Working Memory Deficits in Schizophrenia Are Associated With the Rs34884856 Variant and Expression Levels of the NR4A2 Gene in a Sample Mexican Population: A Case Control Study

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Research article

Keywords: Schizophrenia, NR4A2 genetic variants, NR4A2 mRNA levels, Cognition, Endophenotype, Working memory

Posted Date: August 7th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-53517/v1

Version of Record: A version of this preprint was published on February 9th, 2021. See the published version at https://doi.org/10.1186/s12888-021-03081-w.

Abstract

Background

Cognitive functions represent useful endophenotypes to identify the association between genetic variants and schizophrenia. In this sense, the *NR4A2* gene has been implicated in schizophrenia and cognition in different animal models and clinical trials. We hypothesized that the *NR4A2* gene is associated with working memory performance in schizophrenia. This study aimed to analyze two variants and the expression levels of the *NR4A2* gene with susceptibility to schizophrenia, as well as to evaluate whether possession of *NR4A2* variants influence the possible correlation between gene expression and working memory performance in schizophrenia.

Methods

The current study included 187 schizophrenia patients and 227 controls genotyped for two of the most studied *NR4A2* genetic variants in neurological and neuropsychiatric diseases. Genotyping was performed using High Resolution Melt and sequencing techniques. In addition, mRNA expression of *NR4A2* was performed in peripheral mononuclear cells of 112 patients and 118 controls. A group of these participants, 54 patients and 87 controls, performed the working memory index of the WAIS III test.

Results

Both genotypic frequencies of the two variants and expression levels of the *NR4A2* gene showed no significant difference when in patients versus controls. However, patients homozygous for the rs34884856 promoter variant showed a positive correlation between expression levels and auditory working memory. In these patients, a decrease of *NR4A2* mRNA expression was related to working memory impairment.

Conclusions

Our finding suggested that changes in expression levels of the *NR4A2* gene could be associated with working memory in schizophrenia depending on patients´ genotype in a sample from a Mexican population.

Background

Cognitive deficits are a core feature of schizophrenia [1], and which are present in up to 98% of patients [2]. Several studies show that schizophrenia and cognitive dysfunction manifest considerable heritability between 70–90% and 24–55%, respectively [3, 4]. In particular, cognitive functions, such as working memory, have been proposed as endophenotypes related to schizophrenia [5]. Endophenotypes are intermediate, measurable, heritable and independent traits of the disease status (present both in the prodromal stage and in patients with remission) [6].

Schizophrenia is a heterogeneous, severe, and disabling mental illness that affects 1% of the world's population and is caused by interactions between environmental and genetic factors [7]. It has been considered a neurodevelopmental disorder with alterations in different neurotransmitters, such as dopamine, GABA, and glutamate [8]. However, dopamine plays an essential role in research of this disease. Different genes involved in the neurodevelopment of dopaminergic neurons can be considered candidate susceptibility genes for schizophrenia and cognitive deficits [9, 10], for instance, the gene that codes for the NR4A2 nuclear receptor. This transcription factor regulates the expression of genes involved in the development, survival, and phenotype of dopaminergic neurons [11, 12]. The *NR4A2* gene (also known as Nurr1) has been related to schizophrenia etiology and cognitive function [9, 13–17]. Notably, in relation to this gene, Nurr1 heterozygous (+/-) mice show cognitive impairment and pharmacological responses consistent with a model for schizophrenia [18, 19]. This correlation between *NR4A2* deficiency and cognitive skills has been found in other animal models for Alzheimer´s disease and attention-deficit hyperactivity disorder [17, 20] as well as its particular role in memory tasks as reported in preclinical studies [16, 21–24].

Different clinical studies have analyzed genetic variants and gene expression levels in peripheral blood mononuclear cells (PBMC), both as potential biomarkers for central nervous system disorders [25, 26]. In this way, several genetic variants of the *NR4A2* gene have been analyzed, giving rise to non-reproducible results in different populations [27–32]. The two genetic variants analyzed in the present study have been associated with other neurological, psychiatric disorders, and addiction, which are also related with dysfunction of the dopaminergic system. In particular, the rs35479735 intronic 6 variant has been associated with Parkinson's disease in Asian, Caucasian, and Mexican populations [33–35]. In addition, the rs34884856 promoter variant has been associated with alcohol dependence in people with Mexican ancestry [36]. Moreover, Ancin et al. 2013 [31] identified an association between different variants of *NR4A2* to those analyzed in the current study, and cognition (sustained attention) in Caucasian schizophrenia patients. Furthermore, decreased expression of the *NR4A2* gene was found in the dorsolateral prefrontal cortex (DLPFC) of patients with this disorder [13, 37, 38]. Likewise, the expression of genes related to inflammation, metabolism, and neuroprotection from PBMC could reflect alterations in the activity of brain regions [39, 40]. However, the relationship of *NR4A2* expression in PBMC with gene variants in schizophrenia remains unclear.

