Association Study Between Serotonin Transporter Gene and Fluoxetine Response in Mexican Patients With Major Depressive Disorder

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Objective: Pharmacogenetic studies have identified genetic variants associated with fluoxetine response in patients with major depression disorder (MDD). The serotonin transporter gene is the principal site of action of selective serotonin reuptake inhibitors. Previous studies analyzing *SLC6A4* gene variants are inconsistent and differ among populations. The aim of the present study was to analyze the association between 5-HTTLPR/rs24531 triallelic polymorphism and fluoxetine response in Mexican patients with MDD.

Methods: We analyzed a sample of 150 patients with MDD. Fluoxetine response was assessed according to a reduction in the Hamilton Depression Rating Scale and Montgomery Depression Rating Scale scores of 50% or more at 8 weeks from baseline. In addition, we analyzed the genotype and allele distribution between responder and nonresponder patients in a subgroup of very severe depression patients.

Results: We did not find association between fluoxetine responders and 5-HTTLPR/rs25531 variants ($P = 0.0637$). However, in the analysis of severe depression at baseline (Hamilton Depression Rating Scale ≥ 25), we observed a high frequency of low activity alleles (S/L_G) in nonresponders patients ($P = 0.0102$).

Conclusions: Our findings showed an association between low activity alleles of *SLC6A4* gene and fluoxetine nonresponse in patients with severe depression.

Key Words: *SLC6A4* gene, pharmacogenetics, response, depression, severe depression

(*Clin Neuropharm* 2018;00: 00–00)

Pharmacogenetic studies can help identify DNA sequence variation among individuals, known as polymorphisms (single-nucleotide polymorphisms [SNPs], variable number of tandem repeats, insertion-deletions, and duplications), in genes encoding the target molecules or pathways with which drugs interact, or those encoding candidate genes that predict clinical response and adverse effects that can be transferred to clinical practice.[1]

Evidence suggests a serotoninergic dysfunction in the development of major depression disorder (MDD). The serotonin transporter is the prime target of action of the selective serotonin reuptake inhibitors (SSRIs), which are used as a first-line treatment for MDD.[2] Serotonin transporter is a monoamine transporter protein that regulates the presynaptic recycling of serotonin and it is mainly expressed in presynaptic membranes and intracellular compartment of neurons. In addition, these proteins are expressed in glial cells of central nervous system and in periphery cells, such as blood platelets and lymphocytes.[3] The SSRI bind to serotonin transporter and inhibit the reuptake of serotonin increasing their synaptic availability, which makes them beneficial to MDD.[2]

Serotonin transporter is codified for the *SLC6A4* gene, and it has been the most widely studied in relation to antidepressant response in several psychiatry disorders. The *SLC6A4* gene is located at position 17q11.1-q12. A functional polymorphism identified in the promoter region is called 5-HTTLPR, and it is characterized for an insertion/deletion of 44 bp that is associated with differential levels of gene transcriptional activity. Another variant, an SNP rs25531, located in the sixth nucleotide within the 1 of 2 extra 20 to 23 bp repeats in the L allele of the ins/del polymorphism involves a substitution of G to A in a 16-repeat variant and designated as L_A and L_G, defines, together with the S allele, a triallelic system (5-HTTLPR/rs25531). Interestingly, the L_G variant contains an AP2 binding site that reduces its expression levels, showing a similar activity to the S allele compared with the L_A variant, which has a high expression level.[4]

There are ethnic differences in the allele distributions of 5--HTTLPR/rs25531 variants across populations that could be clinically important.[5] Genetic studies in whites have reported that L carriers show a better response to SSRIs compared with the S carriers.[6–9] In contrast, studies in Asian populations reported contradictory findings.[10,11] Previously, we reported association between L_A variant of triallelic 5-HTTLPR/rs25531 polymorphism and remission treatment to fluoxetine in a sample of 72 patients with MDD.[12]

Interestingly, an association between 5-HTTLPR polymorphism and response rates in 2 ethnic patient groups were studied, although differences in allele frequencies among the ethnic groups were observed, the study did not find any association with response to sertraline.[13]

We hypothesized that genetic variants of the SLC6A4 gene are associated with fluoxetine response in patients with MDD.

The goal of the present study analyzed the association between 5-HTTLPR/rs24531 triallelic polymorphism and fluoxetine response in Mexican patients with MDD.

