ORIGINAL RESEARCH ARTICLE

Dopamine DRD4 receptor polymorphism and attention deficit hyperactivity disorder

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A polymorphism in the dopamine receptor 4 gene (DRD4) has been related to novelty seeking, Tourette's syndrome, and attention deficit hyperactivity disorder (ADHD). The variability is in a 48-bp repeat in exon 3 of the gene (a transmembrane region). This study examined the relation of the 7-repeat (ie, high-risk) allele to questionnaire-based diagnoses of ADHD (both combined type and inattentive type). Several positive findings were obtained for ADHD-inattentive type. In an association test, the 7-repeat allele occurred more frequently in children with ADHD-inattentive type than in control children. In genetically discordant sibling pairs, the sibling with a greater number of 7-repeat alleles displayed more inattentive symptoms than his/her co-sibling with fewer 7-repeat alleles. For ADHD-combined type, the 7-repeat allele frequency was greater than that in the control sample. However, a quantitative transmission disequilibrium test yielded no significant linkage of the 7-repeat allele with hyperactive-impulsive symptoms. A categorical TDT yielded no significant findings, but the number of transmissions was small, especially for ADHD-inattentive type.

Keywords: attention deficit hyperactivity disorder; DRD4; dopamine; genetics; linkage

Introduction

A polymorphism in the dopamine receptor 4 gene (DRD4) is important because of its possible relationship to behavioral traits. This polymorphism is a 48-bp repeat in the gene's third exon, where the transcribed protein spans the nerve cell's membrane.¹ Variability arises from the most common 2, 4, and 7-repeat alleles as well as from base pair substitutions within the repeats. This polymorphism shows considerable racial/ethnic heterogeneity.² Averaged over populations world-wide, the most common allele is a 4-repeat allele (frequency = 63.4%), with the second most common being the 7-repeat allele (20.6%). However, the 7-repeat allelic frequency is only 1% in the Japanese, and their most common *rare* allele is the 2-repeat. The frequency of the 7-repeat rises to nearly 80% in Sunui and Ticuna peoples of South America. Its frequency in mixed Europeans is 21%, whereas in African Bantus, the ethnic origin of many African-Americans, it is 19%. Rarer alleles of size 2–11 repeats also occur. To account for the large DRD4 variability across different populations, the locus may have a high

mutation rate resulting in new haplotypes and may be selectively neutral.

Although some findings have been negative, associations have been reported between the DRD4 7-repeat allele and behavioral traits. Two research groups found an increase in the personality trait of novelty seeking in individuals possessing one or two copies of the 7-repeat allele.^{3,4} Benjamin *et al* extended the analysis to siblings discordant for the number of 7-repeats ($n = 60$ sibling pairs); the siblings with a greater number of 7-repeats had higher novelty seeking and extraversion. A partial replication of the original finding also found that the 7-repeat allele increased novelty seeking in female but not male subjects.⁵ In contrast, the association of novelty seeking with DRD4 failed to replicate in two studies.^{6,7} In one study,⁷ Euro-American females and African-American substance abusers with the 7-repeat allele had *lower* levels of novelty seeking. In addition to normal personality traits, associations have been sought with behavioral disorders. By a linkage disequilibrium test, the 7-repeat allele has been associated with Tourette's syndrome in large family kinships.⁸ In a sample of 113 Finnish alcoholics and their ethnically matched controls, this DRD4 mutation was not associated with alcoholism.⁹ In a study of schizophrenics and controls, there was a statistical trend towards an elevation of the 7-repeat in schizophrenics.¹⁰ The positive evidence is sufficient to make DRD4 a candidate gene for psychopathology.

Association and linkage of attention deficit hyperac-

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tivity disorder (ADHD) with this 48-bp repeat polymorphism in DRD4 has also been examined in a number of studies. In the first, LaHoste¹¹ found an association between the 7-repeat allele and ADHD in a comparison of children with the ADHD-combined type diagnosis and matched controls. Subsequent studies have used both case control and within-family tests to examine association and linkage of DRD4 and ADHD. Although two studies have yielded negative results from case controls and within-family analyses,^{12,13} another two studies have constructively replicated and extended the findings of LaHoste and colleagues. Bailey and her colleagues¹⁴ found linkage between ADHD and DRD4 using both the transmission disequilibrium test (TDT) and an affected sib-pair analysis, and Swanson *et al*¹⁵ found association and linkage between a refined ADHD phenotype and DRD4 using a haplotype relative risk test.

