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Association and Linkage of the Dopamine Transporter Gene and Attention-Deficit Hyperactivity Disorder in Children: Heterogeneity owing to Diagnostic Subtype and Severity

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Summary

Attention-deficit hyperactivity disorder (ADHD) affects ~3%–5% of children in the United States. In the current psychiatric nomenclature, ADHD comprises three subtypes: inattentive, hyperactive-impulsive, and combined. In this study, we used four analytic strategies to examine the association and linkage of the dopamine transporter gene (DAT1) and ADHD. Our sample included 122 children referred to psychiatric clinics for behavioral and learning problems that included but were not limited to ADHD, as well as their parents and siblings. Within-family analyses of linkage disequilibrium, using the transmission disequilibrium test (TDT), confirmed the 480-bp allele as the high-risk allele. In between-family association analyses, levels of hyperactive-impulsive symptoms but not inattentive symptoms were related to the number of DAT1 high-risk alleles. Siblings discordant for the number of DAT1 high-risk alleles differed markedly in their levels of both hyperactive-impulsive and inattentive symptoms, such that the sibling with the higher number of high-risk alleles had much higher symptom levels. Within-family analyses of linkage disequilibrium, using the TDT, suggested association and linkage of ADHD with DAT1 and that this relation was especially strong with the combined but not the inattentive subtype. The relation of DAT1 to ADHD increased monotonically, from low to medium to high levels of symptom severity. Our results replicate and extend previous findings of the association between the DAT1 gene and childhood ADHD. This represents one of the first replicated relations of a candidate gene and a psychiatric disorder in children.

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Introduction

Attention-deficit hyperactivity disorder (ADHD) is a persistent syndrome that consists of extreme levels of motor activity, restlessness, impulsiveness, and inattentiveness. ADHD affects ~3%–5% of children in the United States, with more boys than girls being diagnosed (American Psychiatric Association 1994). In the current psychiatric nomenclature (American Psychiatric Association 1994), ADHD includes three subtypes—inattentive, hyperactive-impulsive, and combined—based on surpassing symptom thresholds on inattentive and/or hyperactive-impulsive problem dimensions. ADHD is one of the most common disorders in child clinical populations. Children with ADHD are at heightened risk for lower educational attainment, lower income, and underemployment (Mannuzza et al. 1993), as well as for dropping out of school, adult criminality, and substance abuse (Loeber and Dishion 1983; Lilienfeld and Waldman 1990; Mannuzza et al. 1993) by virtue of the frequent overlap between ADHD and oppositional defiant disorder (ODD), conduct disorder (CD), and antisocial behavior (Lilienfeld and Waldman 1990; Biederman et al. 1991). As such, ADHD and associated disorders such as CD not only are disruptive to the lives of affected children and their families; they also create serious social problems because they incur substantial societal costs owing to criminal justice, health care, and employment problems.

Despite the seriousness of ADHD, little is known about its causes. In recent years, however, it has become apparent that genetic influences are an important part of the etiology of ADHD. Family (Biederman et al. 1990) and adoption (Morrison and Stewart 1973) studies have suggested that ADHD is familial and that genetic influences may contribute to its etiology. Recent twin studies in both reading-disabled (Gillis et al. 1992) and general population samples (Silberg et al. 1996), using different assessment methods and different age groups, converge on the conclusion that ADHD is moderately to highly heritable. For example, a recent twin study from the Colorado Reading Project (Willcutt et al. 1995) esti-

mated the heritability of ADHD to be 0.80 in both reading-disabled and control samples, and a recent Australian twin study (Waldman et al. 1994) estimated the heritability of ADHD to be 0.89 in a volunteer sample from the general child population. There was no evidence for shared environmental influences (i.e., environmental characteristics experienced in common by family members that make them similar behaviorally) on ADHD in either study, and the remaining 11%–20% of the ADHD variance was due to nonshared environmental influences (i.e., environmental characteristics experienced uniquely by family members that make them different behaviorally).

Although quantitative genetic studies such as those cited above are helpful in providing a summary estimate of the magnitude of genetic and environmental influences on the liability to ADHD, it is important to move beyond the abstract variance components generated by behavior genetic studies, to examine specific genetic and environmental risk factors. Although one group of investigators obtained results from a segregation analysis that suggested a gene of major effect for ADHD (Faraone et al. 1992), most researchers have suggested that ADHD is polygenic (i.e., that a large number of genes, each of relatively small effect, likely influence children's risk for ADHD). Several molecular genetic studies of ADHD have focused on genes that are involved in dopaminergic function, because of the central role of dopamine in motor activity and reward-seeking behaviors. Following reports of an association between the dopamine D4 receptor gene (DRD4) and the personality trait of novelty-seeking in adults (Benjamin et al. 1996; Ebstein et al. 1996), which is similar to aspects of impulsivity seen in ADHD children, an association of DRD4 and ADHD in children was reported (LaHoste et al. 1996). Association of the dopamine transporter gene (DAT1) and ADHD also has been reported (Cook et al. 1995). Although this association was not replicated in a relatively small, less severely affected sample (LaHoste et al. 1995), it was subsequently replicated by an independent research team (Gill et al. 1997). Association of ADHD and DAT1 is of particular interest, given that the psychostimulant medications that are the most frequent treatments of choice for ADHD (e.g., methylphenidate and dextroamphetamine) exert their pharmacological effects in part by inhibiting the dopamine transporter and thus keeping a greater quantity of dopamine active in the synaptic cleft for a longer period of time (Amara and Kuhar 1993). Further evidence suggesting the importance of DAT1 for ADHD comes from a knockout-gene study in mice (Giros et al. 1996). In this study, mice that were homozygous for deactivation of the DAT1 allele were five to six times more active and had dopamine remain active in the synaptic cleft 100 times longer than heterozygous and wild-type mice.

