Dopamine DRD4 gene polymorphism as a risk factor for epilepsy in autism spectrum disorder

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Abstract

Background: Epilepsy can co-occur in Autism Spectrum Disorder (ASD) patients and impact the individual’s behavior, socialization, and language development. Polymorphisms in the dopamine D4 receptor gene - DRD4 - have been studied as a potential biomarker for co-occurrence and severity of these disorders. In this study, we evaluated the prevalence of the polymorphisms of the DRD4 gene, its effects on clinical characteristics of ASD, and its association with epilepsy in ASD patients. Clinical investigations of 53 ASD patients aged between 2 and 16 years old were conducted to assess their health history and comorbidities. Genotyping of these patients was performed through the molecular analysis of the DRD4 gene polymorphisms.

Results: ASD and control subjects did not differ in the prevalence of DRD4 gene polymorphisms. However, heterozygous samples for DRD4 polymorphism of 7 repetitions presented greater association with epilepsy. Combining homozygous and heterozygous samples for DRD4 polymorphism of 7 repetitions resulted in no significant difference. The relative risk for epilepsy in heterozygous for DRD4 polymorphism with 7 repetitions increased 3.5-fold.

Conclusion: ASD patients with DRD4 gene polymorphism of heterozygous alleles with 7 repetitions are associated with a higher frequency of epilepsy.

Introduction

Autism Spectrum Disorder (ASD) is a disorder of child development characterized by deficits in communication and social interaction associated with patterns of behavior, interests or restricted and repetitive activities [1]. Individuals with ASD may present language and/or cognitive impairment, including Intellectual Disability. Furthermore, they also can have associated comorbidities, such as epilepsy and anxiety disorders, behavioral problems and aggressiveness, among others [2–4].
The co-occurrence of ASD and epilepsy is well established, and is mainly associated with increased age, worse cognitive levels, reduced language development, history of regression in development, and more severe symptoms of ASD [5]. The mean prevalence of epilepsy in children with ASD between 2 and 17 years old is 12.5%, less than 10% in children under 10 years old and cumulative of 26.5% in teenagers up to 17 years old [6]. The association between ASD and epilepsy can be explained as both resulting from altered cortical and subcortical connections on a large scale. Molecular and cellular mechanisms are also involved in the increase of epileptogenesis, thus affecting social behavior and changes in language. These factors can be directly influenced by genes associated with early brain development and the release of neurotransmitters, thus disturbing neuronal inhibitory–excitatory balance [7–9].

Although the etiology of ASD has not yet been fully elucidated, its genetic influence is clear. Several genes are listed as possible candidates for the vulnerability to develop ASD [10]. Most of them with extremely complex neurobiological explanations, but none has yet proved to be a single determinant [11–13]. Genetic influence does not follow a specific pattern or a Mendelian inheritance, but contribute to the patients’ phenotype and may be associated with specific comorbidities. Among these, the DRD4 gene (NCBI Gene ID: 1815, OMIM: 126452) that encodes the dopamine D4 receptor comprises one of the most variable regions of the genome. It is located in the chromosome 11 (11p15.5) and is composed of 4 exons and a variable number of tandem repeats (VNTR) in the third exon. Figure 1 shows DRD4 gene structure elements, as well as the amplicon region investigated in this study.

There are seven repetitions of the VNTRs in the dopamine D4 receptors that increase the length of the alleles in the third intracellular loop, thus impairing the activation of the receptor. With the lower sensitivity of the D4 receptor to dopamine, activation of regions where it is highly expressed, such as the prefrontal cortex, anterior cingulate, and limbic areas [14–16], becomes less facilitated. The lower effectiveness provided by the polymorphism may be related, for example, to the decrease in the amount of D4 receptors in individuals with ASD [17].

The 7-fold VNTR present in the DRD4 gene has been associated with repetitive behaviors in autistic children as well as increased risk of autism symptoms in children and teenagers with Attention Deficit and Hyperactivity Disorder (ADHD). It has also been associated with other developmental disorders, such as schizophrenia, oppositional defiant disorder, and separation anxiety [14, 18–20]. In ASD, DRD4 polymorphism has been studied as a potential biomarker for severity and co-occurrence of associated symptoms, higher rates of behavioral problems and repetitive behaviors, and neuropsychiatric disorders [18,21].

