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Association between 5-HTTLPR polymorphism, suicide attempt and comorbidity in Mexican adolescents with major depressive disorder

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Introduction. The World Health Organization reports that suicide is among the leading causes of death for young people. Depression is the most frequently related disorder with suicidal behaviors. There is increasing evidence that suicidal behavior has a strong genetic contribution. Several studies report an association between the genotype "SS" and the "S" allele of the 5-HTTLPR polymorphism of the serotonin transporter gene and suicidal behavior. The aim of the study was to determine the association of variants of the serotonin transporter gene with suicidal attempt and comorbidity in depressed adolescents.

Methods. The frequencies of ss genotypes and s allele were compared between a sample of 200 adolescents with a diagnosis of depression and the antecedent of a suicide attempt who were evaluated with K-SADS-PL and a group of 235 healthy controls. Genotyping of the 5-HTTLPR polymorphism was performed by PCR.

Results. Analysis of the frequencies of genotypes and alleles showed a statistically significant difference between the groups (Genotypes: x2=11.1, df=2, p=0.004, Alleles: x2=11.9, df=1, p=0.0009). There were no associations with comorbid disorders.

Conclusions. The results support the hypothesis that the serotonin transporter gene, specifically the s allele and the ss genotype of the 5-HTTLPR polymorphism, are related to the history of depression and suicide attempt in adolescents.

Keywords: Adolescents, Suicide, Depression, Genes

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Asociación entre el polimorfismo 5-HTTLPR, el intento suicida y la comorbilidad en adolescentes mexicanos con trastorno depresivo mayor

Introducción. De acuerdo a la Organización Mundial de la Salud el suicidio se encuentra en las principales causas de muerte en niños y adolescentes. La depresión es el trastorno más asociado a la conducta suicida. Existe cada vez mayor evidencia respecto a que la conducta suicida tiene una fuerte contribución genética. Varios estudios reportan una asociación entre el genotipo "SS" y el alelo "S" del polimorfismo 5-HTTLPR del gen del transportador de serotonina y la conducta suicida. El objetivo del estudio fue establecer la asociación de las variantes del gen del transportador de serotonina con el intento suicida y la comorbilidad en pacientes adolescentes deprimidos.

Metodología. Se compararon las frecuencias de genotipo "SS" y del alelo "S" entre una muestra de 200 adolescentes evaluados con la entrevista semi-estructurada K-SADS-PL y una muestra de 235 controles sanos. La genotipificación del polimorfismo 5-HTTLPR se realizó mediante PCR.

Resultados. El análisis de las frecuencias de genotipos y alelos mostró diferencia estadísticamente significativas entre los grupos (Genotipos: x^2=11,1, gl=2, p=0,004; Alelos: x^2=11,9, gl=1, p=0,0009). No existió asociación con los trastornos comórbidos.

Conclusiones. Los resultados apoyan la hipótesis de que el gen del transportador de serotonina, específicamente el alelo s y el genotipo ss del polimorfismo 5-HTTLPR, se encuentran relacionados con la historia de depresión e intento suicida en adolescentes.

Palabras clave: Adolescentes, Suicidio, Depresión, Genes

INTRODUCTION

The World Health Organization estimated that by 2020 approximately 1.5 million people will die by suicide and 15 to 30 million will make a suicide attempt[1]. This organization also establishes that suicide is among the five main causes of death in youngsters between 15 and 19 years of age and is the sixth cause of death for those between 5 to 14 years of age[2]. There is enough evidence that the frequency of suicide in children, adolescents, and young adults is increasing around the world, for example in the United States the number of deaths by suicide in the adolescent population increased dramatically (up to 300%) over the last decades[3]. In Mexico a total of 4,388 people across the country died from suicide during 2007, with a mortality rate of 4.12 per 100000, representing a 275% increase respect to that reported in 1970[4]. In this regard, Pérez-Amezcua conducted a national cross-sectional study in 12,424 adolescents attending to public high schools belonging to the 32 states of Mexico and found that 47% had suicidal ideation at some point in their lives and 9% had attempted suicide[5].

Recent data indicate that up to 50% of the mental disorders have an adolescent onset, and that diagnostic delay has been associated with a poor outcome[6]. In this regard, the main risk factors known to be associated with suicidal behavior in this age group include depression, hopelessness, dysfunctional families, substance abuse, school failure and harassment[7]. In particular, depression is associated with this behavior[8,9], more than half of the affected population will present an attempt, and 10% died by suicide within 15 years of being diagnosed[10,11].

Several efforts were conducted in the last decade to establish the genetic bases of suicidal behavior, for example, genetic studies demonstrated the existence of genetic factors involved in the development of suicidal behavior, estimating a 30% -55% [12-14], while the remaining component can be explained by environmental factors.