In the present study, we sought to replicate the relation between DAT1 and childhood ADHD, as well as to extend it in a number of ways, using four different analytic methods. First, we examined this relation using both between-family and within-family analyses, each having their own distinct advantages. Second, we examined whether the association and linkage of DAT1 and ADHD differed across the DSM-IV ADHD subtypes (American Psychiatric Association 1994), given that they emphasize inattentive and hyperactive-impulsive symptoms to different degrees and that differential association and linkage might indicate specificity of DAT1's behavioral effects. Third, we examined whether the association and linkage of DAT1 and ADHD differed as a function of symptom severity, given that severity has been claimed to be a moderator of the association between dopamine genes and other psychiatric disorders (e.g., the dopamine D2 receptor gene [DRD2] and alcoholism; Neiswanger et al. 1995). Fourth, we conducted analyses to investigate the mode of action of DAT1 with respect to ADHD to examine whether DAT1 acts as a dominant or recessive gene. Results from all four analytic methods clearly suggest association and linkage between DAT1 and ADHD, with some specificity for the hyperactive-impulsive symptoms as compared with the inattentive symptoms of the disorder.

Subjects and Methods

Subjects

The sample consisted of 122 families in which there was a child (i.e., a proband) who had been assessed and/or treated for attention-deficit disorders, related behavioral disorders, and/or learning problems at a specialty clinic or by psychiatrists in private practice. The siblings in 41 of these families also participated in the study, as did available and cooperating parents in all of the families. Data collection in this study was conducted at two research sites: Tucson, Arizona, and Atlanta, Georgia. The study protocol was approved by the institutional review boards of both the University of Arizona and Emory University, and appropriate informed consent was obtained for all subjects. At the Tucson site, boys who had ADHD and/or a related disorder were identified through psychiatrists in private practice. Whenever possible, a brother of the clinic-referred child was also sampled. At the Atlanta site, clinic-referred male and female children (i.e., probands) were sampled through the Center for Learning and Attention Deficit Disorders at the Emory University School of Medicine. At this site, brothers and sisters of the probands were also sampled whenever possible. We designated as probands clinic- or private psychiatrist–referred children who originally brought a family to the attention of our study. Although

Table 1

Descriptive Statistics on Background Characteristics and Behavior Problem Scales for Probands, Siblings, and Twins

	Probands	Siblings	Twins
Age	9.26 (2.75)	9.51 (3.37)	8.53 (2.96)
Sex (% male)	74	79	49
Ethnicity (% white/black/Hispanic)	68/12/4[a]	68/15/2[b]	82/11/1[c]
Hyperactive/impulsive	2.14 (1.10)	1.02 (1.03)	.71 (0.81)
Inattentive	2.32 (.83)	1.12 (1.04)	.82 (.95)
Oppositional defiant disorder	1.93 (1.08)	1.09 (.92)	.92 (.84)
Conduct disorder	.48 (0.50)	.30 (.41)	.13 (.22)
Depression/dysthymia	.61 (0.53)	.28 (.40)	.15 (.30)
N	111–117	37–41	709–756

[a] The remaining 16% of the probands were of mixed ethnicity.
[b] The remaining 15% of the siblings were of mixed ethnicity.
[c] The remaining 6% of the twins were of mixed ethnicity.

probands most often were referred for a disruptive behavior disorder (i.e., ADHD and/or ODD or CD), we did not have access to their original psychiatric diagnoses.

The total sample for analyses contained 117 families of clinic-referred children (by combining the Tucson and Atlanta sites), because probands in 5 of the 122 families were excluded from analyses, owing to missing genotypic and/or phenotypic data. Diagnostic and demographic data on probands and their siblings in the sample are shown in tables 1 and 2. For purposes of comparison, data from a nonreferred twin sample representative of the general Georgia child population are also presented in tables 1 and 2. All but one of the probands met the criteria for an ADHD diagnostic subtype based on our questionnaire (see description below), and the remaining proband met criteria for ODD. The average age of the probands was 9.26 years (SD = 2.75 years), and 74% of the probands were male. About two-thirds (68%) of the probands were of full Caucasian ethnicity, with 12% being of full African American ethnicity, 4% of full Hispanic ethnicity, and the remaining 16% of mixed ethnicity. The proband and sibling samples were very similar in age, sex, and ethnicity.

