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Association between *BDNF* Val66Met polymorphism and generalized anxiety disorder and clinical characteristics in a Mexican population

A case–control study

Thelma Beatriz González-Castro, PhD[a], Sherezada Pool-García, MD[b], Carlos Alfonso Tovilla-Zárate, PhD[c,*], Isela Esther Juárez-Rojop, PhD[d], María Lilia López-Narváez, MD[e], Ana Frésan, PhD[f], Alma Delia Genis-Mendoza, PhD[g], Nonanzit Pérez-Hernández, PhD[h], Humberto Nicolini, PhD[g,*]

Abstract

The aim of the present case–control study was to explore the association between *BDNF* Val66Met (rs6265) polymorphism and generalized anxiety disorder in Mexican individuals, and whether this polymorphism plays a role in the symptomatology of anxiety.

A total of 212 subjects were included in the study. Around 75 patients with generalized anxiety disorder were diagnosed by psychiatrists based on the DSM-IV instrument and 137 unrelated subjects psychiatrically healthy were used as comparison group. The subclinical symptomatology in patients was assessed with the State-Trait Anxiety Inventory. *BDNF* rs6265 genotypes were analyzed using the polymerase chain reaction end-point method.

The association between *BDNF* Val66Met with the risk for generalized anxiety disorder was evaluated using 4 inheritance models. The present study showed that carrying the Met allele confers increased risk for the presence of generalized anxiety disorder ($\chi^2 = 4.7$, $P = .03$; OR (95%) 1.96 (1.05–3.56)) when patients with generalized anxiety disorder were compared with the comparison group.

Our results provide evidence of an association between the Val66Met polymorphism of the *BDNF* gene and generalized anxiety disorder in a Mexican population. However, no association was observed between this polymorphism and the symptomatology of anxiety.

Abbreviations: BDNF = brain-derived neurotrophic factor, BMI = body mass index, DSM-IV = Diagnostic and Statistical Manual of Mental Disorders, HDRS = Hamilton Depression Rating Scale in Spanish, HWE = Hardy–Weinberg equilibrium, PCR = polymerase chain reaction, SD = standard deviations, SNP = single nucleotide polymorphism, STAI = State-Trait Anxiety Inventory.

Keywords: BDNF, gene, generalized anxiety disorder, Mexican population

1. Introduction

Anxiety disorders are associated with impairments in academic, professional, social, and family functions of patients; these disorders are also related to the development of several psychopathologies.[1–3] Literature provides evidence that family history is one of the best established risk factors for anxiety disorders, with heritability estimates ranging from 20% to 40%.[4–6] Therefore, there is an urgent need to identify objective indicators or risk factors of anxiety disorders in order to understand their link to underlying biological mechanisms and treatments.[3,7] In this sense, the disruption in the regulation of emotion as a defining feature of anxiety disorders has been investigated. As a result, anxiety disorders have been associated with alterations in volume of amygdala and hippocampus of related prefrontal regions.[8,9] On the other hand, the brain-derived

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[a] *División Académica Multidisciplinaria de Jalpa de Méndez, Universidad Juárez Autónoma de Tabasco, Jalpa de Méndez, Tabasco, México,* [b] *Hospital General de Comalcalco, Secretaría de Salud, Comalcalco, Tabasco, México,* [c] *División Académica Multidisciplinaria de Comalcalco, Universidad Juárez Autónoma de Tabasco, Comalcalco, Tabasco, México,* [d] *División Académica de Ciencias de la Salud, Universidad Juárez Autónoma de Tabasco, Villahermosa, Tabasco, México,* [e] *Hospital General de Yajalón "Dr. Manuel Velazco Suarez", Secretaría de Salud, Yajalón, Chiapas, México,* [f] *Subdirección de Investigaciones Clínicas, Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, Ciudad de México, México,* [g] *Servicios de Atención Psiquiátrica (SAP), Instituto Nacional de Medicina Genómica (INMEGEN), Ciudad de México, México,* [h] *Departamento de Biología Molecular, Instituto Nacional de Cardiología Ignacio Chávez, Ciudad de México, México.*

