SciMedCentral

JSM Bioinformatics, Genomics and Proteomics

Research Article

Evaluation of Polymorphisms in the *TH*, *DNTBP1* and *DRD2* genes in Ecuadorian Schizophrenic Patients

Jennyfer M. García-Cárdenas[1], Alejandro Cabrera-Andrade[1], Andrés López-Cortés[1], Samantha Vargas-Luna[2], Fabián Porras-Borja[3], David Moscoso[4], José Luis Ibáñez[4], Erika Pasquel[4], Carolina Salazar[1], Santiago Guerrero[1], Ana Karina Zambrano[1], Patricia Guevara-Ramírez[1], Diana Abigail González[1], Paola E. Leone[1], César Paz-y-Miño[1]*

[1]*Centro de Investigación Genética y Genómica, Facultad de Ciencias de la Salud Eugenio Espejo, Universidad Tecnológica Equinoccial*
[2]*Facultad de Ingeniería y Ciencias Agropecuarias, Biotecnología, Universidad de las Américas*
[3]*Laboratorio de Citogenética, Centro Especializado en Genética Médica (CEGEMED), Ministerio de Salud Pública*
[4]*Instituto Psiquiátrico Sagrado Corazón*

***Corresponding author**
Centro de Investigación Genética y Genómica, Facultad de Ciencias de la Salud Eugenio Espejo, Universidad Tecnológica Equinoccial, Av. Mariscal Sucre and Mariana de Jesús, Ecuador; Tel: +5932990800 ; Email: cesar.pazymino@ute.edu.ec

Submitted: 27 October 2017

Accepted: 14 December 2017

Published: 16 December 2017

Keywords
- Schizophrenia
- Dopamine
- Multigenic complex disease
- Ancestry

Abstract

Schizophrenia is a serious mental disorder affecting 1% of the world's population. The development of the disease is related to both genetic and environmental factors. Several studies have identified a number of candidate genes with key molecular components involved in neurotransmission pathways, as is the case of *DRD2*, *TH* and *DNTBP1* genes. Results from several studies are often controversial or inconclusive. We aimed to determine the frequency of the following SNPs: rs1801028, rs6275, rs6277, rs1079597, rs6356, rs76240471, rs2070762, rs2619522, rs1011313, and rs760761in Ecuadorian schizophrenic individuals and to identify any association between these variants and the disease. Statistical analysis showed no association between the studied polymorphisms and schizophrenia. Besides of these already known 10 polymorphisms, we found 2 new SNPs which are not reported in the NCBI-SNP database, and they are located at position c.113.425.553 and c.2.165.110, and no correlation was found either. Even though we did not establish any positive associations, our findings are in line with those from other countries including India, China, Japan, Korea, Spain, Australia, and so on. We suggest that population heterogeneity, phenotypic heterogeneity and genetic-environmental interactions and most importantly the small sample size may have contributed to the failure of replication of association studies.

ABBREVIATIONS

DRD2: Dopamine Receptor D2; TH: Tyrosine Hydroxylase; DNTBP1: Dystrobrevin Binding Protein 1; PANSS: Positive and Negative Syndrome Scale

INTRODUCTION

Schizophrenia is a severe and chronic mental disease characterized by impaired brain function and deterioration of cognition, behavior, emotion, motivation, perception and socio-occupational performance [1,2]. It was ranked among the top 25 leading causes of disability worldwide in 2013 [3]. It affects 21 million people around the world; the estimated prevalence of schizophrenia is set between 0.7% and 1.5% worldwide [4]. According to global epidemiology research the estimated prevalence in men and women is 0.57% and 0.36%, respectively. However, in women, schizophrenia's onset usually occurs between 20-30 years, whereas in men between 16-25 years of age [5].

