Interaction between polymorphisms in SLC6A4 and BDNF on major depressive disorder in a sample of the argentinean population

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ABSTRACT
The dysfunction in the serotoninergic neurotransmission has been classically associated with major depressive disorder (MDD); however, other pathways and processes seem to have a role in this illness, such as neurogenesis and related molecules: the Brain-Derived Neurotrophic Factor (BDNF) and the Apolipoprotein E (APOE). There are many reports that indicate an association between certain polymorphism in these genes and MDD. The aim of our study was to analyze the possible association between MDD and polymorphisms in HTR2A (5-hydroxytryptamine receptor 2A), BDNF and APOE genes in a sample of the Argentinian population previously studied for 2 polymorphisms in SLC6A4 (Solute Carrier Family 6 Member 4) gene. Five polymorphisms were studied (rs6311 and rs6313 in HTR2A; rs429358 and rs7412 in APOE, and rs6265 in BDNF) in 95 MDD patients and 107 non-related controls. No statistically significant differences were observed between groups when analyzing the association with a single marker using logistic regression; however, when a possible combinatorial effect of the polymorphisms (including previously studied polymorphisms in SLC6A4 gene) was analyzed using a dominant model for the risk alleles, the genotypes L/S_10/12_G/A (OR=3.57(95%CI=1.43-8.93); p=0.004, adjusted p-value=0.01) in SLC6A4 and BDNF genes and L/S_10/12_T/C_3/3_G/A in SLC6A4, HTR2A, APOE and BDNF genes (OR=5.99(95%CI=1.66-21.56); p=0.002, adjusted p-value=0.07), were more prevalent in patients than in controls (20% vs. 6% and 15% vs. 3%, respectively).

Even though it is necessary to replicate these findings in a larger population, our results suggest a possible interaction between molecules involved in neurogenesis (BDNF and APOE), serotoninergic neurotransmission (SLC6A4 and HTR2A) and the pathogenesis of MDD.

Key words: major depression disorder, polymorphism, serotonin, BDNF, APOE

INTERACCIÓN ENTRE POLIMORFISMOS EN LOS GENES SLC6A4 Y BDNF EN DEPRESIÓN MAYOR EN UNA MUESTRA DE LA POBLACIÓN ARGENTINA

RESUMEN
La disfunción en la neurotransmisión serotoninérgica ha sido clásicamente asociada con el trastorno depresivo mayor (TDM); sin embargo, otras vías y procesos parecerían tener un rol en esta enfermedad, como la neurogénesis y moléculas asociadas: el factor neurotrófico derivado del cerebro (BDNF) y la apoliproteína E (APOE). Existen reportes en los que se establecen asociaciones entre polimorfismos en estos genes y el TDM. El objetivo de nuestro trabajo fue analizar la posible asociación entre el TDM y polimorfismos en los genes HTR2A (receptor 5-hidroxixitamina 2A), BDNF y APOE en una muestra de la población argentina previamente estudiada para 2 polimorfismos en el gen SLC6A4 (transportador soluble familia 6 miembro 4).

Se estudiaron 5 polimorfismos (rs6311 y rs6313 en HTR2A; rs429358 y rs7412 en APOE; rs6265 en BDNF) en 95 pacientes con TDM y 107 controles no relacionados. No se observaron diferencias significativas entre grupos al analizar la asociación por regresión logística con un único marcador; cuando se analizó el posible efecto combinatorio de polimorfismos (incluyendo los previamente estudiados para el gen SLC6A4) usando un modelo dominante para los alelos de riesgo, los genotipos L/S_10/12_G/A (OR=3.57(95%CI=1.43-8.93); p=0.004, valor-p-ajustado=0.01) en SLC6A4 y BDNF y L/S_10/12_T/C_3/3_G/A en SLC6A4, HTR2A, APOE y BDNF (OR=5.99(95%CI=1.66-21.56); p=0.002, valor-p-ajustado=0.07), fueron más prevalentes en pacientes que controles (20% vs. 6% y 15% vs. 3%, respectivamente).

Si bien es necesario replicar estos hallazgos en una población más grande, nuestros resultados sugieren una posible interacción entre moléculas involucradas en la neurogénesis (BDNF y APOE), la neurotransmisión serotoninérgica (SLC6A4 y HTR2A) y la patogenia de la depresión mayor.

