Effect of the polymorphism $BDNF$ rs6265 G/A in Mexican outpatient children with autism spectrum disorders

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ABSTRACT

Introduction. The study of autistic spectrum disorders (ASD) at the genetic level is extremely important to understand their origin. In Mexico, there are few works addressed from this perspective. Objective. We investigated the role of the Brain Derived Neurotrophic Factor ($BDNF$) gene variant rs6265 G/A for single nucleotide polymorphism analysis in Mexican children with ASD using a case-control association design. Method. We made a pilot study by case-control analysis adjusting by gender, age, and ancestry. Results. Our study found no association between the $BDNF$ rs6265 gene polymorphism and ASD ($p = .419$, $OR = 1.597$ ($1.514, 4.967$)). Discussion and conclusion. Worldwide, the results of case-control association studies with the rs6265 of $BDNF$ are controversial and do not always replicate. This may be due to the ethnicity of our population and additional factors not studied in the present work. Our study suggests that the SNP rs6265 is not contributing for ASD susceptibility in Mexican population.

Keywords: $BDNF$, autism spectrum disorders, single nucleotide polymorphism, Mexican population.

RESUMEN

Introducción. El estudio de los trastornos del espectro autista a nivel genético es de suma importancia para entender su origen. En México existen pocos trabajos abordados desde esta perspectiva. Objetivo. Investigamos el papel de la variante del gen rs6265 G/A del factor neurotrófico derivado del cerebro ($BDNF$) para el análisis del polimorfismo de un solo nucleótido en niños mexicanos con TEA por medio de un diseño de asociación de casos y controles. Método. Realizamos un estudio piloto mediante un análisis de casos y controles ajustando por género, edad y ancestría. Resultados. Nuestro estudio no encontró asociación entre el polimorfismo del gen $BDNF$ rs6265 y TEA ($p = .419$, $OR = 1.597$ ($1.514, 4.967$)). Discusión y conclusión. A nivel mundial, los resultados de estudios de asociación caso-control con el rs6265 de $BDNF$ son controvertidos y no siempre se replican. Esto puede deberse a la etnicidad de nuestra población y a otros factores no estudiados en el presente trabajo. El estudio sugiere que el SNP rs6265 no contribuye a la susceptibilidad al TEA en población mexicana.

Palabras clave: $BDNF$, trastornos del espectro autista, polimorfismo de un solo nucleótido, población mexicana.
INTRODUCTION

Autism spectrum disorders (ASD) are a series of neuropsychiatric disorders classified as neurodevelopmental disorders according to the Diagnostic and Statistical Manual of Mental Disorders 5 (DSM-5), which can be detected at an early age. Recent reviews estimate that worldwide one child in 160 suffers from an autism spectrum disorder (World Health Organization, 2013). The gender ratio is five males per every female and is more common in Caucasians than in African Americans or Latinos (Centers for Disease Control and Prevention, 2014). To date, in Mexico there is only one study about the epidemiology of these disorders, which states that the estimated prevalence is .87%, similar to other reported worldwide figures (Fombonne et al., 2016). According to the demand for clinical care at the Hospital Psiquiátrico Infantil in Mexico City, the pervasive developmental disorders are among the five leading causes of demand for care (Márquez-Caraveo, 2009).

Although epidemiological studies have identified several risk factors, none of them has proved necessary or sufficient for the development of ASD. Understanding the interaction of genes and environment in autism is still at an early stage (Lai, Lombardo, Chakrabarti, & Baron-Cohen, 2013). Autism is perhaps the psychiatric condition with the highest heritability, which is as high as 90% (Bourgeron, 2015; Díaz-Anzaldúa & Díaz-Martínez, 2013). Autism studies have been conducted in different populations, Caucasian for the most part. From these studies, it has been determined that genes involved in normal brain development are important candidate genes (Ingram et al., 2000).