The selected nature of the sample with respect to ADHD can be seen clearly in tables 1 and 2. The rates of ADHD diagnoses, especially the combined and inattentive subtypes at the medium- and high-severity levels, are much higher in probands than in the siblings or twins. (The one exception to this is the hyperactive-impulsive subtype). Probands were rated higher on hyperactive-impulsive and inattentive symptoms than on ODD, CD, and depression/dysthymia symptoms (the means for each symptom dimension in table 1 represent the mean rating for each symptom on a 0–4 scale, thus permitting the means to be equated across symptom dimensions that differ in their number of component symptoms).

Although tables 1 and 2 illustrate the selected nature of the proband sample for ADHD, they also illustrate the considerable overlap and covariation of ADHD with other disorders. In contrast to a pure ADHD sample, the probands (and to a lesser extent their siblings) had higher numbers of symptoms and rates of diagnoses than the unselected twin sample, not only for ADHD but for ODD, CD, and depression/dysthymia as well. Given this overlap with ODD, CD, and depression, our results are more generalizable than results from a pure ADHD sam-

Table 2

Rates of Disruptive Behavior Disorder Diagnoses for Probands, Siblings, and Twins

Diagnosis	Probands	Siblings	Twins
Any type ADHD:			
Low-severity	.98	.64	.40
Medium-severity	.85	.32	.18
High-severity	.66	.22	.09
Combined-type ADHD:			
Low-severity	.79	.32	.18
Medium-severity	.53	.15	.06
High-severity	.35	.07	.02
Inattentive-type ADHD:			
Low-severity	.16	.22	.13
Medium-severity	.25	.12	.08
High-severity	.23	.10	.05
Hyperactive/impulsive-type ADHD:			
Low-severity	.03	.10	.09
Medium-severity	.07	.05	.04
High-severity	.08	.05	.02
Oppositional defiant disorder:			
Low-severity	.88	.71	.48
Medium-severity	.69	.29	.14
High-severity	.45	.17	.05
Conduct disorder:			
Low-severity	.38	.27	.13
Medium-severity	.22	.12	.02
High-severity	.14	.07	.01
N	111–117	37–41	709–756

NOTE.—Diagnoses of depression/dysthymia are omitted because of their low rate of occurrence.

ple, because ADHD has been shown to overlap substantially with these disorders both in clinically referred and in nonreferred samples (Lilienfeld and Waldman 1990; Biederman et al. 1991).

Procedures

The Emory Diagnostic Rating Scale.—Probands and their siblings were rated by their parents on the Emory Diagnostic Rating Scale, which was developed by one of us (I.D.W.) to assess symptoms of the major DSM-IV (American Psychiatric Association 1994) childhood psychiatric disorders. These include symptoms of the disruptive behavior disorders (i.e., CD, ODD, and the inattention and hyperactivity-impulsivity symptom dimensions of ADHD), as well as symptoms of internalizing disorders (major depression/dysthymia and anxiety disorders, such as generalized anxiety disorder, social phobia, simple phobia, separation anxiety disorder, panic disorder and agoraphobia, obsessive-compulsive disorder, tics and Tourette disorder, and post–traumatic stress disorder). Each symptom of these disorders was translated into a rating-scale item on which children were rated by their parents on a 0–4 scale, with 0 meaning not at all characteristic of their child and 4 meaning very much characteristic of their child. In this study, scores on the hyperactive-impulsive and inattentive symptom dimensions of ADHD were analyzed. The 0–4 scores for each symptom were summed for each of the items making up the hyperactive-impulsive and inattentive symptom dimensions, yielding symptom-scale scores for each proband and sibling. For purposes of comparison, similar symptom-scale scores were created for ODD, CD, and depression/dysthymia.

This measure yields questionnaire-based diagnoses of these disorders in addition to continuous symptom-scale scores. The symptom scales permit quantitative assessments of behavior because they distinguish severity and number of symptoms over a broad range. Continuous trait scales are better for population-association analyses and discordant sib-pair analyses because they use all available information. On the other hand, within-family analyses of linkage disequilibrium such as the TDT require designating children as "affected" versus "unaffected." For this purpose, questionnaire-based diagnoses were derived from cut-off scores on the continuous symptom dimensions. Probands and their siblings were assigned an ADHD subtype diagnosis if they surpassed the standard diagnostic thresholds (i.e., $\geqslant$6 of 9 symptoms) on the inattention and/or hyperactivity-impulsivity symptom dimensions. Children who were above threshold on the first of these symptom dimensions were diagnosed with ADHD-inattentive subtype, children who were above threshold on the second symptom dimension were diagnosed with ADHD–hyperactive-impulsive subtype, and children who were above threshold on both symptom dimensions were diagnosed with ADHD-combined subtype. Scores of 1, 2, or 3 were alternately used to indicate the presence of a symptom in making diagnoses, thus permitting diagnoses to be made at three different levels of symptom severity.