Palabras clave: depresión mayor, polimorfismos, serotoninina, BDNF, APOE

INTRODUCTION

One of the most widely investigated etiological hypotheses about MDD involves a deregulation in the serotonin (5-HT) pathway. Serotonin transporter (5-HTT) encoded by SLC6A4 gene presents two functional polymorphisms that affect its expression in a temporal and tissue-specific manner: 5-HTTLPR (rs4795541, L/S alleles) and 5-HTTVNTR (rs57098334, 9/10/12 alleles)\(^1\). The 5-HTTLPR polymorphism lies in the promoter region of the 5-HTT encoding gene (SLC6A4), with a 43 base-pair (bp) insertion/deletion generating two 5-HTTLPR alleles: S allele (14 bp repeats) and L allele (16 bp repeats)\(^2\).

Further, an A/G nucleotide substitution in the L allele (rs25531) renders the 5-HTTLPR triallelic (L\(_g/\)L\(_g/\)L\(_s\), L\(_g/\)L\(_s/\)L\(_s\), L\(_s/\)L\(_s/\)L\(_s\)) the L\(_g\) allele is supported to be functionally similar to the S allele\(^3\). Studies suggest that the L allele (vs. S) may have higher transcriptional activity, which affects synaptic 5-HT clearance\(^4\).

A complex relation appears to exist between the 5-HTTLPR polymorphism and MDD risk, with S allele carriers having a greater risk for MDD development in the context of adversity\(^5\) (reviewed in\(^\text{it}\)\)). Their involvement in MDD has previously been demonstrated in a sample of Argentinean patients\(^6\) as well as in other populations. Moreover, an association between 5-HTTLPR genotype and paralimbic structures volume was found for the MDD group: the left thalamus proper volume was greater for L\(_g/\)L\(_g/\) homozygotes compared with L\(_s/\)S heterozygotes. L\(_g/\)L\(_g/\) homozygotes also had a greater left putamen volume than S/S homozygotes and L\(_s/\)S heterozygotes. The thalamus plays an important role in regulating the expression and experience of emotion and has been implicated in MDD pathophysiology\(^7\). Several cortical and subcortical regions that have been reported to play a role in the regulation of mood are known to receive projections from or to innervate the cingulate and putamen nuclei. It is thus plausible that disruption of the connections to the limbic system might initiate vulnerability to affective dysfunction\(^8\). Furthermore, 5-hydroxytryptamine receptor 2A encoded by HTR2A gene, also involved in the serotonin (5-HT) pathway, shows two variants rs2070040 (G/A alleles) and rs6313 (T/C alleles), with differences in the expression profile and implicated in MDD, schizophrenia, the treatment response to antidepressants and suicidal ideation\(^9\). Tightly related to serotonin is the secretion of Brain Derived Neurotrophic Factor (BDNF) since both influence neurogenesis and synaptic plasticity\(^10\), processes implicated in the development and maintenance of depression\(^11\). The neurotrophin hypothesis of depression posits that deficiencies in central BDNF lead to cell death in the hippocampus and prefrontal cortex, which in turn contribute to depression\(^12\).

In this sense, several studies have demonstrated the differential expression of BDNF gene in brain regions involved in depression, including down-regulation in amygdala\(^13\). The Val66Met polymorphism (rs6265, G/A alleles) encoded by this gene affects BDNF availability and there exist lot of evidence that may be involved in MDD\(^14\). The A allele decreases packaging and release of BDNF, it has been associated with lower hippocampal N-acetyl-aspartate levels (i.e. neuronal loss or dysfunction) and increased susceptibility to stress\(^15,16\).

In support of functional connectedness between BDNF and serotonin, BDNF has been found to influence the structural plasticity and survival of central serotonergic neurons in animal models, e.g.,\(^17\) and administration of BDNF enhances 5-HT neurotransmission and increases 5-HT metabolism in the brain\(^18\). Furthermore, there is evidence to suggest that BDNF may facilitate or perhaps mediate responsiveness to treatment with Selective Serotonin Reuptake Inhibitors (SSRIs; e.g.,\(^18\) reviewed in\(^\text{it}\)\)).

Also an association between Val66Met polymorphism and MDD patients’ response to antidepressant drugs and remission was found. In this sense the authors suggest that SSRI should be recommended for Val/Val patients and that, conversely, SNRI/TCA should be recommended for Met patients\(^19\).