MATERIALS AND METHODS

Sample

Patients with MDD were recruited from affective disorders clinic of Instituto Nacional de Psiquiatría Ramon de la Fuente Muñiz in Mexico City. All patients were diagnosed with MDD according to *Diagnostic and Statistical Manual of Mental*

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Conflicts of Interest and Source of Funding: The authors have no conflicts of interest to declare.
This study was supported by FOSISS CONACYT Grant 261459 and Cátedras CONACYT Grant 1683.
Funding was provided by "Consejo Nacional de Ciencia y Tecnología" (CONACYT) Grant FOSISS 261459 to B.C. Some of the equipment used in the present study was kindly financed by CONACYT (Cátedras CONACYT Grant 1683) to C.B.P.

DOI: 10.1097/WNF.0000000000000315

Disorders IV, Text Revised, criteria by *the* Mini-International Neuropsychiatric Interview.[14]

The study was approved by the institutional ethics review board. Informed consent form was obtained of all participants. The study was designed as a naturalistic pharmacogenetic study to analyze genetic variants that are predictive of clinical outcome in relationship to fluoxetine treatment.

Inclusion criteria were patients with MDD with a minimal baseline score of 18 in the Hamilton Depression Rating Scale (HDRS, 21-item version), who started treatment with fluoxetine, aged 18 to 65 years. All participants were Mexican Mestizos with a family background of 3 generations born in Mexico. Exclusion criteria were pregnancy, suicidal risk, concomitant Axis I psychiatric disorder, psychotic symptoms, and significant medical or neurological illness.

Clinical Assessment

The psychiatric diagnostic evaluation was performed through a clinical interview using the Mini-International Neuropsychiatric Interview.[11] The severity of depression was evaluated using 2 established clinician-rated scales: the 21-item HDRS, most widely used measures of depression severity in research and clinical practice,[15] and the Montgomery-Asberg Depression Rating Scale (MADRS),[16] considered the most accurate and internally valid reflection of depression severity. All evaluations were conducted by experienced psychiatrists. The 2 severity depression scales were used to evaluate fluoxetine response by calculating percentage reductions in total scores from baseline to endpoint.

Procedure

Pharmacological treatment was administered in naturalistic conditions, with the treating physicians changing medication dosages at their own discretion in a range between 20 and 40 mg/day according to clinical response over the 8-week treatment duration.

Treatment response was defined as a 50% decreased in scores on the 21-item HDRS and MADRS from baseline to endpoint.

Finally, to determine whether the efficacy of fluoxetine treatment in patients with very severe MDD is associated with genetic variants of 5-HTTLPR/rs25531 triallelic polymorphism, we analyzed the genotype and allele distribution between responder and nonresponder patients. We defined very severe depression at baseline as HDRS score 25 or higher. In addition, we performed an additional analysis defined by very severe depression at baseline as MADRS of 31 or higher.[17–20]

Genetic Analysis

DNA was extracted from whole blood using the Flexigene DNA kit (Qiagen, Minneapolis, Minn). The 5-HTTLPR was genotyped using the primers and the conditions previously reported.[21] Polymerase chain reaction products were resolved on 1.5% high-melt agarose gels and visualized under UV illumination after ethidium bromide staining. Allele sizes were determined by comparison with a 50-bp DNA ladder. SNP rs25531 was subsequently analyzed with a TaqMan SNP Genotyping Assay-by-Design using the conditions described by Gudayol-Ferre et al.[22]

Statistical Analysis

The analysis was performed using the χ^2 test to compare genotype frequencies between responders and nonresponders. One-way analysis of variance and Student *t* test were used to compare demographic and clinical characteristics of the sample.

The power analysis was performed using the program QUANTO V.1.2 (http://hydra.usc.edu/gxe). The sample had a power of 0.98 to detect a 2-fold increased risk, assuming an additive genetic model, a risk allele frequency of 0.46, a population prevalence of 0.1, and α level of 0.05.

RESULTS

A total of 181 patients with MDD were enrolled. One hundred fifty patients completed the study (35 males and 115 females). Eighteen patients dropped out before the end of the study. Thirteen cases were excluded: 9 because of loss of follow up, 3 because of adverse effects, and 1 because of noncompliance with treatment.

