Genetic interaction analysis for DRD4 and DAT1 genes in a group of Mexican ADHD patients


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ABSTRACT

Attention deficit hyperactivity disorder (ADHD) is a clinically complex and multifactorial psychiatric disorder of inattention, hyperactivity and impulsivity. Family, twin and adoption studies suggest a genetic influence in the etiology of ADHD. Two variable number of tandem repeats (VNTR) polymorphic systems have been frequently associated with this disorder: the 7 repeat (R) allele in exon 3 of the dopamine receptor D4 (DRD4) and the 10R allele located in the 3′ untranslated region (UTR) of the dopamine transporter (DAT1). We conducted a case–control association study between ADHD and these polymorphisms in a group of adolescent inhabitants of the metropolitan area of Mexico City. In addition, we evaluated the interaction between these genes, the disorder and its associated psychiatric comorbidities. No positive association between ADHD and the 7R allele of DRD4 or the 10R allele of DAT1 was observed; however, compared to controls, patients with internalized comorbidities had a lesser frequency of genotypes with the 7R allele of DRD4 and the 10/10 genotype of DAT1. A logistic regression analysis showed that the simultaneous absence of the 10/10 DAT1 and 7/7 DRD4 genotypes predicts membership to the group of ADHD patients with internalized comorbidities (e.g. anxiety, depression). Our results highlight the importance of cross-ethnic research and the possibility of a distinct genetic basis that underlies the type of comorbidities associated with ADHD. This result should be considered in terms of the study design, and further replication is necessary in an independent sample.

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Attention-deficit hyperactivity disorder (ADHD) is a common childhood-onset psychiatric syndrome of hyperactivity, impulsivity and impaired sustained attention. This disorder has a prevalence of 4–12% in the school-aged population with 50–80% of patients experiencing symptoms that persist through adolescence and into adulthood [34]. Social dysfunction and skill deficits associated with ADHD may have a significant impact on the labor and academic life of affected individuals [18]. It has been estimated that 20–55% of ADHD patients express other externalized psychiatric disorders (e.g. conduct disorder, oppositional defiant disorder), whereas approximately 10–50% express internalized (e.g. anxiety, depression) comorbidities, leading to the conceptualization of ADHD as a clinically heterogeneous entity [2,17,34]. Furthermore, it has been reported that these characteristics are aggregated in families [7], leading to some authors to propose that the study of these comorbidities may help in the classification of different ADHD subtypes [7,13,22].

Attention-deficit hyperactivity disorder is regarded as a heritable, complex and multifactorial disorder that involves the interaction of biological and environmental factors [1,36]. Pharmacological studies, animal models and brain images have all demonstrated that catecholamine neural pathways are relevant for understanding the neurobiological basis of this disorder. Specifically, it has been proposed that alterations in the cerebral dopamine system partially explain the cardinal symptoms and effectiveness of pharmacological treatments [5,15,39]. Thus, genes encoding dopamine-related proteins have been evaluated in relation with ADHD [12,15,24].

In particular, two polymorphic systems have been the focus of many published studies: a variable number of tandem repeats (VNTR) in exon III of the dopamine D4 receptor gene (DRD4) and the VNTR located on the 3′ untranslated region (3′-UTR) of the dopamine transporter gene (SCL6A3 or DAT1) [15,16,36]. Several metaanalyses have concluded that the 7 repeat (R) allele of DRD4 yield a small but statistically significant effect towards the risk of...
developing ADHD [15,28,30]. The evidence suggesting an association with the 10R allele of DAT1 is less consistent; one metaanalysis supports this association [40] while others do not [28,30].

It has also been suggested that ADHD is polygenic in nature [15,18]. For this reason, interaction analyses may be used as a way of better understanding how genes influence this disorder. To date, only a few reports have explored the genetic interaction between DRD4 and DAT1, as most studies have evaluated the independent effect of these genes [10,19,32,33].

In this way, the aim of the present study was to explore the possible interaction among the aforementioned genes, ADHD, and the psychiatric comorbidities associated with this disorder.

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics and Scientific Committees of the National Psychiatric Institute “Ramon de la Fuente” (INPRF) in Mexico City. All procedures were performed with the adequate understanding and written consent of the subjects.

One hundred and five individuals (12–18 years old) who met diagnostic criteria for ADHD were recruited from the Clinic for Adolescents at the INPRF.