Studies in Latin America suggest that the overall prevalence of schizophrenia is 3% which is much higher than in other continents with specific country prevalence at 2.9% in Argentina, 0.6% in Peru, 1.8% in Puerto Rico, 0.7% in Mexico and 1.4% in Colombia [6-8]. In Ecuador until 2008 it was reported that 38% of patients admitted to psychiatric hospitals corresponded to patients with schizophrenia, schizophrenic and delusional disorders. While the number of outpatients with the diagnosis of schizophrenia prevails in 21.7% in relation to other psychological conditions [9].

This disorder is a multigenic complex disease; with a heritability of 65% to 85%.The genetic component is the most

predominant factor in the etiology of schizophrenia. Although the biological basis of this disease is unknown, several genetic studies have been conducted to identify key molecular components involved in neurotransmission. We have investigated multiple polymorphisms in two important neurotransmission roads: dopaminergic (*DRD2* and *TH*) and glutamatergic (*DNTBP1*) [10-12].

Most results suggest that the *Dopamine Receptor D2* gene (*DRD2*, Chr11q22-23) and its polymorphisms are considered to be associated with behavioral, psychiatric and neurologic disorders such as addiction, Parkinson's disease and schizophrenia [13]. This gene codes for the family of D2 receptors which are grouped into two big groups D1 and D2; D1 family is made up of D1 and D5 receptors; while D2 family by D2, D3, and D4 receptors. It has been suggested that rs1801028 (c.932C> G Serine311Cysteine), rs6275 (c939G> A, His313His) rs6277 (c.957C> T, Proline319Proline) and rs1079597 (intron variant, c.-31-882G> A) polymorphisms generate a change in the conformation of the receptor, which produces a deregulation in neurotransmission. It generates dopaminergic hyperactivity in the mesolimbic area producing over-stimulation of dopamine D2 receptors leading to the appearance of positive symptoms, meanwhile hypoactivity in the mesocortical area triggers an understimulation in D1 receptors generating negative, cognitive and affective symptoms [13-16] .

The *Tyrosine Hydroxylase* gene (*TH*, Chr11p15.5) has a role in the dopaminergic pathway, since it encodes the Tyrosine Hydroxylase enzyme, which is the limiting factor in the dopamine synthesis reaction [17-19]. Polymorphisms present in the gene such as rs76240471 (c360G> A, Arg120Arg) rs2070762 (intron variant, c.1334+127A>G), and rs6356 (C>T, Valine81Methionine) generate changes in the scaffolding of the enzyme, thereby reducing the functionality of the enzyme by increasing the levels of dopamine production in the brain generating the symptomatology of the disease [20-22].

The *Dystrobrevin Binding Protein 1* gene (*DNTBP1*, Chr6p22.3) plays a role in synaptic vesicle trafficking and in neurotransmitter release. It is presynaptically located in glutamatergic neurons in the hippocampus. It is believed that this gene could play a role in glutamate signal transduction in the hippocampus formation [23]. Also, it is involved in the regulation of cell surface exposure of *DRD2* and modulates prefrontal cortical activity via the dopamine/D2 pathway [24]. Polymorphisms like rs1011313 (intron variant, c.-22+4543A>G), rs760761 (intron variant, c.-83+412G>A) and rs2619522 (intron variants, c.-232-1278A>C) produce a protein variant, thus reducing normal levels of glutamate, which directly affects normal levels of dopamine in the brain [11,25].

Considering the significant role played by *DRD2, TH* and *DNTBP1* polymorphisms involved in the symptomatology and response to antipsychotics in this disabling disease a molecular characterization of schizophrenia is necessary. Even more when several results are often controversial or inconclusive. It is imperative to start characterizing Ecuadorian population where no molecular data had been previously reported [17,26-29]. In the present study, we investigated the possible involvement of these 10 polymorphisms in schizophrenia.

