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RESEARCH ARTICLE

PRKCA and *TCF4* Genetic Variants in Anxiety Symptoms and Generalized Anxiety Disorder in a Sample of Colombian Subjects Selected on the Basis of High Anxiety Scores

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Abstract:

Objective:

There are several anxiety disorders leading to a high burden of disease around the world, including Generalized Anxiety Disorder (GAD). The heritability of GAD suggests that genetic factors play an important role in its development; however, further research in this area is needed in Latin America. This study aimed to analyze the possible association between two single nucleotide polymorphisms (SNPs), rs2244497 and rs1452789, located in the *PRKCA* and *TCF4* genes with anxiety symptoms and GAD based on high anxiety scores in a sample of selected Colombian subjects.

Methods:

We evaluated 303 participants using the Hospital Anxiety and Depression Scale (HADS) and Zung's Self-Rating Anxiety Scale (ZSAS). Subjects with high scores in both scales (according to established cut-off points) participated in a psychiatric evaluation for the diagnosis of GAD. TaqMan assays were employed to genotype the SNPs, and statistical analyses were performed using logistic and linear regression.

Results:

In a sample of Colombian subjects selected on the basis of high anxiety scores, we found a significant association between the rs2244497 SNP in the *PRKCA* gene and higher scores in anxiety symptoms, where people carrying the T/T genotype had the highest scores for HADS scale. However, we did not observe this association in people diagnosed with GAD. In addition, the SNP in *TCF4* (rs1452789) did not have an association with anxiety symptoms or GAD diagnosis.

Conclusion:

This study contributes to the analysis of the molecular basis of anxiety disorders in selected Latin American samples. However, further studies are necessary to understand the role of rs2244497 SNP in the *PRKCA* gene and the risk for higher scores in anxiety symptoms.

Keywords: Anxiety disorder, Genetics and genomics, SNPs, Neurosciences, Diagnosis, Colombia.

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1. INTRODUCTION

Constant worrying and physical symptoms of tension are some of the manifestations of anxiety disorders [1]. Feelings of anxiety are usually future-oriented, such that perceived future threats activate physiological responses [2]. According to the National Institute of Mental Health (NIMH), anxiety is a normal part of life; many people worry about different aspects of their future, such as money, health, and family, among others [3]. However, in some cases, it is hard for people to regulate their anxiety, such that it worsens over time and, under certain conditions, leads to specific anxiety disorders [4 - 7], which are among the most common psychiatric disorders [8, 9]. Before the COVID-19 pandemic, the global prevalence of

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anxiety disorders was approximately 4.05% [10]. According to a recent study, anxiety disorders increased by 25.6% due to COVID-19 [11]. In 2015, for individuals between 18 and 44 years old, the prevalence of one or two anxiety symptoms in Colombia was 31.9%, while the prevalence of 5 or more anxiety symptoms was 6.7%; this represents a considerable burden for the health system [12].

Anxiety disorders include generalized anxiety disorder (GAD), panic disorder (PD), social anxiety disorder (SAD), and specific phobias [3]. GAD is one of the most chronic and prevalent of these disorders, with an early age of onset and symptoms that can extend throughout a person's life span, being characterized by uncontrollable and excessive worry present in different areas of life, restlessness, fatigue, irritability, and inability to concentrate or sleep, among others [13]. GAD has a heritability of around 35%, according to the meta-analysis of twin studies carried out in 2021 [14], which suggests that the genetic components play an important role in the development of this disorder [15]. It should be noted that further studies are necessary to understand the etiology of this disorder since GAD leads to a high burden of disease and has the potential to complicate the presentation of other psychiatric disorders [16].

Different studies have analyzed genetic factors related to GAD through two approaches, the genome-wide association studies (GWAS) and the candidate gene studies, finding several polymorphisms associated with this disorder in several populations [17]. In 2017, a GWAS found that several SNPs were associated with GAD symptoms in Latin American subjects in the United States; nevertheless, none of these polymorphisms reached the necessary significance threshold for a genome-wide study ($p < 5 \times 10^{-8}$) [18]. In contrast, a candidate gene study carried out in Mexican individuals found that *BDNF*-rs6265 confers an increased risk for developing GAD in this population [19]. Furthermore, other SNPs in different genes have been analyzed in relation to GAD, for instance, in the serotonin transporter (*SLC6A4*) [20], CD300 molecule like family member f (*CD300LF*) [21], and norepinephrine transporter (*SLC6A2*) [22]. It is important to highlight that there is an urgent need to study the genetic aspects of anxiety disorders in Latin American countries, including the Colombian population, due to the existence of risk factors specific to certain ancestries and taking into account the severe underrepresentation of Latin American countries in genetic studies of anxiety disorders. As an example of this, Fatumo *et al*. [23] recently found that the number of samples from Latin America in the GWAS catalog is very low (less than 2 percent), and van der Walt *et al*. [24] also identified, in a systematic review of GWAS for anxiety disorders, very low participation of individuals from Latin America.