At 8-week treatment, 103 patients (68.7%) showed a reduction in total score of the HDRS and MADRS of 50% or more

TABLE 1. Demographic and Clinical Characteristics of Patients With MDD

Variable	Responder (n = 103)	Nonresponder (n = 47)	Analysis
Sex, female, %	73 (70.9)	42 (89.4)	$\chi^2 = 5.2$, $P = 0.0217$
Married/cohabiting, %	40 (38.9)	18 (38.3)	$\chi^2 = 0.004$, $P = 0.95$
Current smoker, %	21 (20.4)	11 (23.4)	$\chi^2 = 0.17$, $P = 0.675$
Unemployed, %	22 (21.4)	8 (17.0)	$\chi^2 = 0.38$, $P = 0.538$
First episode, %	27 (26.2)	14 (29.8)	$\chi^2 = 0.21$, $P = 0.649$
Age, years	36.2 ± 10.2	33.7 ± 11.5	$F = 1.82$, $P = 0.180$
Age at first major episode of depression, years	21.0 ± 11.9	22.8 ± 10.9	$F = 0.74$, $P = 0.392$
Education, years	12.6 ± 3.3	12.6 ± 3.2	$F = 0.003$, $P = 0.955$
No. depressive episodes	2.9 ± 2.2	2.6 ± 1.9	$F = 0.43$, $P = 0.897$
Score on HDRS			
Baseline	25.8 ± 5.5	27.1 ± 4.9	$F = 1.74$, $P = 0.189$
Endpoint	6.4 ± 3.7	18.6 ± 7.2	$F = 1.87$, $P = 0.0000$
Change from baseline to endpoint, %	75.2	31.4	
Score on MADRS			
Baseline	29.6 ± 5.3	30.2 ± 6.7	$F = 0.32$, $P = 0.570$
Endpoint	7.9 ± 4.3	19.9 ± 8.2	$F = 1.37$, $P = 0.0000$
Change from baseline to endpoint, %	73.3	34.1	

TABLE 2. Association Analysis Between Triallelic Polymorphism and Pharmacological Response to Fluoxetine

	Genotypes			Alleles	
	SS, SL_G, L_GL_G	SL_A, L_AL_G	L_AL_A	S, L_G	L_A
Responders (n = 103)	32 (0.31)	53 (0.51)	18 (0.18)	117 (0.57)	89 (0.43)
Nonresponders (n = 47)	21 (0.45)	22 (0.47)	4 (0.08)	64 (0.68)	30 (0.32)

and were classified as responders, whereas 47 patients (31.3%) were nonresponders.

In terms of demographic and clinical characteristics of the sample, we observed differences in the analysis of endpoint scores of HDRS and MADRS between the two groups (Table 1). There were also significant differences by sex between responders and nonresponder groups ($\chi^2 = 5.2$, $df = 1$, $P = 0.0217$).

Analysis of the 5-HTTLPR/rs25531 triallelic polymorphism did not show deviation from the Hardy-Weinberg equilibrium ($\chi^2 = 0.298$, $df = 2$, $P = 0.584$). In Table 2, we showed the genotype and allele frequencies in fluoxetine responders and nonresponders. We observed a high frequency of low activity alleles (S, L_G) in fluoxetine nonresponders compared with the responders (68% vs 57%); however, there was no differences in allele ($\chi^2 = 3.4$, $df = 1$, $P = 0.0637$) or genotype analysis ($\chi^2 = 3.6$, $df = 2$, $P = 0.165$).

An additional analysis was performed comparing the percentage reduction of HDRS and MADRS scores from week 0 to week 8 among the 3 genotype groups. Analysis of variance did not show statistical differences between genotype groups (data not shown).

Finally, to determine whether the efficacy of fluoxetine treatment in patients with severe depression is associated with genetic variants of 5-HTTLPR/rs25531 triallelic polymorphism, we analyzed the genotype and allele distribution between responder and nonresponder groups (Table 3). In the analysis of severe depression at baseline (HDRS ≥ 25), our sample showed a high frequency of low activity genotypes in nonresponders compared with the responder patients (52% vs 26%; $\chi^2 = 7.8$, $df = 2$, $P = 0.0202$). In addition, there were differences in allele frequencies between nonresponder and responder patients with severe depression ($\chi^2 = 6.6$, $df = 1$, $P = 0.0102$) (Table 3).