[*] *Correspondence: Humberto Nicolini, Servicios de Atención Psiquiátrica (SAP), Instituto Nacional de Medicina Genómica (INMEGEN). Periférico Sur 4809, Arenal Tepepan, 14610. Ciudad de México, México (e-mail: hnicolini@inmegen.gob.mx), Carlos Alfonso Tovilla-Zárate, División Académica Multidisciplinaria de Comalcalco, Universidad Juárez Autónoma de Tabasco, Ranchería Sur, Cuarta Sección, C.P. 86650, Comalcalco, Tabasco, México (e-mail: alfonso_tovillaz@yahoo.com.mx).*

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neurotrophic factor (BDNF) is a member of the neurotrophin family of growth factors and it is concentrated in brain regions such as hippocampus and amygdala.[10,11] Therefore, the *BDNF* gene has been involved in the pathophysiology of anxiety disorders.[12,13]

With regard to studies analyzing the association between neuropsychiatric disorders and a genetic background, the most commonly used variant is the *BDNF* gene in humans. This gene comprises a single nucleotide polymorphism (SNP, rs6265) in which there is substitution of the amino acid valine (Val) to methionine (Met) at codon 66 (Val66Met).[1,2,14] Furthermore, researchers had found that Met carriers were showed stronger neural responses to the emotional faces test and, in consequence, these authors suggested that this allele could affect important neural functions that correlate to anxiety and other psychopathologies.[15–17] Conversely, a number of studies have also found that the Val allele confers a risk for anxiety in case–control studies and family based investigations.[4,18,19] In this sense, we performed an exploratory study and the aim of the present work was: to study the association between the *BDNF* Val66Met polymorphism and generalized anxiety disorder by performing a case–control study and to evaluate the association between *BDNF* Val66Met polymorphism and clinical characteristics such as depression and subclinical anxiety in a Mexican population.

2. Material and methods

2.1. Participants in the study

In the present study, a total of 75 patients (13 males, 62 females) were recruited; the mean age was 44.72 (SD 15.67) years old (range 18–80). Whereas in the comparison group (n = 137; males = 62 and females = 75) the mean age was 35.34 (SD 14.53) years old, with the same age range (18–80 years old). Patients were recruited at the General Hospital of Comalcalco in the city of Comalcalco in the state of Tabasco. All subjects were born in the municipality of Comalcalco. The comparison group was recruited at random when they attended the Carracci Medical Group in Mexico City.

2.2. Ethics statement

All subjects signed an informed consent to participate in the study. Previous to signing, the subjects received detailed explanation of the objective of the study. Their participation was voluntary and they did not receive any economic remuneration. This study was approved by both the DAMC-UJAT Ethics and Research Committee and the Ethics Committee of the General Hospital of Comalcalco (INV/259/C/1011).

2.3. Clinical evaluation

The patient group was diagnosed with generalized anxiety disorder between January and December, 2014. The diagnosis for the patients was based on the criteria of Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) using the Mini-International Neuropsychiatric Interview. All subjects in the study were evaluated by a trained psychiatrist.

2.4. Assessment procedure for clinical variables

In the present study, sociodemographic characteristics such as gender, age, and level of education, were collected using a structured questionnaire. All subjects were measured for height in meters (m) and weight in kilograms (kg) using a digital scale Tanita (Tanita Corporation, Clearbrook, IL). The body mass index (BMI) of each person was calculated as his/her weight (kg) divided by his/her height squared (m^2).