The protein kinase C alpha (*PRKCA*) gene encodes a protein that is part of a family of serine- and threonine-specific protein kinases that influence many cellular processes, such as cellular development, proliferation, and differentiation, among others [25]. Variants in this gene have been previously associated with the risk for psychiatric disorders and neurological diseases, such as bipolar disorder, Alzheimer's disease, and post-traumatic stress disorder [26 - 29]. Another gene associated with neuropsychiatric disorders is transcription factor 4 (*TCF4*), which encodes a protein that is a member of the basic helix-loop-helix family of transcription factors, which has an important role in different processes in nervous system development [30]. Genetic variants located in this gene have been associated with schizophrenia, bipolar disorder, major depression, and post-traumatic stress disorder [30 - 34].

The possible influence of the *PRKCA* and *TCF4* on anxiety disorders, particularly on GAD, has not been explored previously. Notwithstanding, there are two polymorphisms located in both genes, the rs2244497 C/T and rs1452789 T/A, respectively, which have been associated with neuroticism, a personality trait related to anxiety disorders [35 - 36]. Our hypothesis was that these novel SNPs in two candidate genes might be associated with levels of anxiety symptoms. Therefore, we tested this hypothesis in order to analyze the possible association between these variants in *PRKCA* and *TCF4* genes and anxiety symptoms and GAD in a Colombian sample.

2. MATERIALS AND METHODS

2.1. Participants

About 303 Colombian individuals living in the capital city of Bogota were included in this study. All subjects were over 18 years old, with a mean age of 25.2 ± 8.4 years. About 200 subjects were females (66.01%).

2.2. Phenotypic Characterization and Classification of Individuals

We assessed the levels of anxiety symptoms using the Hospital Anxiety and Depression Scale (HADS), specifically, the subscale HADS-A [37] and Zung's Self-Rating Anxiety Scale (ZSAS) [38]. We used previously established cut-off points of 8 for the HADS-A and 36 for the ZSAS [39 - 40]. Scores above these cut-off points suggest clinical levels of anxiety. For this study, the Cronbach's alpha for the HADS-A was 0.8647, and for ZSAS was 0.9920. According to the results of both scales, individuals were classified into two different groups as follows: participants with high scores in anxiety symptoms (HADS-A ≥ 8, and ZSAS ≥36) and participants with low scores in anxiety symptoms (HADS-A < 8 and ZSAS < 36). Then, individuals who showed scores above cutoff points in both scales were invited to a clinical evaluation by a psychiatrist to confirm a possible GAD diagnosis. The psychiatric evaluation included a structured diagnostic interview using the Spanish version of the Mini-International Neuropsychiatric Interview. These interviews took place *via* remote settings (due to the pandemic), following MINI guidelines [41].

2.3. SNP Genotyping

In this study, we employed a candidate gene approach. We selected the SNPs in *PRKCA* (rs2244497) and *TCF4* (rs1452789) using data from the GWAS catalog and Genome Aggregation Database (gnomAD); moreover, we considered the following selection criteria: minor allele frequency in

samples from the Latin American Population (MAF ≥ 0.1) and previous reports of association with other diseases or disorders [35 - 36]. In addition, the RegulomeDB (https://regulomedb .org/regulome-search) web server was used for exploring the classification of variants based on their functional location in a regulatory region [42].

Isolation of DNA from peripheral blood samples was performed through the salting-out method [43]. DNA concentration was determined in a Qubit fluorometer (Thermo Fisher Scientific, MA, USA), and the DNA samples were normalized at 10ng/μL. The genotyping was carried out using TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA), and employed the following pre-designed probes: *PRKCA* (C_370967_10) and *TCF4* (C_9617300_10). For the qPCR reaction, we used the following reagents: 2ul (20ng) of genomic DNA, 1X TaqMan® Genotyping Master Mix, 1X TaqMan assay and water in a total volume of 10 μL. The reaction was carried out in a CFX96 Touch Real-Time PCR system (BioRad, Hercules, CA, USA). qPCR protocol included a 10 min denaturation step at 95ºC (1 cycle), 95 °C for 15 s, and 60 °C for 90 s (50 cycles).

2.4. Statistical Analysis

We used the Plink v1.9 and SNPStats programs [43 - 45]. First, for the 303 participants, we determined the allelic and genotypic frequencies, and then, the Hardy-Weinberg equilibrium (HWE) was assessed, considering the 5% level of significance [46]. Second, we performed a linear regression test to evaluate the possible association between the polymorphisms and the scores in anxiety symptoms. On the other hand, to analyze the association of the SNPs with the psychiatric diagnosis, we compared the cases of GAD (n=50) and controls (participants with scores below the cut-off points, n=101) using a logistic regression test. The regression tests were adjusted by age and sex, and a *p*-value lower than 0.05 was considered statistically significant. In addition, effect sizes were determined to describe the strength of the association using the Hedges' g measure [48].