To our knowledge, there is a lack of studies on the relation of the *NR4A2* gene with working memory deficits in schizophrenia, as well as the need to prioritize diversity in human genomics research [41] in the field of Psychiatry. Therefore, we investigate the association between the *NR4A2* gene with working memory deficit in schizophrenia in the Mexican population, which is under-represented in human genomic research. We hypothesized that the *NR4A2* gene is associated with working memory performance in schizophrenia in a sample Mexican population. This study aimed to analyze the possible association between two variants and the expression levels of the *NR4A2* gene with susceptibility to schizophrenia, as well as to evaluate whether possession of NR4A2 variants could impact the correlation between gene expression and working memory ability in schizophrenia.

Methods

Participants

This study included 187 (111 males and 76 females) patients diagnosed with schizophrenia at the Instituto Nacional de Neurología "Manuel Velasco Suárez" and Hospital Psiquiátrico Fray Bernardino Álvarez from March 2012 to December 2017. Inclusion criteria: patients confirmed by two psychiatrists, following Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (DSM-IV) criteria [42] and the Composite International Diagnostic Interview (CIDI) Version 21 [43], and with at least one year of evolution. Exclusion criteria included: patients with comorbidities due to toxic substance use (except nicotine) during 3 months prior to recruitment, or other serious organic or neurological diseases.

The control group consisted of 227 unrelated participants (127 males and 100 females) recruited from March 2011 to December 2017 by announcement from population of Mexico City´s metropolitan area. This group was screened by psychiatrists and completed the Mini-International Neuropsychiatric Interview (MINI) [44] to rule out any personal history of neuropsychiatric disorder. Control volunteers were without history of substance abuse (except nicotine), and with no family history from of schizophrenia, or other neurological, or psychiatric disorders.

Both the patients and control group met the criteria of Mexican mestizo [45]. The analysis did not include ancestral-informative markers (AIMs). However, standard criteria were used to define the Mexican mestizo, and to keep the effects of population stratification to a minimum. According to the National Institute of Anthropology and History of Mexico, Mestizos are defined as individuals born in Mexico, having a Spanish-derived last name, with family antecedents of Mexican ancestors back at least to the third generation [46]. The study was performed in accordance with principles of the 1964 Helsinki Declaration and its later amendments. The institutional ethics committee of the Instituto Nacional de Neurología y Neurocirugía, "Manuel Velasco Suárez" and Hospital Psiquiátrico Fray Bernardino Álvarez approved the study protocol, and written informed consent was obtained from all individual participants included in the study. The experimental design is show in Fig. 1.

The 187 schizophrenia patients and 227 controls were genotyped for two of the most studied *NR4A2* genetic variants in neurological and neuropsychiatric diseases (see below; molecular analysis).

Clinical and Working Memory Evaluation

The working memory function was evaluated in a group of patients and controls (54 patients with paranoid schizophrenia and 87 controls) of the total of participants using the Wechsler Adult Intelligence Scale (WAIS-III) standardized for the Mexican population [47]. This scale was conducted in literate patients and controls. In addition, it was further confirmed that they were not undergoing any treatment associated with drowsiness and that they met the conditions required to obtain a correct cognitive evaluation. The severity of the schizophrenia symptoms was evaluated in the same patients by means of the Positive and Negative Syndrome Scale (PANSS) [48]. Clinical evaluation and cognitive tests (WAIS-III) were carried out between 2012–2017 by psychiatry and psychology experts, previously trained in the application of both tests. Because the present study aimed to evaluate working memory in schizophrenia, we applied from the complete WAIS-III assessment only the tests that assess working memory. The experimental design is show in Fig. 1.

To evaluate the association between the *NR4A2* gene and working memory in schizophrenia, the working memory index (WMI) and its three subtests (Digit Span, Arithmetic, and Letter-Number Sequencing) that compose it were analyzed with genetic variants and the expression levels of the *NR4A2* gene. The Backward Digit Span (BDS) task that is part of the Digit Span subtest was also considered for analysis. The WMI score was obtained from WAIS-III.

Molecular Analysis

Genotyping

Genomic DNA was extracted from peripheral blood using the standard salting out procedure [49]. Two single nucleotide variants (SNV) of *NR4A2* were genotyped in patients and the control group: rs35479735 at intron 6 and rs34884856 in the promoter of *NR4A2*. Genotyping was performed using High Resolution Melt (HRM) and sequencing techniques. The oligonucleotide design and conditions of both genotyping methods have been previously described [35].

mRNA Expression Levels of NR4A2

NR4A2 expression levels were evaluated in 112 patients and 118 control individuals. *NR4A2* mRNA extraction was performed in PBMC by the organic technique using the TRIzol reagent, following the manufacturer's instructions. Quantification of the expression levels of the *NR4A2* gene was carried out by qRT-PCR, as previously reported [35].