To analyze the association between *BDNF* Val66Met and clinical variables, we tested for depression and subclinical anxiety. To measure depression the Hamilton Depression Rating Scale in Spanish (HDRS) was used.[20,21] The HDRS in Spanish has been validated and the Spanish version is widely used in research studies as well as in public health service in Tabasco. The HDRS comprises 17 items designed to assess the severity of depression with scores ranging from 0 to 52.[22] To assess subclinical anxiety, we used the State-Trait Anxiety Inventory (STAI).[23] This instrument has been validated in Spanish and this version has shown viability and validity in its use.[24,25] The STAI questionnaire evaluates the state of anxiety (Y-1) and the trait of anxiety (Y-2) grouped in 2 subscales. Each subscale has 20 items scored on a 4-point Lisker scale with values from 1 to 4.

2.5. BDNF Val66Met (rs6265) genotyping

Genomic DNA was extracted from peripheral blood leukocytes using the modified Lahiri and Nurnberger[26] method. The *BDNF* Val66Met (rs6265) was genotyped using a polymerase chain reaction (PCR) end-point method. As it has been previously reported we used 5 μL of final volume.[27] We used 5 exonuclease TaqMan genotyping assays (C_11592758_10). The intensity of the fluorescence was measured with a 7500 Real-Time PCR system using the v2.1 software (Applied Biosystems). We performed the genotyping blind to patients or comparison group. As a quality control, we genotyped 20 samples in duplicate.

2.6. Statistical analysis

Data are presented as numbers with frequencies and percentages for categorical variables. The demographic description (years of age, years of schooling) and anthropometric variable (body mass index) are continuous variables and are presented as means and standard deviations (SD). We tested the Hardy–Weinberg equilibrium (HWE) using a Pearson's goodness-of-fit chi-squared test. We used the chi-squared test to compare genotype and allele frequency between the group of patients and the comparison group. The same analysis was used in a comparison between *BDNF* Val66Met and anxiety disorder was carried out by gender. Association analyses among *BDNF* Val66Met polymorphism and demographic, anthropometric and clinical (HDRS, STAI-Y1, STAI-Y2) characteristics were performed with variance analysis (ANOVA), using the SPSS statistical software version 20.0. Significance level was set at $P < .05$.

3. Results

3.1. Characteristics of the population in the study

The socio-demographic features of cases and comparison groups are summarized in Table 1. We observed predominance in the number of females in the group of patients. Characteristics as age, level of education and body mass index between cases and controls were statistically different (Table 1).

3.2. Allele and genotype frequencies

In the present study, the genotype frequencies of *BDNF* Val66Met in patients (*P*-value = 054) and control (*P*-value = .77) groups satisfied the HWE.

Table 1

Socio-demographic characteristics of the comparison group and anxiety disorder patient group (cases group).

	Overall sample		Comparison group		Cases group		Statistic		
	n	%	n	%	n	%	χ^2	*df*	*P*-value
Gender									
Male	75	35.4	62	45.3	13	17.3	*16.52*	*1*	*< .05*
Female	137	64.6	75	54.7	62	82.7			
Socio-economic level									
High	0		0		0		*2.16*	*1*	*.09*
Middle	82	38.7	48	35	34	45.3			
Low	130	61.3	89	65.0	41	54.7			
	Average (SD)	Range	Average (SD)	Range	Average (SD)	Range	*t*	*df*	
Age, years	38.66 (15.62)	18–80	35.34 (14.53)	18–80	44.72 (15.81)	18-80	*−4.35*	*210*	*< .01*
Education (years of schooling)	11.88 (0–24	0–24	14.54 (3.36)	2–24	7.01 (5.64)	0–18	*12.15*	*210*	*< .01*
Body mass index	26.45 (4.89)	18.3–48.3	25.57 (4.77)	18.3–43.25	28.04 (4.73)	18.67–48.32	*-3.60*	*210*	*< .01*

Table 2

Distribution of genotype and allele frequencies of BDNF Val66Met (rs6265) in patients with generalized anxiety disorder (cases group) and comparison group (control group).