The BDNF gene has been proposed as a candidate gene that may importantly influence psychiatric disorders. It is involved in the development and function of the brain as in the neuronal regulation of survival, proliferation, and differentiation, as well as in synaptic plasticity processes (Yang et al., 2012). The BDNF gene is located on chromosome 11p13 and spans 70 kb, including 11 exons (Pruunsild, Kazantseva, Aid, Palm, & Timmusk, 2007). An identified change of guanine to adenine non synonymous SNP, in position 196 of exon 2 (G196A), results in a substitution of amino acid valine to methionine at codon 66 (Val66Met), changing the non synonymous SNP, in position 196 (Sheikh, Aid, Palm, & Timmusk, 2010). This may result in disruption of activity dependent on BDNF secretion (Zhai et al., 2013).

Recently, Bryn et al. (2015) found a significant elevation of plasma BDNF levels in patients with ASD compared to the control group. Many experimental studies strongly suggest an involvement of BDNF in ASD. Direct evidence supporting the participation of BDNF in autism comes from several studies showing that BDNF levels in the blood, serum, and brain are increased in autistic children compared to controls (Halepoto et al., 2014). It has been observed that BDNF levels in the blood reflect BDNF levels in the brain. This molecule is trophic for serotonergic neurons and abnormalities in serotonin levels are the most common biochemical findings in ASD (Ricci et al., 2013). Studies suggest that macrocephaly is mediated by increased anabolic activity in the central nervous system, reflected by increased levels of BDNF. In addition, children with ASD showed impaired circulation in the CNS, leading to hypoxia in brain regions such as the temporal lobe (Broek et al., 2014).

Therefore, as a candidate gene, BDNF, is very important in the study of ASD. It was decided to study the SNP rs6265 in Mexican patients diagnosed with this disorder through a pilot case-control study. To date, there is no association study of this SNP in Mexican children with ASD. With the recent formation of the Consorcio Mexicano de Investigación en Genética del Autismo (CMIGA), where various institutions of the country are involved, we intend to join efforts to establish a national network. With this joint sample of cases, will have a greater power of interpretation of the results that come to generate, representing as much as possible to the Mexican population.

METHOD

Characteristics of the samples

The sample consisted of 85 ASD patients (12 females and 73 males) selected in Mexico at the CMIGA. Participants were patients from 2-18 years of age who accepted to participate along with their parents who underwent an autism diagnostic interview (ADI-R) (Lord, Rutter, & Le Couteur, 1994) and signed an informed consent. The project was approved by the Ethics Committee at the Carracci Medical Group, in accordance with the Helsinki Declaration.

The control group consisted of 70 controls (10 females and 60 males) without past psychiatric history in which psychopathology was discarded using psychometric instru-
Table 1
Description sample of genotypic and allelic distribution of rs6265 SNP in BDNF gene in cases and controls of Mexican origin

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases</th>
<th>Controls</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>GG</td>
<td>57</td>
<td>67.44</td>
</tr>
<tr>
<td>GA</td>
<td>22</td>
<td>25.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>6</td>
<td>6.98</td>
</tr>
<tr>
<td>Total</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>136</td>
<td>80.23</td>
</tr>
<tr>
<td></td>
<td>p = .419, OR = 1.597 (5.14 – 4.967)</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>34</td>
<td>19.77</td>
</tr>
<tr>
<td>Total</td>
<td>170</td>
<td></td>
</tr>
</tbody>
</table>

Note: n indicates number of individuals; p > .05 shows no statistically association; OR indicates odds ratio of the study. Source: Case-control association study ASD vs. controls.

RESULTS

Patients had an age range of 2 to 18 years and 15 to 72 for the control counterparts. From the 85 cases of autism, 85.54% were male, and for controls, 85.71%. Therefore, samples in case-control analysis were paired by gender.

SNP analysis

The genotype distribution was in HWE for the variant analyzed in this study in cases and controls. The genotype, allelic distribution, and frequencies found for the BDNF SNP in the ASD and control groups are shown in Table 1. Statistical analysis showed no association between BDNF polymorphism rs6265 and autism, as seen in table 1.