3. RESULTS

3.1. Psychological Evaluation

The phenotypic evaluation using HADS-A and ZSAS scores showed that 137 individuals exceeded the cut-off points in both anxiety scales (HADS-A ≥ 8 and ZSAS ≥36), suggesting the presence of clinical levels of anxiety (high scores), and 101 participants presented scores below the cut-off points (low scores). About 65 subjects exceeded the cut-off point for one scale only. The psychiatric evaluation of subjects with high scores confirmed the diagnosis of GAD in 50 participants, while 54 did not have clinical evidence for this disorder. Moreover, for 33 subjects, the psychiatric assessment suggested other diagnoses. Sex and age distribution among the groups are shown in Table **1**.

For the analysis of the association between the SNPs and anxiety symptom levels, all participants were included. On the other hand, for the association analysis with GAD, only people with a confirmed diagnosis and subjects with low scores in both scales -controls-, were considered.

3.2. Genetic Characterization

In a sample of Colombian subjects selected on the basis of high anxiety scores for rs2244497 in *PRKCA*, the allele frequencies were 0.34 for allele C and 0.66 for allele T; the genotypic frequencies were C/C (0.10), C/T (0.48), and T/T (0.42). For *TCF4* rs1452789, allelic frequencies were 0.33 for allele T and 0.67 for allele A. The genotypic frequencies were A/A (0.43), A/T (0.49), and T/T (0.09). Both SNPs, rs2244497 and rs1452789, were in Hardy–Weinberg equilibrium, with *p* values of 0.2 and 0.09, respectively.

3.3. Association of Polymorphisms and Scores from Anxiety Scales

We analyzed the association between polymorphisms and scores on each anxiety scale using different genotypic models. We observed a significant association between the rs2244497 in the *PRKCA* gene with HADS-A scores under codominant, dominant, and overdominant models (Table **2**). These results showed that carriers of the T/T genotype have higher scores than C/T and C/C carriers (p = 0.012). In contrast, for the ZSAS scores, we did not observe any significant differences; the mean score for each genotype was T/T (40.54), C/T (39.11), and C/C (39.21). On the other hand, for the rs1452789 of *TCF4*, we did not find significant associations between scores of HADS-A and the different genotypes; the scores according to each genotype were A/A (7.98), A/T (8.02), and T/T (7.62). Similarly, significant differences in the ZSAS scores were not identified, where the mean for each genotype was A/A (39.84), A/T (39.99), and T/T (37.65). Finally, the sum of both scales showed only a significant association with rs2244497 in the *PRKCA* gene (p =0.003) under a dominant model, where carriers of the genotype T/T had higher scores than C/T-C/C, and the mean scores were 49.90 and 46.52, respectively.

Table 1. Demographic variables and scores for HADS and ZSAS.

-	All Participants			High Scores			Low Scores			GAD		
Sex	**All**	**Female**	**Male**	**All**	**Female**	**Male**	**All**	**Female**	**Male**	**All ***	**Female**	**Male**
N	n=303	n=200	n=103	n=137	n=105	n=32	n=101	n=52	n=49	n=50	n=42	n=8
%	100%	66%	44%	45.2%	76.6%	23.4%	33.3%	51.5%	48.5%	-	84%	16%
Age means (SD)	25.19 (8.40)	25.14 (8.36)	25.27 (8.51)	24.58 (7.99)	24.29 (8.15)	25.53 (7.50)	25.72 (8.43)	26.62 (8.57)	24.78 (8.27)	23.62 (7.28)	23.5 (7.2)	21.5 (3.1)
HADS-A mean (SD)	7.97 (4.33)	8.59 (4.39)	6.77 (3.99)	11.71 (2.88)	11.86 (2.98)	11.22 (2.50)	3.99 (2.05)	4.04 (2.05)	3.76 (2.06)	12.2 (3.13)	12.07 (3.45)	12.00 (3.21)

(Table 1) contd.....

-	All Participants			High Scores			Low Scores			GAD		
ZSAS mean (SD)	39.72 (9.81)	42.25 (10.07)	34.82 (7.10)	47.49 (7.64)	49.03 (7.53)	42.44 (5.64)	29.67 (3.35)	30.02 (3.34)	29.31 (3.36)	49.46 (8.29)	50.57 (8.01)	44.13 (7.57)

Notes: Hospital Anxiety Depression Scale – Anxiety Subscale (HADS-A), Zung's Self-Rating Anxiety Scale (ZSAS), High scores: Anxiety symptoms (HADS-A ≥8 and ZSAS ≥36), Low scores: No anxiety symptoms (HADS-A <8 and ZSAS <36), GAD: Generalized anxiety disorder diagnosis by psychiatry, *subjects derived from the high scores group.

Table 2. Association between the rs2244497 in *PRKCA* gene and scores of HADS-A.

