting interaction between polymorphisms. Scale weights were applied to account for the presence of both participants who had and those who had not been screened for externalizing symptomology. Participants were only included in analyses involving each of the three polymorphisms under investigation if they had been successfully genotyped for the analysis-relevant polymorphism. For this reason, the number of participants included in analogous analyses differs between the three polymorphisms.

Results

Table 1 describes the percentage of individuals affected and the mean number of symptoms exhibited by the participants in our study for each disorder, subtype, and summary variable that we examined. Notably, while other studies have often found the hyperactive-impulsive subtype of ADHD occur at a much lower rate than the inattentive

and combined subtypes (Baeyens et al., 2006), in our sample all three subtypes occurred at similar rates. This discrepancy may have arisen because we did not make use of the DSM-IV age-of-onset criterion when assigning subtype diagnoses, or because the MTFS is a community-based, rather than clinical, sample.

Previous research largely implicates the 10R allele of the DAT1 VNTR, the 7R allele of the DRD4 48bp VNTR, and the Long allele of the DRD4 120-bp tandem repeat polymorphism in relation to disinhibited behavior and psychopathology. **Table 2** shows the number of participants who possessed 0, 1, or 2 of the designated risk allele for each polymorphism we examined, as well as the results of tests of Hardy-Weinberg equilibrium for each polymorphism.

We first conducted tests in order to determine whether an association exists in our sample between the possession of these putative risk alleles and the diagnosis of DSM-III-R defined ADHD, ODD, and CD, as well as DSM-IV subtypes of ADHD (Hyperactive, Inattentive, or Combined). We also tested for associations between dopamine gene risk alleles and the possession of at least one DSM-III-R diagnosis (EXT DX). The results of these analyses - as well as the percentage of individuals with each genotype affected by each disorder - appear in **table 3**. Because we studied the relationship of each polymorphism to multiple externalizing phenotypes, we must interpret the result of statistical testing more conservatively. We consider p-values of .01 or less likely to be indicative of a genuine association, with p-values between .01 and .05 of merely nominal interest. We found possession of the DAT1 VNTR 10R putative risk allele to be associated with increased risk for DSM-III-R diagnosis of ADHD, and DSM-IV diagnosis of the hyperactive-impulsive ADHD subtype, but no other categorical

diagnoses. Variation of the DRD4 120-bp tandem duplication polymorphism was not related to any phenotypic outcome in our sample, but the 7R allele of the 48-bp VNTR was marginally related to heightened risk for DSM-IV combined subtype ADHD, as well as DSM-III-R ADHD. We also detected a gene by sex interaction in relation to the influence of the DRD4 48-bp VNTR upon conduct disorder (B(SE) = .79(.35), p = .024), so that the 7R allele was associated with risk for the development of CD only in males. We found no other evidence for gene-sex interactions, and the results of these tests are not shown. We observed no significant interaction ($p > .05$) between the three polymorphisms in relation to the diagnoses of any of the disorders we investigated, and these results are also not shown.

Next, we conducted tests in order to determine whether the possession of dopamine gene risk alleles was associated with an increased number of the symptoms of DSM-III-R defined ADHD, ODD, and CD, or DSM-IV ADHD subtypes. We also tested to see if an association exists between these risk alleles and the total sum of the symptoms of DSM-III-R ADHD, ODD, and CD (EXT SX). The results of these analyses are shown in **table 4** - along with the mean number of the exhibited symptoms of each disorder, split by genotype. 10R risk alleles at the DAT1 VNTR contributed to a marginal increase in both the hyperactive-impulsive and inattentive symptom dimensions - regarded individually - as well as the overall symptoms of DSM-III-R ADHD, and total number of DSM-III-R externalizing disorder symptoms. Individuals who carried the 7R allele of the DRD4 48-bp VNTR exhibited increased levels of symptoms of inattention, but not of any other measures of disinhibitory symptomology. DRD4 120-bp tandem duplication genotype was not related to any observable symptom differences. We found

no evidence for gene-sex interactions between genotypes and symptom counts, nor for gene-gene interactions between the polymorphisms, and the results of these analyses are not shown.

Discussion

Conflicting reports have been put forth regarding whether genetic influence upon childhood disinhibitory psychopathology is largely shared, so that individual disorders are most properly regarded as alternate manifestations of the same underlying etiological foundation (Rhee et al., 2008), or else whether there exist distinct familial (and potentially genetic) influences capable of exerting unique effects sufficient to justify the categorization of phenomenologically distinct syndromes, such as the combination of ADHD with conduct problems, as genuinely etiologically distinct disorders (Faraone, Biederman, & Monuteaux, 2000). Further, past studies of the DAT1 and DRD4 genes have not been able to conclusively determine the specificity of the effects of polymorphisms in these genes upon externalizing behavioral phenotypes, so that it remains uncertain whether the influence of variation at these polymorphisms, if any, is exercised solely upon risk for ADHD (or even isolated ADHD trait dimensions or subtypes), or instead extends to other measures of behavioral disinhibition as well, such as the comorbid diagnosis of ODD or CD with ADHD. Our study addresses both of these questions.