We observed in nonresponder patients with severe depression at baseline (MADRS ≥ 31) a high frequency of low activity genotype compared with fluoxetine responders (55% vs 32%); however, it did not show significant differences ($\chi^2 = 5.5$, $df = 2$, $P = 0.062$). The analysis of allele frequencies showed statistical differences between responders and nonresponder patients with severe depression ($\chi^2 = 4.8$, $df = 1$, $P = 0.028$) (Table 3).

DISCUSSION

The *SLC6A4* gene variants have been analyzed for association with antidepressant treatment response showing contradictory findings. Studies in white population showed association between L allele and better response[6,8,23]; in contrast, in Asian patients, a high frequency of the S allele was observed in responders.[10,11] A previous study in Mexican patients did not show association between 5-HTTLPR/rs25531 polymorphism and fluoxetine response in a small sample size of patients with MDD[22]; however, an association between the L_A allele and remission was reported after 12 weeks of treatment with fluoxetine in patients with MDD.[12]

In the present study, we found an overall response to fluoxetine rate of 68.3%, in agreement with randomized clinical trials reporting 30% to 40% of nonresponder patients. Interestingly, we observed sex differences in the fluoxetine response. Female patients showed an increased favorable response compared with male patients. Several studies suggest that females respond better to serotoninergic antidepressant than males.[24] In addition, it has been suggested that sex differences in the pharmacological response may be associated with clinical and biological factors, such as physiological and behavioral characteristics, comorbidity, menopause, pregnancy, and adherence to pharmacological treatment.[24]

Our study did not find association between *SLC6A4* gene variants and antidepressant response. This result is consistent with those of previous studies.[25–27] It is interesting that we observed in the present study a trend toward higher frequency of low activity alleles in the fluoxetine nonresponder patients; however, this trend may be related to the small sample size in the current study.

Treatment response is a complex phenotype that requires an accurate assessment. The inconsistency in the findings among different studies may be the result of heterogeneity in the phenotype definition with a crucial impact in the pharmacogenetic findings.[28–30] The identification of clinical characteristics that reduces the heterogeneity might increase the possibility to identify the gene variants involve in the treatment response.[29]

Several studies reported that the severity of depression affects the efficacy of antidepressant treatment[31–33]; therefore, we

TABLE 3. Genotype and Allele Frequencies of 5-HTTLPR/rs25531 Polymorphism in Patients With Severe Depression According to HDRS and MADRS at Baseline

	Genotypes			Alleles	
	SS, SL_G, L_GL_G	SL_A, L_AL_G	L_AL_A	S, L_G	L_A
HDRS ≥ 25					
Responders (n = 59)	15 (0.26)	35 (0.59)	9 (0.15)	65 (0.55)	53 (0.45)
Nonresponders (n = 33)	17 (0.52)	15 (0.45)	1 (0.03)	49 (0.74)	17 (0.26)
MADRS ≥ 31					
Responders (n = 44)	14 (0.32)	23 (0.52)	7 (0.16)	51 (0.58)	37 (0.42)
Nonresponders (n = 22)	12 (0.55)	10 (0.45)	0	34 (0.77)	10 (0.23)

analyzed the association between 5-HTTLPR/rs25531 and response in a subgroup with severe depression. In our sample, most patients showed severe depression (HDRS ≥ 25) at baseline (61.3%). The analysis of 5-HTTLPR/rs25531 polymorphism showed a high frequency of low activity alleles in nonresponder patients. We also analyzed the 5-HTTLPR/rs25531 polymorphism in severe depression according to MADRS of 31 or higher at baseline showing a high frequency of low activity alleles in nonresponder patients; however, this difference in frequency was not significant.

It has been shown that MADRS have a better capacity to differentiate between responders and nonresponders to antidepressant treatment compared with the HDRS.[34] Therefore, the discrepancies in the findings reported among studies should be related with the assessment scales used for the definition of treatment response.

Limitations of the study were the small sample size; fluoxetine plasma levels were not determined in the present study, medication compliance was not controlled in the naturalistic design of the study, the absence of a washout period after previous treatments, and inclusion of only patients treated with fluoxetine as the sole SSRI.

In conclusion, our findings showed an association between low activity alleles of *SLC6A4* gene and fluoxetine nonresponse in patients with severe depression. Future studies should consider multiple polymorphisms of the *SLC6A4* gene and greater homogeneity of the samples phenotypes (eg, greater severity of baseline depressive symptomatology).

ACKNOWLEDGMENTS

The authors thank the families and patients who participated in this study.

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