Genotypic Model	N	HADS-A Mean (SD)	*p*-value	Hedges g
Codominant	T/T=128 C/T=146 C/C=29	8.75 (4.41) 7.42 (4.10) 7.28 (4.25)	0.012	T/T *vs* C/T= 0.313 C/T *vs* C/C=0.034 C/C *vs* T/T=0.335
Dominant	T/T=128 C/T-C/C=175	8.75 (4.41) 7.39 (4.10)	0.003	0.321
Overdominant	T/T-C/T=274 C/C=29	8.48 (5.79) 7.28 (4.25)	0.035	0.212

Notes: HADS-A: Hospital Anxiety Depression Scale-Anxiety subscale, SD: Standard deviation. Data analysis was adjusted by sex and age.

Table 3. Allelic and genotypic distribution in cases of GAD and controls of polymorphism in *PRKCA* and *TCF4* genes.

Polymorphism	Alleles and Genotypes	GAD Cases n=101	Controls n=50	*p*-value
rs2244497 (*PRKCA*)	C	0.37	0.27	0.055
	T	0.63	0.73	
	C/C	0.09	0.04	0.220
	C/T	0.56	0.46	
	T/T	0.35	0.50	
rs1452789 (*TCF4*)	A	0.70	0.64	0.299
	T	0.30	0.36	
	A/A	0.49	0.38	0.440
	A/T	0.43	0.52	
	T/T	0.09	0.10	

Notes: Data analysis was adjusted by sex and age.

The second analysis compared 50 cases (GAD-diagnosed individuals) and 101 controls (subjects with scores below the cut-off points). By assessing the allele distribution using a logistic regression adjusted by sex and age, we did not find any significant association with the rs2244497 in the *PRKCA* gene ($p = 0.055$) or rs1452789 in the *TCF4* gene ($p = 0.299$). Similar results were detected when we compared cases and controls under different genotypic models (Table **3**).

According to the RegulomeDB, the variant rs2244497 in *PRKCA* has a rank of 3a, which shows evidence for being a transcription binding site, and a probability score of 0.71; this indicates that there is a high probability it will be a regulatory variant [42]. The rs1452789 in *TCF4* has a rank of 4 and a probability score of 0.61.

4. DISCUSSION

In this study, we analyzed for the first time the association between two polymorphisms and anxiety symptoms and GAD in a sample of Colombian subjects selected based on high anxiety scores. We observed that carriers of the T/T genotype had higher anxiety levels than other genotypes of rs2244497 in the *PRKCA* gene; however, the individual analysis of each scale showed that this association is only with HADS-A scores and not with ZSAS. Moreover, when comparisons were done for GAD cases and controls, we did not find any significant results. On the other hand, *TCF4*-rs1452789 did not show evidence to be involved in the anxiety phenotypes in the individuals of the analyzed population.

Here, 137 (45.21%) individuals showed high levels of anxiety based on the results from the HADS-A and ZSAS analyses; in this group, 76,64% were females (n=105). Previously, it has been reported that anxiety symptoms prevalence in Colombia ranged from 25.8 to 52.9% [12,48 - 50], and international reports have shown an anxiety prevalence between 14 and 25% [8, 9]. However, a meta-analysis detected a prevalence of 46% in people from different countries during the coronavirus pandemic, which suggests an increase in anxiety prevalence around the world [51]. Otherwise, based on a psychiatric examination, 16.5% of individuals were diagnosed with GAD; for this group, 82% were women. In both cases, female individuals were more affected than males, as indicated in previous reports, in which anxiety disorders appear to be 2 to 3 times more prevalent in women than in men [52].

The association analysis only identified, based on high anxiety scores in a sample of Colombian subjects selected, a significant result with the rs2244497 in the *PRKCA* gene. It should be noted that this variant has not been well studied; it is

located in intron 3, and until now, there is no evidence about its functional role. In our results, carriers of the T/T genotype had high levels of anxiety. Interestingly, the allele T of this variant has been associated with neuroticism [35]. This is an important personality trait associated with the development of common mental disorders, including anxiety disorders [53]. On the other hand, although the variant rs1452789 in *TCF4* has also been associated with neuroticism [36], in our study, this was not associated with anxiety symptoms or GAD.

Previously, PRKCA has been implicated in anxiety disorders; for example, a murine model showed that differential expression of the protein kinase C alpha altered neurobiological processes that led to altered anxiety phenotypes [54]. Additionally, there is evidence that this protein has different targets involved in the regulation of emotional behavior, which might result in anxiety-related problems [55]. Another study analyzed the interactome of several anxiety disorders, identifying that *PRKCA* is a key gene expressed in the striatum, an important region for different disorders, including GAD, panic disorder and specific phobias [56]. Interestingly, in an association study, a polymorphism (rs4790904) in *PRKCA* was statistically associated with posttraumatic stress disorder in a population of U.S. veterans [57]. This association highlights the potential role of the genetic variants of *PRKCA* in neuropsychiatric disorders.