Statistical Analysis

The study population is described using means and standard deviations or frequencies and percentages. Mann-Whitney *U* tests for non-parametric data, Student t tests for continuous parametric data, and chi-square tests for categorical parameters were used. The Hardy-Weinberg equilibrium was evaluated for each variant through Pearson's chi-square. Genotype frequencies were analyzed by chi square test and the odd ratio (OR) with 95% confidence intervals (CI) between case and control groups.

For association analysis between genotypes, gene expression and working memory, we used the recessive model considering the minor frequency allele and the risk allele described in various studies [32, 33, 34, 35, 36]. The recessive model postulates that the risk of the disease occurs in the homozygotes for risk allele. Therefore, genotypes were divided in two groups for each variant.

$Log10^{-}$ was used to transform the values of cognitive scores and gene expression levels to approximate normal distributions and to conduct an ANCOVA analysis and linear regression adjusted for age, gender, and educational level.

The association between working memory function, variant genetic and expression levels was analyzed by a two-way ANCOVA. The two fixed factors were diagnosis (schizophrenia vs control) and the genotype (recessive model). The expression levels of *NR4A2* were used as a covariant, as well as demographic data including age, sex and education level. The interaction of diagnosis x genotype, and genotype x expression were obtained. Effects significant for interaction or main effects on genotype were followed by a single effect analysis for genotype in patients and controls separately. In the single effect analysis gender, age and education (self-report of the number of years of study) were used as covariates.

All statistical analyzes were performed using the SPSS Statistics Version 24 software (SPSS Inc., Chicago, IL, USA) and the STATA SE version 12.0 statistical software (Stata Corp, College Station, TX, USA). $p < 0.05$ values were considered statistically significant.

Results

Participant characteristics

Demographic and clinical characteristics of patients and controls included in the study are shown in Table 1. Gender ($p = 0.485$), education ($p = 0.295$), and age ($p = 0.066$) distributions did not show any statistically significant difference between patients and control volunteers.

Table 1
Socio-demographic characteristics, clinical data, and genotype distribution of NR4A2 in schizophrenia patients and control group

Characteristics	**Schizophrenia group (n = 187)**	**Control group (n = 227)**	***p***
Age, year (± SD)	35.44(± 10.07)	38.33 (± 13.17)	0.066[a]
Gender, n (%)			
Male	111 (59.4)	127 (55.9)	0.485[b]
Female	76 (40.6)	100 (44.1)	
Education, year (± SD)	10.98 (± 3.46)	11.52 (± 4.47)	0.295 [a]
Family history of schizophrenia (%)	64 (34.2)	----	
Age of onset, year (± SD)	23.16 (± 7.62)	----	
Disease duration, year (± DS)	12.39(± 9.76)	----	
Typical antipsychotics, n (%)	64 (34)		
SSRI n (%)	17 (9)		
rs34884856 promoter variant			
Allele n (%)	211 (56)	261 (57)	0.757 [b]
2C	163 (44)	193 (43)	
3C			
Genotype n (%)	54 (28.9)	72 (31.7)	0.762 [b]
2C/2C	103 (55.1)	117 (51.5)	
3C/2C	30 (16.0)	38 (16.7)	
3C/3C	0.14	0.43	
HWE (*p*)			
rs35479735 intron 6 variant			
Allele n (%)	168 (45)	211 (46)	0.656 [b]
2G	206 (55)	243 (54)	
3G			

Characteristics	Schizophrenia group (n = 187)	Control group (n = 227)	*p*
Genotype n (%)	32 (17.1)	48 (21.1)	0.504 [b]
2G/2G	104 (55.6)	115 (50.7)	
3G/2G	51 (27.3)	64(28.2)	
3G/3G	0.11	0.89	
HWE (*p*)			

Data are presented as mean ± SD. Underlined allele denotes the minor allele. n, total participants; SD, standard deviation; SSRI, selective serotonin reuptake inhibitors; HWE, Hardy-Weinberg Equilibrium; 3C, insertion C; 2C, deletion C; 3G, insertion G; 2G deletion; [a]Mann Whitney *U* test; [b]Chi-squared test.

Comparison of allele and genotype frequencies between case and control group

The two *NR4A2* genetic variants analyzed in this study have been associated with disorders related to dysfunction of dopaminergic system; their genotypic and allelic frequencies are shown in Table 1. The rs34884856 variant on the promoter (alleles are a cytosine insertion (3C) and a deletion C (2C)), and rs35479735 variant in the intron 6 (a guanine insertion (3G) and a deletion G (2G)), both indels, were analyzed in the group of patients and control participants. These two variants were in Hardy-Weinberg equilibrium in both groups of participants. In particular, 3C minor allele frequency (MAF) for rs34884856 was identified in 44% and 43% of patients and control group, respectively for promoter variant. In addition, 2G MAF for rs35479735 was shown in 45% of study cases and in 46% of control individuals for the intron 6 variant.

Distribution of allelic and genotypic frequencies was similar between patients and control volunteers for both variants. The bivariate logistic regression analysis for the different inheritance models was not significantly associated to schizophrenia for either variant (Supplementary 1).

