



ORIGINAL RESEARCH ARTICLE

# Lack of an association between a dopamine-4 receptor polymorphism and attention-deficit/hyperactivity disorder: genetic and brain morphometric analyses

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**Keywords:** dopamine 4 receptor; VNTR; magnetic resonance imaging; replication studies; case-control studies

Although the etiology of attention-deficit/hyperactivity disorder (ADHD) is likely multifactorial, family,<sup>1</sup> adoption,<sup>2</sup> and twin studies<sup>3</sup> suggest that genetic factors contribute significantly. Polymorphisms of the dopamine 4 receptor (DRD4) affect receptor binding,<sup>4</sup> and one allele with seven tandem repeats in exon 3 (DRD4\*7R) has been associated with ADHD.<sup>5,6</sup> We examined this putative association in 41 children with severe ADHD and 56 healthy controls who were group matched for ethnicity and sex. The frequency of the DRD4\*7R allele did not vary by diagnosis (0.220 vs 0.205 in patients and controls, respectively). Behavioral and brain anatomic MRI measures, previously found to discriminate patients from controls,<sup>7</sup> did not differ significantly between subjects having and those lacking a DRD4\*7R allele. These data do not support the reported association between DRD4\*7R and the behavioral or brain morphometric phenotype associated with ADHD.

Converging lines of evidence implicate central dopaminergic systems in the pathophysiology of ADHD.<sup>8</sup> LaHoste *et al*<sup>5</sup> reported an increased frequency of the DRD4\*7R allele in 39 children with ADHD and concluded that the gene encoding the DRD4 may contribute to the expression of symptoms of ADHD. The same group has confirmed their finding using the haplotype relative risk method.<sup>6</sup> However, the literature regarding the association of D4 receptor polymorphisms and psychiatric illness is confusing because of the failure to replicate preliminary reports in different populations. For example, the association between DRD4\*7R and the putatively dopaminergic trait of Novelty Seeking was reported in two separate studies,<sup>9,10</sup> but was not replicated in three others.<sup>11-13</sup> In addition, DRD4\*7R

**Table 1** Frequencies of dopamine 4 receptor alleles in 41 children with ADHD and 56 healthy controls

	2	3	4	5	6	7	Total (2N)
Control	0.027	0.027	0.723	0.018	0	0.205	112
ADHD	0.073	0.012	0.683	0	0.012	0.220	82

was reported to be in linkage disequilibrium with Tourette syndrome,<sup>14</sup> which is frequently associated with ADHD, but two further reports failed to confirm this association.<sup>15,16</sup>

We tested the reported association between ADHD and DRD4\*7R using a sample of patients with severe ADHD, defined in part by the willingness of parents and probands to participate in a drug treatment study that requires leaving their regular school to attend a research day program for 3 months.<sup>17</sup> There were no significant differences between patients and psychiatrically screened controls in the frequency of the DRD4\*7R allele ( $\chi^2 = 0.06$ , d.f. = 1,  $P = 0.81$ ) nor in any other of the DRD4 alleles (see Table 1). At least one D4 allele with 7 repeats was found in 41% of ADHD subjects and 39% of controls. Moreover, patients and controls did not differ significantly in frequency of individual genotypes (see Table 2) ( $\chi^2 = 9.35$ , d.f. = 8,  $P = 0.31$ ), in the frequency of genotype 4,4 vs genotype 4,7 ( $\chi^2 = 1.31$ , d.f. = 1,  $P = 0.25$ ), or in the frequency of 'short' (2-5 repeats) vs 'long' (6 or 7 repeats) alleles ( $\chi^2 = 0.19$ , d.f. = 1,  $P = 0.66$ ). Because the patient population had a high degree of comorbidity, the frequency of DRD4\*7R in the 12 ADHD patients without comorbid diagnoses was examined (0.208) and found to be identical to that of the patient group as a whole and to that of controls. The same frequency (0.207) was found in the 29 patients (80%) who have a first degree relative with presumptive ADHD. Finally, we also limited an analysis to white-non-Hispanic subjects, since DRD4 frequency varies markedly in different ethnic groups.<sup>18</sup> The frequency of DRD4\*7R in the 31 ADHD patients was 0.161, vs 0.238 in the 42 controls.

LaHoste *et al*<sup>5</sup> reported that their subjects who had at least one DRD4\*7R allele had greater severity of ADHD symptoms. We compared behavioral measures and anatomic brain morphometry in the 17 patients who have at least one DRD4\*7R allele vs the 24 who do not. These two subgroups did not differ significantly in age, socioeconomic status, sex, or handedness. The presence of the DRD4\*7R allele was not associated with significantly greater severity on parent or teacher rat-

**Table 2** Dopamine 4 receptor genotypes in 41 children with ADHD and 56 healthy controls

	2,4	3,4	4,4	4,6	2,7	3,7	4,7	5,7	7,7
Control	1	2	31	0	2	1	16	2	1
ADHD	5	1	17	1	1	0	15	0	1

ings of hyperactivity or conduct,<sup>19</sup> intelligence test scores,<sup>20</sup> math and reading achievement scores,<sup>21</sup> or global assessment of impairment<sup>22</sup> (see Table 3).

