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Polymorphism rs754203 in Gene CYP46A1 Is a Potential Suicide Risk Biomarker in Major Depressive Disorder

María Fernanda Serna-Rodríguez [1], Oscar Cienfuegos-Jiménez [2], Ricardo Martín Cerda-Flores [3], Iván Alberto Marino-Martínez [2], Mario Alberto Hernández-Ordoñez [4], José Alfonso Ontiveros-Sánchez de la Barquera [5] and Antonio Alí Pérez-Maya [1,*]

[1] Departamento de Bioquímica y Medicina Molecular, Facultad de Medicina. Universidad Autónoma de Nuevo León, Monterrey, N.L., México. CP. 64460.
[2] Centro de Investigación y Desarrollo en Ciencias de la Salud de la Universidad Autónoma de Nuevo León. Monterrey, N.L., México. CP. 64460
[3] Facultad de Enfermería, Universidad Autónoma de Nuevo León, Av. Dr. José Eleuterio González 1500, Mitras Centro, Monterrey,N.L., México. CP 64460.
[4] Departamento de Medicina Forense, Hospital Universitario "Dr. José Eleuterio González", Universidad Autónoma de Nuevo León, Av. Francisco I. Madero y Av. Gonzalitos s/n, Mitras Centro, Monterrey, N.L., México. CP 64460.
[5] Departamento de Psiquiatría, Hospital Universitario "Dr. José Eleuterio González", Universidad Autónoma de Nuevo León, Av. Francisco I. Madero y Av. Gonzalitos s/n, Mitras Centro, Monterrey, N.L., México. CP 64460.
* Correspondence: bioquimicomty@gmail.com

Abstract: Background: Currently, completed suicide, suicide ideation, suicide behavior, and suicide attempts are major public health problems worldwide. Major Depressive Disorder (MDD) is one of the most common mental disorders associated with an increased risk of suicide. Since the relationship between suicide and cholesterol levels is still controversial, in this study, we explore the association between SNPs rs754203 and rs4900442 of *CYP46A1* with suicide risk in Mexican patients with major depressive disorder. **Methods:** We evaluated 188 unrelated suicide completers and compared them to 144 non-suicidal individuals (controls) and 126 MDD patients. Genotypes were analyzed using the Real Time-polymerase chain reaction method and two allele-specific probes to detect specific SNP targets. A chi-square test was used to identify a possible risk genotype or allele type for suicide. **Results:** Statistical analysis showed significant differences between completed suicide and controls in their allelic and genotypic frequencies in rs754203 SNP. The genotype G/G of *CYP46A1* rs754203 was significantly associated with suicide. Also, the G allele was associated with an increased risk of suicide (OR= 1.370, 95% CI= 1.002-1.873). No differences in either genotype distribution or allele frequencies of *CYP46A1* rs4900442 were observed. **Conclusions:** The results of the current study report the first association between G allele carriers (A/G + G/G) of rs754203 and increased risk for suicide, especially in males.

Keywords: suicide; risk factor; polymorphism; CYP46A1; depression

1. Introduction

Suicide can be defined as an intentional attempt by a person to end his own life (Nock et al., 2008). Currently, completed suicide, suicide ideation, suicide behavior, and suicide attempts are major public health problems around the world. Recently a study has calculated a suicide prevalence of 8.76 per 100,000 inhabitants in Mexico and previous reports have estimated 804,000 annual suicides worldwide (Cabello-Rangel et al., 2020). Major Depressive Disorder (MDD) is one of the most common mental disorders, affecting over 320 million people worldwide, and has been associated with an increased risk of suicidal ideation (SI), suicide attempts (SA), and completed suicide (Liu et al., 2022; Xu et al., 2022).

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Even though serotoninergic genes are typically considered the main constituents of suicidal behavior at the molecular level (Wang et al., 2009), recent discoveries have suggested other previously unknown non-serotoninergic parameters that can also be used as markers. Non-serotoninergic candidates include Catechol-*O*-methyltransferase (*COMT*) (Bellivier et al., 1998), the brain derived neurotrophic factor (*BDNF*) (Zarrilli et al., 2009), and genes related to cholesterol metabolism (Knowles et al., 2018).