Comparison of NR4A2 **mRNA peripheral expression between schizophrenia patients and the control group as well as with** NR4A2 **genetic variants**

The mRNA expression levels of *NR4A2* were not significantly different between the patient and control groups ($p = 0.766$), or between the different genotypes of the two variants in patients or controls. (Supplementary 2). Likewise, expression levels showed no differences by sex ($p = 0.158$), drug treatment ($p = 0.224$), or family history ($p = 0.528$) in the schizophrenia group.

Analysis on the relation of working memory performance and the rs34884856 promoter variant

For association analysis between genotypes and working memory, we used the recessive model considering the minor frequency allele and the risk allele described in various studies [32, 33, 34, 35, 36]. The recessive model postulates that the risk of the disease occurs in homozygotes for the risk allele. Therefore, genotypes were divided in two groups for the rs34884856 promoter variant: 3C/3C homozygous, and 2C carriers ("3C/2C + 2C/2C"), for the rs35479735 intronic 6 variant: 3G/3G homozygous, and 2G carriers (3G/2G + 2G/2G).

The genotypes of the rs35479735 intronic 6 variant (recessive model) did not show significant differences for sociodemographic, cognitive or clinic evaluations in schizophrenia patients and the control group (Supplementary material 3).

Table 2 shows both sociodemographic and clinical variables for recessive model genotypes of the rs34884856 promoter variant. No significant differences were found between genotypes for variables such as age, gender, and education in patients and controls. Furthermore, no significant differences in clinical variables such as age of onset, family history of schizophrenia and treatment between 3C/3C homozygous and carriers of the 2C allele were found in patients.

Table 2
Socio-demographic and clinical characteristics of patients and the control group to the rs34884856 promoter variant

	Schizophrenia group			Control group		
	3C/3C n = 12	3C/2C + 2C/2C n = 42	*P*	3C/3C n = 21	3C/2C + 2C/C n = 66	*p*
Age, year (± SD)	31.67 (9.16)	33.81 (9.86)	0.479[a]	37.90 (13.0)	38.97 (11.75)	0.800[a]
Gender, n (%)						
Male	8 (66.7)	27 (64.3)	0.582[b]	10 (47.6)	33 (50)	0.849[c]
Female	4 (33.3)	15 (35.7)		11 (52.4)	33 (50)	
Education, year (± SD)	10.58 (2.57)	12.07 (2.97)	0.123[a]	14.14 (3.73)	13.31 (4.30)	0.409[a]
Family history of schizophrenia (%)	5 (41.7)	12 (28.6)	0.485[b]			
Age of onset, year (± SD)	20.08 (8.86)	23.26 (6.77)	0.074[a]			
Disease duration, year (± DS)	11.58 (8.27)	10.74 (9.84)	0.525[a]			
Typical antipsychotics, n (%)	5 (33.4)	14 (41.7)	0.284[b]			
SSRI n (%)	1 (8.3)	5 (11.9)	0.718[b]			
PANSS						
Positive symptoms	21.00 (7.50)	20.45 (7.98)	0.994			
Negative symptoms	26.64 (26.64)	23.17 (8.56)	0.128			
General symptoms	41.71 (14.01)	37.90 (10.91)	0.228			
PANSS total	90.36 (26.86)	81.52 (23.59)	0.242			

Data are presented as mean ± SD; SD, standard deviation; n, total participants; 3C, insertion C; 2C, deletion C; SSRI, selective serotonin reuptake inhibitors; PANSS, positive and negative syndrome scale; recessive model, 3C/3C vs 3C/2C + 2C/2C.[a]Mann-Whitney *U*, [b]Fisher test, [c]Chi-squared test.

Table 3 shows the ANCOVA analysis conducted for the WMI, working memory subtests (Arithmetic, Digit Span, and Letter-Number Sequencing) and Backward Digit Span (BDS) task between patient and control groups according to rs34884856 promoter variant. This analysis showed that performance scores on the BDS task, subtests and WMI were significantly lower in patients compared to healthy controls (Table 3). Further, no genotype effect was identified for performance on any of the subtests, BDS task or WMI.

Table 3
Comparisons among working memory test by diagnostic, genotypic groups and interaction analysis for promoter variant