On the other hand, stressful situations leading to depression produce changes in the brain that may result in an increased vulnerability to develop Alzheimer’s Disease (AD). Polymorphisms in APOE gene have been associated with AD and is also a possible candidate in affective disorders\(^20\). Apolipoprotein E (ApoE) is a cholesterol transporter glycoprotein, synthesized mainly in the liver. ApoE is involved in myelin formation and regeneration after neuronal injury or during the development of neurons\(^21\). It has three major isoforms (ε2, ε3, and ε4) from two polymorphisms (rs429358 and rs7412)\(^22\) which determine 6 different genotypes (ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, and ε4/ε4); the ε3 allele is the most prevalent in the general population. The ApoE ε4 allele has been strongly associated with increased risk of Alzheimer’s disease (AD) in both familial and sporadic forms\(^22\) while the presence of the ε2 allele might be a protective factor for AD development\(^23,24\). A meta-analysis showed a protective effect of ε2 against depression in the Caucasian population\(^22\). Also it was reported that a deleterious effect of depressive symptoms on cognitive decline is magnified by the presence of the APOE ε4 allele. Specifically, cognitive decline increased significantly among participants with one or more copies of the APOE ε4 allele and a higher number of depressive symptoms than either one individually\(^25\).

Considering inconsistencies in reported results and taking into account that as far as we know there is no local data, the goal of this research was to extend the analysis of our previous findings to other polymorphisms in different genes to clarify the relevance of these variations in a sample of Argentinean population who suffers from MDD versus a healthy control group.
MATERIAL AND METHODS
Subjects from a previous study analysing the influence of 5-HTTLPR and 5-HTTVNTR polymorphisms of the serotonin transporter gene (SLC6A4) on major depressive disorder in a sample of Argentinean population were used. In brief, subjects suffering from MDD (n = 95, 74 women and 21 men; 57.6 ±13.3 years old) and unrelated healthy controls (n = 107, 81 women and 26 men; 47.1 ± 14.2 years old) from the same geographical area were recruited. All the subjects signed informed consent. This protocol was approved by Hospital Italiano de Buenos Aires Bioethic board. Subjects were interview by professional experts and grouped based on diagnostic criteria from the DSM-IV, HAM-D, MINI structured diagnostic interview and self-administered Beck scale. Only those patients with HAM-D greater than 18 points were included in this research. Patients with other axis I or II disorders were excluded. Control individuals were evaluated with the same methodology as patients. Subjects with history of psychiatric disorders or chronic diseases were excluded.

Blood was drawn by venipuncture in the morning with no fasting requirements, Genomic DNA was extracted from 200 ul blood samples using a commercial DNA isolation kit (QIAamp DNA blood mini kit, Qiagen), according to the manufacturer’s instructions.

Polymorphisms rs6311 and rs6313 were studied for HTR2A gene; rs429358 and rs7412 for APOE gene; and rs6265 for BDNF gene by PCR-RFLP with primers and restriction enzymes previously described.

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. Hardy-Weinberg equilibrium was calculated using R v2.14.1 (http://www.r-project.org/). The measurement of Linkage Disequilibrium and the analysis of association were done using UNPHASED v3.1.4 (http://homepages.lshtm.ac.uk/frankdudbridge/software/unphased/). Significant associations were supervised by 1,000 permutation tests.

For the evaluation of gene-gene interactions, we used the multifactor dimensionality reduction method (MDR v1.0.0) available at http://www.epistasis.org/mdr.html. P-values lower than 0.05 were considered significant. All analyses were corrected by gender and age.

RESULTS
Table 1 shows the distributions of allelic and genotypic frequencies in patients and controls. The frequencies were in Hardy-Weinberg equilibrium. We evaluated LD bet-
ween rs6311 and rs6313 polymorphisms in HTR2A gene which indicates a linkage between both polymorphic loci (D'=0.87 and r=0.72) and therefore all statistical analyses were performed only on the rs6313 polymorphism. When single marker association under different inheritance models was evaluated, no statistical difference was observed between patients and controls. However, since a combinatorial effect of single polymorphisms is considered to be possible, the contribution of genotypes was also analysed. For this analysis data from our previous study was included.