	Cases	Control	Cases	Control	Cases	Control
rs6265	n (%)	n (%)	Male		Female	
Genotype frequency	75	137	n (%)	n (%)	n (%)	n (%)
Val-Val	61 (81.3)	93 (67.9)	11 (84.6)	42 (67.7)	50 (80.6)	51 (68.0)
Val-Met	13 (17.3)	39 (28.5)	2 (15.4)	18 (29.0)	11 (17.7)	21 (28.0)
Met-Met	1 (1.3)	5 (3.6)	0	2 (3.3)	1 (1.7)	3 (4.0)
	ns		ns		ns	
Allele frequency						
Val	135 (90.0)	225 (82.1)	24 (92.3)	102 (82.2)	111 (89.5)	123 (82.0)
Met	15 (10.0)	49 (17.9)	2 (7.7)	22 (17.8)	13 (10.5)	27 (18.0)
Total	150	274	26	124	124	150
	OR (95%) 1.96 (1.05–3.63)		OR (95%) 2.58 (0.56–11.77)		OR (95%) 1.84 (0.92–3.18)	

Bold values signifies risk for generalized anxiety disorder.

The distribution of alleles and genotypes in the overall sample, patient group and comparison group subdivided by gender is presented in Table 1. No significant association was observed between the study group and the comparison group for genotype ($\chi^2=4.57$, $P=.10$). However, a statistical significance was observed for allele [$\chi^2=4.7$, $P=.03$; OR (95%) 1.96 (1.05–3.56)]. When we performed an analysis by gender, no differences were observed in the frequencies for genotype and alleles (Table 2). In addition, the inheritance hypothesis was tested according to 4 models: codominant, dominant, recessive and overdominant. However, none of the inheritance models was associated with an increased risk for developing anxiety disorders (Table 3).

3.3. Clinical characteristics by genotype

Finally, the present study analyzed the socio-demographic and clinical characteristics of the patients distributed by genotypes (Table 4). However, no significant differences were observed when we analyzed the socio-demographic characteristics, presence of depression, or characteristics such as state and trait of anxiety in patients. These results suggest that *BDNF* Val66Met

Table 3

Polymorphism risk analysis as function of the inheritance model in patients with generalized anxiety disorder (cases group) and comparison group (control group) considering the BDNF rs6265 polymorphism.

Inheritance model	Genotype	Cases n	Control n	OR	95%CI	*P*-value
Codominant	Val-Val	61	93	1.00		*.27*
	Val-Met	13	39	1.74	0.65–4.67	
	Met-Met	1	5	4.71	0.37–60.72	
Dominant	Val-Val	61	93	1.00		*.15*
	Val-Met + Met-Met	14	44	1.97	0.77–5.06	
Recessive	Val-Val + Val-Met	74	132	1.00		*.23*
	Met-Met	1	5	4.17	0.33–52.90	
Overdominant	Val-Val + Met-Met	62	98	1.00		*.33*
	Val-Met	13	39	1.62	0.61–4.33	

Table 4

Socio-demographic and clinical characteristics of patients distributed according to the BDNF rs6265 polymorphism.

Characteristics (Mean ± SD)	Val-Val (n = 61)	Val-Met (n = 13)	Met-Met (n = 1)	Overall sample	F	*P*-value
Education Level, years	7.03 ± 5.99	7.00 ± 4.08	6.00	7.01 ± 5.64	0.01	.98
Age, years	44.41 ± 15.58	45.23 ± 17.75	57	44.72 ± 15.81	0.31	.73
Body mass index	27.68 ± 5.01	29.34 ± 2.80	33.3	28.04 ± 4.73	1.30	.27
Depression (HDRS)	15.69 ± 6.90	14.92 ± 5.22	15.00	15.55 ± 6.57	0.07	.92
STAI trait	35.59 ± 8.73	37.92 ± 10.36	44.00	36.11 ± 8.99	0.74	.47
STAI state	35.52 ± 9.21	39.54 ± 11.96	36.00	36.23 ± 9.71	0.91	.40

HDRS = Hamilton Depression Rating Scale in Spanish, STAI = State-Trait Anxiety Inventory.

does not influence depression or characteristics of anxiety in patients with anxiety disorders (Table 4).