Other studies focused on the coding genes for the enzymes, transporters and serotonin receptors[15,16] showed an association between the serotonin transporter gene (SLC6A4) and suicidal behavior[17]. The serotonin transporter (5-HTT) gene, which is found on chromosome 17 on location 17q11.1-q12, is one of the major genes known to influence serotonergic transmission. The gene has long (L) and short (S) variants, which are identifiable by the insertion or deletion of 44 base pairs close to the beginning of the gene's transcription site. Recent studies found that individuals with the shorter variant were more likely to exhibit suicidal behavior[18], aggressiveness and impulsiveness[19,20]. Schild et al[21] conducted a study analyzing the frequency of suicide in different countries and found an ethnicity-based interaction between national suicide rates and 5-HTTLPR allele frequency, with the s allele acting as a protective factor in Caucasian and as a risk factor in non-Caucasian populations. Regarding studies in adolescent samples, the one carried out by Cicchetti and collaborators[22] showed that those who were carriers of the "S" allele and had a history of child abuse had a stronger association with suicidal behavior compared with the "L" allele carriers. In addition, a study conducted with 1,030 Americans as part of the National Longitudinal study of adolescent health, showed that the carriers of the "S" allele who had poor family support had greater severity of depressive symptoms and a higher risk for develop suicide attempts compared with carriers of allele "L"[23]. However, the findings regarding the participation of the 5-HTTLPR gene SLC6A4 aren't conclusive [24].

In Mexico, there are no reports of genetic association in adolescent samples with a diagnosis of depression and a history of suicide attempt. The objective of this study was to determine the association of polymorphic variants of the serotonin transporter gene in Mexican adolescents with major depressive disorder and suicide attempt and with their comorbid disorders.

METHODS

The recruitment of patients was carried out in the inpatient and outpatient services of the Child Psychiatric Hospital in Mexico City. We included all adolescents who had a diagnosis of depression according to the Diagnostic and Statistical Manual of Mental disorders, fourth edition revised text (DSM IV-TR) and a suicide attempt in the last 6 months. The acceptance to participate in the study was carried through the signing of the informed consent and assent by the patients and their parents. The study was approved by the hospital ethics committee.

Patients were evaluated with the diagnostic interview semi-structured Schedule for affective disorders and schizophrenia for school-age children-present and lifetime version (K-SADS-S-PL)[25]. This interview confirmed the diagnosis of depression, determined the type and number of comorbid diagnoses and the occurrence of the suicide attempt during the current depressive episode. Assessment of the suicide attempt was done with the five items concerning suicidal ideation and behavior of the screening interview of the K-SADS-PL. Were taken into account when all the informants (patients and parents) agreed on the intention of dying.

After completing the interview, a blood sample was taken to obtain the DNA and analysis of the 5-HTTLPR polymorphism, by PCR using the conditions described by Camarena et al.[26]

The control group consisted of 235 individuals older than 25 years, with no current or past history of a mental disorder who were evaluated with the through the psychiatric interview DIS (Diagnostic Interview Schedule)[27]. The sample was part of the genomic bank of the pharmacogenetics department of the National Institute of Psychiatry "Ramón de la Fuente Muñiz".

STATISTICAL ANALYSIS

The statistical analysis was performed using the program SPSS 18.0 (Statistical Package for the Social Sciences, Chicago, USA). Frequencies and percentages were obtained for categorical variables and mean and standard deviation for continuous variables. As a hypothesis test in the comparison of groups, Chi-Square (X2) was used to evaluate the differences in allele frequency between the patient and the control group; as well as the differences in the frequency of comorbid disorders among the carriers of the different alleles. Also, one-way ANOVA was performed to compare age and number of comorbid diagnoses. The level of statistical significance was set at $p \leq 0.05$.

RESULTS

We included 200 patients with a diagnosis of depression and a history of suicide attempt, 64% were female (n=128). Their mean age was 14.3 ± 1.6 years (range 11-18 years). At the time of the study, 76.5% (n=153) of the sample had at least one comorbid disorder (range 1 to 4), the most frequent were dysthymia (28.5%), generalized anxiety disorder (18.5%), oppositional defiant disorder (20.5%) and attention deficit hyperactivity disorder (19.5%). Comorbidity with alcohol use disorders was found in 2.5% of the sample.

Figure 1 shows the methods used in the latest suicide attempt reported by patients.

