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Attention deficit/hyperactivity disorder children with a 7-repeat allele of the dopamine receptor D4 gene have extreme behavior but normal performance on critical neuropsychological tests of attention

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Contributed by Michael I. Posner, February 17, 2000

An association of the dopamine receptor D4 (DRD4) gene located on chromosome 11p15.5 and attention deficit/hyperactivity disorder (ADHD) has been demonstrated and replicated by multiple investigators. A specific allele [the 7-repeat of a 48-bp variable number of tandem repeats (VNTR) in exon 3] has been proposed as an etiological factor in attentional deficits manifested in some children diagnosed with this disorder. In the current study, we evaluated ADHD subgroups defined by the presence or absence of the 7-repeat allele of the DRD4 gene, using neuropsychological tests with reaction time measures designed to probe attentional networks with neuroanatomical foci in D4-rich brain regions. Despite the same severity of symptoms on parent and teacher ratings for the ADHD subgroups, the average reaction times of the 7-present subgroup showed normal speed and variability of response whereas the average reaction times of the 7-absent subgroup showed the expected abnormalities (slow and variable responses). This was opposite the primary prediction of the study. The 7-present subgroup seemed to be free of some of the neuropsychological abnormalities thought to characterize ADHD.

Dopamine plays an important role in normal attention (1) and disorders of attention (2, 3). Recently, this role of dopamine has stimulated molecular genetic studies (4) of attention deficit/hyperactivity disorder (ADHD), the most prevalent psychiatric disorder of childhood recognized in the United States. The dopamine receptor genes (5) have been investigated in other psychiatric disorders (e.g., schizophrenia; see refs. 6 and 7), and the background from this work set the stage for our molecular genetic investigations of ADHD.

In our program of research, we adopted a candidate gene approach, focusing on the dopamine receptor D4 (DRD4) gene on chromosome 11p15.5. This gene has a polymorphism in a coding region—a variable number of tandem repeats of a 48-base pair sequence in exon 3 (8) that codes for variation in the third intracellular loop of the D4 receptor, which may have functional significance. *In vitro* studies suggest that the receptor encoded by the DRD4 7-repeat allele may be subsensitive to endogenous dopamine compared with the receptor encoded by the 2-repeat allele (9), although this apparently is not due merely to the length of the third intracellular loop (10). Initially, in our clinical studies we used population-based (11) and family-based (12) association designs, which suggested that the DRD4 7-repeat allele is associated with ADHD, but with a small relative risk (about 1.5). A review of the recent literature (4) revealed that two independent groups have confirmed this association in children (13, 14), but one group did not (15). The pattern of replication has held up in several other case studies not yet published.

The presence of the DRD4, 7-repeat allele is not a necessary condition (about half of the ADHD cases did not have a 7-repeat allele) (11, 12) or a sufficient condition (about 20% of ethnically matched control subjects did have a 7-repeat allele) (11). This is not surprising because it is generally assumed that ADHD has many causes and that any clinical sample is likely to be heterogeneous rather than homogeneous with respect to etiology (see ref. 16). Still, an etiological role of the DRD4 7-repeat allele is tantalizing, for several reasons, including a long history of dopamine theories of ADHD (see ref. 17); the location of the variable length 48-bp repeat in a coding region of the gene (5–10); and the neuroanatomical foci of the gene product, D4 receptors, in cortical areas including the anterior cingulate gyrus (6), a brain region that plays an important role in normal attention (refs. 18–21; also see ref. 1) and abnormal attention (ref. 22; also see refs. 3 and 16).

In the framework of a multidisciplinary research program, the present study was designed by a cross-national group (XNAT), from the Netherlands, China, Israel, Canada, and multiple sites in the United States. There were two specific aims of this XNAT project: to select a theoretically based set of neuropsychological tests to distinguish ADHD and control subjects on quantitative measures of attention and to use the selected test battery to contrast patterns of abnormalities in subgroups of ADHD children defined by the presence and absence of the DRD4 7-repeat allele.