Cholesterol is the main constituent of cell membranes and is involved in cell signaling multiple pathways in many cell types. Alterations in cholesterol metabolism have been typically linked to cardiovascular disease, neurodegenerative disease, and neoplasia (Sirianni et al., 2019). Interestingly, even though high cholesterol and triglyceride levels have been historically associated with most of the previously mentioned illnesses, in psychiatry it has been suggested that low lipids could be associated with suicide (De Berardis et al., 2012). More specifically, low levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) can presumably be good predictors of suicide attempts in old and middle-aged patients (González-Castro et al., 2021). Moreover, cholesterol levels could be related to aggressive behavior (Hjell et al., 2020), suggesting a direct link between the metabolism of lipids and neuronal signaling. Indeed, the relationship between cholesterol and serotoninergic signaling could explain the previously mentioned discoveries (Sun et al., 2015). Since serotoninergic parameters are well-established markers of suicide, cholesterol levels can be considered as an indirect surrogate that can be evaluated easily in patients with suicide risk.

A recent study suggested that the suicide-attempt risk increase in low cholesterol level patients is 123%. On the other hand, an 85% risk increase of suicide completion was found in the same patients under this condition (González-Castro et al., 2021). Interestingly, reduced-cholesterol levels have been also associated with depression in rat models (Sun et al., 2015). Nevertheless, some authors have questioned this association. A study made on suicide attempters has found no correlation between low cholesterol levels and suicide risk but an inverse correlation in men (De Leon et al., 2011). Thus, further evaluation is necessary to support or reject this theory.

As part of a natural hypothesis derived from the aforementioned observations, there is great interest in exploring the role of enzymes involved in cholesterol homeostasis. A key enzyme in the brain-cholesterol metabolism is a 24-hydroxylase enzyme encoded by Cytochrome P450 Family 46 Subfamily A Member 1 (*CYP46A1*) gene. This enzyme manages the cholesterol turnover in the central nervous system (CNS). In the brain, dead-neuron-derived cholesterol is converted into 24S-Hydroxycholesterol (24S-OH-Chol) by CYP46A1. Being the main oxysterol in the brain, 24S-OH-Chol has been proposed as a metabolite that could be directly linked to neurodegenerative damage, however, several studies treat this association as controversial (Gamba et al., 2021).

The identification of Single Nucleotide Polymorphisms (SNPs) of this gene could be attractive to promote CYP46A1 as a parameter of suicide behavior. SNP rs754203 of *CYP46A1* has been associated with mild cognitive impairment in type 2-diabetes patients (Shi et al., 2021). Moreover, another SNP of interest is rs4900442, which has been associated with neurodegeneration and Alzheimer's disease (AD) in certain populations (Jia et al., 2016).

Since the relationship between suicide and cholesterol levels is still controversial, in this study, we explore the association between SNPs rs754203 and rs4900442 of *CYP46A1* with suicide risk in Mexican patients with major depressive disorder.

2. Methods

2.1. Participants and samplings

This study was conducted from August 2017 to August 2022. We present data from three cohorts: completed suicide victims, MDD patients with and without suicidal behavior, and controls. All subjects were Mexicans. Suicide victim samples were collected during the autopsy performed at the Department of Forensic Medicine at the "Dr. Jose Eleuterio Gonzalez" University Hospital, Autonomous University of Nuevo Leon, Monterrey.

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All patients were consecutively recruited from the Psychiatry Department at the "Dr. Jose Eleuterio Gonzalez" University Hospital. The suicidal group was recruited from the emergency room and inpatients whose SA and SI were confirmed by a psychiatrist. The non-suicidal group was recruited from psychiatric inpatients. Participants were diagnosed with MDD by a trained psychiatrist with a structural interview based on the Diagnostic and Statistical Manual 5 (DSM-V) criteria. Controls had no lifetime history of mental illness or suicidal behavior. All participants understood and signed an informed consent form detailing the research goal, procedures, caveats, and safeguards. The study was approved by the Institutional Ethics Research Committee of the Hospital Universitario "Dr. José Eleuterio Gonzalez"- Universidad Autónoma de Nuevo León (UANL) in Monterrey, Mexico (protocol registration number BI18-00002). The study was conducted under the principles of Helsinki's statement.

Peripheral blood (6mL) was collected into BD Vacutainer® blood collection tubes with ethylenediaminetetraacetic acid (EDTA) by routine venipuncture, labeled with an anonymized ID number, and stored at 4°C in a locked freezer until the time of future processing.

