Evidence of an association between 10/10 genotype of DAT1 and endophenotypes of attention deficit/hyperactivity disorder


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KEYWORDS
DAT1; Endophenotype; Executive function; Genotype; Variable number tandem repeats; Attention deficit hyperactivity disorder

Abstract
Introduction: Genetic variance of attention deficit hyperactivity disorder (ADHD) is a strong determinant of this disorder. The 40 bp variable number tandem repeat (VNTR) located in the 3′ untranslated region (UTR) of DAT1 gene increases the expression of the dopamine transporter. Therefore, DAT1 has been associated with susceptibility to ADHD.
Objective: To determine the association between the VNTR of DAT1 and the phenotype of ADHD or its endophenotypes in a sample of children aged between 6 and 15 years from Bogotá.
Subjects and methods: We selected 73 patients with ADHD and 54 controls. WISC test was applied in all subjects and executive functions were assessed. The VNTR of DAT1 was amplified by polymerase chain reaction. Data regarding population genetics and statistical analysis were obtained. Correlation and association tests between genotype and neuropsychological testing were performed.
Results: The DAT1 polymorphism was not associated with ADHD (P=.85). Nevertheless, the 10/10 genotype was found to be correlated with the processing speed index (P<.05). In the hyperactivity subtype, there was a genotypic correlation with some subtests of executive function (cognitive flexibility) (P≤.01). In the combined subtype, the 10/10 genotype was associated with verbal comprehension index of WISC (P<.05).

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Introduction

Attention deficit hyperactivity disorder (ADHD) is one of the most common neuropsychiatric problems in school-aged children. Its worldwide prevalence ranges from 4% to 19%.\(^1\)\(^2\) This disorder accounts for a sizeable portion of all child psychiatry and neurology consults in specialist centres. It affects boys more than girls by a ratio of 3:1. Furthermore, it has an impact on the individual’s academic performance, peer relations, and future life, since some symptoms may remain into adulthood.

Several factors that contribute to the disease’s aetiology have been described. Since these factors may be environmental, psychological, and genetic, ADHD is regarded as a multifactorial disease. Its estimated heritability of 76% indicates that genetic variance contributes greatly to development of the phenotype. Several studies have been designed to search for candidate genes.\(^3\)\(^-\)\(^5\) Genes that may be involved in the disorder have been identified based on the action mechanism of certain drugs used to lessen the symptoms. These genes may act on the pathways related to the dopaminergic, acetylcholinergic, and serotonergic systems on the one hand, or code for transmembrane proteins on the other. Research has shown that genes coding for the dopamine transporter (DAT1), dopamine receptors (DRD2, DRD3, DRD4, and DRD5), the serotonin transporter (5-HTT), serotonin receptors 1B and 2A, the catechol-O-methyltransferase enzyme (COMT), the monoamine oxidase enzyme (MAO), and the protein associated with synaptosome 25 (SNAP25 protein) can be linked to increased susceptibility to the disorder.\(^4\) Faraone et al. examined the ORs from several case-control studies and family-based allele transmission studies in their review of the literature on genetics in ADHD. They concluded that variants of the DRD4, DAT1, DRD5, DBH, SNAP-25 and 5-HTT genes were in fact linked to increased susceptibility for developing this disorder.\(^5\)

One of the best-studied candidate genes is DAT1, which codes for the DAT receptor. This gene began to be studied when researchers observed that many of the drugs used to treat ADHD act by blocking the action of the dopamine transporter. This increases dopamine availability at the synaptic cleft, which in turn contributes to alleviating the symptoms.\(^6\)\(^-\)\(^8\) Different studies have shown that polymorphisms of the DAT1 gene, such as the variable number tandem repeat (VNTR) of 40bp located in the 3′ UTR, may be regarded as risk factors since they increase expression of the dopamine transporter.\(^9\)\(^-\)\(^14\) The genotypes for this VNTR are determined by the number of 40-bp repeats in each of

Conclusions: A correlation was found between DAT1 VNTR and the subtest “processing speed index”\(^1\)\(^-\)\(^3\) of WISC and the subtest “cognitive flexibility”\(^1\)\(^-\)\(^3\) of executive functions. To our knowledge, this is the first report to assess DAT1 gene in a Colombian population.