MATERIALS AND METHODS

Participants and Samples

The sample used in this study consisted of 20 Ecuadorian unrelated schizophrenic patients from Instituto Psiquiatrico Sagrado Corazon. All of the participants were Ecuadorian - mestizos of which 6 were females and 14 males with a mean age of 58.95 years. The clinical diagnosis was made according to the International Statistical Classification of Diseases and Related Health Problems (ICD-10) by the World Health Organization. In addition, further psychological tests, PANNS, Hamilton tests of anxiety and depression and Plutchik for risk of suicide, aggressiveness and impulsivity, were done. Normal healthy control samples came from the Centro de Investigaciones Genetica y Genomica of the Universidad Tecnologica Equinoccial (CIGG-UTE) DNA biobank. The controls were 36 unrelated Ecuadorian–mestizos who lacked neurological or psychiatric diseases and family history of psychiatric diseases. They were 21 females and 15 males with a mean age of 40.03 years. Approval was obtained from Universidad Central del Ecuador ethics committee and all subjects gave informed consent. Relevant data were obtained from the clinical records such as the age of onset of psychosis, type of schizophrenia and family history.

Genotyping

Genomic DNA was prepared from peripheral blood, using the Pure Link T Genomic DNA Kit (Invitrogen, Carlsbad, CA) followed by DNA quantification using Nano Drop 2000 (Thermo Scientific, Waltham, MA). *DRD2, DNTBP1* and *TH* polymorphism genotypes were determined using polymerase chain reaction (PCR). PCR was performed in a final volume of 25 µL containing 13ng/µL of DNA template, 15.5 µL H_2O Milli-Q, 10 mM of forward and reverse primers, 1.5 mM MgCl2, 5 X buffer (Green Go Taq Flexi Buffer), 0.2 µM of each deoxynucleotide triphosphate (dNTP), and 2.5 U Taq Platinum DNA polymerase (Invitrogen, Carlsbad, CA). The PCR was done with an initial denaturation step for 5 min at 94ºC, 35 cycles with a 45 s denaturation step at 94ºC, an annealing step at primer-specific temperatures (Table 1) for 45 s and an elongation step at 72ºC for 1 m, with final elongation at 72ºC for 3 min. PCR products were evaluated by electrophoresis on ethidium bromide-stained agarose gels; the sizes of the PCR products were 433bp for rs1801028 and rs6277, 218bp for rs1079597 2, 286bp for rs2070762, 404bp for rs6356, 208bp rs760761, 208bp for rs1011313, and 266bp for rs2619522. Finally, the fragments were purified with Agentcourt Cleanseq (Beckman Coulter, Miami, FL), sequenced using a Big Dye® Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems TM, Austin, TX), and analyzed with Seq-Scape Software v2.6. (Applied BiosystemsTM, Austin, TX).

Statistical analysis

Descriptive statistical analysis was done. The Hardy–Weinberg equilibrium and Chi square ($\chi 2$) test were used to determine the correspondence between the genotype and allele frequencies between patients and control group. All information obtained from the subjects was compiled into a

Table 1: PCR primers and annealing temperature.

SNPs	PRIMERS	T°
rs1801028 and rs6277	Fw: CTGATGCCTGGGAACTTGTC	61°C
	Rv: AATGGGACCTTTCACAGACC	59°C
rs1079597	Fw: CCTGCCAAACCTCATCATCT	60°C
	Rv: GGAGGTTGCAATAGGCAAGA	60°C
rs6356	Fw: ATCCCCTGCCTCTGTGTGCCAT	69°C
	Rv: TCAGGAACTCAGCCCACACAGC	67°C
rs2070762	Fw: AGAGCTTCAGTGACGCCAAG	61°C
	Rv: ACGTCCTCACAAAGCTCAGG	60°C
rs2619522	Fw: TGCAGCTCTAGCCCATTTATC	59°C
	Rv: TTGCAGCAAAACAGTACTCTCC	60°C
rs1011313	Fw: TGCTCAGATCAATTGAAGGAA	58°C
	Rv: TGGCTTATATGTGTCCAGCA	58°C

Table 2: Allele distribution and Hardy Weinberg equilibrium analysis.