Test	Schizophrenia group		Control group		Diagnosis		Genotype		Diagnosis x genotype		Genotype x expression	
	3C/3C	3C/2C + 2C/C	3C/3C	3C/2C + 2C/C	F	*p*	F	*p*	F	*P*	F	*p*
WMI	71.33 ± 9.88	78.47 ± 14.21	97.62 ± 12.92	88.89 ± 17.43	37.047	**0.000***	0.251	0.617	6.412	0.638	1.864	0.175
Arithmetic	4.67 ± 2.06	5.57 ± 2.55	8.5 ± 2.27	7.92 ± 3.25	21.419	**0.000***	0.025	0.875	1.531	0.218	1.080	0.301
DS	4.92 ± 1.24	6.21 ± 2.07	6.8 ± 2.06	6.47 ± 1.92	6.136	**0.015**	1.386	0.241	2.82	0.095	1.836	0.178
BDS task	6.75 ± 1.66	7.21 ± 1.81	7.57 ± 2.06	7.06 ± 1.71	13.788	**0.000***	1.859	0.175	4.99	**0.027**	6.991	**0.009***
LNS	4.67 ± 2.01	6.17 ± 2.34	8.48 ± 1.94	6.86 ± 3.10	7.727	**0.000***	0.151	0.698	6.159	**0.014**	0.896	0.346

Two-way ANCOVA analysis. The two fixed factors were the genotype (recessive model) and the diagnosis (schizophrenia vs control). The expression levels NR4A2 was used as a covariant, as well as demographic data including age, sex, and education level. The interaction of diagnosis x genotype, and genotype x expression were obtained. Data are presented as mean ± SD. WMI, working memory index; DS, total Digit Span; BDS, Backward Digit Span; LNS, Letter-Number Sequencing; * Bonferroni correction < 0.01. The WMI and its three subtests (DS, Arithmetic, and LNS) that compose it were analyzed. The BDS task that is part of DS subtest was also considered for analysis.

Working memory and expression levels of the NR4A2 gene

We found a significant interaction effect for diagnosis (schizophrenia vs control) x genotype (recessive model, "3C/3C" vs "3C/2C + 2C/2C") on the BDS task ($p = 0.027$) and Letter-Number Sequencing ($p = 0.014$), and a significant genotype x expression effect on the BDS task ($p = 0.009$, Bonferroni correction). Correlation analysis showed that BDS test was correlated with expression levels *NR4A2* only in the patients ($p = 0.05$). Also, patients homozygous for the rs34884856 promoter variant (3C/3C) showed a positive correlation between expression levels and auditory working memory (Fig. 2). This graph shows that only in 3C/3C rs34884856 patients, a decrease of *NR4A2* mRNA expression was related to working memory impairment in schizophrenia ($p = 0.022$).

Discussion

Schizophrenia is a mental illness with cognitive deficits that are a core feature on the disease, and characterized by dysfunction in the dopamine system. Therefore, it is relevant to mention that the *NR4A2* gene has been considered as a candidate gene for schizophrenia due to its relevant role in the dopamine system.

The NR4A2 (NURR1) transcription factor regulates the expression of important genes in the development and phenotype of dopaminergic neurons [1, 2, 50]. In addition, it participates in the modulation of metabolic, inflammatory, and cognitive processes [23, 24, 51–54]. Alteration in the *NR4A2* gene has been proposed as animal model of schizophrenia [18, 19]. Furthermore, different animal models and clinical studies support the importance of the *NR4A2* gene in cognitive processes [17, 20, 21]. In particular, several studies support the important role of NR4A2 in memory [16, 18, 21–24]. Various studies have considered this gene as a potential therapeutic target and biomarker in neurological and psychiatric disorders [55–58].

It is important to mention that this is the first study to analyze the association of two genetic variants of the *NR4A2* gene (rs34884856 in promoter and rs35479735 in intron 6) and *NR4A2* mRNA expression with working memory deficits in

schizophrenia.

Genetic association study between NR4A2 variants and schizophrenia

This study did not identify any association between the genetic variants analyzed and the risk of schizophrenia. Genetic association studies of *NR4A2* variants with this mental disorder in different populations have produced inconclusive and controversial results [27–30, 32, 59]. This could be due to the heterogeneity of the disease diagnosis, the different populations included, and the sample sizes analyzed in different studies. The two genetic variants analyzed in this study have been associated with other neurological, and psychiatric disorders and addiction, which are diseases with altered dopamine function. In particular, the rs35479735 intronic variant has been associated with Parkinson's disease in Asian, Caucasian, and Mexican populations [33–35]. In addition, the rs34884856 promoter variant has been associated with alcohol dependence in people with Mexican ancestry [36]. It should be highlighted that the genotypic frequencies observed in our study are similar to those identified in Asian populations and different from those reported for Caucasian populations in diseases associated with dopamine dysfunction such as schizophrenia [29, 33, 34].

Analysis of NR4A2 mRNA peripheral expression levels between schizophrenia patients and the control group as well as with NR4A2 genetic variants

In contrast to other studies [13, 37, 38] where a decreased mRNA expression level of *NR4A2* was identified in brain tissue (DLPFC) of schizophrenia patients, our study showed no significant differences in such expression levels in the mRNA obtained from peripheral blood. It should be noted, however, that the current study analyzed a different type of biological sample (blood vs cerebral cortex tissue). It has been reported that peripheral expression of this gene was found to be significantly decreased in Parkinson's disease (PD), which is a disease associated mainly with the degeneration of dopaminergic neurons, as well as with aging in different populations [32, 35, 60, 61]. In addition, we did not find an influence of the genetic variants analyzed on the expression levels of the *NR4A2* gene in schizophrenia patients as seen in PD patients [35]. In this neurodegenerative disease it has been previously reported that the levels of expression of the *NR4A2* gene were decreased in 3C homozygous compared to the other genotypes of the rs34884856 promoter variant [35].