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an individual’s two alleles. The most common types world-
wide are the 9-repeat allele (allele 9) and the 10-repeat allele (allele 10). The 10/10 genotype, meaning the geno-
type with two 10-repeat alleles of 40bp, has been shown
to be associated with ADHD. However, this association has
not been replicated in all studies analysing this genetic vari-
ant. As a result, experts do not yet agree on how much this
polyorphism influences susceptibility to the disorder.\textsuperscript{15–17}

In light of the discrepancies between results from studies
analysing susceptibility genes, researchers have proposed
studying endophenotypes, which may represent changes
in biochemical, neuropsychological, or cognitive functions.
Indirectly, endophenotypes are a sign of specific pathophy-
siological processes or subphenotypes of the disease.\textsuperscript{18} For
example, executive function anomalies are common in ADHD
patients, and they may be regarded as an endophenotype of
the disorder.\textsuperscript{19} On this basis, we may conclude that studying
these anomalies may help identify genetic variants related
specifically to the changes in executive functions that are
typical in ADHD.

To date, no Colombian studies have examined the associ-
ation between the \textit{DAT1} gene and ADHD endophenotypes.
The purpose of our study was therefore to determine if
there is an association between the 40-bp VNTR in the 3’
UTR region of \textit{DAT1} and the ADHD phenotype, subtypes, and
endophenotypes (mainly executive function), in a Colum-
bian population sample.

Subjects and methods

Subjects

We selected children aged 6 to 15 from various public and
private schools in Bogotá, Colombia. Subjects were evalu-
ated using the DSM-IV checklist for ADHD, the Behavior
Assessment System for Children (BASC), and the revised
Wechsler Intelligence Scale for Children (WISC-R). Cases
were selected according to results on the DSM-IV check-
list and the BASC scale. Children who met 6 or more of
the 9 criteria for inattention and/or hyperactivity on the
DSM-IV checklist and who tested at or below the 85th per-
centile in the attention problems and hyperactivity domain
of the BASC scale, according to teacher and parent rat-
ings, were considered cases. The WISC-R test was applied
to exclude children with intellectual disability, defined as a
total score of less than 70. Cases were classified as hyper-
active/impulsive, inattentive, or combined ADHD subtypes
based on test results.

A control group of children not suspected of having ADHD
was formed; these children were also assessed with the DSM-
IV checklist and BASC scale. Controls were also screened
for cognitive disability; we excluded individuals with scores
lower than 70 on the WISC-R, and those with poor aca-
demic performance (in cases in which the test could not
be administered for operational reasons). Executive func-
tions in both cases and controls were examined using ENI
(\textit{Evaluación Neuropsicológica Infantil}), a Spanish-language
neuropsychological assessment for children.\textsuperscript{20}

This study complies with ethical principles for medical
research on human subjects as set forth by the Helsinki
Declaration and the World Medical Association (Seoul, 2008)
and Resolution 008430 of 1993, establishing the scien-
tific, technical, and administrative guidelines for medical
research in Colombia. The study protocol was assessed and
approved by our hospital’s research ethics committee. All
participating children (cases and controls) agreed to partic-
ipate in the study; parents signed informed consent forms
permitting them to participate and authorising researchers
to use the children’s DNA samples.

DNA extraction

In cases and controls, we collected 5 mL of peripheral venous
blood or buccal swab samples, depending on how difficult
it was to draw blood samples from these children. DNA
extraction was performed using a conventional desalting
protocol; molecule quality was assessed in 1.2% agarose gels
stained with ethidium bromide. DNA was quantified using
a Nanodrop\textsuperscript{®} spectrophotometer. Prior to being used, DNA
from cases and controls was stored at −20\textdegree C at the DNA bank
maintained by the genetics unit at Universidad del Rosario
(Bogotá, Colombia).

Genotyping

The 40-bp VNTR polymorphism in the 3’ UTR end of the \textit{DAT1}
gene was analysed using a polymerase chain reaction (PCR)
method and previously described specific primers\textsuperscript{9} that
amplified a fragment measuring between 160 and 640bp,
according to the number of VNTRs present in each case
or control. The smaller allele (2 repeats) corresponded to
a molecular weight of 160bp, whereas the larger allele
(13 repeats) had a molecular weight of 640bp.