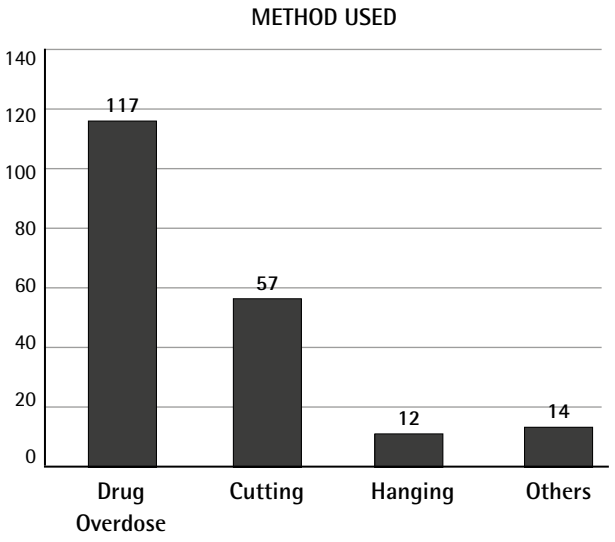

Figure 1 | *Suicidal method used*

The frequencies of genotypes and alleles showed a significant difference between the patients and controls (x^2=11.1, gl=2, p=0.004; x^2 =11.9, gl=1, p=0.0009, respectively) (Table 1).

Table 2 shows the demographic and clinical characteristics of the patients, as well as the association of the genotypes with the main comorbidities found.

DISCUSSION

The main objective of the present study was to determine the association of polymorphic variants of the serotonin transporter gene in Mexican adolescents with a diag-

Table 1 | **Genotypes and alleles frequency of 5-HTTLPR polymorphism**

	Genotypes Frequency			Alleles Frequency	
	SS	SL	LL	S	L
Cases (n=200)	81 (0.40)	89 (0.45)	30 (0.15)	251 (0.63)	149 (0.37)
Controls (n=233)	66 (0.28)	106 (0.46)	61 (0.26)	238 (0.51)	228 (0.49)

Genotypes: x^2=11.1, gl=2, p=0.0043
Alleles: x^2=11.9, gl=1, p=0.0009

Table 2 **Demographic and clinical characteristics of subjects who carried different genotypes**

Characteristic	LL N=33	SL N=89	SS N=78	Statistics
Female	63.3%	60.7%	69.3%	x^2=0.96, p=0.61
Mean age (SD)	14.6 (1.5)	14.1 (1.6)	14.3 (1.6)	F=0.98, p=0.37
Mean comorbid disorders (SD)	1.2 (0.9)	1.1 (0.8)	1.2 (1)	F=0.61, p=0.54
Generalized anxiety disorder	16.7%	19.1%	18.5%	x^2=0.08, p=0.95
Dysthymia	14%	42.1%	43.9%	x^2=0.374, p=0.82
Attention Deficit Hyperactivity Disorder	20%	20.2%	18.5%	x^2=0.08, p=0.95
Negative Defiant Disorder	26.7%	20.2%	18.5%	x^2=0.89, p=0.63

nosis of depression and suicide attempt. The results showed that there is a difference in the frequency of the different alleles of this gene between the patient and the control group, but no association was observed between the alleles and other clinical variables.

Regarding the clinical characteristics of the patients included in the study, our results are similar to those reported in the international scientific literature where it has been observed that suicide attempts are more frequent in females[28]. Regarding the methods used there were also no differences with studies that analyze this variable. In general, non-violent methods such as drug overdose are usually preferred in suicide attempts[29,30].

The literature has reported that 40 to 70% of depressed adolescents have two or more comorbid diagnoses, being the most frequent dysthymia, anxiety disorders, attention deficit hyperactivity disorder, disruptive behavior disorders and substance abuse disorder[31]. Our findings were consistent with those reports since 83.5% of the sample showed comorbidity.

The results support previous descriptions regarding an association between genotype "SS" and the "S" allele of the 5-HTTLPR polymorphism with suicidal behaviour[16,19,32], particularly with the Li study[23], which associated the presence of the "S" allele with depressive symptoms and higher suicide risk in adolescents. The differences between the present results and those of the Zalsman[33] and Cichetti[22] studies, which, despite using similar methods, did not find an association with suicidal behavior, can be explained by the larger number of patients examined in our study and the differences in the populations, since it has been demonstrated that there are allelic variations among the ethnic groups[34]. No differences were found in the frequency of comorbid disorders among the groups carrying the different genotypes, which resembles previous reports that have studied this association[11,35].

The present results should be examined taking into account limitations such as the sample size and the fact that the controls were not age-matched. Future genetic studies could use case-parent trio studies to determine this association. Given the suicidal behavior heterogeneity and its hereditary complexity, the need to find demonstrable intermediate phenotypes that may make it possible to establish links between genes and suicide behaviors (endophenotypes). The main candidate endophenotypes of suicidal behaviors are neuropsychological, personality traits, neurochemistry and neuroimaging[36].

In conclusion, the higher frequency of "SS" genotype and "S" allele in the study population supports the hypothesis that the serotonin transporter gene (SLC6A4) plays an important role in the development of suicidal behavior in adolescents with a diagnosis of depression, regardless of the presence of psychiatric comorbidity.

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CONFLICTS OF INTEREST

The authors declare not to have conflicts of interest.

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