It should be noted that in this study, the rs2244497-*PRKCA* was only associated with HADS-A scores and not with ZSAS. Both scales are used to measure anxiety symptoms, and in our analysis, we evidenced a positive correlation between their scores (Spearman's Rho: 0.784; p= 0.000); this was similar to other studies [58]. However, there might be some differences that could explain the association with only HADS-A. For instance, the type of symptoms assessed by each scale; the HADS-A is focused on evaluating psychological symptoms [39], whereas the ZSAS covers both somatic and psychological symptoms [40]. This difference suggests that rs2244497 might influence the psychological aspects of anxiety but not the somatization. In contrast, in spite that the assessment time and the number of items are differents between both scales, these seem not to affect anxiety detection rates [58, 59].

Although we observed a significant association with levels of anxiety symptoms for GAD, we did not observe the same results. Several reasons can explain these findings: first, the low number of individuals with a GAD diagnosis decreases the statistical power of our study. Second, the etiology of GAD might involve other pathways different from those involved in anxiety symptoms, and finally, we only analyzed the association with two single polymorphisms, and we did not perform any assessment of gene-gene or gene-environment interaction, which might also provide evidence about the etiology of GAD [17].

As we mentioned above, in this study, the main limitation was the sample size, particularly, for the group of people with GAD. In addition, the sample was selected on specific criteria and was not randomly chosen. This work was performed during the COVID-19 pandemic, and this limited our capacity to include more participants. Future studies should include larger samples and other novel SNPs in candidate genes.

CONCLUSION

In summary, in a sample of Colombian subjects selected on the basis of high anxiety scores, our results suggest that the *PRKCA* gene has an association with levels of anxiety symptoms, but it is not associated with GAD. However, further studies are necessary to elucidate the complete relationship between *PRKCA* and GAD. Moreover, our work is the first genetic analysis of GAD in the Colombian population and one of the first genetic studies of anxiety symptoms in samples from Latin American populations.

AUTHORS' CONTRIBUTION

DF, MLTG and YGG contributed to the conceptualization, methodology, validation, and review. DALR and LUM contributed to formal analysis and original draft preparation. SB, LM and WV contributed to administering and interpreting the psychological and psychiatric tests of this study. LUM, LM, BJQ, SZ, WV, YGG and MLTG participated in the formal analysis, investigation, and review. MLTG and YGG contributed to editing the work. All authors (DALR, LUM, BJQ, SZ, WV, LM, YGG, SB, DF and MLTG) contributed to critically revising the work, approved the final version of the article to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

LIST OF ABBREVIATIONS

GAD	=	Generalized Anxiety Disorder
SNPs	=	Single Nucleotide Polymorphisms
HADS	=	Hospital Anxiety and Depression Scale
HWE	=	Hardy-Weinberg Equilibrium

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The Ethics Committee of the Universidad Antonio Nariño approved the study (protocol code 27052019-1).

HUMAN AND ANIMAL RIGHTS

No animals were used that are the basis of this study. All the human procedures were conducted in accordance with the Declaration of Helsinki principles.

CONSENT FOR PUBLICATION

Informed consent was obtained from all participants of this study.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding author, [M.L.T-G] upon reasonable request.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Craske MG, Stein MB, Eley TC, *et al.* Anxiety disorders. Nat Rev Dis Primers 2017; 3(1): 17024. [http://dx.doi.org/10.1038/nrdp.2017.24] [PMID: 28470168]

[2] Stein MB, Sareen J. Generalized anxiety disorder. N Engl J Med 2015; 373(21): 2059-68. [http://dx.doi.org/10.1056/NEJMcp1502514] [PMID: 26580998]

[3] Vogel DA. Anxiety disorders. In: Adolescent Medicine. 2008; pp. 297-302. [http://dx.doi.org/10.1016/B978-032304073-0.10038-X]

[4] Addis SG, Nega AD, Miretu DG. Depression, anxiety and stress levels among chronic disease patients during COVID-19 pandemic in dessie town hospitals, ethiopia. Open Psychol J 2021; 14(1): 249-57. [http://dx.doi.org/10.2174/1874350102114010249]

[5] Garit JR, Rodríguez RC, Surita YP, *et al.* Peculiarities of the relationships between anxiety, psychological skills, and injuries in cuban athletes. Open Psychol J 2023; 16(1): e187435012212060. [http://dx.doi.org/10.2174/18743501-v15-e221207-2022-60]

[6] Kustimah K, Kumalasari AD, Meindy N. When do College students seek mental health services? Open Psychol J 2023; 16(1): e187435012304120.