Association between auditory working memory performance and the rs34884856 promoter variant

Working memory is one of the most widely researched cognitive functions as a cognitive endophenotype of schizophrenia given that it reflects prefrontal cortex alterations [62, 63]. Working memory refers to the mechanisms or processes involved in the control, regulation, and keeping of relevant information active for the execution of complex cognitive tasks [64]. This cognitive function is defined as a system for both temporal storage and manipulation of information, with it participating in key cognitive processes, such as language comprehension, reading, and reasoning [65].

In the present study, an association between the rs34884856 promoter variant of the *NR4A2* gene and auditory working memory in schizophrenia patients was identified. The analysis for the recessive model of this variant (3C/3C *versus* "3C/2C + 2C/2C") showed significant differences in the scores for BDS; 3C homozygous patients were lower when compared to 2C allele carriers (Table 3, $p = 0.027$). This is supported by a study that showed the association of genetic variants of this gene with sustained attention in schizophrenia patients in a Caucasian population [31]. Nevertheless, this association was not identified in the control group. However, both the genetic variants analyzed and the cognitive functions evaluated in that previously study are different from those analyzed in our study. It is noteworthy that in our study the relationship between the promoter variant and the working memory functions identified in schizophrenia patients were different from the control group for the BDS task.

Working memory subtests can be classified as verbal and nonverbal. In this classification, the left side of DLPFC is more related to verbal tests and the right side of DLPFC is more related to visual spatial tests. Information input divides working memory into auditory (for instance, Backward Digit Span) and visual spatial. It has been noted that the prefrontal cortex regions involved in simple information storage are different from the regions involved in actively manipulating stored information [66].

The difference found between patients and control group regarding the promoter variant can be related to the way in which information is received for processing, and to the affected brain regions in patients. For example, working memory subtests can be classified as verbal and nonverbal. In this classification, the left side of DLPFC is more related to verbal tests and the right side of DLPFC is more related to visual spatial tests. Information input divides working memory into auditory (for instance, BDS) and visual spatial. It has been noted that the prefrontal cortex regions involved in simple information storage are different from the regions involved in actively manipulating stored information [66]. In addition, the left hemisphere shows greater pathological alterations in schizophrenia compared to the right hemisphere [67].

Our findings are consistent with studies evaluating other genetic variants that have shown these inconsistencies related to the effects of the allele between different tests and pathological conditions. One of the most widely researched variants demonstrating this effect is the BDNF genetic variant (Val66met) [68, 69]. In this case, the Met allele has a differential effect between patients and controls regarding the same cognitive function.

In addition, the importance of the *NR4A2* gene in relation with metabolism and neuroprotection of dopaminergic neurons allows us to understand the association identified with cognitive endophenotypes of a working memory construct. In order to provide support for the aforementioned, various studies with genes related to dopaminergic metabolism have showed its association with cognitive endophenotypes [70].

Association between cognitive functions and expression levels of the NR4A2 **gene**

This is the first study to identify a positive correlation between *NR4A2* gene expression levels and working memory function in schizophrenia patients. Our results are consistent with previous studies that show that the *NR4A2* gene dosage is significantly related to cognitive function in animal models of schizophrenia [18, 19]. The association of NR4A2 deficiency and reduction in performance on cognitive tasks has been reported for Alzheimer´s disease and attention-deficit hyperactivity disorder animal models [17, 20], as well as with reports of its important role in diverse memory tasks in preclinical studies [16, 21–24]. NR4A2 is also involved in neurodevelopmental disorders and cognitive deficits as reported in clinical trials [14, 15].

We identified a significant positive correlation of *NR4A2* mRNA expression levels with performance on the BDS task in 3C/3C patients (rs34884856 promoter variant). The association between *NR4A2* mRNA expression and better cognitive performance was found in this task of working memory.

In particular, the analysis on the BDS task demonstrated a key association when adjusting variables that affect cognition, such as age, gender, and education level. Our main result was the association of performance on the BDS with the rs34884856 promoter variant and the expression levels of the *NR4A2* gene in schizophrenia patients. For instance, the BDS task allows the evaluation of alterations in auditory working memory, which is related to the left DLPFC.

Our results are consistent with the relationship between cognitive deficits in schizophrenia and dysfunction in different brain regions related to cortex, such as the DLPFC, medial prefrontal cortex, and visual cortex [63]. Therefore, a decrease in dopaminergic neurotransmission in those brain regions could be related to the cognitive deficits in this psychiatric disorder, in particular, the decrease in the *NR4A2* gene in the left DLPFC (area involved in working memory, mainly auditory) in schizophrenia patients.