In this study, we examine association and linkage of the DRD4 gene's 7-repeat allele with ADHD. The dopaminergic pathways in the brain are thought to be essential for the experience of reward.¹⁶ ADHD children appear to be over-responsive to reward and under-responsive to punishment. Further, the drug methylphenidate (Ritalin) used in the treatment of ADHD, inhibits the action of the dopamine transporter protein, making dopamine more available for intercellular communication. It is likely that dopaminergic activity plays some causal role in the predisposition to ADHD, with a lack of sensitivity to dopamine being one plausible cause of ADHD. In this regard, the 7-repeat mutation has been found to reduce the response of cells to dopamine, as the neurotransmitter induced less activity of adenylyl cyclase in cells with the 7-repeat allele.¹⁷ Although other neurotransmitter pathways are probably involved as well, considerable evidence now ties the dopaminergic pathways to ADHD.

This study is a constructive replication and extension of LaHoste's study of ADHD. Our first strategy, following LaHoste *et al*, was to compare the frequency of the 7-repeat allele in ADHD children and controls. Our second strategy was to examine *within-family* association and linkage. One within-family association test compared siblings discordant for the number of 7-repeat alleles. Unlike LaHoste *et al*, we also possessed data on parental genotypes. Thus, our second within-family analysis examined the transmission of the 7-repeat allele from heterozygous parents to their children using both categorical and quantitative transmission disequilibrium tests (TDT).^{18,19} Compared to the case control method, the TDT is less susceptible to biases resulting from population heterogeneity.

Subjects and methods

Subjects

Data were collected at two research sites: Tucson, Arizona and Atlanta, Georgia. At the Tucson site, children in the control sample were ascertained through a list of families in the general population with at least one male child 8–12 years old generated by Multi-Media, a

market research firm. Any second male sibling 6–16 years old was also sampled. Clinic-referred boys in Tucson were identified through psychiatrists in private practice. Whenever possible, a brother was also sampled. At the Atlanta site, only clinic-referred children (both males and females) were sampled through the Center for Learning and Attention Deficit Disorders (CLADD) at Emory University's School of Medicine. At this site, siblings were also sampled whenever possible.

Genotyping

Except for difficult cases genotyped with a new primer set, the DRD4 repeat polymorphism was genotyped using standard methods.¹ Genotyping was completed at the University of Arizona's *Laboratory of Molecular and Systematic Evolution*, a multi-user lab facility. Genotypes were available for 168 children from 123 families visiting CLADD in Atlanta or a psychiatric office in Tucson and 71 children from 53 general population families. Genotyping of DRD4 was also completed on 155 mothers and 122 fathers. The sample of 71 non-referred children and siblings is hereafter identified as the *control sample*; the sample of 168 referred children and their siblings from psychiatric settings is hereafter identified as the *clinic sample*. For all 239 children (191 males, 48 females), the average age was 10 years (s.d. = 2.8 years).

Ethnic ancestry

Ethnic ancestry was determined from the ancestry of the children's biological father and maternal grandparents, as reported by the children's mother. Four ethnic groups were distinguished: Caucasian, African-American, Hispanic, and Asian. A percentage of genes identical by descent was attributed to different ethnic/racial groups. For example, a subject with one African-American maternal grandparent, one Hispanic maternal grandparent, and a Caucasian biological father would score as 0.25 (or 25%) African-American, 0.25 Hispanic, and 0.50 Caucasian. The proportions of children with 100% ancestry were: African-American, 8.5%, Hispanic, 4.3%, and Caucasian, 71.8%.

Instruments and interview

At the Tucson site, an interviewer visited each family in their homes. After obtaining informed consent, the mother was interviewed about household composition. The mother and father next received questionnaires for completion. The family session ended with the collection of cheek cells using a water-mouthwash procedure. At the Emory site, a few families were seen at CLADD, whereas the majority of families were seen in their homes. The data collection session followed much the same sequence as in Tucson.