Background

The diagnosis of ADHD is based on subjective reports by parents and teachers of symptoms of inattention, hyperactivity, and

Abbreviations: ADHD, attention deficit/hyperactivity disorder; DRD4, dopamine receptor D4; ODD, oppositional defiant disorder; RT, reaction time; TDT, transmission disequilibrium test.

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impulsivity (23). In the U.S., this diagnosis usually results in pharmacological treatment with stimulant medication, such as methylphenidate or amphetamine. Currently, ADHD is diagnosed and treated with stimulant medications in about 2 million children per year in the U.S. and less frequently but increasingly around the world (ref. 24; also see ref. 16 for a review).

A major site of action of stimulant drugs is the dopamine synapse (see refs. 5 and 6). Amphetamine and methylphenidate stimulate the release and/or block the re-uptake of dopamine (see refs. 25 and 26), which increase the levels of extracellular dopamine in the synaptic space, although presynaptic regulation may change this over time (27). At clinical doses, this results in decreased activity, inattention, and impulsivity (i.e., decreased symptoms of ADHD). These pharmacological properties of the stimulants were influential in the development of site-of-action theories of ADHD, which focus on possible abnormalities in dopamine pathways of the brain and suggest that the stimulants may correct or compensate for the core deficits of the disorder (see ref. 17; also see refs. 3 and 16). In previous reviews of the neuroanatomical and molecular genetic bases of ADHD (2, 4, 16, 28), we proposed a variant of the dopamine theory of ADHD based on reduced activity in the mesolimbic dopamine pathway due to multiple factors, including the possibility that the DRD4 7-repeat allele might code for subsensitive DA receptors in the frontal lobes and produce underactivity in the neural networks involved in executive functions (3, 20, 29).

Because there are so many (>50,000) genes expressed in brain, it is typically assumed that an initial report of an association with any one gene will be a false positive (30). However, when multiple replications of the same association are reported with family-based association methods, the assumption of a false positive association is discounted. In the literature, multiple replications of a candidate gene association for a psychiatric disorder is unusual (see ref. 30), but this has occurred for the DRD4 gene in studies of ADHD (see ref. 4).

This success may be due to the use of relevant theories [i.e., the neuroanatomical network theory (1, 31) and the DA theory of ADHD (17)], which led us to select DRD4 as a candidate gene. These theories also suggest investigations of performance on specific neuropsychological tests that depend on function of D4-rich brain regions. In the present study of children with ADHD, we hypothesized that, in comparison to a control group, the 7-present subgroup would show larger abnormalities than the 7-absent subgroup in performance of the selected neuropsychological tests of attention. The XNAT group developed a protocol that was approved by the Institutional Review Board of the University of California at Irvine for a study of neuropsychological and molecular genetic bases of ADHD.

Methods

Our ADHD subjects were drawn from a cohort of ADHD subjects ($n = 96$) and control subjects ($n = 48$) at the University of California at Irvine, one of the six sites of the Multimodality Treatment Study of Children with ADHD (MTA); (32). The ADHD children were diagnosed by psychiatric interviews and questionnaires about psychopathological behavior that are components of a research assessment battery for ADHD (see ref. 33). The inclusion criteria for our clinical subjects included a DSM-IV diagnosis of ADHD-Combined Type, including the endorsement of at least six of nine symptoms of inattention and six of nine symptoms of hyperactivity/impulsivity. The control subjects were unscreened volunteers from classrooms in the same schools and grades of the ADHD subjects in the MTA study. The volunteer rate in each classroom was over 50% of the enrolled students, with one control child selected from randomly selected classrooms. A restriction on gender was imposed to provide matching to the ADHD group for the percentage of males and females.