The goal of this study was to determine whether polymorphisms in the dopamine transporter and dopamine receptor D4 genes were related to measures of childhood disinhibitory psychopathology, and if so, whether this relationship was universally effected, or specific to certain measures of disinhibition. We found that, in our sample,

the 10R allele of the DAT1 40-bp VNTR, and the 7R allele of the DRD4 48-bp VNTR were associated with detectable differences in only a limited set of externalizing disorder diagnoses and symptoms, and that these effects varied between the two genes.

ADHD is the phenotype most frequently studied in relation to variation in both DAT1 and DRD4 polymorphisms. In finding an at least marginal association between risk for ADHD diagnosis, increased ADHD symptom count, and both the 7R allele of the DRD4 48-bp VNTR and the 10R allele of the DAT1 40-bp VNTR, we replicated the findings of several previous studies (Li et al., 2006; Yang et al., 2007). However, we also uncovered dissimilarities between the influences of the two polymorphisms upon the subtypes and trait dimensions of ADHD. Among DSM-IV ADHD subtypes, the 10R allele of the DAT1 VNTR was associated only with increased risk for diagnosis of the hyperactive-impulsive subtype, echoing the findings of previous research (Waldman et al., 1998); meanwhile, the 7R allele of the DRD4 48-bp VNTR was tentatively associated only with the combined subtype. Among measures of ADHD symptoms, DAT1 risk alleles were marginally related to the increased exhibition of both hyperactive-impulsive and inattentive symptoms, while DRD4 VNTR risk alleles were related only to symptoms of inattention, again similar to the conclusions of earlier studies (Rowe et al., 1998). That both the DAT1 and DRD4 VNTRs display a degree of incongruity in their relationship to subtype diagnoses versus ADHD trait dimension symptom counts, implies that the effects of these polymorphisms upon ADHD-related measures may differ between the normal range and the clinical range of ADHD affectedness – and also attests to the utility of including both diagnostic and dimensional measures of psychopathological presentation.

We observed no main effects of DRD4 or DAT1 variation upon either the diagnoses or the symptoms of ODD and CD, perhaps suggesting that in at least these two often-studied candidate genes, genetic influence may be uniquely exerted upon one manifestation of disinhibited psychopathology – that is, upon the diagnosis and symptomology of ADHD – without necessarily impacting other externalizing phenotypes. The effects of DAT1 and DRD4 polymorphisms, though, were not entirely constrained to ADHD-related phenotypes: individuals with the 10R allele of the DAT1 VNTR exhibited increased levels of EXT SX, the summed total of DSM-III-R ADHD, ODD, and CD.

This study must be interpreted in light of several limitations. First, we examined only a very small number of polymorphisms within the DAT1 and DRD4 genes. These polymorphisms do not account for all genetic variation within DAT1 and DRD4 genes, and may therefore fail to detect additional actually existing effects of variation in these genes upon externalizing psychopathology. Studies including a larger number of polymorphisms may do a better job of detecting such effects; for example, several studies including multiple single nucleotide polymorphisms across the DAT1 gene have found a relationship between DAT1 genetic variation in polymorphisms other than the 40-bp VNTR and ADHD (Li et al., 2006).

Second, we limited our analyses to the diagnoses and symptoms of DSM-III-R and DSM-IV defined childhood externalizing disorders. Mounting evidence suggests that DSM-defined psychopathology may not provide the most valid representation of natural psychopathological constructs, and that multivariate techniques for the identification of latent clusters or dimensions, such as latent class analysis (Todd et al., 2005; Acosta et

al., 2008) or principal components analysis (Dick, 2008) may be capable of more accurately representing the categorical or dimensional presentation of externalizing symptoms.

In our sample, DRD4 48-bp VNTR genotype frequencies were not in Hardy Weinberg equilibrium. Genotyping error is the likely explanation, since previous studies of this polymorphism have observed genotyping error caused by the dropout of minor alleles (Eisenberg et al., 2007), and concordance rates below 100% for genotype calling using multiple DNA samples taken from the same individuals (Hamarman, Fossella, Ulger, Brimacombe, & Dermody, 2004). Some studies have re-genotyped all samples initially called as homozygotes, in order to confirm that allelic dropout did not occur (Dreber et al., 2009), but we have not yet taken such corrective measures.