The mother provided demographic data on a short questionnaire, and both parents rated their children for behavioral problems on the Emory Diagnostic Rating Scale (*viz*, EDRS). This questionnaire was developed by Dr Waldman and his students to assess symptoms of the major DSM-IV childhood disorders. These include

symptoms of the disruptive behavior disorders (ie, CD, ODD, and the inattention and hyperactivity-impulsivity symptom dimensions of ADHD), symptoms of internalizing disorders (specific phobias, separation anxiety, generalized anxiety, social phobia, obsessions and compulsions, major depression/dysthymia, and panic and agoraphobia), and symptoms of Tourette's syndrome (ie, vocal and motor tics). Each symptom of these disorders was translated into an item on which children were rated by their parents on a 0–4 scale, with 0 being not at all characteristic of their child and 4 being very much characteristic of their child.

In this article, we examine the relation of ADHD with a 48-bp repeat polymorphism in exon 3 of the dopamine receptor DRD4 gene. Continuous symptom dimensions were constructed for DSM-IV ADHD hyperactive/impulsive and inattentive symptoms. For each dimension, the symptom levels based on the mother's and father's ratings were averaged to produce a more reliable composite symptom rating. This procedure was justified by the high correlation of mother's and father's ratings (hyperactive, $r = 0.80$; impulsive $r = 0.72$; and inattentive, $r = 0.83$). This average was then divided by four to express the mean symptom count as a proportion of the highest possible score (eg, a mean symptom count of 2 yields a score of 0.50).

Other analyses, such as the transmission disequilibrium test (TDT),¹⁸ require designating children as 'affected' vs 'unaffected.' For this purpose, questionnaire-based diagnoses were derived from symptom thresholds in the DSM-IV. The presence of specific symptoms was first established, and then a DSM-IV ADHD questionnaire-based diagnosis was assigned if the number of symptoms surpassed the standard diagnostic threshold (*viz.* six or more of nine symptoms on the inattention and/or hyperactivity-impulsivity symptom dimensions). If a child surpassed a diagnostic threshold on the basis of either the mother's or father's ratings, a questionnaire-based diagnosis was made.

In the clinic sample, children who were above threshold on the inattentive dimension, but not the hyperactive-impulsive dimension, were diagnosed with the ADHD-inattentive subtype ($n = 37$); those who were above threshold on the hyperactive-impulsive dimension but not the inattention dimension were diagnosed with the ADHD-hyperactive/impulsive subtype ($n = 11$); and those who were above threshold on both symptom dimensions were diagnosed with the ADHD-combined subtype ($n = 70$). Thus, 70% of the clinic sample received an ADHD questionnaire-based diagnosis.

In the control sample, five, two and six children received the questionnaire-based diagnoses of ADHD-inattentive type, ADHD-hyperactive/impulsive subtype, and ADHD-combined type, respectively. Thus, 18% received a questionnaire-based diagnosis, a prevalence greater than the 3–6% typically observed in the general population. The higher 18% prevalence may reflect in part the self-selected nature of families where parents were told during recruitment that the study concerned ADHD and may reflect in part the use of a

questionnaire-based diagnosis. The diagnosed control children were dropped from analyses that compared diagnosed clinic sample children with non-diagnosed controls. We did not add the diagnosed controls to the diagnosed clinic children because the methods of ascertainment were different.

Table 1 presents demographic information on the sample subdivisions. All 71 children in the control sample were male; about 30% of the 168 children in the clinic sample were female. Those 118 clinic children with any questionnaire-based diagnosis of ADHD were younger, by 1 year and 6 months, than the 13 diagnosed controls. There was also a wider age range in the clinic samples. With regard to ethnicity by descent, the only statistically significant difference among samples was the greater mean level of African-American ancestry in the clinic samples. Parental education was measured on a scale from 1 = 8th grade or less, to 8 = advanced degree (eg, MD, PhD). The samples' means were from about 6 = some college, to 7 = graduated from college or university. Although one difference was statistically significant (control diagnosed vs control not diagnosed), the magnitude of educational differences among the samples was small. The majority (81.5–90%) of the clinic children were sampled at Emory University. Because the diagnosis was questionnaire-based, it necessarily follows that the children with a diagnosis had higher symptom levels than the unaffected children, both clinic and control. Although not significantly different because of the small sample size, the control questionnaire-diagnosed children had more than twice the symptom means as the control non-diagnosed children. Their means, however were lower than those of the clinic diagnosed children. About equal numbers of parents were successfully genotyped in the four samples.