[7] Siswadi AG, Shabrina A, Djunaidi A, Iskandarsyah A. The role of perceived social support and resilience in predicting the Mental Health of Healthcare Professionals during the COVID-19 pandemic: A study from indonesia. Open Psychol J 2023; 16(1): e187435012212220. [http://dx.doi.org/10.2174/18743501-v15-e221222-2022-69]

[8] Vos T, Barber RM, Bell B, *et al.* Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: A systematic analysis for the Global Burden of Disease Study 2013. Lancet 2015; 386(9995): 743-800. [http://dx.doi.org/10.1016/S0140-6736(15)60692-4] [PMID: 26063472]

[9] Bandelow B, Michaelis S. Epidemiology of anxiety disorders in the 21st century. Dialogues Clin Neurosci 2015; 17(3): 327-35. [http://dx.doi.org/10.31887/DCNS.2015.17.3/bbandelow] [PMID: 26487813]

[10] Javaid SF, Hashim IJ, Hashim MJ, Stip E, Samad MA, Ahbabi AA. Epidemiology of anxiety disorders: Global burden and sociodemographic associations. Middle East Current Psychiatry 2023; 30(1): 44. [http://dx.doi.org/10.1186/s43045-023-00315-3]

[11] Santomauro DF, Mantilla Herrera AM, Shadid J, *et al.* Global prevalence and burden of depressive and anxiety disorders in 204 countries and territories in 2020 due to the COVID-19 pandemic. Lancet 2021; 398(10312): 1700-12. [http://dx.doi.org/10.1016/S0140-6736(21)02143-7] [PMID: 34634250]

[12] Ministry of Health and Social Protection, Colciencias. National Mental Health Survey 2015. Volume 1. Bogotá. 2015. Available from:https://www.minjusticia.gov.co/programas-co/ODC/Publicaciones/Publicaciones/CO031102015-salud_mental_tomoI.pdf

[13] Showraki M, Showraki T, Brown K. Generalized anxiety disorder: Revisited. Psychiatr Q 2020; 91(3): 905-14. [http://dx.doi.org/10.1007/s11126-020-09747-0] [PMID: 32383134]

[14] Penninx BWJH, Pine DS, Holmes EA, Reif A. Anxiety disorders. Lancet 2021; 397(10277): 914-27. [http://dx.doi.org/10.1016/S0140-6736(21)00359-7] [PMID: 33581801]

[15] Hettema JM, Neale MC, Kendler KS. A review and meta-analysis of the genetic epidemiology of anxiety disorders. Am J Psychiatry 2001; 158(10): 1568-78. [http://dx.doi.org/10.1176/appi.ajp.158.10.1568] [PMID: 11578982]

[16] Saha S, Lim CCW, Cannon DL, *et al.* Co-morbidity between mood and anxiety disorders: A systematic review and meta-analysis. Depress Anxiety 2021; 38(3): 286-306. [http://dx.doi.org/10.1002/da.23113] [PMID: 33225514]

[17] Gottschalk MG, Domschke K. Genetics of generalized anxiety disorder and related traits. Dialogues Clin Neurosci 2017; 19(2): 159-68. [http://dx.doi.org/10.31887/DCNS.2017.19.2/kdomschke] [PMID: 28867940]

[18] Dunn EC, Sofer T, Gallo LC, *et al.* Genome□wide association study of generalized anxiety symptoms in the Hispanic Community Health Study/Study of Latinos. Am J Med Genet B Neuropsychiatr Genet 2017; 174(2): 132-43. [http://dx.doi.org/10.1002/ajmg.b.32448] [PMID: 27159506]

[19] González-Castro TB, Pool-García S, Tovilla-Zárate CA, *et al.* Association between BDNF Val66Met polymorphism and generalized anxiety disorder and clinical characteristics in a Mexican population. Medicine 2019; 98(11): e14838. [http://dx.doi.org/10.1097/MD.0000000000014838] [PMID: 30882674]

[20] You JS, Hu SY, Chen B, Zhang HG. Serotonin transporter and tryptophan hydroxylase gene polymorphisms in Chinese patients with generalized anxiety disorder. Psychiatr Genet 2005; 15(1): 7-11. [http://dx.doi.org/10.1097/00041444-200503000-00002] [PMID: 15722951]

[21] Kaufmann FN, Lago N, Alí-Ruiz D, *et al.* Sex-dependent role of CD300f immune receptor in generalized anxiety disorder. Brain Behav Immun-Health 2020; 11: 100191. [http://dx.doi.org/10.1016/j.bbih.2020.100191]

[22] Chang HA, Fang WH, Tzeng NS, *et al.* Age-dependent association of polymorphisms in the promoter and 5′-untranslated region of the norepinephrine transporter gene with generalized anxiety disorder. J Affect Disord 2020; 270: 124-30. [http://dx.doi.org/10.1016/j.jad.2020.03.059] [PMID: 32339102]

[23] Fatumo S, Chikowore T, Choudhury A, Ayub M, Martin AR, Kuchenbaecker K. A roadmap to increase diversity in genomic studies. Nat Med 2022; 28(2): 243-50. [http://dx.doi.org/10.1038/s41591-021-01672-4] [PMID: 35145307]

[24] van der Walt K, Campbell M, Stein DJ, Dalvie S. Systematic review of genome-wide association studies of anxiety disorders and neuroticism. World J Biol Psychiatry 2023; 24(4): 280-91. [http://dx.doi.org/10.1080/15622975.2022.2099970] [PMID: 35815422]