A three-stage process was used to establish the ADHD diagnosis. The initial step included a clinical screening for any current psychiatric disorder through the Mini International Neuropsychiatric Interview for children and adolescents (M.I.N.I. KID), a structured interview that renders a presumptive diagnosis of mental disorders based on Diagnostic Statistical Manual IV Revised (DSMIV-R) criteria. Subsequently, a certified psychiatrist by way of a clinical interview confirmed the diagnosis and documented other relevant clinical issues of the patient. Lastly, a definitive diagnosis of ADHD and associated comorbidities was obtained through the application of the Brief Psychiatric Rating Scale for Children (BPRS-C) by a child/adolescent psychiatrist. It is important to mention that the parents of probands were also interviewed at the second and third stages of evaluation in order to corroborate the clinical data.

Participants were excluded if the expressed symptoms were better explained by the presence of another psychiatric disorder, specifically pervasive developmental disorder.

The patients were divided into categories according to the comorbidities identified: externalized, including patients with oppositional defiant disorder, conduct disorder and substance abuse (n = 48); and internalized, including those expressing at least one internalized disorder such as dysthymia, major depressive disorder, phobic and anxiety disorders (n = 53). Five patients did not present any comorbidity.

The control group included participants from a recent epidemiological survey of mental health in adolescents developed by the INPRF [6]. Three thousand and five subjects (between the ages of 12 and 18) living in the metropolitan area of Mexico City were interviewed face to face by personnel trained in the use of the Adolescent-Composite International Diagnostic Interview-Computer Assisted Personal Interview (CIDI-CAPI). This inquiry generates a Psychiatric diagnosis based on the DSM IV-R and ICD-10 (International Statistical Classification of Diseases and Related Health Problems) criteria. The 1261 participants who did not fulfill diagnostic criteria for a psychiatric disorder were categorized as non-cases. From the 883 DNA samples available, we chose the first 105 that were age and sex matched with the case group. Eleven additional samples (10% of the sample) were randomly selected, sequenced and included to minimize the loss of information due to genotyping problems.

In addition, it was possible to obtain blood samples from the parents of 30 probands, which were subsequently analyzed in a family association study.

Blood samples from the patients were drawn by venipuncture and DNA was extracted by a conventional phenol-chloroform method. For the control group, mouthwash samples were obtained and DNA was extracted using the Puregene DNA purification Kit (GENTRA).

Amplification of the DRD4 exon 3 VNTR was performed using conditions reported by our group [3] and primers described by Lichter [29]. The DAT1 3′-UTR VNTR was performed as reported by Kang et al. [23]. Genotyping was determined through agarose gel electrophoresis. In order to assure the correct length of the alleles, bands were compared with standards of known molecular weight and/or with the group of samples previously sequenced. Gels were read in a blind fashion by two different evaluators. Almost all samples were genotyped at least twice in order to ensure their correct identification; doubtful genotypes were excluded from the analysis.

Hardy–Weinberg equilibrium (HWE) was tested with the HW subroutine of the genetic LINKAGE program [31].

Allele and genotype frequencies were analyzed with χ²-test. A logistic regression analysis was used to evaluate the independent and interactive effect of the simultaneous absence of the 10/10 genotype of DAT1 and 7/7 genotype of DRD4 in ADHD patients and associated comorbidities. These analyses were run with SPSS software (v. 11). Bonferroni correction was then carried out for positive results and the significance level was set at 0.025 (0.05/2, two polymorphic systems).

Finally, the transmission disequilibrium test (TDT) was performed using the 1.7.3 version of the FBAT program [26].

Table 1 shows allelic frequencies for both polymorphic systems. As expected, genotype frequencies in control subjects were in HWE (DRD4: χ² = 4.18, d.f. = 15, p = 0.997; DAT1: χ² = 0.095, d.f. = 1, p = 0.757). Since our controls were derived from an epidemiological study, the allelic data reflected in this group should approach to the real frequencies for the adolescent population of Mexico City. It is worth to note that the allele frequencies of the DRD4 polymorphism in exon 3 were comparable with our previous results in an independent control group [3]. Moreover, to our knowledge, this is the first report that documents the genetic variability of the DAT1 3′-UTR polymorphism in Mexicans.

### Table 1

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Alleles</th>
<th>Statistics</th>
<th>Alleles</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n = 113)</td>
<td>85.4</td>
<td>14.6</td>
<td>83.3</td>
<td>16.7</td>
</tr>
<tr>
<td>ADHD (n = 105)</td>
<td>83.3</td>
<td>16.7</td>
<td>χ² = 0.35, d.f. = 1, p = 0.55</td>
<td></td>
</tr>
<tr>
<td>Externalized (n = 49)</td>
<td>89.0</td>
<td>14.3</td>
<td>χ² = 0.005, d.f. = 1, p = 0.94</td>
<td></td>
</tr>
<tr>
<td>Internalized (n = 52)</td>
<td>80.8</td>
<td>19.2</td>
<td>χ² = 0.81, d.f. = 1, p = 0.36</td>
<td></td>
</tr>
</tbody>
</table>

Numbers in cells represent percentages. The statistical analyses showed correspondence to the comparison between the groups represented in the corresponding row vs. controls.