Characteristics of DAT1.—The chromosomal location of DAT1 is 5p15.3. Studies of allele frequencies in different populations (viz., ethnic groups) have revealed a 40-bp repeat at this VNTR with a range of 3–11 repeats, with the 9 and 10 repeats (i.e., 440 bp and 480 bp) being most frequent (Vandenbergh et al. 1992). Allele frequencies are highly similar across Caucasian and Hispanic populations, with the frequency of the 10-repeat allele at ~71% and the 9-repeat allele at ~27% in both groups (Doucette-Stamm et al. 1995). Allele frequencies in an African American population were similar for the 10-repeat allele (72%) but differed for the 9-repeat (17%) and for rarer alleles (12%) (Doucette-Stamm et al. 1995).

Consistent with previous studies, the 10-repeat allele was the most frequent (69%) in our sample, followed by the 9-repeat allele (29%). A number of rare alleles made up the remaining 2% of the alleles present in the clinic-referred children. Genotypic frequencies were consistent with Hardy-Weinberg equilibrium. As in previous studies (Cook et al. 1995), the 10-repeat allele was considered the high-risk allele in analyses, whereas the 9-repeat allele and all other alleles were combined and treated as low-risk alleles. This assumption of high-risk status was tested and confirmed by means of the TDT.

DNA extraction, genotyping, and scoring.—DNA collection, extraction, and amplification of the DAT1 locus were performed by use of previously published procedures (Vandenbergh et al. 1992; Rowe et al. 1998). Buccal cells were collected in 30 ml of 4% sucrose mouthwash swished vigorously in the mouth for 1 min and then were delivered on ice within 48 h to the laboratory. Cells were pelleted at 2,000 *g* for 10 min, the DNA was immediately extracted with a QIAmp Tissue kit (Qiagen) by use of the manufacturer's protocols for crude-cell lysates, and the samples were preserved in TE (10 mM Tris Hcl, 1 mM EDTA).

The DAT1 locus was amplified on an MJ PTC100 thermal cycler (MJ Research) in a two-step protocol with an initial 1 min denaturing step at 94°C, followed by 28 cycles of 10 s at 94°C and 30 s at 74°C and a final extension of 2 min at 72°C using the primers 5-TGT GGT GTA GGG AAC GGC CTG AG-3′ and 5′-CTT CCT GGA GGT CAC GGC TCA AGG-3′ (Vandenbergh et al. 1992).

The 10-μl reaction mixture consisted of 10 mM Tris-HCl (pH 8.3), 25 mM KCl, 3.0 mM $MgCl^2$, 200 μM dNTPs, 0.5 μM of each primer, 250 μg ml^{-1} BSA, 2% (w/v) sucrose (density-increasing agent), 0.1 mM Cresol

Red (gel-loading dye), 50 ng of genomic DNA, and 0.3 units of *Taq* polymerase (Stratagene *Taq*2000, Stratagene) that had been previously incubated with 0.06 μg *Taq* polymerase antibody (Clonetech TaqStart). After amplification, the reaction mixture was electrophoresed on a 2% agarose gel and was subsequently stained in 1X SYBR green (FMC).

Genotypes were determined from pictures of UV-illuminated stained gels by at least two researchers. Ambiguous or unidentifiable results were reamplified and rescored, as were a random sample of 5% of the probands. Samples that continued to amplify poorly were eliminated from the study population.

Analytic Strategies

We examined the association between DAT1 and ADHD using both between-family and within-family analytic methods. The between-family association methods consisted of examination of the relation between the number of high-risk alleles (i.e., 0, 1, or 2) and the levels of ADHD symptoms. Between-family association analytic methods have played a significant role in the detection of associations between candidate genes and disorders (Plomin et al. 1994) and have a number of advantages, which include high statistical power (Nothen et al. 1993; Plomin et al. 1994), ease of communication, and similarity to classical case-control epidemiological methods. Nonetheless, these methods also have a number of disadvantages, the most important being that associations between disorders and candidate genes may be due to either the causal effects of those genes or population heterogeneity. In particular, spurious association may be found because the affected and control populations differ in both the frequency of the disorder and the frequency of the high-risk allele. Given the high degree of population heterogeneity in allele frequencies for certain genes with potential relevance to psychiatric disorders (e.g., the DRD2 gene; Barr and Kidd 1993), this is a probable confounding factor in samples drawn from the general U.S. population. It also is important to realize that any source of population stratification, not simply ethnic heterogeneity, may result in artifactual inferences regarding association (Ewens and Spielman 1995).

Within-family tests of linkage disequilibrium (Spielman et al. 1993; Schaid and Sommer 1994) avoid confounding due to population stratification, because full siblings must belong genetically to the same ethnic/racial group (Schaid and Sommer 1994). One elegant and simple example of a within-family test of linkage disequilibrium, the TDT (Spielman et al. 1993), is based on the detection of unequal transmission of particular alleles by heterozygous parents to affected children. The Mendelian expectation under the null hypothesis of no linkage or association is that either allele carried by a heterozygote has a 50:50 chance of transmission to an affected child. If the allele actually plays a role in the development of the behavioral trait, however, then its transmission should exceed 50%.