[25] Skalhegg BS, Tasken K. Specificity in the cAMP/PKA signaling pathway. differential expression, regulation, and subcellular localization of subunits of PKA. Front Biosci 2000; 5(1): d678. [http://dx.doi.org/10.2741/Skalhegg] [PMID: 10922298]

[26] Hahn CG, Friedman E. Abnormalities in protein kinase C signaling and the pathophysiology of bipolar disorder. Bipolar Disord 1999; 1(2): 81-6. [http://dx.doi.org/10.1034/j.1399-5618.1999.010204.x] [PMID: 11252663]

[27] Carroll LS, Williams NM, Moskvina V, *et al.* Evidence for rare and common genetic risk variants for schizophrenia at protein kinase C, alpha. Mol Psychiatry 2010; 15(11): 1101-11. [http://dx.doi.org/10.1038/mp.2009.96] [PMID: 19786960]

[28] Alfonso SI, Callender JA, Hooli B, *et al.* Gain-of-function mutations in protein kinase Cα (PKCα) may promote synaptic defects in Alzheimer's disease. Sci Signal 2016; 9(427): ra47. [http://dx.doi.org/10.1126/scisignal.aaf6209] [PMID: 27165780]

[29] Bian YY, Yang LL, Zhang B, *et al.* Identification of key genes involved in post-traumatic stress disorder: Evidence from bioinformatics analysis. World J Psychiatry 2020; 10(12): 286-98. [http://dx.doi.org/10.5498/wjp.v10.i12.286] [PMID: 33392005]

[30] Forrest MP, Hill MJ, Quantock AJ, Martin-Rendon E, Blake DJ. The emerging roles of TCF4 in disease and development. Trends Mol Med 2014; 20(6): 322-31. [http://dx.doi.org/10.1016/j.molmed.2014.01.010] [PMID: 24594265]