DISCUSSION AND CONCLUSION

We observed that the females-males ratio was approximately 5:1, matching up closely with research data reported for other populations, which indicates that there is a gender ratio of four males for every female with autism spectrum disorder in Mexican children. Differences were found in the genotypic and allelic frequencies between patients with ASD and the control group for the rs6265 SNP. AA genotype and A allele were more frequent in cases than in controls (Table 1). The case-control association study analysis did not found a significant p value, suggesting no association between ASD and rs6265. This means that this polymorphism is not relevant for the genetic susceptibility for ASD in Mexican population.

Although some groups have already conducted research on the association of polymorphisms of the BDNF gene and autism, this is the first study performed in Mexican population diagnosed with ASD. Studies in other populations (Cheng et al, 2009; Nishimura et al., 2007; Yoo, Yang, Cho, Park, & Kim, 2014) showed controversial results, because in some cases a significant association with rs6265 and ASD was found and in some others there was none. Interestingly, the most reported variation is in BDNF serum levels, where differences between cases and controls were found (Bryn et al, 2015; Kasarpalkar, Kothari, & Dave, 2014; Meng et al., 2016). Hippocampal neurons transfected with BDNF protein Met variant show decreased secretion induced depolarization (Zhai et al, 2013). Furthermore, in recent years, Magnetic Resonance Imaging (MRI) has revealed that the structural polymorphism rs6265 BDNF could influence the morphology of the human brain (Yang et al, 2012). Allelic variants at this locus have also shown association with mood disorders, obsessive-compulsive disorder and many other neurodevelopmental and neurodegenerative disorders (Kim et al, 2011).

Evidence in variation of abnormal cortical volume and surface area in ASD patients was different to controls as-

ments that included the Beck Depression Inventory, the Impulsiveness Scale Plutchik, the AUDIT Questionnaire, and the Fagerström Test.

Genotyping

Genomic DNA was extracted from blood lymphocytes or mouth swab using a commercial kit following the manufacturer’s protocol (Qiagen, Puregene). BDNF rs6265 polymorphism genotyping was done using TaqMan methodology. The genotypes were analyzed by the TaqMan method specific for allelic discrimination using the QuantStudio7 Detection System (Thermofisher scientific) according to the manufacturer’s protocols with the reaction mixture comprising 10ng genomic DNA, 2XTaqMan Universal Mastermix, and nuclease free water. PCR cycle was as follows: 95°C for 10min for denature, 40 cycles of PCR at 95°C/15sec, 60°C/1min.

Statistical analysis

A case-control analysis was done calculating p and OR values, p < .05 was considered significant. Association analysis was done under a logistic regression model, adjusted by age and gender implemented in Plink V 1.07. Patients had an age range of 2 to 18 years and 15 to 72 for the control counterparts. From the 85 cases of autism, 85.54% were male, and for controls, 85.71%. Therefore, samples in case-control analysis were paired by gender.

Evidence in variation of abnormal cortical volume and surface area in ASD patients was different to controls as-
associated with rs6265 (Raznahan et al., 2009). Other studies that have evaluated the association of this polymorphism and ASD have been studied in different populations. Yoo et al. (2014) conducted a family-based study evaluating four variants of BDNF gene in the Korean population. In a similar study in Japan, Nishimura et al. (2007) evaluated 25 variants. In China, Cheng et al. (2009) evaluated this association in a case and control study with four BDNF variants. These studies found no significant association with Val66Met and ASD. In Mexico, there are no previous studies evaluating this polymorphism. However, Márquez et al. (2013) conducted a single nucleotide polymorphism case and control study and found a significant association of OCD and Val66Met in the Mexican population. Recently, our group found an association with rs6265 and body mass index on bipolar patients (Morales-Marín et al., 2016). All these evidences show the involvement of this gene in psychiatric diseases.

This work is preliminary with a small sample for which additional research is needed in a larger sample to validate our findings. In addition, only one SNP of a single candidate gene was evaluated. The BDNF gene contains several polymorphisms; if more SNPs are evaluated, a haplotype study can be done which would be a useful and powerful tool in determining genetic associations. In future research, we will look to determine BDNF serum levels, gene expression, and methylation to elucidate the molecular effect of the neurotrophin related with ASD aetiology.

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Conflict of interests
The authors declare they have no conflict of interests.

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