4. Discussion

We studied the association between the *BDNF* Val66Met polymorphism and generalized anxiety disorder patients and healthy controls. Subsequently, we investigated whether the *BDNF* Val66Met polymorphism may influence socio-demographic and clinical characteristics, as well as the state and trait of anxiety in a Mexican population. To our knowledge, this is the first study examining the impact of this SNP in a Mexican community sample. Anxiety disorders have attracted special attention for their high prevalence. Due to their predominance, these disorders may serve as predictors for the development of other mental diseases such as bipolar disorder.[4,28] Some evidence suggests that genetic factors may influence the vulnerability to develop anxiety disorders. The *BDNF* gene has been implicated in the pathophysiology of these disorders given that it plays a key role in memory and anxiety in the brain.[4,29,30]

Anxiety disorders were the sixth leading cause of disability in terms of YLDS (years of life lived with disability), in high- or middle-income countries.[31] Moreover, in another report was observed a global current prevalence of anxiety disorder of 7.3% (4.8%–10.9%) and ranged from 5.3% (3.5%–8.1%) in African cultures to 10.4% (7.0–15.5%) in Euro/Anglo cultures.[32] In Mexico the anxiety disorder has been addressed as one of the principal disorders of the ICD-10. In fact, the National Survey of Psychiatric Epidemiology (ENEP) analyzed by Medina-Mora et al,[33] was shown that about 28.6% of the population presented some of the 23 disorders of the ICD-10 at some time in their life. By type of disorders, the most frequent were those of anxiety (14.3% at some time in life), followed by substance use disorders (9.2%) and affective disorders (9.1%). In fact, anxiety disorders are more frequently observed in females (prevalence = 1.6) than in males (prevalence = 0.7) according with the data.[33]

The present study showed that carrying the Met allele confers an increased risk for having an anxiety disorder in the population selected from the southeast of Mexico. The Met allele of this SNP has been associated with volume reduction in areas involved in the regulation of cognitive and emotional behavior such as hippocampus, prefrontal cortex, and amygdala in the brain.[34,35] In agreement with our findings, Moreira et al[18] showed a significant association between the Met allele and risk of generalized anxiety disorder. Tocchetto et al.[36] investigated the association of the *BNDF* polymorphism and anxiety disorders in children and adolescents, and logistic regression analysis showed that carrying one copy of the Met allele confers increased risk of presenting an anxiety disorder. Jiang et al.[14] tested the relationship of this variant in single allele-based association studies with psychiatric diagnoses and personality measures. The Tridimensional Personality Questionnaire was administered to measure anxious temperament (harm avoidance and novelty seeking). They reported that individuals with the Met 66 allele were associated with increased harm avoidance and this allele was most abundant in individuals with both anxiety disorders and major depression. Furthermore, other results of genetic association in previous studies showed evidence that *BDNF* plays an important role in the pathophysiology of anxiety.[37,38]

When we assessed the association between the *BDNF* Val66Met polymorphism and socio-demographic and clinical characteristics of patients, it was not possible to observe any association between *BDNF* in the clinical data of Mexican patients and generalized anxiety disorder. The *BDNF* Val66Met polymorphism has been associated with obesity in patients suffering from a psychiatric disease.[39] Therefore, the lack of association reported in various studies could be explained considering that *BDNF* may be acting in conjunction with other "hypothetical" genes or with genes that contribute to the clinical characteristics of certain psychiatric diseases.[40,41] Second, it is important to take into account that the frequency of *BDNF* alleles differs among populations.[40,42]

Finally, some limitations of this study should be mentioned. First, the sample size is relatively small; therefore, these data need to be confirmed in a much larger sample in order to have a strong supportive evidence of the findings. Second, we did not include the early experience of patients and other environmental factors. *BDNF* Val66Met could be related to these characteristics of patients. Third, we did not consider other factors that could exert an influence on symptoms, such as psychiatric comorbidity which may represent one challenge for genetic studies.[36,43] Finally, we recognized there is genetic heterogeneity among Mexican population. Then, to reduce ethnic variation, we only included individuals with Mexican descendance (Mexican parents and Mexican grandparents). Hence, at the light of these limitations caution must be taken by readers when generalizing our results.