Table 1. Demographics, ADHD symptoms, and psychometric test scores of the groups

	ADHD, $n = 44$	Control, $n = 21$
Demographics		
Age, months	141.2	134.3
% Medicated	54.5	0.0
% Male	79.5	81.0
% Caucasian	68.2	71.4
% Non-Black Hispanic: Mexican	06.8	14.3
% African-American	02.3	00.0
% Asian: Japanese	00.0	04.8
% Other	22.7	09.5
Parent-SNAP		
Inattention	2.3	0.4
Hyperactivity/impulsivity	2.1	0.2
ODD	1.6	0.3
Teacher-SNAP		
Inattention	2.3	0.7
Hyperactivity/impulsivity	2.2	0.2
ODD	1.5	0.4
Psychometric tasks		
WIAT reading	98.7	113.9
WIAT math	98.7	112.0
WIAT spelling	97.4	110.0
WISC block design	10.9	11.9
WISC vocabulary	10.3	11.9

To participate in the present study, in addition to the MTA consent process, written consent of parents and assent of children was obtained from 44 ADHD children and 21 control children to undergo 2 days of neuropsychological assessment in the XNAT protocol. As part of the MTA assessment, extreme parent and teacher ratings (0, "not at all"; 1, "just a little"; 2, "pretty much"; and 3, "very much") of ADHD items on the Swanson, Nolan, and Pelham (SNAP) rating scale (33) were required. The average rating per item can be used to document severity of symptoms in both domains for ADHD (inattention and hyperactivity/impulsivity), and a SNAP score above 2.0 is considered severe. Ratings were also obtained for another disruptive behavior disorder of childhood, oppositional defiant disorder (ODD), that is often comorbid with ADHD. As expected, the overall ADHD group had higher average symptom ratings than the control group for parent ratings of inattention (2.27 vs. 0.43) and hyperactivity/impulsivity (2.07 vs. 0.21), as well as for ODD (1.61 vs. 0.35). The teacher ratings also verified symptom severity of inattention (2.32 vs. 0.65), hyperactivity/impulsivity (2.0 vs. 0.35), and ODD (1.45 vs. 0.21). Only children in the ADHD group (57%) were being treated with stimulant medication during the time when they participated in this study, but children being treated with stimulants did not take medication for at least 24 h before the administration of the neuropsychological tests. Because of the recruitment strategy of the MTA study, the ADHD group was slightly but significantly older than the control group (141 vs. 134 months). No significant differences were found (see Table 1) when the ADHD and control groups were compared on gender (80% of the ADHD and 81% of the control group were boys) and ethnicity (e.g., 68% of the ADHD group and 71% of the control group were Caucasian).

To address our first aim, we selected three neuropsychological tasks for the XNAT battery. Directed by the neuroanatomical network theory of attention proposed by Posner and Raichle (1, 31), we chose tasks to probe the functions of three brain regions implicated in the attentional deficits in ADHD (anterior cingulate, right dorsolateral prefrontal, and posterior parietal). We

also used controlled experimental procedures to maximize the degree to which the ADHD subjects were cooperating and following the instructions of these tasks, which has been crucial in neuropsychological evaluation of other patient groups (see ref. 34). This was accomplished by making frequent checks of subjects while they were performing the tasks (with redirection provided by the experimenter, if necessary) and by giving frequent rest periods.

We administered the following tasks: (*i*) a color-word task (35) to probe the executive function network linked to anterior cingulate brain regions and to conflict resolution (20); (*ii*) a cued-detection task (36) to probe the orienting and alerting networks linked to posterior parietal and frontal brain regions and to shifting and maintenance of attention (37); and (*iii*) a go-change task (38, 39) to probe the alerting network (and the ability to initiate a series of rapid response in a choice reaction time task), as well as the executive network (and the ability to inhibit a response and re-engage to make another response) (40). Within-task conditions were designed to probe hypotheses about specific attentional deficits of ADHD children, but an overall measure of performance was developed (see below) and used in this paper.

To address the second aim of the XNAT project, we genotyped as many of our ADHD subjects as possible. Of these 44 ADHD families recruited and consented for the neuropsychological assessment, 32 also provided written consent and assent for the collection of blood samples for genetic analysis. DNA was extracted, and PCR assays previously reported (see refs. 7, 8, 11, and 12) were used to amplify the 48-bp VNTR of the DRD4 gene. Of the 32 ADHD subjects, 40.6% (13/32) had at least one 7-repeat allele and were assigned to the 7-present subgroup, and 59.4% (19/32) did not have a 7-repeat allele and were assigned to the 7-absent subgroup. The percentage of ADHD subjects with the 7-present genotype was slightly lower than the percentage (about 50%) reported in our previous studies (11, 12).