Gene	SNPs	Genotype	No. of individuals	Allelic frequency	HWE P value
DRD2	rs1801028 G→C	G/G	46	0.9	P > 0.05
		G/C	9		
		C/C	1	0.1	
	rs6275 G→A	G/G	17	0.6	P > 0.05
		G/A	31		
		A/A	8	0.4	
	rs6277 G→A	G/G	41	0.8	P > 0.05
		G/A	13		
		A/A	2	0.2	
	rs1079597 C→T	C/C	18	0.6	P > 0.05
		C/T	30		
		T/T	8	0.4	
	NRA* T→C	T/T	19	0.6	P > 0.05
		T/C	29		
		C/C	8	0.4	
TH	rs6356 C→T	C/C	10	0.42	P < 0.05*
		C/T	27		
		T/T	19	0.58	
	rs76240471 G→A	G/G	53	0.97	P < 0.05*
		G/A	3		
		A/A	0	0.03	
	rs2070762 A→G	A/A	5	0.3	P < 0.05*
		A/G	24		
		G/G	27	0.7	
	NRB C→T	C/C	49	0.94	P > 0.05
		C/T	7		
		T/T	0	0.06	
DNTBP1	rs2619522 C→A	C/C	0	0.05	P > 0.05
		C/A	6		
		A/A	50	0.95	
	rs1011313 C→T	C/C	16	0.4	P > 0.05
		C/T	12		
		T/T	28	0.6	
	rs760761 G→A	G/G	46	0.9	P > 0.05
		G/A	10		
		A/A	0	0.1	

* Significant deviation from the equilibrium

Table 3: Allele distribution and Hardy Weinberg equilibrium..

Gene	Genotype	Affected N (%)	Controls N (%)	OR	95% CI	P
DRD2 rs1801028 G→C	G/G	15 (80)	31 (86.1)	1	Reference	
	G/C	5 (25)	4 (11.1)	0.4	0.09 – 1.7	0.4^{NS}
	C/C	0 (0)	1 (2.8)	-	-	-
	G/C + C/C	5 (25)	5 (13.9)	0.5	0.12 – 1.9	0.5^{NS}
DRD2 rs6275 G→A	G/G	6 (30)	11 (30.6)	1	Reference	
	G/A	10 (50)	21 (58.3)	1.2	0.3 – 4.0	1.0^{NS}
	A/A	4 (20)	4 (11.1)	0.6	0.1 – 3.0	0.7^{NS}
	G/A + A/A	14 (70)	25 (69.4)	1.9	0.8 – 3.9	1.0^{NS}
DRD2 rs6277 G→A	G/G	14 (70)	27 (75)	1	Reference	
	G/A	6 (30)	7 (19.4)	0.6	0.2 – 2.2	0.5^{NS}
	A/A	0 (0)	2 (5.6)	-	-	-
	G/A + A/A	6 (30)	9 (25)	0.8	0.2 – 2.6	0.9^{NS}
DRD2 rs1079597 C→T	C/C	10 (50)	8 (22.2)	1	Reference	
	C/T	8 (40)	22 (61.1)	3.4	1.0 – 11.8	0.09 ^{NS}
	T/T	2 (10)	6 (16.7)	3.8	0.6 – 23.9	0.2^{NS}
	C/T + T/T	10 (50)	28 (77.8)	3.5	1.1 – 11.4	0.04*
DRD2 NRA T→C	T/T	10 (50)	9 (25)	1	Reference	
	T/C	8 (40)	21 (58.3)	2.9	0.9 – 9.8	0.2^{NS}
	C/C	2 (10)	6 (16.7)	3.3	0.5 – 20.9	0.2^{NS}
	T/C + C/C	10 (50)	27 (75)	2.9	0.7 – 12.3	0.1^{NS}
DNTBP1 rs760761 G→A	G/G	16 (80)	30 (83.3)	1	Reference	
	G/A	4 (20)	6 (16.7)	0.8	0.2 – 3.3	0.7^{NS}
	A/A	0 (0)	0 (0)	-	-	-
	G/A + A/A	4 (70)	6 (16.7)	0.8	0.2 – 3.3	0.7^{NS}