One of the main limitations of the present study was the sample size for cognitive analysis. However, no significant differences concerning characteristics that could affect cognitive function were found between the different genotypic groups in either patients or controls. In addition, the functional effect of the rs34884856 promoter variant of the *NR4A2* gene has not yet been described. Therefore, this genetic variant could be in linkage disequilibrium with another polymorphism that has a functional effect on the expression levels of this gene and thus, on cognitive function.

Conclusion

In conclusion, we identified in this study a significant genetic effect of the relationship of the rs34884856 promoter variant and the expression levels of the *NR4A2* gene with working memory endophenotypes in schizophrenia patients. Our findings suggest that

decreased *NR4A2* gene expression could be associated with a deficit in auditory working memory in schizophrenia patients depending on their genotype in a sample from a Mexican population.

Abbreviations

AIMs
Ancestral-informative markers; BDS:Backward Digit Span; CIDI:Composite International Diagnostic Interview; CI:Confidence intervals; DLPFC:Dorsolateral prefrontal cortex; DS:Total Digit Span; HRM:High Resolution Melt; MAF:Minor allele frequency; MINI:Mini-International Neuropsychiatric Interview; LNS:Letter-Number Sequencing; OR:Odd ratio; PANSS:Positive and Negative Syndrome Scale; PBMC:Peripheral blood mononuclear cells; qRT-PCR:Real-Time Quantitative Reverse Transcription Polymerase Chain reaction; SNV:Single nucleotide variants; SSRI:Selective serotonin reuptake inhibitors; WAIS-III:Wechsler Adult Intelligence Scale III; WMI:Working memory index; 3C:insertion C; 2C:deletion C.

Declarations

Ethical approval and consent to participate

The institutional ethics committee of the Instituto Nacional de Neurología y Neurocirugía, “Manuel Velasco Suárez” and Hospital Psiquiátrico Fray Bernardino Álvarezapproved the study protocol, and written informed consent was obtained from all individual participantsincluded in the study.The study was performed in accordance with The Code of Ethics of the World Medical Association (Helsinki Declaration of 1964, as revised in 2018).

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interest

The authors declare that they have no competing interests

Funding

This research was funded partially by National Council of Science and Technology of Mexico (CONACyT) grant, Project number SALUD-2008-01-87095. The funders had no involvement in the preparation or writing up of this research.

Authors´ contributions

Ruiz-Sánchez E participated in writing and designing the protocol, participated in planning, performed genetic experiments, statistical analyses, managed the literature searches and wrote the draft and final version of the manuscript. Jiménez-Genchi J recruited participants, collected data, instructed author Alcántara-Flores on protocols, supervised cognitive test, and reviewed the manuscript. Alcántara-Flores Y M participated in the organization of data collection, performed cognitive test, and reviewed the manuscript. Castañeda-González C J recruited participants and revised critically the manuscript. Aviña-Cervantes C L coordinated the inclusion of patients and performed psychiatric assessment. Yescas P participated in the collection and processing of samples,and reviewed the manuscript. González-Valadez M del S performed inclusion of patients and performed psychiatric assessment. Martínez-Rodríguez N supervised statistical analyses, and reviewed the manuscript. Ríos-Ortiz A performed psychiatric evaluation and cognitive test. González-González M participated in data interpretation, and contributed to final version of the manuscript. López- Navarro M E performed cognitive test and reviewed the manuscript. Rojas P obtained funding, conceived and designed the research, participated in writing the protocol, participated in planning, recruited participants, reviewed drafts and participated in writing the final version of the article. All authors read and approved the final manuscript.

Acknowledgements

This work was submitted in partial fulfillment of the requirements for the PhD degree by Elizabeth Ruiz Sánchez, who is a doctoral student from the Programa de DoctoradoenCienciasBiomédicas, Universidad Nacional Autónoma de México (UNAM). We are deeply grateful to all patients and healthy controls participating in this study. The authors thank Dr. Robyn Elizabeth Hudson for her valuable comments.