Results

To address our first specific aim, we compared the performance of the total ADHD group ($n = 44$) and the control group ($n = 21$) on the color-word, cued-detection, and go-change tasks. We used an overall summary score to reflect the average speed across all of the specific conditions of each task, and for each task we calculated the average reaction time (RT) and standard deviation (SD) of RT for each subject. A simple t test (equal variances not assumed) was used to contrast the groups on each of two summary scores (RT and SD) for each task.

The sensitive measures of speed (RT) and variability (SD) of performance revealed large group differences (see Fig. 1). Across all conditions of each task, the ADHD group was slower (longer mean RTs) and more variable (greater within-subject SD of RTs) than the control group. For each ADHD versus control group comparison on RT and SD summary scores, the t tests were significant. In addition to performing a significance test, the magnitude of the ADHD-control group differences were estimated by calculating effect sizes (the standardized mean difference between groups) for the RT and SD summary scores, which varied from moderate to large across the three tasks in the XNAT battery: color-word, RT = 0.49 ($P = 0.054$) and SD = 0.72 ($P = 0.005$); cued-detection, RT = 0.68 ($P = 0.009$) and SD = 0.89 ($P = 0.001$); go-change, RT = 0.86 ($P = 0.002$) and SD = 0.80 ($P = 0.003$).

These findings confirm the sensitivities of the quantitative traits measured by the XNAT battery, at least for contrasts of a refined phenotype of ADHD used for the MTA study (severe cases with the combined type) and a control group. These data do not support the view that these ADHD children have a hasty style of responding that results in fast and inaccurate (i.e., impulsive) responses; rather, they suggest that ADHD children have an inefficient style of responding that results in slow and inaccurate (i.e., inefficient) responses.

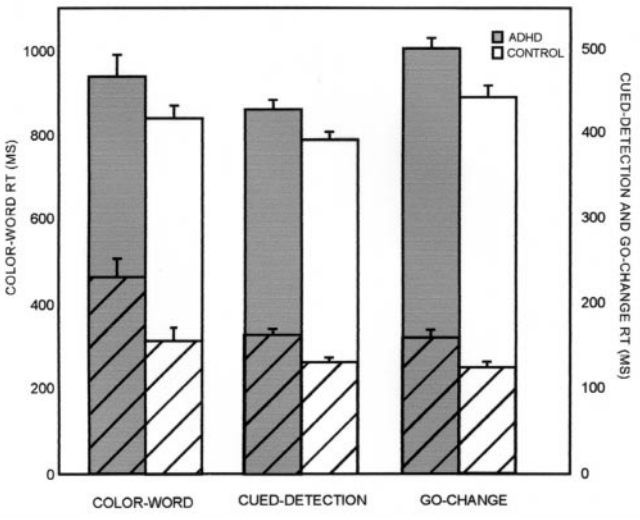

Fig. 1. Comparison of total ADHD group and the control group on the XNAT battery

To address the second specific aim, we compared the 7-present ($n = 13$) and 7-absent ($n = 19$) subgroups defined by the DRD4 7-repeat genotype. These two subgroups did not differ on the severity of ADHD symptoms of inattention or hyperactivity/impulsivity, which is not surprising, because the entry criteria for the MTA study required that all subjects have severe symptoms of ADHD. Also, the 7-present and 7-absent subgroups did not differ in terms of average age, intellectual ability (as measured by two subtests of an IQ test), academic achievement, medication treatment, gender ratio, or ethnicity. The 7-present subgroup did have somewhat higher ratings of ODD, but these subgroup differences were not significant at $P < 0.05$.