Results

Between individual analyses

Our association tests depend on the existence of allelic variability. Table 2 shows the allele frequencies in clinic children with ADHD questionnaire-based diagnoses vs in control children without any ADHD diagnosis (n persons = 58). Too few individuals had a diagnosis of ADHD-hyperactive/impulsive type for analysis. In both the combined ($n = 70$) and inattentive ($n = 37$) ADHD types, the 4-repeat and 7-repeat alleles were the most common alleles. Both ADHD types had greater 7-repeat frequencies than the controls (23% and 27% vs 12.9%, respectively).

We next evaluated whether genotypes containing the 7-repeat allele were more frequent in diagnosed children vs unaffected children. We grouped genotypes as O/O, O/7, and 7/7, where O is any allele other than the 7-repeat allele. Because the 7/7 homozygote was rare, we scored the genotypes as O/O = 0, O/7 = 1, and 7/7 = 1 for the chi-square and correlational analyses below, thus assuming genetic dominance.

Table 3 shows the genotypic frequencies in the different samples. In the ADHD-combined and inattentive

Table 1 Demographic characteristics and mean symptom counts by questionnaire-based diagnostic subgroups

	<i>Clinic diagnosed*</i>	<i>Clinic no diagnosis</i>	<i>Control no diagnosis</i>	<i>Control diagnosed*</i>
Sex (% male)	70%	76%	100%	100%
Age†	9.4 ^a (4–18)	9.8 (4–17)	9.8 (7–13)	10.9 ^b (6–16)
Hispanic†	0.05	0.06	0.10	0.14
Black†	0.15 ^a	0.17 ^a	0 ^b	0.04 ^c
White†	0.74	0.72	0.85	0.83
Years education, Mother†	6.2	6.6	6.7 ^a	5.9 ^b
Years education, Father†	6.0	6.1	6.2	5.7
Tucson site	19.5%	10%	100%	100%
Emory site	81.5%	90%	0%	0%
Hyperactive-impulsive symp†	0.54 ^a	0.14 ^b	0.12 ^b	0.34 ^c
Inattentive symp†	0.60 ^a	0.17 ^b	0.15 ^b	0.41 ^c
% Both parents genotyped	62%	72%	79%	69%
<i>n</i>	118	50	58	13

*Any questionnaire-based diagnosis.

†Statistically different means (Tukey's Studentized Range Test, when F was significant overall) are designated by different superscript letters; means sharing a letter do not differ significantly.

Table 2 Allelic frequencies in clinic and control samples

<i>Allele</i>	<i>Control No diagnosis^a</i>		<i>Clinic ADHD combined type</i>		<i>Clinic ADHD inattentive type</i>	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
2	13	11.2	10	7.1	3	4.1
3	4	3.4	9	6.4	2	2.7
4	80	69.0	84	60	48	65
5	1	0.9	3	2.1	0	0
6	1	0.9	2	1.4	0	0
7	15	12.9	32	23	20	27
8	0	0.0	0	0	0	0
9	2	1.7	0	0	1	1.4
<i>n</i> (alleles)	116		140		74	

^aDiagnoses were questionnaire-based.

Table 3 Frequency of 7-repeat allele in affected vs controls

	<i>n</i>	<i>O/O</i>	<i>Genotype</i>		$\chi^2(1)^a$
			<i>O/7</i>	<i>7/7</i>	
Control	71	0.83	0.15	0.03	
Control ^b	58	0.78	0.19	0.04	
<i>Compared with control, no questionnaire-based diagnosis^a</i>					
ADHD-CT	70	0.57	0.40	0.03	5.9**
ADHD-INAT	37	0.57	0.32	0.11	4.6*

^aO/7 genotype grouped with 7/7 genotype. O=other allele, 7=seven-repeat allele.

^bControl individuals surpassing the diagnostic threshold for any disorder removed.

* $P < 0.05$; ** $P < 0.01$.

types, the frequency of 7-repeat containing genotypes was about double (43% for both) that in the 58 controls without questionnaire-based diagnoses. The inattentive subtype also had an excess of 7/7 homozygotes (11% vs 4% in controls). The significant chi-square tests in Table 3 establish an association between the 7-repeat allele and the two ADHD diagnoses.