Anatomically, DRD4 has been localized in primates to several regions implicated in the pathophysiology of ADHD<sup>23</sup> including cerebral cortex and globus pallidus.<sup>24</sup> We tested whether the presence of the DRD4\*7R allele was associated with the brain morphometric differences within the ADHD group ( $n = 38$ ). There were no significant differences ( $P > 0.45$ ) in the predicted direction for total cerebral volume, caudate volume, caudate asymmetry, globus pallidus volume, or globus pallidus asymmetry. Since total cerebral volume correlated significantly with caudate volume ( $r = 0.68$ ,  $n = 37$ ,  $P < 0.0001$ ), we performed analyses of covariance with total cerebral volume as the covariate. The results remained unchanged except that the tendency for globus pallidus volume to be larger in the patients with a DRD4\*7R allele, ie, in the opposite direction to that predicted, became a trend ( $F = 3.17$ ,  $d.f. = 1, 31$ ,  $P = 0.08$ ).

One limitation of this report is the high degree of lifetime comorbidity, which is typical of ADHD samples. However, DRD4\*7R frequency also did not differ in the sub-sample without comorbid diagnoses. Another potential concern was statistical power. Assuming the findings of LaHoste *et al*<sup>5</sup> we had power  $> 0.80$  to detect a critical effect size<sup>25</sup> of 0.19 at  $\alpha = 0.05$  assuming sampling from the same population.

The importance of these negative findings should not be overstated. The most important limitation of this study is its case-control design, which makes it vulnerable to population stratification effects that are known to be substantial in DRD4.<sup>18</sup> There are now two independent reports of an association between DRD4\*7R and ADHD using a family-based approach,<sup>6</sup> (also see this issue, Rowe *et al*), and no negative studies using that method have yet to be published. However, the modest nature of the positive findings to date, combined with

our lack of any supportive data from phenomenology or from brain anatomy, suggest that, at best, DRD4 may be one of several genes implicated in ADHD. Since a number of groups are actively collecting larger family-based samples for candidate gene studies and for genome-based scans, the role of DRD4 in ADHD is likely to be elucidated in the near future.<sup>26</sup>

## Materials and methods

### Subjects

Children with ADHD ( $n = 41$ ), mean age  $9.7 \pm 2.6$  years) were determined to be stimulant responders in a double-blind stimulant study described elsewhere<sup>17</sup> with the exception of one 16-year-old female who participated only in a brain imaging study. Inclusion criteria were a history of impairing hyperactive, inattentive, and impulsive behaviors in at least two settings (home, school, or day program), and a Conners Teacher Hyperactivity rating above the 95th percentile for age and sex norms.<sup>19</sup> The DSM-III-R diagnosis of ADHD was confirmed by a Diagnostic Interview for Children and Adolescents—Revised (DICA-R)<sup>27</sup> interview with a parent and Conners Parent and Teacher Rating Scales.<sup>19</sup> Behavioral measures for patients were obtained after at least 4 weeks off psychoactive medications. Exclusion criteria were a full-scale WISC-R IQ  $< 80$  and evidence of medical or neurological disorders. Although subjects were excluded if they had any other current Axis 1 psychiatric disorders requiring specific treatment, lifetime comorbid diagnoses were made in 29 patients. These were oppositional defiant disorder ( $n = 12$ ); conduct disorder ( $n = 4$ ); anxiety disorders ( $n = 5$ ); enuresis ( $n = 7$ ); encopresis ( $n = 7$ ); tic disorders not otherwise specified ( $n = 7$ ); specific learning disorders ( $n = 4$ ); and depressive disorder not otherwise specified ( $n = 1$ ). Familial ADHD, defined as the presence of at least one other first-degree relative with ADHD, was present in