PCR was performed under the following conditions: Master
Mix (6.25\textmu L Promega\textsuperscript{®}), forward primer 0.5\textmu L and
reverse primer 0.5\textmu L (10 PM/\mu L), DNA 150ng, ultrapure
water 5.25\textmu L, and 2\textmu L dimethyl sulfoxide at 10%. The
thermal cycler programme was as follows: denaturing at
94\textdegree C during 5min, followed by 30 cycles of denaturing at
94\textdegree C for 45s, annealing at 61\textdegree C for 45s, and extension
at 72\textdegree C for 45s, with a final extension cycle of 10min at
72\textdegree C (Agilent\textsuperscript{®} thermal cycler). Each PCR setup included
a negative control for non-specific amplification so as to dis-
card any contaminated samples. As positive controls, we
used samples that had previously been genotyped in the 3’
UTR region of \textit{DAT1}. All amplified products were analysed
in 3% agarose gels stained with 3% ethidium bromide, using
a 50bp molecular weight size marker. Two co-researchers,
working independently, performed genotyping by viewing
the amplified product in gel, and they calculated allele fre-
dencies by direct counting. The genotype was assigned by
comparing results to the amplified band profile obtained
using the molecular weight pattern, keeping in mind that
the allele with fewer repetitions (2) had a weight of 160bp.

Statistical analysis

SNPStats software\textsuperscript{21} was used to determine population-wide
genetic statistics for allele frequency, genotype frequency,
and Hardy-Weinberg equilibrium. We calculated the ORs for
Table 1: Comparison of demographics for cases and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (n = 73)</th>
<th>Controls (n = 54)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>6–13 (SD 1.64)</td>
<td>6–15 (SD 2.31)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>58 (79.5%)</td>
<td>32 (59.4%)</td>
</tr>
<tr>
<td>Female</td>
<td>15 (20.5%)</td>
<td>22 (40.6%)</td>
</tr>
<tr>
<td>ADHD subtypes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inattention</td>
<td>26 (35.6%)</td>
<td></td>
</tr>
<tr>
<td>Hyperactivity-impulsivity</td>
<td>10 (13.7%)</td>
<td></td>
</tr>
<tr>
<td>Combined</td>
<td>37 (50.7%)</td>
<td></td>
</tr>
</tbody>
</table>

For flexibility, IV between matched gene function, which results in no error rate being observed. The population was in Hardy-Weinberg equilibrium (P = .78). The most frequent genotype in both cases and controls was 10/10 (two 10-repeat alleles); frequencies were 62.8% and 62.2%, respectively.

Table 1

<table>
<thead>
<tr>
<th>Allele frequency [proportion]</th>
<th>Genotype frequency [proportion]</th>
<th>NG</th>
<th>HWE (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele 9</td>
<td>50 [0.203]</td>
<td>2 [0.008]</td>
<td>9/9</td>
</tr>
<tr>
<td>Allele 10</td>
<td>194 [0.788]</td>
<td>38 [0.308]</td>
<td>77 [0.626]</td>
</tr>
<tr>
<td>Allele 13</td>
<td>2 [0.008]</td>
<td>16 [0.666]</td>
<td>0 [0]</td>
</tr>
<tr>
<td>Controls (n = 54)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total population</td>
<td>6 [0.048]</td>
<td>7 [0.291]</td>
<td>16 [0.666]</td>
</tr>
<tr>
<td>Individual subtypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inattention (n = 26)</td>
<td>9 [0.187]</td>
<td>1 [0.041]</td>
<td>7 [0.291]</td>
</tr>
<tr>
<td>Hyperactivity-impulsivity (n = 10)</td>
<td>2 [0.111]</td>
<td>2 [0.222]</td>
<td>7 [0.777]</td>
</tr>
<tr>
<td>Combined (n = 37)</td>
<td>18 [0.243]</td>
<td>14 [0.378]</td>
<td>21 [0.567]</td>
</tr>
</tbody>
</table>

HWE: Hardy-Weinberg equilibrium; NG: no genotyping.