5. Conclusions

The findings of this study showed that the Met allele of the *BDNF* Val66Met polymorphism may be involved in the generalized anxiety disorder in a sample of Southeast Mexican population. Similarly, no association between this polymorphism and clinical characteristics such as depression and subclinical anxiety was established in this Mexican population. However, due to the above mentioned limitations, our results must be interpreted with caution. The conduction of similar studies with larger samples is needed to confirm the present findings.

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Author contributions

Conceptualization: Carlos Alfonso Tovilla Zárate, Ana Fresán, Nonanzit Pérez-Hernández, Humberto Nicolini.
Data curation: Carlos Alfonso Tovilla Zárate, Humberto Nicolini.
Formal analysis: Carlos Alfonso Tovilla Zárate, Humberto Nicolini.
Funding acquisition: Carlos Alfonso Tovilla Zárate, Humberto Nicolini.
Investigation: Sherezada Pool García, Carlos Alfonso Tovilla Zárate, Humberto Nicolini.
Methodology: Thelma Beatriz González-Castro, Sherezada Pool García, Carlos Alfonso Tovilla Zárate, Ana Fresán, Alma Delia Genis-Mendoza, Nonanzit Pérez-Hernández, Humberto Nicolini.
Project administration: Carlos Alfonso Tovilla Zárate, Isela Esther Juárez-Rojop, Humberto Nicolini.
Resources: Sherezada Pool García, Carlos Alfonso Tovilla Zárate, Isela Esther Juárez-Rojop, María Lilia López-Narváez, Humberto Nicolini.
Software: Carlos Alfonso Tovilla Zárate, Humberto Nicolini.
Supervision: Thelma Beatriz González-Castro, Carlos Alfonso Tovilla Zárate, Isela Esther Juárez-Rojop, María Lilia López-Narváez, Nonanzit Pérez-Hernández, Humberto Nicolini.
Validation: Carlos Alfonso Tovilla Zárate, Humberto Nicolini.
Visualization: Carlos Alfonso Tovilla Zárate, Humberto Nicolini.
Writing – original draft: Carlos Alfonso Tovilla Zárate, Humberto Nicolini.
Writing – review & editing: Carlos Alfonso Tovilla Zárate, Humberto Nicolini.