Abbreviations: NS: No Significative; OR: Odds Ratio; CI: Confidence Interval

database, and statistical analyses were carried out using SPSS v.22 (SPSS, Chicago, Illinois). To determine the risk of developing schizophrenia for each polymorphism, the odds ratio (OR) and 95% confidence interval (CI) were also calculated. The data were analyzed using a 2 × 2 contingency table.

RESULTS

In this study, 20 affected people were included, in relation to gender; women represented 30% (n = 6), and men 70% (n = 14). When analyzing antecedents of mental illnesses such as: Alzheimer's, schizophrenia and non-specific pathologies of close relatives, they were present in 80% of the subjects. Regarding the age of psychosis onset, in women the average age was 38.4 years and men 38.5 years. At the moment of relating the polymorphisms to the presence of family history, and age of psychosis onset, no statistically significant association was found ($p > 0.05$).

For genetic analysis, we first performed the Hardy-Weinberg equilibrium test, and significant deviation from the equilibrium was observed only for rs6356, rs76240471 and rs2070762, as such, no further analyses of these were done (Table 2). The observed genotypes and the allele frequency in the studied population are also shown in Table 2. Besides, we found two new polymorphisms on *DRD2* and *TH* gene (no records found in the NCBI-SNP database). For the *DRD2* gene, this new polymorphism was located at position c.113.425.553, which was amplified by the rs1079597 primer (Table 1). Hereinafter we refer to it as NRA (No Reported A). The second new variant is located on position c.2.165.110, which was amplified by the rs2070762 primer and named as NRB (No Reported B). Both of them are located on intronic sequences. Given the small sample size in the genotype - schizophrenia association analysis, we took into account Fisher's exact test where expected count cell was less than 5 (Table 3).

Given that some genotypes were absent, the OR could not be calculated. That was the case for rs1801028 (C/C), rs6277 (A/A), NRB (C/T and T/T), rs2619522 (C/C), rs1011313 (C/C) and rs760761 (A/A). For the rest of the SNPs no positive association with schizophrenia was found (Table 3).

DISCUSSION

We performed a case-control association study for schizophrenia in the Ecuadorian population using 10 SNPs present in 3 candidate genes, the implication of which in the disease etiology had been previously reported. We were able to detect two new polymorphisms which are located in two different genes on intronic sequence whose clinical significance is unknown. NRA seems to be equally distributed in patients and controls ($p>0.05$). On the other hand, we think it is important to highlight that we were not able to find NRB T/T genotype in any of the 56 individual of the studied population. We suggest increasing the

sample size in order to detect this missing genotype, determine its frequency and that of other analysed SNPs

When comparing our output with other reports, it has been noted that studies across populations have not had a clear cut off. For instance, Betcheva et al., (255 cases and 556 controls); Glatt & Jönsson (3707 cases and 5363 controls); Kukreti et al., found a positive associations [30-32]. But our results agree with those reported in other populations such as Japan (241 cases and 201 controls), and Taiwan (1214 cases and 616 controls) [33-35].

There are several reasons that indicated the causes for the failure of replication of association studies. One of them was demonstrated by Li et al., who conducted a genome wide association analysis in Chinese individuals. They found a strong directional consistency of schizophrenia risk alleles across ancestry groups. This indicates that risk loci were shared across ancestral populations. Meaning that findings in Caucasian samples could not explain all genetic susceptibility variants in other populations [36]. This gives relevance to population studies and even more to those, which have a very mixed background such as Ecuadorians. The average mestizo population is 61.7% Amerindian, 34.6% Caucasians and 8.7% Afro-descendants [37-41].