The TDT has certain advantages over other within-family association tests, such as the affected family–based control (Thomson 1995) and haplotype-based haplotype relative risk methods (Schaid and Sommer 1994; Spielman and Ewens 1996). These advantages include greater statistical power, robustness against artifacts induced by population stratification, the provision of a test of linkage in the presence of association, and the ability to include in a test of linkage (but not association) multiple affected siblings from a family without having to correct for nonindependence. Given these features, we relied on within-family analyses using the TDT as a primary source of evidence regarding the association and linkage of DAT1 and ADHD. We also analyzed data from two-parent families only, omitting the TDT families with data from only one parent, to avoid biases incurred by inclusion of such families (Curtis and Sham 1995). The conventional TDT is a 1-df McNemar χ^2 test, as follows: $\chi^2_{TDT} = (b - c)^2/(b + c)$, where b is the number of times the high-risk allele was transmitted and c is the number of times the low-risk allele was transmitted.

Results

Between-Family Association of DAT1 and ADHD Symptoms

The continuous scores on both the hyperactive-impulsive and inattentive symptom scales in probands ($n = 117$ with complete data) were regressed on the number of DAT1 high-risk alleles (0, 1, or 2). This analysis examines the association of DAT1 with the two ADHD symptom dimensions in clinic-referred children across the families in the study. As such, any association found may not reflect linkage disequilibrium because the results may be confounded by ethnic differences across these families. As shown in figure 1, the regression of hyperactive-impulsive symptom scores on the number of DAT1 high-risk alleles was linear, whereas the regression of inattentive symptom scores on the number of DAT1 high-risk alleles was somewhat curvilinear. The number of DAT1 high-risk alleles was significantly related to the number of hyperactive-impulsive symptoms ($t = 1.87$, one-tailed $P = .032$) but not to the number of inattentive symptoms ($t = 1.10$, one-tailed $P = .137$). The number of DAT1 high-risk alleles explained 3.6% of the variance in hyperactive-impulsive symptoms and 1.1% of the variance in inattentive symptoms. To examine the effects of ethnic stratification on these results, we controlled for

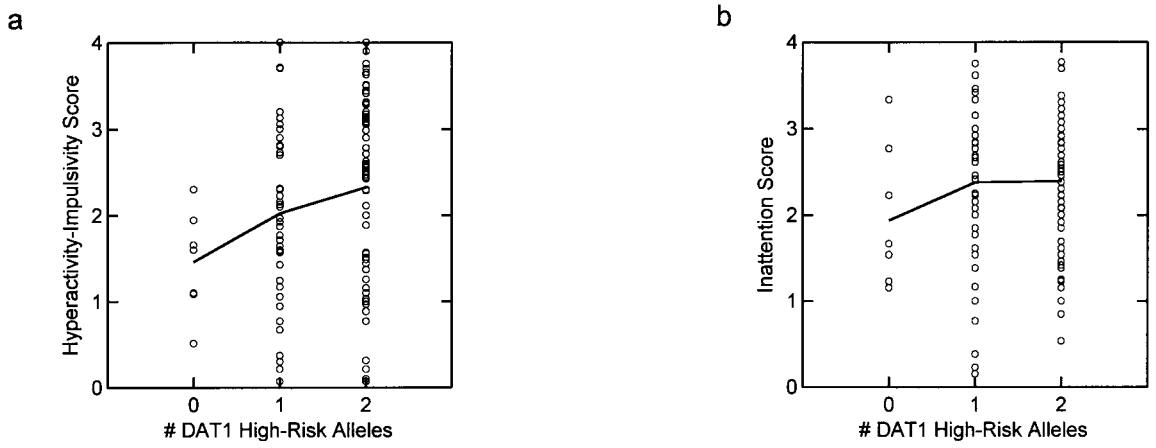

Figure 1 *a*, Hyperactivity-impulsivity symptom scores by the number of DAT1 high-risk alleles. *b*, Inattention symptom scores by the number of DAT1 high-risk alleles.

ethnicity in a subsequent between-family association analysis. We did this by partialling out three variables that represented the percentage of Caucasian, African American, and Hispanic ethnic heritage, by regressing the ADHD symptom scores on them before we examined the effects of the number of DAT1 high-risk alleles. Controlling for ethnicity had virtually no impact on the results: the number of DAT1 high-risk alleles was still significantly related to the number of hyperactive-impulsive symptoms ($t = 1.87$, one-tailed $P = .032$) but not to the number of inattentive symptoms ($t = 1.20$, one-tailed $P = .116$).