We next examined associations using the continuous symptom variables instead of the categorical diagnoses. Table 4 presents correlations of demographic variables with the two symptom dimensions and DRD4 genotypes, coded as above. For the most part, these associations were weak, suggesting that the modest demographic differences among samples (see Table 1) were not serious methodological confounds. Females were more inattentive ($r = 0.13$; where male = 0, female = 1) than males, but not more hyperactive. The absence of large sex differences is probably a result of the ascer-

Table 4 Correlations of demographic variables with ADHD symptom counts and DRD4 7-repeat allele containing genotypes

Demographic variable	Hyperactive-impulsive	Inattentive	DRD4 genotype
Sex	0.04	0.13*	0.05
Age	-0.30**	-0.05	-0.04
Hispanic	-0.08	-0.04	0.01
Black	-0.01	0.00	0.10
White	0.03	-0.04	-0.16*
Mother's education	0.02	-0.01	-0.06
Father's education	-0.14*	-0.13	-0.09

$n = 195-239$.

* $P < 0.05$; ** $P < 0.01$.

tainment process, where the girls were all found through psychiatric referrals and would be high scorers on the symptom dimensions as a consequence. The strongest relationship, accounting for 9% of the variance, was a decrease in hyperactive-impulsive symptoms with age. With regard to ethnicity by descent, fewer Whites had genotypes containing the 7-repeat allele ($r = -0.16$). This suggests that ethnicity should be statistically controlled when associating DRD4 with the symptoms dimensions.

Table 5 presents the correlations of DRD4 with the continuous symptom dimensions. A positive association of the 7-repeat allele and both symptom types was found in the total sample. In the total sample, DRD4 explained about 4% of symptom variation. In the sample with any ADHD diagnosis, the associations with the 7-repeat allele were stronger for inattentive than for hyperactive-impulsive symptoms. In the control sample, no associations were observed.

In another analysis in Table 5, DRD4 was regressed on three variables: the percentage of Euro-Caucasian ancestry, the percentage of African-American ancestry, and the percentage of Hispanic ancestry. This regression equation explained 3% of the variance in the DRD4 variable ($F = 2.6$, $P = 0.06$). The residuals

from this regression were correlated with the symptom dimensions (ie, r_{adj}). The adjusted correlations were approximately equal to the unadjusted ones. Thus, there was no evidence that ethnic stratification had produced the positive association of the 7-repeat allele with inattentive or hyperactive/impulsive symptoms.

The association of hyperactive-impulsive symptoms with age led to another analysis in Table 5. Hyperactive-impulsive symptoms were first residualized on age, and then the correlation with residualized DRD4 was repeated. The magnitude of association was quite similar to that obtained for hyperactive-impulsive symptoms without any age adjustment, indicating that the age association was also not a serious methodological confound.

Despite the findings controlling for racial/ethnic heterogeneity, more subtle forms of stratification remain an alternative explanation for DRD4 associations. Thus, within-family tests of linkage disequilibrium were conducted, where significant associations with a genetic marker depend on the presence of genetic linkage, which controls for population heterogeneity.

Within-family analyses of disequilibrium

We took two approaches to test for within-family association and linkage: (1) comparing ADHD symptom levels in sibling pairs discordant for their DRD4 genotype; and (2) the transmission disequilibrium test (TDT).

In the clinic sample, 12 sibling pairs were discordant for the number of 7-repeat alleles. In ten sibling pairs, one child was genotype 7/O (O = other allele) and the other was genotype O/O. In two pairs, the contrast was 7/7 vs 7/O. Signed differences were computed on symptom counts, where the symptom count for the sibling with fewer 7-repeat alleles was subtracted from the count for the sibling with more 7-repeat alleles. For inattentive symptoms, the mean difference was positive and statistically significant (inattentive $M = 0.21$, $s.d. = 0.31$; $t = 2.4$, $P < 0.05$). For hyperactive-impulsive symptoms, the mean difference was positive but failed to reach significance ($M = 0.10$, $s.d. = 0.29$, $t = 1.2$, $n.s.$). In nine of the 12 sibling pairs, the child possessing the greater number of 7-repeat alleles had the greater symptom count for inattentive symptoms.