**Table 3** Effects of DRD4\*7R allele on behavioral and brain anatomic measures in children with ADHD

	With DRD4*7R			Without DRD4*7R			<i>t</i>	<i>P</i>
	Mean	<i>s.d.</i>	<i>n</i>	Mean	<i>s.d.</i>	<i>n</i>		
Teacher hyperactivity rating <sup>a</sup>	1.39	0.68	17	1.65	0.65	24	-1.25	0.22
Teacher conduct rating <sup>a</sup>	0.27	0.46	17	0.66	0.71	24	-2.03	0.05
Parent hyperactivity rating <sup>a</sup>	2.15	0.94	15	2.23	0.58	24	-0.29	0.77
Parent conduct rating <sup>a</sup>	1.04	0.72	15	1.43	0.60	24	-1.84	0.07
Children's global assessment of functioning <sup>b</sup>	47.4	5.74	17	43.7	6.09	23	1.95	0.06
WISC-R full scale IQ	109.4	14.7	17	113.0	19.9	24	-0.64	0.53
Woodcock-Johnson reading composite standard score	101.7	15.7	16	101.4	18.9	23	0.04	0.97
Woodcock-Johnson math composite standard score	102.0	17.1	16	102.9	18.5	22	0.13	0.89
<i>Anatomic magnetic resonance imaging measures (ml)</i>								
Total cerebral volume	1068	101	15	1090	117	22	-0.61	0.55
Total caudate volume	9.44	1.3	16	9.38	1.2	22	0.13	0.89
Total globus pallidus volume	2.70	0.48	12	2.41	0.45	20	1.72	0.10

<sup>a</sup>Range 0–3, higher scores indicate greater severity.

<sup>b</sup>Range 0–100, lower scores indicate greater severity.

80% (29 of 36; three were adopted and two lacked complete data).

Healthy ( $n = 56$ ) subjects (mean age  $17.6 \pm 9.1$  years (s.d.), range 6–52) were recruited from the community. Screening for all subjects included structured telephone interview and an in-person assessment, which included physical and neurological examinations and structured psychiatric interviews (DICA-R or the Schedule for Affective Disorders and Schizophrenia—Lifetime<sup>28</sup> for adult probands). These were supplemented by parent and teacher rating scales as described elsewhere.<sup>7</sup> Family psychiatric history for first- and second-degree relatives was ascertained from one or both parents or from the proband themselves if they were over 18. Individuals with physical or neurological abnormalities, or a history of psychiatric illness; or those with any first-degree relatives, or greater than 20% of second-degree relatives with major psychiatric disorders, were excluded.

This protocol was approved by the NIMH Institutional Review Board and written informed consent was obtained from all parents or adult probands, with written informed assent obtained from all children. Forty-two of the control subjects were white-non-Hispanic, two were Asian American, nine were African American, and three were Hispanic. Among the ADHD group, 31 were white-non-Hispanic, five were African American, and five were Hispanic. The racial distribution did not differ significantly between groups.

#### Laboratory methods

DNA was extracted from Epstein-Barr transformed lymphoblast cell lines as described elsewhere.<sup>29</sup> The DRD4 exon 3 VNTR polymorphic site was amplified with primers D4-3 (5'-GCG ACT ACG TGG TCT ACT CG-3') and D4-42 (5'-AGG ACC CTC ATG GCC TTG-3') with modifications from the method of Lichter *et al.*<sup>30</sup> PCR reactions were prepared in 25- $\mu$ l volumes, each containing 400 ng DNA, 0.32  $\mu$ M of each primer, 1 $\times$  Buffer No. 5 (Stratagene, La Jolla, CA, USA), 200  $\mu$ M dATP, dTTP, dCTP, 100  $\mu$ M dGTP, 100  $\mu$ M 7-deaza-GTP (Boehringer-Mannheim, Indianapolis, IN, USA), and 2.5 U Taq polymerase (Boehringer-Mannheim) in 10% DMSO. Amplification was performed in a Perkin-Elmer 9600 thermocycler with a 4-min denaturation at 94°C, followed by 40 cycles of 94°C for 20 s, 54°C for 20 s, 72°C for 40 s, with a final extension at 72°C for 10 min. PCR products were electrophoresed on a 2% agarose gel.

Anatomic brain images obtained with the same 1.5 Tesla GE Signa magnetic resonance imaging scanner were available for 38 subjects in each group. A variety of semi-automated and operator-based techniques were utilized to quantify total cerebral volume and the volumes of the caudate and globus pallidus as described elsewhere.<sup>7</sup> All raters were 'blind' to subject characteristics.

#### Statistical analysis

Differences in allele frequency were tested by comparison to the chi-square distribution. Differences in

behavioral ratings, demographic characteristics, and brain morphometric measures between those having and those lacking the DRD4\*7R allele were tested by *t*-test. Significance level for this replication study was set at 0.05 with all comparisons two-tailed.

#### Acknowledgements

Amy Krain, Julie Liu, and Maureen Tobin recruited and screened control subjects. Barbara Keller, PhD and Diana Dahlgren, PhD performed psycho-educational evaluations of patients. Yolanda Vauss, MS obtained cognitive batteries in control subjects.

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Received 24 March 1998; revised and accepted 1 May 1998