Finally, apart from population heterogeneity we have to add phenotypic heterogeneity, interweaving diagnostic criteria and misclassification, overestimated risk in the initial study, small sample size, influence of multiple loci (causal heterogeneity) and genetic–environmental interactions as the reasons of different results of population studies [30].

CONCLUSION

Schizophrenia is a complex disorder; several studies across the world have been done in order to identify a rare variant that directly affects gene function. Results have not been clear, in some populations certain polymorphism has been associated with this disorder but not in other populations and ethnicities. Even though, we could not find any association, this study is important since it is the first to genetically characterize the Ecuadorian schizophrenic patients. From this base line we will be able to increase sample size as well as molecular variants.

ACKNOWLEDGEMENTS

The authors are grateful to Instituto Psiquiatrico Sagrado Corazon for providing the diagnosis and samples.

REFERENCES

1. Li T, Zhang F, Liu X, Sun X, Sham PC, Crombie C, et al. Identifying potential risk haplotypes for schizophrenia at the DTNBP1 locus in Han Chinese and Scottish populations. Mol Psychiatry. 2005; 10: 1037-1044.
2. Owen MJ. Implications of genetic findings for understanding schizophrenia. Schizophr Bull. 2012; 38: 904-907.
3. Chong HY, Teoh SL, Wu DB, Kotirum S, Chiou CF, Chaiyakunapruk N. Global economic burden of schizophrenia: a systematic review. Neuropsychiatr Dis Treat. 2016; 12: 357-373.
4. Vázquez GH. La esquizofrenia empieza en la adolescencia? Psicodebate 5 Psicol Cult y Soc. 2005:81-90.
5. Fuentealba-Herrera R. Esquizofrenia y Tiroiditis de Hashimoto. Descripción de un Caso Clínico. Rev Chil Psiquiatr Y Neurol La Infanc Y Adolesc. 2010.
6. Rodríguez JJ, Kohn R, Aguilar-Gaxiola S. Epidemiología de Los Trastornos Mentales En América Latina Y El Caribe. Washington: Organización Panamericana de la Salud; 2009.
7. Lopes da Silveira J, Lazzarini de Oliveira R, Magalhaes-Viola B, Marques da Silva T, Miranda-Machado R. Schizophrenia and the use of alcohol and other drugs: epidemiological profile. Northeast Netw Nurs J. 2014;15(3).
8. Díez-Martín J, Hoenicka J, Martínez I, Aragüés M, Rodríguez-Jiménez R, Jiménez-Arriero MA, et al. COMT Val158Met polymorphism and schizophrenia in a series of Spanish patients. Med Clin (Barc). 2007; 41-44.
9. World Health Organization. Report On The Mental Health System In Ecuador. 2008.
10. Yasuno F, Suhara T, Okubo Y, Sudo Y, Inoue M, Ichimiya T, et al. Low dopamine d(2) receptor binding in subregions of the thalamus in schizophrenia. Am J Psychiatry. 2004; 161: 1016-1022.
11. Bertolino A, Fazio L, Caforio G, Blasi G, Rampino A, Romano R, et al. Functional variants of the dopamine receptor D2 gene modulate prefronto-striatal phenotypes in schizophrenia. Brain. 2009; 132: 417-425.
12. Sáiz PA, García-Portilla MP, Arango C, Morales B, Arias B, Corcoran P, et al. Genetic polymorphisms in the dopamine-2 receptor (*DRD2*), dopamine-3 receptor (DRD3), and dopamine transporter (SLC6A3) genes in schizophrenia: Data from an association study. Prog Neuro-Psychopharmacology Biol Psychiatry. 2010; 34: 26-31.
13. Doehring A, Kirchhof A, Lötsch J. Genetic diagnostics of functional variants of the human dopamine D2 receptor gene. Psychiatr Genet. 2009; 19: 259-268.
14. Bertolino A, Fazio L, Caforio G, Blasi G, Rampino A, Romano R, et al. Functional variants of the dopamine receptor D2 gene modulate prefronto-striatal phenotypes in schizophrenia. Brain. 2009; 132: 417-425.
15. Spurlock G, Williams J, Mc Guffin P, Aschauer HN, Lenzinger E, Fuchs K, et al. European Multicentre Association Study of Schizophrenia: A study of the *DRD2* Ser311Cys and DRD3 Ser9Gly polymorphisms. Am J Med Genet - Neuropsychiatr Genet. 1998; 81: 24-28.
16. Yanguas P. Antipsychotics for schizophrenia: paradigm of psychiatric medications Antipsychotics for schizophrenia: the paradigm of psychiatric drugs. Buenos Aires. 2015; 11: 115-128.
17. Ankenman R, Salvatore MF. Low dose alpha-methyl-para-tyrosine (AMPT) in the treatment of dystonia and dyskinesia. J Neuropsychiatry Clin Neurosci. 2007; 19: 65-69.
18. Sánchez-Espinosa MP, Corredor-Rozo LZ, Forero Castro RM. Genética de la esquizofrenia: algunos genes candidatos asociados. Rev LA Asoc Colomb Ciencias Biol. 2015;1(22).
19. Sánchez-Espinosa MP, Corredor-Rozo LZ, Forero Castro RM. Descripción molecular de 26 genes asociados a la esquizofrenia. Cienc En Desarro. 2012;3(1).
20. Breen G, Brown J, Maude S, Fox H, Collier D, Li T, et al. -141 C del/ins polymorphism of the dopamine receptor 2 gene is associated with schizophrenia in a British population. Am J Med Genet. 1999; 88: 407-410.
21. Iwata Y, Matsumoto H, Minabe Y, Osada N, Nakamura K, Sekizawa T, et al. Early-onset schizophrenia and dopamine-related gene polymorphism. Am J Med Genet B Neuropsychiatr Genet. 2003; 116: 23-26.