2.2. Measures and data collection

Demographic information, mental health status, and suicidal behavior characteristics were obtained through face-to-face interviews using a structured questionnaire conducted by a trained psychiatrist. Written informed consent was signed by each participant before the interview, which took about 1.5 h. All participants had complete freedom and the right to answer any questions or terminate the interview at any time.

We designed a questionnaire to obtain demographic information including age, sex, occupation, education level, marital status, religious belief, lifestyle choices (eating habits, drinking alcohol, smoking, exercise), and family history of mental illness or suicide.

To evaluate suicide risk in the participants, we applied the Columbia Suicide Severity Rating Scale, or C-SSRS, which is a suicidal ideation and behavior rating scale created by researchers at Columbia University, the University of Pennsylvania, the University of Pittsburgh, and New York University. This scale consists of four categories: severity and intensity of suicidal ideation, severity, and lethality of past and recent suicidal behavior. C-SSRS was designed to provide definitions of suicidal behaviors and ideation, non-suicidal self-destructive behaviors, as well as to quantify the full spectrum of suicidal ideation and behavior to measure the severity at given periods.

The Mini International Neuropsychiatric Interview (MINI) is a short diagnostic structured interview to evaluate a wide range of psychiatric disorders according to DSM-IV or ICD-10. The MINI focuses mainly on current diagnoses and only explores lifetime diagnoses where it is clinically relevant to the present. We focus on the suicidal ideation section which consists of 7 questions related to suicide risk in the previous month of the interview, and if there has been a suicidal attempt throughout his life.

The Beck Depression Inventory (BDI), which is a 21-question multiple-choice self-report inventory, was applied to measure the severity of depression. Each question had a set of at least four possible responses (0 – 3), ranging in intensity. The scores obtained determine the depression's severity where the cut-off scores go as follows: 0-9 indicates minimal depression, 10-18 mild depression, 19-29 moderate depression, and 30-63 indicates severe depression.

2.3. DNA extraction and genotyping

According to the manufacturer's instructions, DNA was isolated from peripheral blood samples using QIAmp2 DNA Blood Mini Kit. DNA concentration was evaluated by NanoDrop 2000 (Thermo Fisher Scientific Inc., Wilmington, DE, USA). All samples were normalized to 50 ng/µL to perform an initial quality control evaluation of DNA based on PCR amplification of the *TNF* gene which was visualized on a 2% agarose gel stained with GelRed. All samples that passed the quality evaluation were genotyped using a pre-designed Taqman SNP Genotyping Assay for *CYP46A1* rs754203 and rs4900442 in the StepOnePlus™ Real-Time PCR System (Applied Biosystems).

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2.4. Statistical analysis

Descriptive statistics were used to characterize the sample and a t-test or ANOVA was used to examine differences between the three groups. The distribution of genotypes was analyzed using the Hardy-Weinberg Equilibrium (HWE) test. For allele and genotype frequencies odds ratio (OR) with a 95% confidence interval (CI) was calculated. Stratified analysis by sex was carried out. The significance level was set as *P* less than 0.05. Statistical analyses were carried out using SPSS 27.0 statistical package (IBM Corporation).

3. Results

3.1. Clinical and demographic characteristics

A total of 188 suicide completers, 126 MDD patients, and 144 controls were included in the current analysis. The case-control demographic characteristics are presented in Table 1. Among de 188 cases, 90.14% died by suffocation, mostly male (82.97%). The mean age of completed suicide, patients, and controls was 36.42 ± 16.93, 33.10 ± 14.55, and 30.41 ± 12.40 years old, respectively. Control presented a longer education compared to patients (p <0.001). We did not find statistically significant differences in the belief of religion. The BDI score of patients and control were 16.09 ± 12.15 and 4.74 ± 4.14, respectively.

Table 1. Demographic and clinical characteristics of cases and controls.

	Completed suicide (n=188)	**MDD patients** (n=126)	**Controls** (n=144)	**P**
Sex (male/female)	156/32	36/90	69/75	<0.001[a]
Age (years)	36.42 ±16.93	33.10 ±14.55	30.41 ±12.40	0.016 [b]
Education (years)		13.70 ±3.40	16 ±2.73	<0.001 [a]
Belief in religion (%)		95 (49.5)	97 (50.5)	0.083 [a]
BDI score		16.09 ±12.15	4.74 ±4.14	<0.00 [a]

Mean ±SD. MDD: Major Depression Disorder. BDI: Beck Depression Inventory. [a] T-student. [b] Comparison among three groups.