The distributions of the RTs for the three tasks are presented in Fig. 2, and the mean RTs and SDs for the subgroups are presented in Table 2. Instead of showing the predicted abnormality in performance on these neuropsychological tasks (i.e., longer RTs and greater SDs), the subjects in the 7-present subgroup showed no significant abnormalities when compared with the control group. The 7-absent subgroup, on the other hand, showed, on average, longer RTs and greater SDs. Inspection of the distributions of RTs showed that, in the 7-absent subgroup, depending on the task, 2–5 of the 19 subjects (about 17%) had RTs that did not overlap with the RTs of the control group.

Because we predicted that the 7-present subgroup would show the greatest abnormalities, any directional (one-tailed) test would be nonsignificant. However, to clarify the observed group differences, we performed post hoc or exploratory analyses using two-tailed tests. To avoid the loss of power associated with multiple tests and to gain reliability by the Spearman-Brown method of combining tests, an ANOVA framework was used with independent variables specified by Group (7-present, 7-absent, and control) and Task (color-word, cued-detection, and go-change). An analysis was performed separately for the two summary scores from each subject (RT and SD).

The Group × Task ANOVA of RT revealed significant main effects of Group [$F(2, 46) = 3.68$, $P = 0.033$] and Task [$F(2, 92) = 134.88$, $P < 0.001$], but the Task × Group interaction was not significant [$F(4, 92) = 2.09$, $P > 0.089$]. The Group marginal means (average RT across tasks) were compared by two-sided Dunnet's t tests (41) for comparison of the two ADHD sub-