Within-Family Analyses of DAT1 and ADHD Symptoms, Using Genetically Discordant Siblings

Probands and their siblings were discordant for the number of DAT1 high-risk alleles in 12 of the 41 sibling pairs. In a second set of analyses, these genetically discordant siblings were contrasted in their levels of hyperactive-impulsive and inattentive symptoms. In 10 of the 12 sibling pairs, the sibling with the greater number of DAT1 high-risk alleles had higher scores on both symptom dimensions than the sibling with fewer DAT1 high-risk alleles (Wilcoxon signed ranks test: $Z = 2.43$, one-tailed $P = .008$ for hyperactive-impulsive symptoms and $Z = 2.27$, one-tailed $P = .011$ for inattentive symptoms). As shown in figure 2, these genetically discordant sibling pairs differed markedly in levels of hyperactive-impulsive symptoms (paired samples $t = 3.42$, df = 11, one-tailed $P = .003$, effect size = .99 SD) and inattentive symptoms (paired samples $t = 2.75$, df = 11, one-tailed $P = .009$, effect size = .79 SD).

Within-Family Analyses of Linkage Disequilibrium between DAT1 and ADHD, Using the TDT

In a third set of analyses, linkage disequilibrium between DAT1 and the diagnostic subtypes of ADHD across differing symptom severity levels was examined by use of the TDT. We first examined linkage disequilibrium between DAT1 and any ADHD diagnosis because this most closely replicated previous association findings (Cook et al. 1995; Gill et al. 1997). As shown in table 3, the results suggested linkage disequilibrium between DAT1 and any ADHD diagnosis at each level of symptom severity, with the degree of linkage disequilibrium becoming stronger as levels of symptom severity increased. Specifically, the TDT for the low level of symptom severity fell just short of significance at the .05 level, whereas the TDTs for medium and high levels of symptom severity both were significant. The odds of transmission of a high-risk versus a low-risk allele increased monotonically with increasing symptom severity level.

A different pattern emerged for each ADHD diagnostic subtype: linkage disequilibrium with DAT1 was found for the combined but not for the inattentive ADHD subtype (as shown in table 2, there were not enough subjects meeting criteria for the hyperactive-impulsive subtype to conduct TDTs). As shown in table 3, linkage disequilibrium with DAT1 was suggested for the combined subtype across all symptom severity levels, given that all three TDTs were statistically significant. Similar to the results for any ADHD diagnosis, the odds of transmission of a high-risk versus a low-risk allele increased monotonically with increasing symptom severity level. Although the TDTs suggest that linkage disequilibrium was strongest at a moderate level of symp-

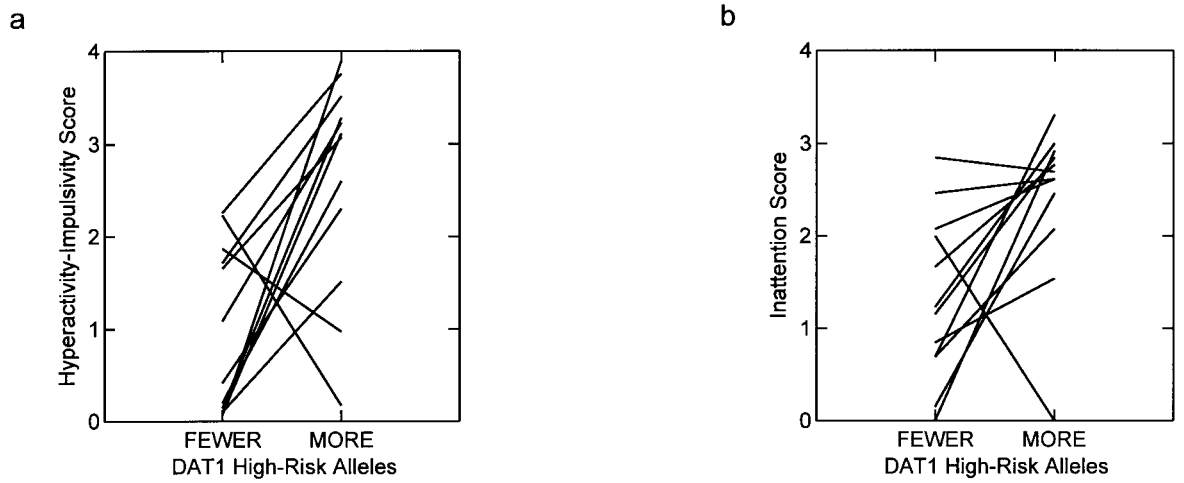

Figure 2 *a,* Hyperactivity-impulsivity symptom scores in siblings discordant for the number of DAT1 high-risk alleles. *b,* Inattention symptom scores in siblings discordant for the number of DAT1 high-risk alleles. Note that siblings in the "MORE" group have one more DAT1 high-risk allele (i.e., 1 or 2) than their cosiblings in the "FEWER" group, who have one fewer DAT1 high-risk allele (i.e., 0 or 1).

tom severity, given the increasing odds ratios with increasing symptom severity, this appears merely to be due to the decreased numbers of allelic transmissions and nontransmissions at the high level of symptom severity. In contrast to the results for the combined subtype, there was no evidence for linkage disequilibrium between DAT1 and the inattentive subtype at any level of symptom severity.