Table 5 Correlations of continuous symptom counts with DRD4 genotypes

Sample	Hyperactivity impulsiveness symptoms		Inattention symptoms		
	r	Race adjusted r_{adj}	Race/age adjusted r_{adj}	r	Race adjusted r_{adj}
Total sample	0.19**	0.19**	0.16**	0.21**	0.20**
Any diagnosis ^a	0.12	0.16*	0.12	0.22**	0.21**
Control sample	-0.14	-0.11	-0.19	-0.10	-0.14

^aDiagnoses were questionnaire-based.

* $P < 0.05$, one-tailed; ** $P < 0.01$, one-tailed.

The second within-family test, the transmission disequilibrium test, also used parental genotypes. In a categorical TDT, a chi-square test is used to determine whether heterozygous parents disproportionately transmit the 7-repeat allele to *affected* offspring.¹⁸ A count is made of the number of heterozygous parents transmitting the 7-repeat allele and the number transmitting any other allele. The formula for the TDT chi-square test is:

$$\chi^2_{\text{TDT}} = (b - c)^2 / (b + c),$$

where b = the number of times the 7-repeat allele is transmitted and c = the number of times any other allele is transmitted. For a test of linkage, siblings constitute independent cases (ie, the laws of Mendelian inheritance apply independently to each sibling) and so can be entered in the analysis without biasing the TDT statistic. Lastly, the TDT may be a biased statistic if both parents do not provide genotypes.²⁰ For this reason, we restricted the analysis to families with genotypic data from both parents.

For the categorical TDT, the number of informative families (ie, those in which at least one parent is heterozygous) were 23 for ADHD-combined type and nine for ADHD-inattentive type (there were too few ADHD-hyperactive/impulsive cases for analysis). In no case did the number of transmissions of the high-risk allele significantly exceed that of the low risk allele. Specifically, the transmission rates were: ADHD-combined type, $b = 15$, $c = 14$, $\chi^2 = 0.03$; and ADHD-inattentive subtype, $b = 8$, $c = 5$, $\chi^2 = 0.7$. Although for both questionnaire-based diagnoses there was an absence of linkage disequilibrium, statistical power was also low. It was especially low for inattentive symptoms, with only 13 total transmissions.

Unlike the categorical TDT, a quantitative transmission disequilibrium test (QTDT)¹⁹ does not require a cut-off threshold that separates affected and unaffected individuals, and thus may be greater in statistical power. As with the categorical TDT, it requires that both parents have been genotyped for DRD4 and that at least one parent must be a heterozygote. The variables X_1 and X_2 are created:

- $X_2 = 0$ if a heterozygous mother does not transmit the 7-repeat allele
- $X_1 = 1$ if a heterozygous mother does transmit the 7-repeat allele
- $X_2 = 0$ if a heterozygous father does not transmit the 7-repeat allele
- $X_2 = 1$ if a heterozygous father does transmit the 7-repeat allele

As a 1 degree of freedom test is appropriate (ie, when there is no hypothesis of genetic imprinting), the variables X_1 and X_2 are summed. The regression is computed as:

$$Y = \beta_0 + \beta_1(X_1 + X_2),$$

where Y is the continuous symptom variable. For the purpose of significance tests and effect size estimation, a standardized β_1 is equivalent to the correlation coef-

ficient, thus we simply correlated each disorder with the sum of X_1 and X_2 . In the total sample, 70 families qualified for the QTDT. This comprised 50 families in the clinic sample and 20 families in the control sample. The QTDT correlation of transmitted 7-repeat alleles with hyperactive/impulsive symptoms was near zero in the total sample ($r = 0.01$) and greater than zero, but not statistically significant, in the clinic sample ($r = 0.13$). There was a positive correlation of 7-repeat allele transmissions with inattentive symptoms in both the total sample ($r = 0.13$, n.s) and clinic sample ($r = 0.18$, $P < 0.1$ one-tailed). Thus, the TDTs failed to provide unequivocal support for linkage disequilibrium between the 7-repeat allele and ADHD, but the QTDT provided evidence suggestive of linkage disequilibrium between DRD4 and inattentive symptoms of ADHD.

Discussion

Our findings were most positive for the questionnaire-based diagnosis of ADHD-inattentive type. For this diagnosis, the frequency of DRD4 genotypes containing the 7-repeat allele was significantly greater in ADHD-inattentive type children than in the control children. In genetically discordant sibling pairs, the sibling with a greater number of 7-repeat alleles displayed more inattentive symptoms than his/her co-sibling with fewer 7-repeat alleles. Although the within-family TDT was non-significant, there were too few total transmissions from heterozygous parents to test rigorously for linkage disequilibrium ($n = 13$ transmissions). The ADHD-combined type also produced greater 7-repeat frequencies than the control children, but no within-family test of association *via* linkage was statistically significant.