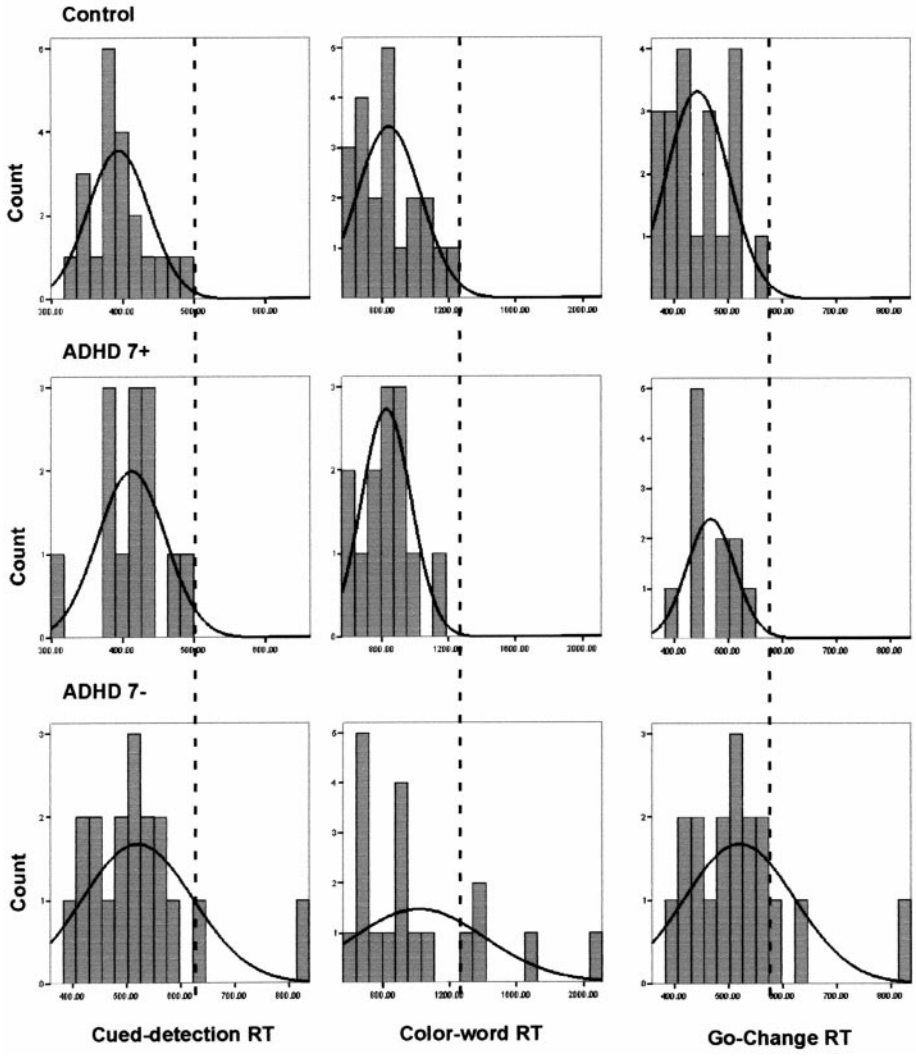

Fig. 2. RT distributions for control, 7-present, and 7-absent groups.

groups to a common control. In our exploratory analysis, this procedure was selected to maximize power by including the largest group (the control group) in each comparison, instead of making a comparison of the smaller ADHD subgroups. The overall difference in RT was significant for the comparison of the 7-absent subgroup to the control group (mean difference = 100 ms, standard error = 40, $P = 0.033$), but the comparison of the 7-present subgroup and the control group was not significant (mean difference = −0.8 ms, standard error = 47, $P = 1.00$). (Because of the smaller sample sizes, the ADHD subgroups differed significantly by one-tailed but not two-tailed tests).

Table 2. Mean RTs and SDs for the summary scores of the neuropsychological tasks of the XNAT battery

	ADHD 7-absent		ADHD 7-present		Control	
	Mean	SD	Mean	SD	Mean	SD
Color-word	1014.5	410.8	828.9	158.6	839.2	197.6
Cued-detection	435.9	86.1	412.4	54.9	394.1	49.1
Go-change	520.7	147.6	468.4	129.1	444.5	113.7
Average	657.0	214.8	569.9	114.2	559.3	120.1

The Group × Task ANOVA of average SD also revealed significant main effects of Group [$F(2, 46) = 4.27$, $P = 0.02$] and Task [$F(2, 92) = 59.50$, $P < 0.001$]. The Group × Task interaction was also significant [$F(4, 92) = 2.71$, $P = 0.035$]. The Dunnet's t tests for comparison to a common control revealed that the 7-absent subgroup differed significantly from the control group for average SD (mean difference = 78.8, standard error = 28.2, $P = 0.015$), but that the 7-present subgroup did not (mean difference = 11.8, standard error = 32.6, $P = 0.913$).

Discussion

This study indicates that the 7-present genotype is not a necessary condition for the manifestation of cognitive abnormalities thought to be characteristic of children with DSM-IV diagnoses of ADHD-combined type. Although the 7-absent subgroup shows clear abnormalities in performance on these neuropsychological tests of attention, the 7-present subgroup was free of critical neuropsychological abnormalities thought to characterize children with psychiatric (DSM-IV) diagnoses of ADHD. This suggests that the 7-repeat allele may identify a subgroup with the behavioral but not the cognitive components of ADHD. These findings led us to reconceptualize the possible association of the DRD4 gene with ADHD and to align our clinical finding with the literature on associations of the DRD4 gene with

normal attention and behavior (for a critical review, see ref. 42). According to this speculation, the 7-repeat allele of the DRD4 gene may be associated with extreme placement on a dimension of personality (e.g., extraversion) or proposed dimensions of temperament, such as novelty seeking (43, 44) or effortful processing (see ref. 45). These children may be easily bored in the absence of highly stimulating conditions (see ref. 46), may show delay aversion and choose to avoid waiting (47), may have a style difference that is adaptive in some situations (48), and may benefit from high activity levels during childhood (e.g., ref. 49).

In contrast, for a complex disorder like ADHD, we suggest that the 7-absent subgroup is most certainly nonhomogeneous, and composed of individuals with other genetic abnormalities or nongenetic etiologies. These could include (*i*) other alterations in the highly variable DRD4 gene itself (see ref. 8) not analyzed in the current study, (*ii*) alterations in other relevant genes such as DAT1, (see ref. 2), and (*iii*) minimal brain damage (MBD) or dysfunction (50–55). We propose that these other etiologies produce both the behavioral abnormalities reflected as symptoms of ADHD and the cognitive abnormalities reflected by longer RTs and SDs in the neuropsychological tests of the XNAT battery. For example, the observed cognitive abnormalities (i.e., slow and variable responses) are very common sequelae of brain injury (56) that may in some instances produce the symptoms of ADHD (57).