3.2. Genotyping and allelic distributions

3.2.1. Genotyping and Allelic Distributions in Completed Suicide and Controls

The distribution of both polymorphisms was fit to HWE in each group. This study found a significant association between rs754203 (*CYP46A1*) and the risk of suicide. Allelic and genotypic frequencies are presented in Table 2. Statistical analysis showed significant differences between completed suicide and controls in their allelic and genotypic frequencies in rs754203 SNP. The genotype G/G of *CYP46A1* rs754203 was significantly associated with suicide (Table 2). Also, the G allele was associated with an increased risk of suicide (OR= 1.370, 95% CI= 1.002-1.873).

No differences in either genotype distribution or in allele frequencies of *CYP46A1* rs4900442 were observed between cases and controls (Table 2).

Table 2. Distribution of genotypes and alleles of *CYP46A1* polymorphisms.

SNP	**Genotype**	**Completed suicide**	**Controls**	**p-Value**	**OR**	**95% CI**
rs754203	A/A A/G G/G	48 (25.5%) 109 (58.0%) 31 (16.5%)	51 (35.4%) 77 (53.5%) 16 (11.1%)	**p=0.04776**	**2.059**	1.001-4.232

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	p-value HWE	p= 0.0203	p= 0.1011			
	Allele					
	A	205	179	**p=0.0484**	**1.370**	1.002-1.873
	G	171	109			
	C/C	25 (13.7%)	25 (17.5%)			
	C/T	102 (55.7%)	69 (58.3%)	p=0.69773	1.143	0.582-2.242
	T/T	56 (30.6%)	49 (34.3%)			
rs4900442	p-value HWE	p= 0.0457	p= 0.9333			
	Allele					
	C	152	119	p=0.9839	1.003	0.733-1.373
	T	214	167			

HWE: Hardy–Weinberg equilibrium, OR: Odds Ratio, CI: Confidence interval.

3.2.2. Genotyping and Allelic Distributions based on gender

We conducted an analysis based on gender due to the evidence that suicide markers seem to be affected by this variable. The distribution of rs754203 polymorphism was fit to HWE in each group. Allelic and genotypic frequencies are presented in Table 3. We found a significant difference between male completed suicide and male controls in their allelic and genotypic frequencies for rs754203 SNP. The heterozygote genotype (A/G) was significantly associated with suicide in males (Table 3). No differences in either genotype distribution or in allele frequencies in females for both polymorphisms were observed.

Table 3. Distribution of genotypes and alleles of *CYP46A1* polymorphisms in MDD patients.

SNP	Genotype	Completed suicide	Controls	p-Value	OR	95% CI
	A/A	37 (24.2%)	26 (38.2%)			
	A/G	91 (59.5%)	32 (47.1%)	**p=0.0335**	**1.998**	1.050-3.802
	G/G	25 (16.3%)	10 (14.7%)			
rs754203	p-value HWE	p= 0.0148	p= 0.9790			
	Allele					
	A	165	84	p=0.1249	1.380	0.914-2.085
	G	141	52			

HWE: Hardy–Weinberg equilibrium, OR: Odds Ratio, CI: Confidence interval.

3.2.3. Genotyping and Allelic Distributions in MDD Patients and Controls

The differences in allelic and genotypic frequencies in MDD patients with controls were compared. However, Table 4 shows no statistically significant associations for both polymorphisms.

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Table 4. Distribution of genotypes and alleles of *CYP46A1* polymorphisms in MDD patients.

SNP	Genotype	MDD patients	Controls	p-Value	OR	95% CI
rs754203	A/A A/G G/G	56 (45.5%) 51 (41.5%) 16 (13%)	51 (35.4%) 77 (53.5%) 16 (11.1%)	p=0.8164	0.911	0.413-2.007
	p-value HWE	p= 0.4203	p= 0.1011			
	Allele A G	128 83	179 109	p=0.3241	0.836	0.586-1.193
rs4900442	C/C C/T T/T	30 (24.6%) 54 (44.3%) 38 (31.1%)	25 (17.5%) 69 (58.3%) 49 (34.3%)	p=0.2066	0.646	0.328-1.275
	p-value HWE	p= 0.2204	p= 0.9333			
	Allele C T	114 130	119 167	p=0.2371	0.813	0.576-1.148

HWE: Hardy–Weinberg equilibrium, OR: Odds Ratio, CI: Confidence interval.