We did not observe an association between the genotype of the VNTR in the 3’ UTR of DAT1 and the ADHD phenotype (P = .85). Neither were there any associations between genotype and the inattentive, hyperactive/impulsive, and combined subtypes (P = .83, P = .40, and P = .44, respectively).

A correlation study for the VNTR genotype in 3’ UTR of DAT1 and the subtests from both the WISC and the executive functions domain of the ENI revealed a statistically significant correlation only between genotype and the processing speed index (PSI) on WISC (P = .043, Table 3). We determined that cases with the 10/10 genotype had lower mean scores on this index than subjects with other genotypes (mean with 10/10 genotype = 88; mean with 9/9 or 9/10 genotype = 95).

The inattention subtype did not show any correlations between genotype and the subtests that were analysed. However, some subtests such as total phonemic word fluency and semantic graphic fluency yielded P-values approaching statistical significance (P = .62 and P = .84, respectively). For the hyperactive/impulsive subtype, the DAT1 genotype was associated with the number of correct responses (P = .011) and errors (P = .015) on the cognitive flexibility subtest, as well as with the number of perseverative answers (P = .001). For the combined subtype, only the WISC verbal comprehension index results showed a significant association with genotype (P = .037).

In addition, we performed an analysis broken down by genotype subgroups to assess the influence of alleles on endophenotypes. This entailed creating a group for genotypes containing the 9-repeat allele (i.e., genotypes 9/9 and 9/10) and another for those lacking the 9-repeat allele (genotypes 10/10 and 10/13). Through this analysis, we detected a group effect on the PSI subtest from the WISC (P = .033); the mean score was higher on this index for cases bearing the 9-repeat allele (94 vs 88). No other correlations were identified.
Table 3  Statistically significant correlations between endophenotypes and VNTR genotype at the 3′ UTR section of DAT1

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Test/endophenotype</th>
<th>Adjusted R²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADHD</td>
<td>Processing speed index (WISC)</td>
<td>0.046</td>
<td>.043</td>
</tr>
<tr>
<td>Hyperactivity-impulsivity</td>
<td>Number of correct answers (cognitive flexibility)</td>
<td>0.623</td>
<td>.011</td>
</tr>
<tr>
<td></td>
<td>Total errors (cognitive flexibility)</td>
<td>0.596</td>
<td>.015</td>
</tr>
<tr>
<td></td>
<td>Number of perseverative responses (cognitive flexibility)</td>
<td>0.753</td>
<td>.001</td>
</tr>
<tr>
<td>Combined</td>
<td>Processing speed index (WISC)</td>
<td>0.096</td>
<td>.037</td>
</tr>
</tbody>
</table>

* p < .05.

Discussion

The dopamine transporter, which is located in the presynaptic region of dopaminergic neurons, regulates dopamine reuptake by presynaptic terminals. DAT1, the gene that codes for this protein, has been examined by numerous association studies in ADHD since it displays multiple features that make it a good candidate gene. Knock-out mice exhibit increased motor activity with dopamine remaining in the synaptic cleft 100 times longer than in normal mice. SPECT and PET studies of patients with disorder reveal higher striatal densities of DAT1. This gene product is the target of the drug methylphenidate, whose action mechanism blocks more than 50% of dopamine transporters at the presynaptic neuron level.

Findings from our study indicate that the 10/10 genotype in patients with ADHD was associated with scores on the processing speed index. This is a test of the subject’s ability to focus attention and scan quickly, and it provides information about attention span and short-term visual memory. Mean processing speed index scores were lower among carriers of this genotype, indicating that these individuals show alterations in this area. Likewise, allele-specific analysis confirmed the correlation between individuals homozygous for the 10-repeat allele and the processing speed index. This finding indicates that this allele is responsible for susceptibility to an altered endophenotype; similarly, findings from the study by Bellgrove et al. show that the 10-repeat allele may contribute to the appearance of a neuropsychological variant.

Likewise, the analysis by ADHD subtype showed that the 10/10 genotype in hyperactive/impulsive individuals was linked to a lower number of correct responses, the total number of errors on the card sorting test, and the number of perseverative answers. Viewed as a whole, these tests provide information about individuals’ ability to anticipate and establish goals, self-regulate during tasks, and carry out tasks efficiently. They also inform us about a subject’s ability to adapt to the requirements in his or her setting. Alterations in these endophenotypes may be linked to this subtype’s propensity for poor adaptation to classrooms and other similar settings that change and require individuals to complete tasks in an effective manner.