Finally, we restricted our analyses of the influence of risk alleles in DAT1 and DRD4 polymorphisms upon externalizing psychopathology to additive models of genetic effect, assuming that individuals homozygous for risk alleles would be at greater risk for behavioral disinhibition than individuals heterozygous for risk alleles. This assumption may not always be warranted. For example, recent research suggests the potential for non-additive effects of DAT1 VNTR genotype, so that individuals with the heterozygous 9R/10R genotype may be at higher risk than those homozygous for either allele for the symptoms of disruptive behavior disorders, including both hyperactive-impulsive and inattentive symptoms of ADHD, as well as symptoms of ODD and CD (Lee et al., 2007).

The results of our study imply that the biological effects that follow from genetic variation in DAT1 and DRD4 are sufficiently focused that a relatively specific set of changes in behavioral outcomes may be observed to follow from the possession of

different genotypes at these polymorphisms - presuming a study has adequate power to detect such phenotypic differences. A variety of ADHD-related neuropsychological measures have already been assessed as endophenotypes in relation to DRD4 and DAT1 genotypes (Kebir, Tabbane, Sengupta, & Joober, 2009). The present study reinforces the notion that such endophenotypes may uncover distinct biological correlates underlying the different facets of externalizing behavior.

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Appendix

Table 1. Diagnostic affectedness and symptom count of childhood disinhibited behavior disorders

Diagnosis	Rate (%)	Mean # SX	(SD)
DSM-IV			
HI ADHD subtype	4.9	1.58	1.97
IA ADHD subtype	5.4	1.82	2.2
C ADHD subtype	4.3	--	--
DSM-III-R			
ADHD	8.3	1.96	2.94
ODD	21.4	2.86	2.01
CD	21.8	1.1	1.57
EXT	34.6	5.91	5.12

IA, Inattentive; HI, Hyperactive-impulsive; C, Combined.
EXT, Rate reflects proportion of sample affected by any DSM-III-R externalizing disorder, while Mean # SX reflects total number of the symptoms of DSM-III-R externalizing disorders.

Table 2. Risk allele genotype distribution, and Hardy Weinberg equilibrium statistics

	Number of risk alleles			HWE X^2	HWE p-value
	0	1	2		(1 d.f.)
DAT1 40-bp	167 (6%)	1013 (38%)	1476 (56%)	.15	.70
DRD4 48-bp	827 (67%)	350 (28%)	56 (5%)	5.66	.02
DRD4 120-bp	69 (2%)	750 (27%)	1967 (71%)	.06	.81

Putative risk alleles, by polymorphism: DAT1 40-bp, 10R; DRD4 48-bp, 7R; DRD4 120-bp, Long.

Table 3. Association analyses between dopamine system gene polymorphism risk alleles and the diagnoses of childhood disinhibited behavior disorders

							% affected, by number of risk alleles		
	N	B	SE	Odds Ratio (95% CI)	p		0	1	2
DAT1 40-bp									
HI ADHD subtype[1]	2656	.55	.18	1.74 (1.22, 2.49)	**0.002***		2.4	3.6	6.0
IA ADHD subtype[1]	2656	.09	.17	1.09 (0.78, 1.54)	0.609		7.2	4.5	5.8
C ADHD subtype[1]	2656	.21	.19	1.24 (0.86, 1.79)	0.254		2.4	4.0	4.5
ADHD[2]	2646	.40	.15	1.50 (1.12, 2.01)	**0.007***		5.4	6.5	9.6
ODD[2]	2650	.07	.09	1.07 (0.89, 1.28)	0.465		19.8	21.2	21.9
CD[2]	2647	-.02	.09	0.98 (0.81, 1.17)	0.800		25.1	21.2	21.5
Any EXT[2]	2652	.04	.08	1.04 (0.89, 1.21)	0.662		34.7	34.1	34.6
DRD4 48-bp									
HI ADHD subtype[1]	1233	.03	.26	1.03 (0.61, 1.73)	0.912		5.4	4.3	8.9
IA ADHD subtype[1]	1233	.19	.27	1.21 (0.71, 2.03)	0.486		4.0	5.4	5.4
C ADHD subtype[1]	1233	.62	.25	1.85 (1.13, 3.02)	**0.014#**		3.1	7.1	7.1
ADHD[2]	1229	.40	.19	1.49 (1.02, 2.17)	**0.038#**		6.8	10.9	12.5
ODD[2]	1229	.10	.15	1.11 (0.83, 1.47)	0.485		20.1	20.6	26.8
CD[2]	1226	-.01	.14	0.99 (0.75, 1.31)	0.927		21.7	21.6	25
Any EXT[2]	1230	.08	.12	1.08 (0.85, 1.37)	0.518		33.9	36.9	37.5
DRD4 120-bp									
HI ADHD subtype[1]	2786	-.16	.17	0.85 (0.61, 1.19)	0.346		5.8	5.7	4.7
IA ADHD subtype[1]	2786	-.03	.17	0.97 (0.69, 1.37)	0.868		5.8	5.7	5.4
C ADHD subtype[1]	2786	-.08	.20	0.92 (0.63, 1.36)	0.686		5.8	4.3	4.1
ADHD[2]	2775	-.16	.14	0.86 (0.65, 1.13)	0.275		11.6	9	7.8
ODD[2]	2777	-.19	.10	0.83 (0.68, 1.00)	0.063		29	23.9	20.7
CD[2]	2774	-.06	.11	0.94 (0.76, 1.17)	0.578		26.1	22.1	21.5
Any EXT[2]	2780	-.17	.09	0.84 (0.71, 1.00)	0.062		40.6	37.6	33.5