References

[1] Kalueff AV, Avgustinovich DF, Kudryavtseva NN, et al. BDNF in anxiety and depression. Science 2006;312:1598–9. author reply 1598-9.
[2] Kumari A, Singh P, Baghel MS, et al. Social isolation mediated anxiety like behavior is associated with enhanced expression and regulation of BDNF in the female mouse brain. Physiol Behav 2016;158:34–42.
[3] Chen J, Yu J, Liu Y, et al. BDNF Val66Met, stress, and positive mothering: differential susceptibility model of adolescent trait anxiety. J Anxiety Disord 2015;34:68–75.
[4] Park MH, Chang KD, Hallmayer J, et al. Preliminary study of anxiety symptoms, family dysfunction, and the brain-derived neurotrophic factor (BDNF) Val66Met genotype in offspring of parents with bipolar disorder. J Psychiatr Res 2015;61:81–8.
[5] Carola V, Gross C. BDNF moderates early environmental risk factors for anxiety in mouse. Genes Brain Behav 2010;9:379–89.
[6] Chang YH, Lee SY, Chen SL, et al. Genetic variants of the BDNF and DRD3 genes in bipolar disorder comorbid with anxiety disorder. J Affect Disord 2013;151:967–72.
[7] Chen ZY, Jing D, Bath KG, et al. Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science 2006;314:140–3.
[8] Frustaci A, Pozzi G, Gianfagna F, et al. Meta-analysis of the brain-derived neurotrophic factor gene (BDNF) Val66Met polymorphism in anxiety disorders and anxiety-related personality traits. Neuropsychobiology 2008;58:163–70.
[9] Golimbet VE, Lebedeva IS, Alfimova MV, et al. [Interaction effects of anxiety and the BDNF Val66Met polymorphism on attention in patients with schizophrenia spectrum disorders]. Zh Nevrol Psikhiatr Im S S Korsakova 2013;113:49–54.
[10] Golimbet VE, Volel BA, Kopylov F, et al. Anxiety and polymorphism Val66Met of BDNF gene–predictors of depression severity in ischemic heart disease. Kardiologiia 2015;55:9–13.
[11] Hunnerkopf R, Strobel A, Gutknecht L, et al. Interaction between BDNF Val66Met and dopamine transporter gene variation influences anxiety-related traits. Neuropsychopharmacology 2007;32:2552–60.
[12] Jamal M, Van der Does W, Penninx BW. Effect of variation in BDNF Val (66)Met polymorphism, smoking, and nicotine dependence on symptom severity of depressive and anxiety disorders. Drug Alcohol Depend 2015;148:150–7.
[13] Janke KL, Cominski TP, Kuzhikandathil EV, et al. Investigating the role of hippocampal BDNF in anxiety vulnerability using classical eyeblink conditioning. Front Psychiatry 2015;6:106.
[14] Jiang X, Xu K, Hoberman J, et al. BDNF variation and mood disorders: a novel functional promoter polymorphism and Val66Met are associated with anxiety but have opposing effects. Neuropsychopharmacology 2005;30:1353–61.
[15] Lang UE, Hellweg R, Kalus P, et al. Association of a functional BDNF polymorphism and anxiety-related personality traits. Psychopharmacology 2005;180:95–9.
[16] Lee SY, Chen SL, Wang YS, et al. COMT and BDNF interacted in bipolar II disorder not comorbid with anxiety disorder. Behav Brain Res 2013;237:243–8.
[17] Martinowich K, Manji H, Lu B. New insights into BDNF function in depression and anxiety. Nat Neurosci 2007;10:1089–93.
[18] Moreira FP, Fabiao JD, Bittencourt G, et al. The Met allele of BDNF Val66Met polymorphism is associated with increased BDNF levels in generalized anxiety disorder. Psychiatr Ggenet 2015;25:201–7.
[19] Arias B, Aguilera M, Moya J, et al. The role of genetic variability in the SLC6A4, BDNF and GABRA6 genes in anxiety-related traits. Acta Psychiatr Scand 2012;125:194–202.
[20] Hamilton M. A rating scale for depression. J Neurol Neurosurg Psychiatr 1960;23:56–62.
[21] Ramos-Brieva J, Cordero A. Validación de la versión castellana de la escala de Hamilton para la depresión. Actas Luso-Esp Neurol Psiquiatr 1986;14:324–34.
[22] Bobes J, Bulbena A, Luque A, et al. Evaluación psicométrica comparativa de las versiones en español de 6, 17 y 21 ítems de la Escala de valoración de Hamilton para la evaluación de la depresión. Med Clin (Barc) 2003;120:693–700.
[23] Spielberger CD, Gorsuch RL, Lushene RE. Manual for the State-Trait Anxiety Inventory (Self-Evaluation Questionnaire). Palo Alto, CA: Consulting Psychologists Press; 1970.
[24] Guillén-Riquelme A, Buela-Casal G. Estructura factorial del Cuestionario de Ansiedad Estado-Rasgo (STAI) para pacientes diagnosticados con depression. Salud Mental 2015;38:293–8.
[25] Rodrigo G, Lusiardo M. Note on the reliability and concurrent validity of the Spanish version of the State-Trait Anxiety Inventory. Percept Mot Skills 1988;67:926.
[26] Lahiri DK, Nurnberger JIJr. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res 1991;19:5444.
[27] Gonzalez-Castro TB, Tovilla-Zarate C, Juarez-Rojop I, et al. Association of the 5HTR2A gene with suicidal behavior: case-control study and updated meta-analysis. BMC Psychiatry 2013;13:25.
[28] Miller S, Hallmayer J, Wang PW, et al. Brain-derived neurotrophic factor val66met genotype and early life stress effects upon bipolar course. J Psychiatr Res 2013;47:252–8.
[29] Matsuo K, Walss-Bass C, Nery FG, et al. Neuronal correlates of brain-derived neurotrophic factor Val66Met polymorphism and morphometric abnormalities in bipolar disorder. Neuropsychopharmacology 2009;34:1904–13.
[30] Frodl T, Schule C, Schmitt G, et al. Association of the brain-derived neurotrophic factor Val66Met polymorphism with reduced hippocampal volumes in major depression. Arch General Psychiatry 2007;64:410–6.
[31] Baxter AJ, Vos T, Scott KM, et al. The global burden of anxiety disorders in. Psychol Med 2014;44:2363–74.
[32] Baxter AJ, Scott KM, Vos T, et al. Global prevalence of anxiety disorders: a systematic review and meta-regression. Psychol Med 2013;43:897–910.
[33] Medina ME, Borges G, Lara C, et al. Prevalencia de trastornos mentales y uso de servicios: Resultados de la Encuesta Nacional de Epidemiología Psiquiátrica en México. Salud Mental 2003;26:1–6.
[34] Miyajima F, Ollier W, Mayes A, et al. Brain-derived neurotrophic factor polymorphism Val66Met influences cognitive abilities in the elderly. Genes Brain Behav 2008;7:411–7.