4. Discussion

To the best of our knowledge, this is the first study to investigate the influence of *CYP46A1* rs754203 and rs4900442 polymorphisms on suicide and MDD. Suicide is a complex, multifactorial behavioral phenotype involving genetic predispositions and distal/proximal environmental factors. Family, twin, and adoption studies have established a genetic basis for suicidal behavior, with heritability of close to 50% (Coon et al., 2018; Dwivedi, 2012; Supriyanto et al., 2011). The search for biomarkers that help detect suicide risk in the early stages is valuable, highlighting the importance of studying suicide and psychiatric genetics. Identifying allelic and genotypic variants through SNPs may help understand the pathophysiology of suicide.

In this study, we found that the frequency in carriers of *CYP46A1* rs754203 G allele (A/G + G/G) increased the risk for suicide, especially in males. On the other hand, there was no difference in the genotype and allele frequencies of rs4900442 between the three groups. This is the first time these SNPs are evaluated in relation to their association with suicide risk, however, both polymorphisms have been previously associated with neurological disorders, such as Alzheimer's disease.

Cytochrome P-450, family 46, subfamily A, polypeptide 1 (CYP46A1, also known as cholesterol 24S-hydroxylase), is the principal enzyme that mediates the conversion of cholesterol to 24S-hydroxycholesterol (24-OHC), the major cholesterol elimination product of the brain cholesterol (He et al., 2012; Jia et al., 2016; Jin et al., 2013). 24-OHC is a neurotoxic product and can induce nerve cell death. CYP46A1 is specially located in neurons among brain regions including the cerebellar cortex, thalamus, and hippocampus (Na et al., 2021; Shi et al., 2021). In addition to the role of regulating cholesterol synthesis, CYP46A1 may control or modulate crucial elements of the neuron function (Burlot et al., 2015). Freemantle et al. (2013) suggest that increased oxysterol, such as 24-OHC, levels in the CNS are also implicated in the neuropathology of depression and suicide. The results of their study suggest that there is an increased turnover of cholesterol to 24-OHC in the prefrontal cortex of suicides and this might have implications for synapse maintenance and loss in the neuropathology.

The rs754203 polymorphism has been reported to participate in the risk of AD, suggesting that it might play a role in mediating the increased risk of AD by causing differences in blood 24-OHC concentration levels (Shi et al., 2021). He et al. (2012) reported that the distribution of CYP46A1 rs754203 genotypes was significantly different in AD patients compared to controls, with the variant allele being having a higher frequency. This SNP is located in the intronic region of *CYP46A1* gene, which does not affect the amino acids sequence or the structure of the protein expressed by the target gene, however, it seems to be involved in the risk of some neurological disorders (Jin et al., 2013). These results have been controversial due to that not everyone can replicate the results, such as Shi et al. (2021) that report no association between rs754203 genotype and 24-OHC plasma levels in type 2 diabetes mellitus (T2DM) patients with risk of developing early cognitive impairment. Neurological diseases have been associated with an increase in suicidal ideation and behavior. This association is particularly strong in the case of AD and other dementias (Alejos et al., 2020).

Lastly, we did not find an association between rs4900442 SNP and suicide or MDD. These null results may be due to the relatively small sample size of patients. Other authors have studied this SNP in relation to other neurological disorders and its role as a genetic risk factor remains controversial due to the differences across ethnicities and the lack of reports on this matter (Jia et al., 2016).

There are several limitations to this study. First, our sample size for the patient group was relatively small. It is possible that the true effects of CYP46A1 genotypes on suicide were not detected in our study. Second, we did not measure 24-OHC levels to evaluate the effect these alleles or genotypes might have on the expression of this metabolite. Additionally, because the pathogenesis of suicide is complex, in addition to genetic factors, different environmental factors could also modify the effects of *CYP46A1* gene in Mexicans with risk of suicide.

In conclusion, the results of the current study report the first association between G allele carriers (A/G + G/G) of rs754203 and increased risk for suicide, especially in males. Further research with larger samples is needed to explore the interaction of *CYP46A1* gene polymorphisms and suicide in MDD patients.

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