[1]DSM-IV diagnosis; [2]DSM-III-R diagnosis. IA, Inattentive; HI, Hyperactive-impulsive; C, Combined. #, significant to $p \leq .01$; * significant to $p \leq .05$ but $> .01$.

Table 4. Association analyses between dopamine system gene polymorphism risk alleles and the symptomology of childhood disinhibited behavior disorders

						Symptom count Mean (SD), by number of risk alleles		
	N	B	SE	Odds Ratio (95% CI)	p	0	1	2
DAT1 40-bp								
Hyperactive-impulsive	2656	.05	.02	1.06 (1.00, 1.11)	**0.029#**	1.4 (1.7)	1.5 (1.9)	1.7 (2.0)
Inattentive	2656	.06	.03	1.06 (1.01, 1.12)	**0.019#**	1.7 (2.2)	1.7 (2.1)	1.9 (2.3)
ADHD	2644	.08	.03	1.08 (1.02, 1.15)	**0.013#**	1.7 (2.6)	1.8 (2.8)	2.1 (3.0)
ODD	2647	.03	.02	1.03 (0.99, 1.08)	0.109	2.6 (1.8)	2.8 (2.0)	2.9 (2.1)
CD	2645	.03	.02	1.03 (0.98, 1.07)	0.226	1.1 (1.6)	1.0 (1.5)	1.1 (1.6)
Total EXT	2650	.07	.03	1.07 (1.01, 1.13)	**0.019#**	5.4 (4.8)	5.6 (4.9)	6.1 (5.3)
DRD4 48-bp								
Hyperactive-impulsive	1233	.07	.04	1.08 (0.99, 1.17)	0.079	1.5 (1.9)	1.9 (2.1)	1.9 (2.3)
Inattentive	1233	.11	.04	1.12 (1.03, 1.21)	**0.010***	1.7 (2.0)	2.1 (2.4)	2.2 (2.3)
ADHD	1227	.08	.05	1.09 (0.98, 1.21)	0.129	1.9 (2.9)	2.4 (3.2)	2.3 (3.3)
ODD	1227	.05	.03	1.05 (0.99, 1.12)	0.110	2.8 (2.0)	2.9 (1.9)	3.3 (2.1)
CD	1224	0	.03	1.00 (0.94, 1.07)	0.997	1.1 (1.7)	1.0 (1.3)	1.4 (1.8)
Total EXT	1229	.08	.05	1.08 (0.99, 1.19)	0.087	5.8 (5.3)	6.3 (4.9)	6.9 (6.1)
DRD4 120-bp								
Hyperactive-impulsive	2786	-.04	.03	0.96 (0.91, 1.02)	0.204	1.8 (2.1)	1.7 (2.0)	1.5 (2.0)
Inattentive	2786	-.04	.03	0.96 (0.91, 1.02)	0.198	2.2 (2.4)	1.9 (2.2)	1.8 (2.2)
ADHD	2773	-.04	.04	0.97 (0.90, 1.04)	0.324	2.2 (3.1)	2.0 (3.0)	1.9 (2.9)
ODD	2774	-.02	.03	0.98 (0.93, 1.03)	0.438	3.2 (2.4)	2.9 (2.1)	2.8 (2.0)
CD	2772	-.04	.03	0.96 (0.91, 1.01)	0.116	1.5 (2.3)	1.1 (1.6)	1.1 (1.6)
Total EXT	2778	-.04	.03	0.96 (0.90, 1.03)	0.223	7.0 (6.6)	6.1 (5.2)	5.8 (5.1)

Total EXT indicates the total sum of the symptoms of DSM-III-R ADHD, ODD, and CD. #, significant to $p \leq .01$; * significant to $p \leq .05$ but $> .01$.