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Figures

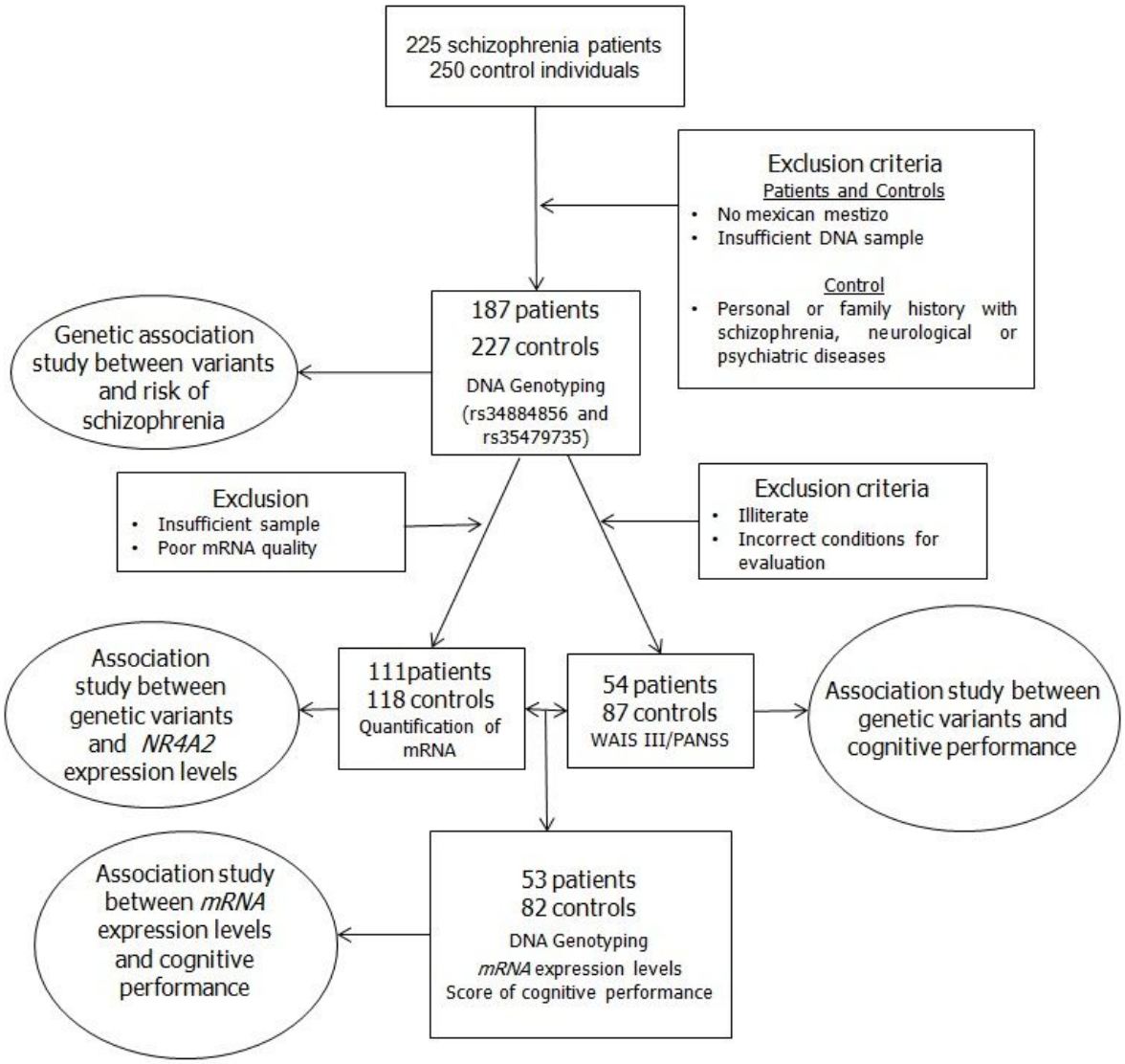

Fig.1

Figure 1

Experimental design

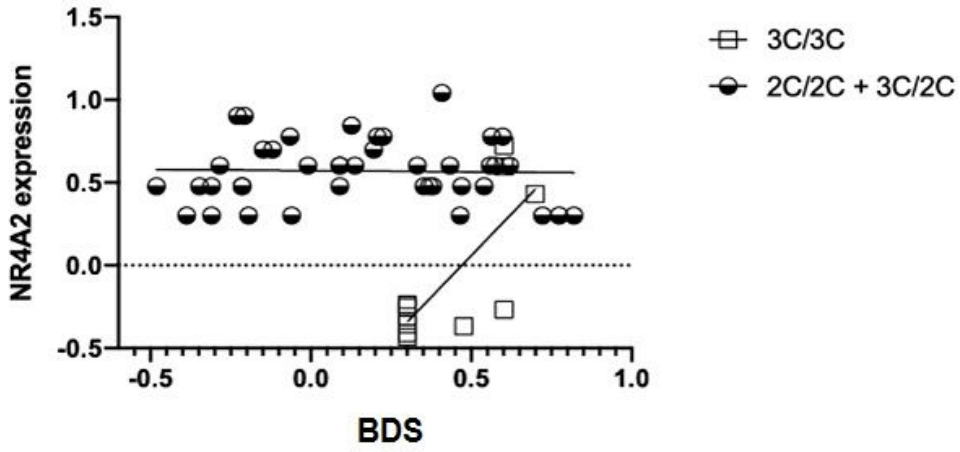

Fig. 2

Figure 2

Correlation between Working Memory and NR4A2 expression levels.Schizophrenia patients homozygous for 3C/3C of rs34884856 promoter variant showed a positive correlation between Backward Digit Span (BDS) and NR4A2 expression levels. In 3C/3C rs34884856 patients, a decrease of NR4A2 mRNA expression was related to working memory impairment in schizophrenia.

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