Of course, there are many limitations of this initial study. We will list a few of these, which provide direction for our next steps in the XNAT research program on ADHD. First and primary is the small sample size for the ADHD subgroups. Despite a relatively large number of subjects in the total ADHD group ($n = 44$), the numbers dwindled because of less than unanimous consent for a blood sample (only 32/44 or about 75%). Even though we have a consistent pattern of performance in our sample, we realize it is likely that this pattern may be different in another sample of ADHD children, and some informal comparisons of 7-present and 7-absent subgroups in typical ADHD samples have not duplicated our results (S. Smalley, personal communication; X. Castellanos, personal communication). However, our sample size is comparable to the sample sizes in the initial candidate gene studies of Alzheimer's disease, which contrasted groups on quantitative traits. In a study of glucose metabolism as a quantitative trait that showed the earliest decline in Alzheimer's patients, Reiman *et al.* (58) screened 235 subjects and accumulated only 11 e4 homozygotes. This small group was compared with a group of 22 control subjects. Plassman *et al.* (59) contrasted only 6 subjects with the e4 genotype with 14 subjects without an e4 allele in a study of hippocampal volume as a quantitative trait. As in these extraordinarily important studies of Alzheimer's disease, the analyses of our small samples of ADHD subjects should be considered exploratory. The primary use of the findings reported here should be to generate hypotheses to be tested by planned comparisons in future studies.

Second, the refined ADHD phenotype we used to select subjects may limit the generalization of this study. The MTA entry criteria (32, 33) selected subjects with ADHD-combined type, with onset of symptoms by the age of 7 years, and with severity of symptoms documented by parent and teacher ratings and confirmed by parent interviews (structured and clinical). Thus, our results may not hold for the other ADHD subtypes (inattentive type or hyperactive/impulsive type) or to ADHD subjects with less pervasive or severe expression of symptoms (see refs. 11 and 12).

Third, in this study, the comparison of the 7-present and 7-absent subgroups was not conditional on the parental genotypes. Even though our data suggest that these DRD4 genotypes may be useful in forming more homogeneous subgroups of children with ADHD, it is possible that the 7-present and 7-absent subgroups differ on other factors than the status of the DRD4 7-repeat allele. The quantitative trait extension of the transmission disequilibrium test (TDT) (e.g., ref. 60) provides a way to control for population stratification and other unknown factors. For the comparison of group performance on RT and SD measures derived from the XNAT battery of neuropsychological tests, a quantitative TDT would provide additional controls. For example, Allison's TDT-Q5 statistic uses genotype as an independent variable, as we did in our analyses, but includes only those subjects who are offspring of heterozygous parents with a 7-repeat allele. Because almost all probands in the 7-present subgroup are informative (there are very few 7-repeat homozygotes), the limiting factor is the number of probands in the 7-absent subgroup who had parents with a 7-repeat allele that was not transmitted. Therefore, a large sample, such as the total MTA sample of 579 ADHD subjects, would be necessary to accumulate a modest subsample of the informative cases for a TDT evaluation of the hypothesis that these genotypes differ in performance on tests of attention.

Fourth, a number of other genes have been implicated in ADHD, including the dopamine transporter gene (2) and other dopamine receptor genes (42). The independent or interactive effects (see ref. 60) of multiple genes deserve investigation.

It is clear that additional studies will be needed to understand the molecular bases of ADHD. Some investigators are using genome scans and linkage methods in the hope of identifying other genomic regions associated with ADHD. We are focusing on direct DNA sequence analysis of the DRD4 gene region, in an attempt to determine whether specific variants of this highly polymorphic gene (5–10) play important roles in the etiology of ADHD.

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