Our findings replicated those of LaHoste *et al.*¹¹ Specifically, in ADHD-combined type children, LaHoste *et al* found that 49% had at least one 7-repeat allele, whereas only 21% of controls had at least one 7-repeat allele. In our sample, the frequencies were 43% and 23%, respectively. Furthermore, both studies found that the 7-repeat allele increased symptom severity among ADHD children. We found this in our total sample, where DRD4 explained about 4% of the variance in both inattentive and hyperactive-impulsive symptoms. No association was found within the control sample alone, however. Unlike in LaHoste *et al*'s study, the control children were not matched with the ADHD children for demographic traits. Nonetheless, there was little change in the strength of the 7-repeat association with DRD4 after ethnic/racial ancestry was statistically controlled.

In Table 6, our DRD4 allele frequencies are compared to those published elsewhere. The 7-repeat allele frequency in our control sample was 12.9%, which appears discrepant from the 18.2–20% frequency in Table 6's samples drawn from the general population. The frequency of the 4-repeat allele was comparable across samples. Thus, our statistically significant findings could be partly a function of an unusual control group. However, our results were strongest for ADHD-

Table 6 Allelic frequencies in several comparison studies

Allele	Present controls %	Gelernter controls ^a %	Pogue-Geile twins ^b %	Swanson probands ^c %	Present ADHD-inattentive %	Present ADHD-combined %
2	11.2	4.5	7.0	7.7	4.1	7.1
3	3.4	0	2.0	1.8	2.7	6.4
4	69.0	69.7	69.0	63.5	65.0	60.0
5	0.9	4.5	1.0	0	0	2.1
6	0.9	0	0	0	0	1.4
7	12.9	18.2	20.0	27.9	27.0	23.0
8	0	3.0	1.0	0	0	0
9	1.7	0	1.0	0	1.4	0
n (Alleles)	116	66	562	104	74	140

^aCaucasian controls from University of Connecticut Health Center and Medical College of Virginia, adults.⁷^bSame-sex twins 18–27 years old, community sample.⁶^cProbands, interview diagnosis of ADHD without serious comorbid disorders and receiving methylphenidate treatment.¹⁵

inattentive type. In our sample the 7-repeat allele frequency (27%) comes close to exactly matching Swanson's¹⁵ ADHD children's (27.9%), and is greater than the 19.8% weighted average from pooling the control samples in Table 6. The ADHD children in Swanson *et al* were selected for the absence of serious comorbid disorders. Although we did not follow those selection criteria, our ADHD-inattentive type children were, by definition, selected somewhat for an absence of hyperactive-impulsive symptoms. As expected, they also were lower than ADHD-combined type children on symptoms of other disruptive behavior disorders (eg, oppositional defiant disorder, conduct disorder). This makes our result a constructive replication of previous findings on association and linkage between DRD4 and ADHD.

Despite our partially positive results, stronger findings could have been obtained, especially for the TDT analyses. DRD4 has produced highly inconsistent findings for novelty seeking (eg, the wrong allele showing association with novelty seeking, Gelernter;⁷ no association in Pogue-Geile;⁶ vs a partial replication of the original association, Epstein⁵). One possibility is that the number of repeats is in linkage disequilibrium with a functional mutation elsewhere in or near the DRD4 gene. This functional site could be an amino acid replacement within a 7-repeat unit, as the 48-bp repeat has considerable internal heterogeneity, such that Lichter *et al*¹ identified 19 different repeat units, in many of which the amino acid sequences were different. They commented, '... Variation in the same-sized haplotypes also changes the predicted primary sequence of the protein and may have structural and functional effects as well' (p 770). Another explanation is that the 7-repeat allele is in linkage disequilibrium with another functional mutation in the DRD4 gene or its promoter. However, at least for ADHD, the positive within-family association found by Swanson, and our trend in this direction for ADHD-inattentive type, suggest that repeat number provides some functional basis for the association between DRD4 and ADHD. Further

research should focus on similar within-family tests that avoid the methodological confound of population stratification.

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