When risk alleles were analysed under dominant model (5-HTTLPR: SS = LS; 5-HTTVNTR: 10/10 = 10/12; rs6313: CC = TC; Val66Met: AA = GA; APOE 3/3 = 2/3) genotypes combinations L/S 10/12 G/A (OR = 3.57 (95% CI = 1.43-8.93); p = 0.004, adjusted p-value from permutation test p = 0.01) and L/S 10/12 T/C 3/3 G/A in SLC6A4, HTR2A, APOE and BDNF (OR = 5.99 (95% CI = 1.66-21.56); p = 0.002, adjusted p-value p = 0.07) were more prevalent in patients than controls (20% vs. 6%, 15% vs. 3%). The MDR analysis carried out in our sample did not identify evidence of gene-gene interaction between markers in SLC6A4, HTR2A, APOE and BDNF genes. The best overall model obtained was the two locus model SLC6A4-BDNF which had a maximum on the testing balanced accuracy equal to 0.5388 and a cross-validation consistency equal to 10/10; however, a permutation testing (n = 1000) showed a not statistically significant p-value for this model (p>0.05).

**DISCUSSION**

This is the first study investigating the cumulative effect of four of the main genes (BDNF, SLC6A4, HTR2A and APOE) known to be associated with the risk of developing depression in MDD patients and healthy controls, in an Argentinean population. We previously reported an association between polymorphisms in SLC6A4 gene and the risk of major depression.

In the present study, we studied the above mentioned polymorphisms in the same sample of patients and controls and found an association between polymorphisms in SLC6A4 and BDNF genes with MDD. Patients carrying at least one copy of S_10_A (5-HTTLPR, 5-HTTVNTR, and Val66Met polymorphisms respectively) had an increased risk of suffering from MDD. This finding is consistent with previous studies which have correlated SLC6A4 and BDNF genes (S and A alleles) with the modulation of neurophysiological response to aversive and sad stimuli in regions of the brain that are connected to the amygdala, and smaller hippocampal volumes. Amygdala hyperactivity and/or the reduction of hippocampal volumes could represent a neural mechanism underlying increased susceptibility to emotional deregulation and depressive symptoms.

Moreover, it has been shown that healthy subjects carrying at least one copy of S variant show reduced levels of BDNF mRNA in white blood cells. Also, Met allele has been associated with lower availability of BDNF and an interaction between BDNF, 5-HTTLPR and childhood neglect was observed in depressed patients; more specifically, among Met carriers, those with the risk s-allele of the 5-HTTLPR scored significantly higher in BDI-II than L/L homozygotes. On the other hand, a different study found that the probability of each MDD patient to be simultaneously a Val homozygote for BDNF, Met carrier for COMT, and L’ carrier for SLC6A4 was significantly higher in comparison to controls. Taking together, these results suggest that an interaction between alleles of both genes might be synergistically involved in the depressive phenotype. Because gene-gene interactions are difficult to detect using traditional parametric statistical methods, we also performed MDR analysis (method for collapsing high-dimensional genetic data into a single dimension thus permitting interactions to be detected in relatively small sample sizes). Despite the dendogram showed a trend of epistatic interaction between SLC6A4 and BDNF genes (and consistent with the logistic regression analysis) a permutation testing showed a not statistically significant p-value for this model, which reflects that the gene network affecting the interaction between these two genes and MDD might be more complex than previously recognized.
Analysis of a larger sample of patients with MDD would be necessary to identify a more accurate model of the interaction of genes.

We also observed that patients carrying at least one allele of 10_S_C_3_A (SLC6A4, HTR2A, APOE and BDNF respectively) had an increased risk of suffering from MDD. However, this result should be taken with caution because of the sample size and the limited statistical power. Although a replication is needed in a larger sample of Argentinean population with characterized ancestry, the present findings suggest that an interaction of the Val66Met polymorphism in BDNF gene and the 5-HTTLPR and 5-HTTVNTR polymorphisms in SLC6A4 gene may be involved in the pathogenesis of major depressive disorder in a sample of Argentinean population. This interaction might also reflect that differential reactivity to environmental stress could be moderated by both genes.

However, it is still unclear how the coexistence of these variants affects the function of the affective and cognitive control systems.

ACKNOWLEDGES

We specially acknowledged the support of Dr. Pablo Argibay.

We gratefully acknowledged the helpful manuscript revision of Ms. Belén Viano. This research was supported by FUCIBA (Fundación para el desarrollo de Ciencias Básicas), Hospital Italiano de Buenos Aires and CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas) through a scholarship to Sabrina C. Bassi. The funding source did not have a role in the study design; in the collection, analysis or interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

Conflicts of interest: The authors declare that they have no competing financial interests.

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