The DRD4 exon 3 VNTR 48-bp.

a DRD4 exon 3 VNTR 48-bp.
b DAT1 3′-UTR VNTR 40-bp.
Based on previous reports, an excess of the 7R allele of DRD4 and 10R allele of DAT1 in patients compared to controls was expected. We found instead no significant differences for allele and genotype frequencies between groups of comparison (DRD4 genotype: $\chi^2 = 17.18$, d.f. = 13, $p = 0.19$; DRD4 allele: $\chi^2 = 7.59$, d.f. = 5, $p = 0.18$; DAT1 genotype: $\chi^2 = 1.09$, d.f. = 2, $p = 0.57$; DAT1 allele: $\chi^2 = 0.35$, d.f. = 1, $p = 0.55$). The family study did not show a preferential allelic transmission for either of the genes (DRD4: $z = 0.535$, $p = 0.59$; DAT1: $z = 0.2$, $p = 0.8$).

On the other hand, when the ADHD group was analyzed in terms of other expressed comorbidities we observed a small decrement of 7R allele of DRD4 and the 10R allele of DAT1 in addition to a tendency towards an increasing number of 4R alleles in patients with internalized comorbidities, Table 1.

A logistic regression analysis showed that the simultaneous absence of the 10/10 genotype of DAT1 and 7/7 genotype of DRD4 predicts the presence of ADHD with internalized comorbidities ($b = 0.974, p = 0.01$) with an OR of 2.649 (CI 95% 1.175–5.972), Table 2.

Genes that codify proteins involved in neural dopaminergic pathways are interesting candidates for the study of ADHD. A few metanalysis have concluded that, while important variability occurs across studies, the DRD4 exon 3 and the DAT1 3’-UTR polymorphisms render a small effect towards the risk of developing ADHD [14,40]. The majority of association studies have evaluated Caucasian populations and there are few reports in Latin–Americans [10,19,33], making this the first study in subjects of Mexican ancestry.

The allelic frequencies presented in this study were between those reported for Caucasian and Indigenous groups across Latin America (DRD4 [11], DAT1 [23]). These results support the observation that allelic frequencies of both polymorphic systems differ widely across populations [11,23,38], and are in agreement with recent data that show that the population of Mexico City represents a genetic admixture of Caucasian and Indigenous groups [20].

The purported association between the 7R allele of DRD4, the 10R allele of DAT1 and ADHD was not observed in the present study. Previous reports in Latin Americans have failed to show an association with the 10R allele of DAT1, and the literature surrounding the 7R allele of the DRD4 is inconsistent [4,10,19,33]. Possible explanations for this discrepancy include population stratification and the elevated frequency of the risk allele in a relatively small sample. It is also possible that particular genetic variants of specific phenotypes (e.g. ADHD) differ across ethnic groups. This can be seen for example with the association of the 2R allele of DRD4 and ADHD in the Asian population [27], and the lack of association with the 10R allele in the Chinese Han population [39].

To date, there are few studies that have evaluated the genetic interaction between DRD4 and DAT1 in relation to ADHD. While some studies have reported a positive interaction between the 7+ genotype of DRD4 and the 10/10 genotype of DAT1 [10,19,31], others such as Qian et al. did not find the same association [32].

On the other hand, only few reports have evaluated the genetic influences of these polymorphic systems regarding ADHD comorbidities [21,25,37]. For example, an association between the 7R allele of DRD4 and ADHD with externalized disorders has been reported [21,25]. Additionally, a family-based study in a Turkish population showed that patients without the 7R allele were more anxious and depressed than those possessing at least one copy of this variant [37].

In this sense, the present study showed that the simultaneous absence of the 10/10 genotype of DAT1 and 7/7 genotype of DRD4 predicts membership to the group of patients with internalized comorbidities. This finding raises the following question: does the genetic variability associated with patients expressing internalized comorbidities differ from those who express solely externalized disorders? This is a proposal that has yet to be extensively studied. Notwithstanding, a more parsimonious explanation could be that our results represent a false positive association [35].

To our knowledge, this is the first study that explored an interaction between DRD4 and DAT1 in relation to ADHD and its comorbidities; nevertheless, the results presented in this study must be considered as preliminary and merit proper replication.

Future research that includes haplotype analysis of these genes, especially for DAT1 [9], as well as psychosocial and adversity factors (e.g. family dysfunction, cognitive style of coping, parental detachment, etc.) is warranted in order to fully understand this heterogeneous, complex, and multifactorial disorder.

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**References**