[35] Szeszko PR, Lipsky R, Mentschel C, et al. Brain-derived neurotrophic factor val66met polymorphism and volume of the hippocampal formation. Mol Psychiatry 2005;10:631–6.
[36] Tocchetto A, Salum GA, Blaya C, et al. Evidence of association between Val66Met polymorphism at BDNF gene and anxiety disorders in a community sample of children and adolescents. Neurosci Lett 2011;502:197–200.
[37] Mueller SC, Aouidad A, Gorodetsky E, et al. Gray matter volume in adolescent anxiety: an impact of the brain-derived neurotrophic factor Val (66)Met polymorphism? J Am Acad Child Adolesc Psychiatry 2013;52:184–95.
[38] Lau JY, Goldman D, Buzas B, et al. BDNF gene polymorphism (Val66Met) predicts amygdala and anterior hippocampus responses to emotional faces in anxious and depressed adolescents. Neuroimage 2010;53:952–61.
[39] Morales-Marin ME, Genis-Mendoza AD, Tovilla-Zarate CA, et al. Association between obesity and the brain-derived neurotrophic factor gene polymorphism Val66Met in individuals with bipolar disorder in Mexican population. Neuropsychiatr Dis Treat 2016;12:1843–8.
[40] Gonzalez-Castro TB, Nicolini H, Lanzagorta N, et al. The role of brain-derived neurotrophic factor (BDNF) Val66Met genetic polymorphism in bipolar disorder: a case-control study, comorbidities, and meta-analysis of 16,786 subjects. Bipolar Disord 2015;17:27–38.
[41] Schiele MA, Ziegler C, Holitschke K, et al. Influence of 5-HTT variation, childhood trauma and self-efficacy on anxiety traits: a gene-environment-coping interaction study. J Neural Transm 2016;123:895–904.
[42] Wang J, Zhang F, Zhu W, et al. Meta-analysis of the association of brain-derived neurotrophic factor Val66Met polymorphism with obsessive-compulsive disorder. Acta Neuropsychiatr 2015;27:327–35.
[43] Suliman S, Hemmings SM, Seedat S. Brain-Derived Neurotrophic Factor (BDNF) protein levels in anxiety disorders: systematic review and meta-regression analysis. Front Integr Neurosci 2013;7:55.