Interestingly, investigation of the role of dopamine in ASD intensified after observing the beneficial effects of

![Figure 1: DRD4 gene structure. This gene is shown on chromosome 11 (Chr 11) in the p15.5 region. DRD4 gene is composed by 4 exons (black boxes, E1 to E4) and the studied amplicon region is found in exon 3. The position of the start codon ATG and the stop codon TGA are described in E1 and E4, respectively. Grey bar represents 5’-UTR, 3’-UTR and introns numbered 1 to 4 (1-14).](https://www.peertechz.com/journals/journal-of-biology-and-medicine)
antipsychotics (dopaminergic antagonists) on aspects such as hyperactivity and aggressiveness. Studies have reported that different polymorphisms are associated to an increase or decrease in the activity of the dopaminergic system in the prefrontal cortex of autism individuals. The dysfunction of this system may still be related to behaviors of aggressiveness and impulsivity in adults with ASD [22–23].

Thus, the objective of this study was to evaluate the prevalence of polymorphisms of the DRD4 gene and their clinical impact in patients with ASD. We have characterized the genotypic frequency of the DRD4 polymorphisms in order to demonstrate their potential association to ASD through the analysis of transmission and case-control disequilibrium. In addition, we tested the association of DRD4 polymorphisms with specific symptoms of ASD and individual comorbidities.

Materials and methods

This is an observational, analytical, cross-sectional study with prospective data collection. Procedures have been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and were approved by the Independent Ethics Committee (IEC) of the Centro Universitário Autônomo do Brasil – UNIBRASIL, according to the opinion 002/2008 and acceptance of the Term of Informed Consent. All subjects gave their informed consent for inclusion in the study before enrollment.

Patients

Subjects included in the study are patients from the Autism Outpatient Clinic of CENEP – Centro de Neupediatría do Hospital de Clínicas da Universidade Federal do Paraná, with diagnosis of idiopathic ASD and who were followed from January 2012 to December 2014. The control group had 40 healthy individuals who were ethnically- and gender-matched to the ASD group. All samples from the control group were obtained from the DNA bank of the Centro Universitário Autônomo do Brasil – UNIBRASIL.

Patient inclusion and exclusion criteria

Patients diagnosed with idiopathic ASD whose caregivers agreed to participate in the study through A Term of Informed Consent and who performed regular follow-up at the outpatient clinic for at least one year were included in the study. Patients with genetic tests, brain imaging, or dimorphisms characterizing the diagnosis of specific genetic syndromes associated with ASD were excluded from the sample.

Fifty-eight patients were initially selected for participation and blood samples were collected. Subsequently, five patients were excluded from the clinical analysis because they failed to follow-up. Thus, a total of 53 patients whose blood samples were collected were included in the clinical and genetic analysis, and a total of 58 patients were genotyped for ASD vs control comparisons. The exclusion criteria for both groups consisted of the presence, or familial history, of genetic or non-genetic neurological diseases.

Procedures

Peripheral venous blood samples (5ml) were collected from each subject in an EDTA tube. Patient and control samples were submitted to DNA extraction by the method of Lahiri and Nurnberger [24].

All genetic tests were performed in the laboratory of Genetics and Molecular Biology of the Centro Universitário Autônomo do Brasil – UNIBRASIL.

For the DRD4 gene, the alleles were divided as to their functionality since an allele with 7 repetitions reduces dopamine affinity for the D4 receptor and its inhibitory potency to 2 to 3 times lower. Therefore, they were divided in 3 categories: (i) No 7n – alleles without 7 repetitions with inhibitory power at the D4 receptor, (ii) Heterozygous 7n – heterozygous with an allele with 7 repetitions with lower inhibitory power and (iii) Homozygous 7n – homozygous for an allele with 7 repetitions with much lower inhibitory power at the D4 receptor [15].

The dependent variables analyzed were the presence of idiopathic ASD in the analysis of frequencies, and the presence of the following clinical characteristics and associated comorbidities:

- Diagnostic criteria for ASD: stereotypies, inflexibility, sensory dysfunctions;
- Associated characteristics: anxiety, psychomotor agitation, aggressiveness, regression in development;
- ASD specifications: language impairment and cognitive impairment;
- Comorbidities: epilepsy
- Level of impairment: levels 1, 2 or 3;
- Changes in exams: Electroencephalogram and imaging exams (Tomography or Brain Magnetic Resonance);
- Use of medications (mono and polytherapy)

For the DRD4 gene, the genotyping process was performed to define the number of repetitions of the VNTR region presented by individuals in the third exon of the DRD4 gene. The sequence of the primers used was described in Gadow et al., and the amplification by Polymerase Chain Reaction (PCR) agrees with the protocol suggested by the same authors [18].

The analysis of the genotypes was performed in agarose gel electrophoresis (concentration 2.5%) which observed allelic variations of 2 to 11 repetitions [18].

Statistical analysis

Measures of central tendency and dispersion are expressed as mean and standard deviation (mean ± SD) for the symmetrical distribution of continuous variables and in medians, minimum and maximum values (median, minimum – maximum) for the asymmetric distribution variables.