Within-Family Analyses of the Genetic Model for DAT1 and ADHD

In addition to the TDT, we used within-family analyses to examine the genetic model that best characterized the relation between DAT1 and ADHD. We used a set of χ^2 analyses developed specifically to test for recessive or dominant transmission (Schaid and Sommer 1994). As shown in table 3, neither the results for recessive nor for dominant transmission were as strong as the corresponding TDTs. In addition, results of these analyses for any ADHD diagnosis and for the combined subtype were somewhat inconsistent: there was stronger evidence for dominant than for recessive transmission at lower levels of symptom severity but stronger evidence for recessive than for dominant transmission at higher levels of symptom severity. There was no evidence for either recessive or dominant transmission for the inattentive subtype, consistent with the TDT results.

Discussion

Evidence for Linkage Disequilibrium between DAT1 and ADHD

Results of both the between- and within-family analyses furnished evidence for linkage disequilibrium between DAT1 and ADHD. Regression analyses suggested that levels of the hyperactive-impulsive but not the inattentive symptoms of ADHD increased as a function of the number of DAT1 alleles and that this increase was predominantly linear in form (fig. 1). Effect sizes from these between-family analyses suggested that DAT1 explains ~1%–4% of the overall variance in ADHD symptoms and that the relation with hyperactive-impulsive symptoms is stronger and more reliable than that with inattentive symptoms. The relation of DAT1 and the hyperactive-impulsive symptoms remained after we controlled for ethnicity, which suggests that these association results are unlikely to be an artifact of population stratification. Analyses of siblings discordant for the number of DAT1 high-risk alleles also furnished strong evidence for linkage disequilibrium of DAT1 with ADHD, as siblings with a greater number of high-risk alleles had higher ADHD symptom scale scores than their cosiblings. As shown in figure 2, differences between genetically discordant siblings on the symptom scales were quite dramatic, estimated as almost an SD in magnitude, and were greater for the hyperactive-impulsive symptoms than for the inattentive symptoms (effect sizes = .99 and .79 SD, respectively).

Analyses of parent-offspring transmission to affected children (viz., the TDT) also provided evidence consistent with association and linkage between DAT1 and ADHD, since the results for any ADHD diagnosis were statistically significant or nearly so across all levels of symptom severity. It is interesting to note that further analyses by ADHD subtype suggested linkage disequilibrium of DAT1 with the combined but not the inattentive subtype. The fact that evidence for linkage disequilibrium between DAT1 and ADHD emerged from

Table 3

TDT Results (χ^2) by ADHD Diagnosis, Severity Level, and Genetic Model

	ADHD Diagnosis and Severity Level								
	Any Diagnosis			Combined Type			Inattentive Type		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
Transmissions/ nontransmissions	39/24	29/15	22/8	26/13	16/5	11/3	11/10	9/7	7/5
ODDS Ratio	1.63	1.93	2.75	2.00	3.20	3.67	1.10	1.29	1.40
χ^2_{TDT}	*3.57*	4.45	6.53	4.33	5.76	4.57	.05	.25	.33
$\chi^2_{RECESSIVE}$	1.69	2.61	*3.28*	2.14	4.27	*3.27*	.02	.29	.47
$\chi^2_{DOMINANCE}$	*3.07*	*2.78*	1.38	*3.27*	2.25	1.92	.07	.00	.00

NOTE.—Underlined table entries are statistically significant ($P < .05$). Table entries that are italicized are marginally significant ($P < .10$.)

three different sets of analyses provides strong support, in our view, for the etiological role of DAT1 in ADHD. These results constructively replicate earlier findings of association between DAT1 and ADHD (Cook et al. 1995; Gill et al. 1997) and extend those findings by suggesting that linkage disequilibrium of DAT1 with ADHD is somewhat specific to the hyperactive-impulsive symptoms, rather than the inattentive symptoms. This represents one of the first replicated associations of a candidate gene and a psychiatric disorder in children. Linkage disequilibrium of DAT1 with any ADHD diagnosis and ADHD-combined type increased by level of symptom severity, suggesting that DAT1 may influence not only the presence or absence of ADHD, but also the severity of the disorder. Analyses by genetic model were less revealing, given that dominant versus recessive inheritance could not clearly be resolved.