22. Yi Z, Lu W, Fang Y, Yu S. O-66 - Association between tyrosine hydroxylase gene polymorphism and susceptibility to early-onset schizophrenia in the chinese population. Eur Psychiatry. 2012; 27: 1.

23. Trost S, Platz B, Usher J, Scherk H, Wobrock T, Ekawardhani S, et al. The DTNBP1 (dysbindin-1) gene variant rs2619522 is associated with variation of hippocampal and prefrontal grey matter volumes in humans. Eur Arch Psychiatry Clin Neurosci. 2013; 263: 53-63.

24. Escalona M, Álvarez O, Reyes JF. Factores de riesgo genéticos y no genéticos asociados con la esquizofrenia. Correo Científico Médico . 2012;17(2).

25. Escalona M, Álvarez O, Reyes JF. Factores de riesgo genéticos y no genéticos asociados con la esquizofrenia. Correo Científico Médico. 2012;17(2).

26. Cho CH, Kang SG, Choi JE, Park YM, Lee HJ, Kim L. Association between Antipsychotics-Induced Restless Legs Syndrome and Tyrosine Hydroxylase Gene Polymorphism. Psychiatry Invest. 2009; 6: 211-215.

27. Lane HY, Lee CC, Chang YC, Lu CT, Huang CH, Chang WH. Effects of dopamine D2 receptor Ser311Cys polymorphism and clinical factors on risperidone efficacy for positive and negative symptoms and social function. Int J Neuropsychopharmacol. 2004; 7: 461-470.

28. Voisey J, Swagell CD, Hughes IP, Barnes M, Burton SC, van Daal A, et al. A *DRD2* polymorphism predicts PANSS score variability in schizophrenia patients treated with antipsychotics. Psychiatry Res. 2010; 17: 367-368.

