Association between DRD4 Gene and Performance of Children with ADHD in a Test of Sustained Attention

Christian Kieling, Tatiana Roman, Alysa E. Doyle, Mara Helena Hutz, and Luis Augusto Rohde

**Background:** The adoption of neuropsychological tests as endophenotypic measures can provide an increased sensitivity to specific dimensions of attention-deficit/hyperactivity disorder (ADHD).

**Methods:** The association between a variable number of tandem repeats polymorphism at the dopamine D4 receptor gene (DRD4) and the performance of children and adolescents with ADHD in a continuous performance test (CPT) was evaluated. The sample comprised 90 clinically referred children and adolescents with ADHD. Errors of omission and commission in the CPT were computed and the number of 48-base pairs tandem repeats in the exon III of DRD4 was assessed.

**Results:** The presence of a 7-repeat allele was associated with more errors of commission and omission even after adjusting for age. The presence of a 7-repeat allele was associated with more errors of commission and the homozygosity of the 4-repeat allele was related to fewer errors of commission and omission even after adjusting for age.

**Conclusions:** These findings bring further evidence on the role of DRD4 polymorphisms on the performance in sustained attention tasks among children and adolescents with ADHD diagnosis.

**Key Words:** Attention-deficit/hyperactivity disorder, child, continuous performance test, dopamine D4 receptor, genetics, neuropsychology

The molecular basis of attention-deficit/hyperactivity disorder (ADHD) still remains unknown, despite its high heritability estimates (Biederman and Faraone 2005). This may be due to its complex nature, both at the genotypic (multiple genes) and the phenotypic (phenomenological heterogeneity) levels. Supported by neurobiological findings, the most investigated neurotransmitter system is the dopaminergic (Bobb et al 2005). Although there is no clear consensus, a meta-analysis conducted by Faraone et al (2001) evidenced a significant role for the dopamine D4 receptor (DRD4) gene, specifically the 7-repeat allele of the exon III variable number of tandem repeats (VNTR), in the etiology of ADHD.

One hypothesis that could explain the lack of consistency in the literature is the great variability in the phenotypic definition of ADHD based on DSM-IV criteria (American Psychiatric Association 1994). The augmentation of categorical descriptions by quantitative measures strengthens the assessment of genetic underpinnings, affording more straightforward association studies (Gottesman and Gould 2003; Sergeant 2005).

The adoption of neuropsychological tests as endophenotypic measurements might enhance the power of genetic studies since it may provide an increased sensitivity to specific dimensions of ADHD (Doyle et al 2005). Thus far, only a small number of studies have assessed the role of DRD4 polymorphisms and performance on neurocognitive measures in individuals with ADHD. Results have been variable, with one study showing an association between the DRD4 7-repeat allele and an impulsive, error-prone response style (Langley et al 2004) and two studies showing greater inattention, impulsivity, and reaction time anomalies in individuals without the 7-repeat allele (Swanson et al 2000; Manor et al 2002).

Continuous performance tests (CPTs) assess the maintenance of focused attention while responding to target stimuli and the inhibition of responses to non-target stimuli. In a recent meta-analytic review, Frazier et al (2004) showed that CPT scores achieved the largest effect size for the diagnosis of ADHD. The aim of this study was to evaluate the association between the most investigated candidate gene for ADHD (DRD4) and performance on a CPT in a large, well-characterized sample of children and adolescents from Brazil.

**Methods and Materials**

**Subjects**

The sample consisted of 90 children and adolescents referred to the ADHD outpatient clinic at our University Hospital (HCPA). Inclusion criteria were: 1) age between 7 and 17 years; 2) ADHD diagnosis according to DSM-IV; 3) drug naïve patients; and 4) IQ ≥ 70. The only exclusion criterion was the presence of clinical conditions that might interfere with the test, including blindness, deafness, pervasive developmental disorder, and other major neurological disorders. No child, however, met such exclusion criteria. ADHD and comorbid diagnoses were achieved through a three-stage process: 1) evaluation with a semi-structured interview (Schedule for Affective Disorders and Schizophrenia for School-Age Children, Epidemiological Version – K-SADS-E; Orvaschel 1985), modified to assess DSM-IV criteria and applied to parents by trained research assistants, whose inter-rater reliability for the ADHD diagnosis had been previously evaluated (kappas from .77 to 1.00, p < .001); 2) discussion of each diagnosis derived through the K-SADS-E in a clinical committee chaired by a senior psychiatrist (LAR); and 3) clinical evaluation of ADHD and comorbid conditions using DSM-IV criteria by a child psychiatrist who had previously received the K-SADS-E results. When a diagnostic disagreement occurred in the three-stage process, priority was given for diagnoses obtained through clinical interviews (Rohde et al 2005). Severity of ADHD symptoms was assessed with the Swanson, Nolan, and Pelham (SNAP-IV) rating scale (Swanson et al 2001). Parents provided written informed consent and children or adolescents gave verbal assent to participate. The Ethical Committee of HCPA approved this investigation.
Neuropsychological Assessment

Full Scale IQ was estimated from vocabulary and block design subtests of the Wechsler Intelligence Scale for Children–Third Edition (Wechsler 1991), administered by trained psychologists. Subjects performed a simplified version of the CPT (Cornblatt et al 1999). Testing was implemented using standardized procedures, including same software and raters. A series of numbers, from 1 to 9, appeared randomly, one at a time, with a fixed interval, on the computer screen. Subjects were required to press a button whenever the number 6 appeared. Omission (missed targets) and commission (incorrect targets) errors were computed and adjusted respectively for the number of times the selected and other digits appeared.