On the other hand, analysis of the combined subtype found that individuals with the 10/10 genotype presented lower verbal comprehension indexes than did other genotypes. This indicates that the 10/10 genotype is associated with altered verbal ability in children with the combined ADHD subtype. Capilla et al. reported that this subtype presents alterations in cognitive flexibility according to magnetoencephalography (MEG) assessments carried out while subjects were performing tasks involving this function.

Similarly, other groups have found associations between the 10/10 genotype of this polymorphism and abnormal results on neuropsychological tests. Loo et al. reported a link between the 10/10 genotype of the DAT1 gene and an increase in the total number of errors, in addition to a higher number of impulsive answers and more variability in response time. In a case-control study, Bellgrove et al. found a link between this genotype and poor performance on attention-related tasks. This study showed that individuals homozygous for allele 10 showed greater response variability in the test of spatial attentional bias; the researchers indicate that the 10-repeat allele of DAT1 may mediate neuropsychological impairment in ADHD. Viewed together, these studies show this polymorphism’s key role in executive function alterations caused by ADHD. They also highlight the importance of understanding the genetic substrate of this disorder, and although this is not possible for the disorder with all of its traits, we see that certain neuropsychological anomalies (endophenotypes) may arise in these patients.

As our results show, and as we stated previously, there is an association between ADHD endophenotypes and the 10/10 genotype of the VNTR of DAT1. However, other alleles associated with ADHD have been described in the same VNTR locus, including 7-repeat and 9-repeat alleles. Barkley et al. identified more marked expression of ADHD symptoms in carriers of the 9/10 genotype than in carriers of the 10/10 genotype. In addition, this study showed that carriers of the 9/10 genotype display poorer academic performance, more strained mother-child relationships, and more significant behaviour disorders. Kim et al. also found the 9/10 genotype to be more frequent in subjects with ADHD and that frequencies for the 9-repeat allele were significantly higher among subjects with ADHD. These authors report that carriers of the 9-repeat allele made more errors of omission on continuous vigilance tasks. Oh et al. found that subjects with the 10/10 genotype made fewer errors of omission on the attention variables test than individuals with genotype 10/10 in the first quarter of the test. This indicates the presence of a protective factor for individuals with the 10/10 genotype.

There may be several explanations for the reports that associate genotypes other than 10/10 with ADHD in different populations. These include inter-population differences in the heritability value; the impact of the psychosocial environment, which is a significant risk factor; and statistical independence for the alleles of the analysed VNTR and other polymorphisms that are functionally responsible.
for the phenotype. An additional explanation is that haplotypes affect susceptibility to the phenotype more than single polymorphisms. The type of analysis also influences results since population stratification may be present in case-control studies, and patients’ phenotypes and the homozygosity status of the parents may affect analyses of the transmission-disequilibrium test. Numerous studies have failed to identify an association between ADHT and DAT1, although this gene is an optimal biological and physiological candidate. It is therefore believed that the gene may act more like a phenotype modifier whose action depends on interactions with other polymorphisms of dopaminergic pathways, such as DRD4.15,17

Findings from this study underscore the importance of knowing the genotype of the VNTR located at region 3’ UTR of DAT1 in Colombian populations, given that carrying the 10/10 genotype has been linked to poorer performance on neuropsychological tests.

The present study’s limitations include its small sample size, which affects the external validity and ability to generalise results. Since the population examined in this study was sampled from a few schools in the city of Bogotá, these results may only be extrapolated to other populations with caution. For future studies, we recommend increasing the sample size on both the local and regional levels, especially in regions where a higher prevalence of the disorder is reported. This will be helpful for identifying low-frequency alleles, which are difficult to detect in small populations.1 We also recommend evaluating more variants of the DAT1 gene and any others that might be masking the relationships reported by earlier studies, and, lastly, completing functional studies to shed light on the biological role of this polymorphism in poor performance on the neuropsychological tests listed here.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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References


Association between DAT1 and ADHD endophenotypes