29. Zuo L, Luo X, Krystal JH, Cramer J, Charney DS, Gelernter J. The efficacies of clozapine and haloperidol in refractory schizophrenia are related to DTNBP1 variation. Pharmacogenet Genomics. 2009; 19: 437-446.

30. Betcheva E, Mushiroda T, Takahashi A, Kubo M, Karachanak S, Zaharieva I, et al. Case control association study of 59 candidate genes reveals the *DRD2* SNP rs6277 (C957T) as the only susceptibility factor for schizophrenia in the Bulgarian population. J Hum Genet. 2009; 54: 98-107.

31. Glatt SJ, Jonsson EG. The Cys allele of the *DRD2* Ser311Cys polymorphism has a dominant effect on risk for schizophrenia: Evidence from fixed- and random-effects meta-analyses. Am J Med Genet - Neuropsychiatr Genet. 2006; 141: 149-154.

32. Kukreti R, Tripathi S, Bhatnagar P, Gupta S, Chauhan C, Kubendran S, et al. Association of *DRD2* gene variant with schizophrenia. Neurosci Lett. 2006; 392: 68-71.

33. Harano M. Ser-311-Cys polymorphism of the dopamine D2 receptor gene and schizophrenia--an analysis of schizophrenic patients in Fukuoka. Kurume Med J. 1997; 44: 201-208.

34. Crawford F, Hoyne J, Cai X, Osborne A, Poston D, Zaglul J, et al. Dopamine *DRD2*/Cys311 is not associated with chronic schizophrenia. Am J Med Genet. 1996; 67: 483-484.

35. Glatt SJ, Faraone SV, Lasky-Su JA, Kanazawa T, Hwu HG, Tsuang MT. Family-based association testing strongly implicates *DRD2* as a risk gene for schizophrenia in Han Chinese from Taiwan. Mol Psychiatry. 2009; 14: 885-893.

36. Li Z, Chen J, Yu H, He L, Xu Y, Zhang D, et al. Genome-wide association analysis identifies 30 new susceptibility loci for schizophrenia. Nat Genet. 2017; 49: 1576-1583.

37. Zambrano AK, Gaviria A, Vela M, Cobos S, Leone PE, Gruezo C, et al. Ancestry characterization of Ecuador's Highland mestizo population using autosomal AIM-INDELs. Forensic Sci Int Genet Suppl Ser. 2017.

38. Paz-y-Miño C, García-Cárdenas JM, López-Cortés A, Cabrera-Andrade A, Guevara-Ramírez P, González DA, et al. Evaluation of ancestral membership proportions and genotype distribution in the perception of Umami taste in Ecuadorian mestizos. Forensic Sci Int Genet Suppl Ser. September 2017.

39. Leone PE, Cabrera-Andrade A, Garcia-Cardenas JM, Gonzalez DA, Guevara-Ramirez P, Lopez-Cortis A, et al. Ancestry study in Ecuadorian population with multiple myeloma. Forensic Sci Int Genet Suppl Ser. 2017.

40. López-Cortés A, Echeverría-Garcés G, Burgos G, Zambrano AK, Cabrera-Andrade A, García-Cárdenas JM, et al. Molecular analysis of ancestry informative markers (AIMs-INDELs) in a high altitude Ecuadorian mestizo population affected with breast cancer. Forensic Sci Int Genet Suppl Ser. September 2017.

41. Paz-y-Miño C, López-Cortés A. Genética Molecular Y Citogenética Humana: Fundamentos, aplicaciones e investigaciones en Ecuador. 2014 edición primera. Quito: Yachay; 2014.

Cite this article

García-Cárdenas JM, Cabrera-Andrade A, López-Cortés A, Vargas-Luna S, Porras-Borja F, et al. (2017) Evaluation of Polymorphisms in the TH, DNTBP1 and DRD2 genes in Ecuadorian Schizophrenic Patients. J Bioinform, Genomics, Proteomics 2(3): 1025.