Even as these results replicate and extend previous findings on DAT1 and ADHD, they raise a number of new questions. The evidence for association between DAT1 and ADHD was much stronger from the genetically discordant sib-pair analyses than from the between-family regression analyses. We think that there are two plausible, non–mutually exclusive reasons for this. First, the within-family analyses control for many other factors that vary across families (e.g., socioeconomic status) and that may influence ADHD symptom scores and their relation to DAT1 and, thus, may have affected the results of the regression analyses. Second, a number of behavior-genetic studies of ADHD (e.g., Thapar et al. 1995) have found evidence for contrast effects in parental ratings of ADHD symptoms, such that parents tend to exaggerate differences in ADHD symptoms between siblings or fraternal twins relative to parental ratings of identical twins. It is possible that such contrast effects influenced parents' ratings of ADHD symptoms in the genetically discordant sib pairs, thus resulting in an overestimate of the degree to which these siblings truly differ. Another unresolved question concerns the magnitude of the relation between DAT1 and ADHD. Although DAT1 accounted for 1.1% of the variance in inattentive symptoms and 3.6% of the variance in hyperactive-impulsive symptoms in the regression analyses, these estimates could be seriously misleading if the marker that we used in DAT1 is a neutral marker that is some distance away from the functional part of the gene. More specifically, the magnitude of the relation between DAT1 and ADHD is underestimated, to a greater or lesser extent, depending on the degree of linkage disequilibrium between the marker we studied and the functional parts of the gene that play a causal role in ADHD.

ADHD as a Complex Trait

Like many physical diseases, and virtually all psychiatric disorders, ADHD can be considered a complex trait from a genetic perspective (Lander and Schork 1994). Given its non-Mendelian transmission pattern, the lack of a simple one-to-one genotype-phenotype relationship, reduced penetrance of any putative liability-increasing alleles, and the presence of phenocopies, ADHD must be approached by use of contemporary molecular genetic analytic methods. These points were clearly in evidence in the current study, in which some children had one or two copies of the DAT1 high-risk allele yet failed to surpass symptom thresholds necessary to merit a diagnosis of ADHD, and there were a few children meeting ADHD diagnostic criteria who had no DAT1 high-risk alleles. Thus, although there appears to be a reliable relation between DAT1 and ADHD, this relation is likely to be relatively small in magnitude. Although there may be a gene of major effect for ADHD (Faraone et al. 1992), it is likely that the genetic influences on ADHD are due to many genes each having a relatively minor effect (e.g.,< 5% of the liability variance). These issues are only compounded by possible genetic heterogeneity, environmental influences, and gene-environment interaction for ADHD.

Two other factors reinforce the consideration of ADHD as a complex trait with respect to its relation with DAT1. First, the frequency of the DAT1 high-risk

allele was ~70% in our sample, similar to results from previous studies. Such a high frequency for a putative high-risk allele raises many questions. These include whether this allele merely is in linkage disequilibrium with a true causal allele of much lower frequency and whether the DAT1 gene might have had some selective advantage in ancestral populations. Even if DAT1 represents one of many genetic risk factors for ADHD, this paradox of the frequency of the high-risk allele requires explanation. Second, the mice knockout-gene results referred to previously also represent a paradox, since the deactivation of DAT1 actually *increased* the mice's hyperactivity levels in the presence of a functional excess of dopamine at the synapse. These results appear to be at odds with what is known regarding the pharmacological treatment of ADHD, in which stimulant medications reduce ADHD symptoms by inhibiting the dopamine transporter and thus increasing the amount of dopamine at the synapse. Unfortunately, our study was not designed to address such questions in the relation of DAT1 to ADHD; hence, they await elucidation in future research.

Limitations of the Present Study and Future Directions

Although the current findings represent an exciting step in the illumination of specific genetic influences on ADHD, there are certain limitations of this study and future directions raised by these findings that deserve mention. First, although 117 clinical families may appear to provide an adequate sample size in between-family association analyses for detecting genes of small effect (e.g., <5% of the liability variance), this yields a relatively small sample for within-family analyses such as the TDT, owing to the restriction of selecting children with at least one heterozygous parent. Thus, although within-family analyses like the TDT have the advantage of being free from statistical artifacts (e.g., spurious association due to population admixture) in the detection of linkage disequilibrium, they place increased demands on sample size relative to between-family association analyses. Second, our examination of symptom severity as a moderator of linkage disequilibrium was hampered somewhat by the fact that we treated as categorical an inherently continuous variable. Extensions of the TDT to examine moderator variables that are continuous as well as categorical in nature (Waldman et al. 1997*a*, 1997*b*) will permit a more sensitive and accurate examination of genetic heterogeneity than is now possible. Third, an important feature of psychiatric disorders as "complex traits" is the fact that they frequently overlap and their symptoms frequently covary, although the sources of such overlap and covariation are not well understood. This phenomenon, often referred to as "comorbidity," has been documented for ADHD, which overlaps substantially with many other childhood psychiatric disorders (e.g., ODD, CD, Tourette syndrome, anxiety disorders; Lilienfeld and Waldman 1990; Biederman et al. 1991). Molecular genetic analyses can shed light on the bases of comorbidity by examining whether distinct disorders share genetic influences in common, as well as whether linkage disequilibrium for a given disorder varies depending on the presence or absence of other disorders. We are currently in the process of investigating such issues for ADHD and related disorders and for DAT1 and other candidate genes. Given the intertwined nature of etiological and nosological issues, these analyses may help to clarify not only the genetic influences on ADHD and related disorders, but also the classification of children's psychiatric disorders.

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