Genotyping

High molecular weight genomic DNA was extracted from whole blood by a salting-out procedure (Lahiri and Nurnberger 1991). The DRD4 exon III region containing the 48-base pair VNTR polymorphism was amplified by polymerase chain reaction as described by Roman et al (1999).

Data Analysis

Comparison among categorical variables was performed using chi-square or Fisher’s exact test. All continuous variables showing a normal distribution were compared between the groups by Student’s t-test; for those variables (e.g., CPT measures) that did not show a normal distribution, non-parametric tests were used (Mann-Whitney U test). The significance level accepted was .05. Potential confounders evaluated were age, sex, ethnic background, ADHD subtype, severity of symptoms (SNAP-IV scores), IQ, setting for data collection, and comorbidity (mood, anxiety, and disruptive behavior disorders). A confounder was defined as a variable associated with both independent and dependent variables (p ≤ .2). Only age, despite significant overlap between genotypic groups, met the criteria for a confounder and was included in adjusted analyses. In order to obtain a normal distribution, CPT scores were ranked before inclusion in the analysis of variance (Montgomery 1997). All tests were two-tailed.

Results

Sample Characteristics

Ninety children and adolescents were assessed. Mean age was 10.9 (SD = 2.8) years, and mean IQ was 94 (SD = 12). Most of the sample was of European ancestry (95.2%) and males (74.4%). The ADHD combined type was the most prevalent (69.7%), and oppositional defiant disorder was the most common comorbid condition (41.1%). Subgroup analyses according to genotype showed that mean age was 9.77 years (SD = 2.66) for children in the 7-repeat group and 11.34 (SD = 2.79) for those without the allele. Children with two 4-repeat alleles had a mean age of 11.35 years (SD = 2.72) while those without homozygosity had a mean of 10.36 (SD = 2.87).

Neuropsychological Performance

There were differences on CPT scores among different genotypic groups after adjusting for age (Table 1). The presence of a 7-repeat allele was associated with more commission errors (F = 4.55, df = 1, p = .036). In the same direction, subjects with two copies of the 4-repeat allele made fewer errors of both commission (F = 5.94, df = 1, p = .017) and omission (F = 5.23, df = 1, p = .025). Severity of symptoms (SNAP-IV scores), albeit not fulfilling statistical criteria for a potential confounder, was included in additional analyses and did not significantly affect any results (data available upon request).

Discussion

Our data suggest an association between the DRD4 genotype and CPT performance in children and adolescents with ADHD. Youths with one or more 7-repeat alleles gave more impulsive responses, while 4-repeat homozygosity was associated with reduced errors of both inattention and impulsivity. Neurochemical mechanisms may underlie such contrast, given that the receptors expressed by the non–4-repeat alleles show a suboptimal response to dopamine (Wang et al 2004).

The present work should be interpreted in the context of some limitations. First, a small number of neuropsychological outcomes were evaluated. Second, we cannot exclude haplotype heterogeneity among alleles with the same number of repeats. However, the robustness of our findings is supported by the conservative data analyses and the fact that ours is a drug-naïve sample. The impact of medication is a potential confounder not frequently considered in previous studies.

Our results provide a constructive replication of a previous study (Langley et al 2004) and go a step further to identify a significant difference in CPT performance according to DRD4 status within the ADHD diagnosis. We not only evaluated the

Table 1. Scores for Errors of Omission and Commission in the Continuous Performance Test According to DRD4 Genotype

<table>
<thead>
<tr>
<th></th>
<th>Score</th>
<th>Unadjusted Analysis</th>
<th>Adjusted Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>Omission errors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>absent</td>
<td>64</td>
<td>.073</td>
<td>.911</td>
</tr>
<tr>
<td>4-repeat homozygosity</td>
<td>48</td>
<td>.070</td>
<td>.911</td>
</tr>
<tr>
<td>present</td>
<td>42</td>
<td>.192</td>
<td>.875</td>
</tr>
<tr>
<td>Commission errors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-repeat allele</td>
<td>26</td>
<td>.019</td>
<td>.100</td>
</tr>
<tr>
<td>absent</td>
<td>64</td>
<td>.006</td>
<td>.165</td>
</tr>
<tr>
<td>4-repeat homozygosity</td>
<td>48</td>
<td>.006</td>
<td>.165</td>
</tr>
<tr>
<td>present</td>
<td>42</td>
<td>.012</td>
<td>.100</td>
</tr>
</tbody>
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aCorrection for age.

DRD4, Dopamine D4 Receptor.
impact of the 7-repeat allele, but also demonstrated the protective effect of the 4-repeat allele. These findings add further evidence on the role of DRD4 polymorphisms on the performance of children and adolescents in sustained attention tasks that cannot be explained by the severity of the ADHD symptoms.

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