[31] Stefansson H, Ophoff RA, Steinberg S, *et al.* Common variants conferring risk of schizophrenia. Nature 2009; 460(7256): 744-7. [http://dx.doi.org/10.1038/nature08186] [PMID: 19571808]
[32] Del-Favero J, Gestel SV, Børglum AD, *et al.* European combined analysis of the CTG18.1 and the ERDA1 CAG/CTG repeats in bipolar disorder. Eur J Hum Genet 2002; 10(4): 276-80. [http://dx.doi.org/10.1038/sj.ejhg.5200803] [PMID: 12032737]
[33] Wray NR, Ripke S, Mattheisen M, *et al.* Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. Nat Genet 2018; 50(5): 668-81. [http://dx.doi.org/10.1038/s41588-018-0090-3] [PMID: 29700475]
[34] Gelernter J, Sun N, Polimanti R, *et al.* Genome-wide association study of post-traumatic stress disorder reexperiencing symptoms in >165,000 US veterans. Nat Neurosci 2019; 22(9): 1394-401. [http://dx.doi.org/10.1038/s41593-019-0447-7] [PMID: 31358989]
[35] Luciano M, Hagenaars SP, Davies G, *et al.* Association analysis in over 329,000 individuals identifies 116 independent variants influencing neuroticism. Nat Genet 2018; 50(1): 6-11. [http://dx.doi.org/10.1038/s41588-017-0013-8] [PMID: 29255261]
[36] Nagel M, Watanabe K, Stringer S, Posthuma D, van der Sluis S. Item-level analyses reveal genetic heterogeneity in neuroticism. Nat Commun 2018; 9(1): 905. [http://dx.doi.org/10.1038/s41467-018-03242-8] [PMID: 29500382]
[37] Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand 1983; 67(6): 361-70. [http://dx.doi.org/10.1111/j.1600-0447.1983.tb09716.x] [PMID: 6880820]
[38] Zung WWK. A rating instrument for anxiety disorders. Psychosomatics 1971; 12(6): 371-9. [http://dx.doi.org/10.1016/S0033-3182(71)71479-0] [PMID: 5172928]
[39] Brennan C, Worrall-Davies A, McMillan D, Gilbody S, House A. The Hospital anxiety and depression scale: A diagnostic meta-analysis of case-finding ability. J Psychosom Res 2010; 69(4): 371-8. [http://dx.doi.org/10.1016/j.jpsychores.2010.04.006] [PMID: 20846538]
[40] Dunstan DA, Scott N. Norms for Zung's self-rating anxiety scale. BMC Psychiatry 2020; 20(1): 90. [http://dx.doi.org/10.1186/s12888-019-2427-6] [PMID: 32111187]
[41] Ferrando L, Bobes J, Gibert J, Soto M, Soto O. 2000. Available from:http://entomologia.rediris.es /pub/bscw.cgi/ d602335/ MINI/Entrevista
[42] Boyle AP, Hong EL, Hariharan M, *et al.* Annotation of functional variation in personal genomes using RegulomeDB. Genome Res 2012; 22(9): 1790-7. [http://dx.doi.org/10.1101/gr.137323.112] [PMID: 22955989]
[43] Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16(3): 1215. [http://dx.doi.org/10.1093/nar/16.3.1215] [PMID: 3344216]
[44] Chang CC, Chow CC, Tellier LCAM, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: Rising to the challenge of larger and richer datasets. Gigascience 2015; 4(1): 7. [http://dx.doi.org/10.1186/s13742-015-0047-8] [PMID: 25722852]
[45] Solé X, Guinó E, Valls J, Iniesta R, Moreno V. SNPStats: A web tool for the analysis of association studies. Bioinformatics 2006; 22(15): 1928-9. [http://dx.doi.org/10.1093/bioinformatics/btl268] [PMID: 16720584]
[46] Wigginton JE, Cutler DJ, Abecasis GR. A note on exact tests of Hardy-Weinberg equilibrium. Am J Hum Genet 2005; 76(5): 887-93. [http://dx.doi.org/10.1086/429864] [PMID: 15789306]
[47] Lenhard W, Lenhard A. Computation of effect sizes. Code 2016. [http://dx.doi.org/10.13140/RG.2.2.17823.92329]
[48] Monterrosa-Castro ÁJ, Ordosgoitia-Parra E, Beltrán-Barrios T. Anxiety and depression identified with the Golberg Scale in university students in the health area. MedUNAB 2020; 23(3): 372-404. [http://dx.doi.org/10.29375/01237047.3881]
[49] Suarez DE, Cardozo AC, Ellmer D, Trujillo EM. Short report: cross sectional comparison of anxiety and depression symptoms in medical students and the general population in Colombia. Psychol Health Med 2021; 26(3): 375-80. [http://dx.doi.org/10.1080/13548506.2020.1757130] [PMID: 32314943]
[50] Osma-Zambrano SE, Lozano-Osma MD, Mojica-Perilla M, Redondo-Rodríguez S. Prevalencia de depresión y ansiedad y variables asociadas en gestantes de Bucaramanga y Floridablanca (Santander, Colombia). MedUNAB 2019; 22(2): 171-85. [http://dx.doi.org/10.29375/01237047.3586]
[51] da Silva ML, Rocha RSB, Buheji M, Jahrami H, Cunha KC. A systematic review of the prevalence of anxiety symptoms during coronavirus epidemics. J Health Psychol 2021; 26(1): 115-25. [http://dx.doi.org/10.1177/1359105320951620] [PMID: 32830577]
[52] Ströhle A, Gensichen J, Domschke K. The diagnosis and treatment of anxiety disorders. Dtsch Arztebl Int 2018; 155(37): 611-20. [http://dx.doi.org/10.3238/arztebl.2018.0611] [PMID: 30282583]
[53] Ormel J, Jeronimus BF, Kotov R, *et al.* Neuroticism and common mental disorders: Meaning and utility of a complex relationship. Clin Psychol Rev 2013; 33(5): 686-97. [http://dx.doi.org/10.1016/j.cpr.2013.04.003] [PMID: 23702592]
[54] Keil MF, Briassoulis G, Gokarn N, Nesterova M, Wu TJ, Stratakis CA. Anxiety phenotype in mice that overexpress protein kinase A. Psychoneuroendocrinology 2012; 37(6): 836-43. [http://dx.doi.org/10.1016/j.psyneuen.2011.09.016] [PMID: 22024111]
[55] Keil MF, Briassoulis G, Stratakis CA. The role of protein Kinase A in anxiety behaviors. Neuroendocrinology 2016; 103(6): 625-39. [http://dx.doi.org/10.1159/000444880] [PMID: 26939049]
[56] Karunakaran KB, Amemori S, Balakrishnan N, Ganapathiraju MK, Amemori K. Generalized and social anxiety disorder interactomes show distinctive overlaps with striosome and matrix interactomes. Sci Rep 2021; 11(1): 18392. [http://dx.doi.org/10.1038/s41598-021-97418-w] [PMID: 34526518]
[57] Liu Y, Rimmler J, Dennis MF, Ashley-Koch AE, Hauser MA, Beckham JC. Association of Variant rs4790904 in Protein Kinase C Alpha with posttraumatic stress disorder in a U.S. Caucasian and African-American veteran sample. J Depress Anxiety 2013; 2(1): S4001. [http://dx.doi.org/10.4172/2167-1044.S4-001] [PMID: 24839585]
[58] Guo C, Huang X. Hospital anxiety and depression scale exhibits good consistency but shorter assessment time than Zung self-rating anxiety/depression scale for evaluating anxiety/depression in non-small cell lung cancer. Medicine 2021; 100(8): e24428. [http://dx.doi.org/10.1097/MD.0000000000024428]
[59] Yue T, Li Q, Wang R, *et al.* Comparison of Hospital Anxiety and Depression Scale (HADS) and Zung Self-Rating Anxiety/Depression Scale (SAS/SDS) in evaluating anxiety and depression in patients with psoriatic arthritis. Dermatology 2020; 236(2): 170-8. [http://dx.doi.org/10.1159/000498848] [PMID: 31434087]