The estimation of the difference of continuous variables of normal distribution was performed by the parametric test, Student-t test and ANOVA (combined categorical and continuous variables). Asymmetrically distributed variables were analyzed by the non-parametric tests of Mann-Whitney and Kruskal-Wallis ANOVA.

Estimates of the difference between categorical variables were performed using Fisher’s exact test and Pearson’s chi-square test.

For all tests a minimum significance level of 5% and a minimum test power of 90% were considered.

**Results**

Genotyping of 58 patients with ASD and of 40 patients of the control group were performed. Alleles of the DRD4 gene which vary between 2 and 8 repetitions in relation to the considered VNTR were found in this study. According to the genotypic characterization of the groups the allele and genotype frequencies were estimated in both populations, and the population was found to be in Hardy-Weinberg equilibrium (ASD: p=1.000; control: p=0.9999), as shown in Table 1.

Comparison of the genotypic and allelic frequencies between the two groups resulted in non-significant values (genotypic: p=0.5685; alleles: p=0.4305), indicating that both genotypes and alleles are similarly distributed among these populations. The frequency observed in this study (adding the ASD and control groups due to the homogeneous distribution of the alleles between them) compared to the mean frequencies observed in the world population did not differ significantly (p= 0.9754).

Table 1 Allelic frequencies were divided according to their gene expression and functionality.

Table 2 The results of the allele comparison with the clinical characteristics of the sample are summarized in Table 3.

Homozygous patients for polymorphism of 7 repetitions for the DRD4 gene presented a higher frequency of association with epilepsy (p = 0.01). When grouped with homozygous for 7 repetitions, no statistical difference was observed (p = 0.09) with Relative Risk (RR) for epilepsy of 3.5 (95% CI = 0.95 to 13.08). Although the sample is small, with only 17 patients with at least one allele with 7 repetitions, this ratio may represent a risk factor for epilepsy in ASD patients. However, studies with at least one allele with 7 repetitions in the ASD group was the same as in the control group due to the homogeneous distribution of the alleles between them) compared to the mean frequencies observed in the world population did not differ significantly (p= 0.9754).

Chi-square test.

The relatively similar distribution of the DRD4 alleles in the ASD and control groups observed in this study was expected since the previous studies suggest that there is an allele ratio of 7 repetition of DRD4 with some specific symptoms of ASD [18,25,26] or with associated comorbidities [20]. However, we did not find significant differences for specific symptoms (repetitive behaviors, anxiety, psychomotor agitation, inflexibility or aggressiveness). This fact may be related to the difficulty in clinically evaluating these symptoms since the analysis is subjective, and these data were not objectively quantified by specific questionnaires to investigate comorbidities. In addition, data analysis was shown to be complex due to sample heterogeneity relative to the degree of impairment and the low age of the sampled population (median of 7 years old). Another key factor hindering the analysis is the small sample size of patients with at least one allele with 7 replicates (n=17).

**Discussion**

In this study, we show that the prevalence of DRD4 gene polymorphisms in ASD group was the same as in the control group. From a clinical perspective, in the ASD group the DRD4 gene polymorphism with the presence of homozygous alleles with 7 repetitions was associated with a higher frequency of epilepsy in the ASD group.

### Table 1: Genotypic and allelic frequencies of the DRD4 gene observed in the ASD and control groups.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n of patients (%)</th>
<th>n of controls (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-2</td>
<td>0 (0)</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>2-4</td>
<td>8 (13.8)</td>
<td>2 (5.0)</td>
</tr>
<tr>
<td>2-7</td>
<td>1 (1.72)</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>3-3</td>
<td>0 (0)</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>3-4</td>
<td>2 (3.45)</td>
<td>3 (7.5)</td>
</tr>
<tr>
<td>4-4</td>
<td>27 (46.55)</td>
<td>21 (52.5)</td>
</tr>
<tr>
<td>4-5</td>
<td>1 (1.72)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>4-6</td>
<td>1 (1.72)</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>4-7</td>
<td>13 (22.42)</td>
<td>7 (17.5)</td>
</tr>
<tr>
<td>4-8</td>
<td>0 (0)</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>7-7</td>
<td>5 (8.62)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Total</td>
<td>58 (100)</td>
<td>40 (100)</td>
</tr>
<tr>
<td>χ²</td>
<td>p=1.0000</td>
<td>p=0.9999</td>
</tr>
</tbody>
</table>

Allele frequencies:

<table>
<thead>
<tr>
<th>Allele</th>
<th>Patient alleles n (%)</th>
<th>Control alleles n (%)</th>
<th>Observed population Frequency</th>
<th>World population frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>9 (7.7)</td>
<td>5 (6.3)</td>
<td>7.14%</td>
<td>8.8%</td>
</tr>
<tr>
<td>3</td>
<td>2 (1.7)</td>
<td>5 (6.3)</td>
<td>3.57%</td>
<td>2.4%</td>
</tr>
<tr>
<td>4</td>
<td>79 (68)</td>
<td>56 (70)</td>
<td>68.88%</td>
<td>65.1%</td>
</tr>
<tr>
<td>5</td>
<td>1 (0.9)</td>
<td>—</td>
<td>0.51%</td>
<td>1.6%</td>
</tr>
<tr>
<td>6</td>
<td>1 (0.9)</td>
<td>1 (1.2)</td>
<td>1.02%</td>
<td>2.2%</td>
</tr>
<tr>
<td>7</td>
<td>24 (20.7)</td>
<td>12 (15)</td>
<td>18.37%</td>
<td>19.2%</td>
</tr>
<tr>
<td>8</td>
<td>—</td>
<td>1 (1.2)</td>
<td>0.51%</td>
<td>0.6%</td>
</tr>
<tr>
<td>Total</td>
<td>116 (100)</td>
<td>80 (100)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chi-square test.
Table 3: Results of genotype analysis with the clinical characteristics of the sample.

<table>
<thead>
<tr>
<th>Assessed Item</th>
<th>Alleles DRD4</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No 7 n (%)</td>
<td>Heterozygous 7 n (%)</td>
<td>Homozygous 7 n (%)</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>Stereotypes</td>
<td>16 (44.4)</td>
<td>3 (25)</td>
<td>1 (20%)</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>Inflexibility</td>
<td>9 (25)</td>
<td>2 (16.7)</td>
<td>1 (20)</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>Anxiety</td>
<td>11 (30.6)</td>
<td>3 (25)</td>
<td>1 (20)</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>Psychomotor agitation</td>
<td>11 (30.6)</td>
<td>2 (16.7)</td>
<td>2 (40)</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>Aggressiveness</td>
<td>4 (11.1)</td>
<td>1 (8.3)</td>
<td>0</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>11 (30.6)</td>
<td>4 (33.3)</td>
<td>3 (60)</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>Level 1</td>
<td>15 (41.7)</td>
<td>9 (75)</td>
<td>2 (40)</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Level 2</td>
<td>8 (22.2)</td>
<td>2 (16.7)</td>
<td>1 (20)</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Level 3</td>
<td>13 (36.1)</td>
<td>1 (8.3)</td>
<td>2 (40)</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Language Impairment</td>
<td>18 (50)</td>
<td>3 (25)</td>
<td>3 (60)</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Cognitive Impairment</td>
<td>19 (52.8)</td>
<td>3 (25)</td>
<td>3 (60)</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Epilepsy</td>
<td>3 (8.3)</td>
<td>2 (16.7)</td>
<td>3 (60)</td>
<td>0.01**</td>
<td></td>
</tr>
</tbody>
</table>

Nonetheless, the potential risk of a greater association of epilepsy and ASD in patients with at least one allele with 7 repetitions needs to be highlighted and further investigated. Traditionally, the occurrence of epilepsy may be explained by the imbalance between excitatory (glutamate) and inhibitory (GABA) neurotransmitters. Currently, the SFARI database presents 943 genes considered to predispose autism in individuals [27]. Among these genes, some include the dopamine and serotonin neurotransmitter systems that are also involved in epileptogenesis. Dopamine D4 receptors belong to the D2-like dopamine receptors and can be found in post-synaptic glutamatergic and GABAergic neurons with an inhibitory action by activating Gi protein. The association between the dysfunctional dopaminergic system and epilepsy is evidenced by pro-convulsants and anti-convulsants agents that bind to dopaminergic receptors. Additionally, animal studies demonstrated that receptors with D2-like action have a crisis-regulating effect, as is the case with D4 [28]. As we have observed in our results, DRD4 polymorphism with 7 repetitions presents a higher association of epilepsy in ASD patients. As this polymorphism makes the D4 receptor less functional by lowering its sensitivity to dopamine, thus decreasing its inhibitory action [29,30], it is likely that epileptogenesis might be increased by an incapability of dopamine to properly regulate glutamatergic and GABAergic systems. Further studies are necessary to understand and help in the development of drugs employed in the search for the well-being of the individuals affected by ASD.

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Author contributions

Conceptualization, Lilian Pereira Ferrari, Salmo Raskin and Sergio A. Antuniak; Methodology, Marlene Wehmuth, Liya Regina Múkami, Karine Bittencourt da Silva Alcantud; Formal Analysis, Adriana de Oliveira Christoff, Henrique Ravanhol Frigeri, Suelen Lucio Boschen; Writing – Original Draft Preparation, All Authors; Writing – Review & Editing, all authors.

References


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