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Body index mass not associated with *DRD4*, *DAT1*, *BDNF*, and *COMT* gene polymorphisms in young adults without depression or anxiety disorders

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ABSTRACT. Depressive and anxiety disorders have been associated with body mass index (BMI), since mesolimbic reward circuits, such as dopamine secretion and the expression of dopamine neuronal receptors, have been related to weight in people with mental issues. However, few studies have analyzed polymorphisms of genes of the dopaminergic system in healthy populations. We evaluated association of BMI with four polymorphisms of genes related to the dopaminergic system, *DRD4*, *DAT1*, *BDNF, and COMT*, in young adults without depression or anxiety disorders. Blood samples of 62 subjects were analyzed for polymorphisms of VNTR *DRD4* and *DAT1* by PCR, and the SNPs of *BDNF* and *COMT* genes by Sanger sequencing, as well as their score in the Beck Depression and Beck Anxiety inventories. We also measured the level of physical activity, height (cm), weight (kg), and calculated BMI. Multivariate linear regression was used to analyze associations between BMI and polymorphisms. Allelic frequencies of the four genes in the sample

studied were in Hardy-Weinberg equilibrium. BMI was higher in male subjects ($p<0.05$). Multivariate linear regression did not show an association between the polymorphisms with BMI after adjusting by sex. The BMI of young adults without depression and anxiety disorders was not found to be associated with polymorphisms of the 3rd exon of *DRD4*, 3'UTR of *DAT1* genes, rs6265 *BDNF*, or rs4680 *COMT*.

Key words: Body index mass; Single nucleotide polymorphism; VNTR gene polymorphism; Mental health

INTRODUCTION

Mental disorders such as anxiety and depression have been associated with higher body mass index (BMI) (Luppino et al., 2010); BMI is an indicator of the health state of a person concerning their weight and adiposity (Stice et al., 2010). In 2016, the BMI of the world population indicated that 39% and 13% were overweight and obese, respectively (World Health Organization, June 9, 2021). BMI can be influenced by mental health and other factors such as diet, physical activity, and genetic factors (40-70% heritability) (Herrera and Lindgren, 2010). Different genetic variants such as variable number tandem repetitions (VNTR) (Ariza et al., 2012; Sikora et al., 2013; González-Giraldo et al., 2018) and single nucleotide polymorphisms (SNPs) (Beckers et al., 2008; Skledar et al., 2012) related to the dopaminergic system have been associated with BMI (Balcioglu and Wurtman, 1998; Fuemmeler et al., 2008) and mental disorders (Alfimova et al., 2014; Hosang et al., 2014; Gafarov et al., 2021).

Among the genes related to the dopaminergic system that have been associated with BMI in different populations are VNTRs of 48-bp repeats in exon 3 of the dopamine D4 receptor gene (*DRD4*) (Ariza et al., 2012; Stice et al., 2010), the VNTR of 40 bp in the UTR-3' region of dopamine transporter gene (*DAT1*) gene (Sikora et al., 2013; González-Giraldo et al., 2018); the SNP Val66Met (rs6265) in human brain-derived neurotrophic factor (*BDNF*) (Beckers et al., 2008); and the SNP Val158(rs4680) in Metcatechol-o-methyltransferase gene (*COMT*) (Kring et al., 2009). However, most studies that have studied the relationship between BMI and polymorphism in *DRD4*, *DAT1*, *BNDF*, and *COMT* genes (Kring et al., 2009; Ariza et al., 2012; Sikora et al., 2013; Zamani et al., 2019) have not taken into account the mental state of the participants, which can be a confounding factor. Therefore, the objective of this study was to determine if BMI is associated with polymorphisms of genes related to the dopaminergic system *DRD4*, *DAT1*, *BDNF*, and *COMT*, in young adults without depression or anxiety disorders.

MATERIAL AND METHODS

We performed a cross-sectional study in the city of Santiago de Cali-Colombia to evaluate association of BMI with four polymorphisms of genes related to the dopaminergic system, *DRD4*, *DAT1*, *BDNF*, and *COMT*, in young adults without depression or anxiety. We recruited a total of 143 subjects, of which 62 met inclusion criteria: 1) undergraduate students, 2) ages between 18 and 25 years. The exclusion criteria were: 1) consumption of

psychoactive substances such as marijuana, cocaine, hallucinogens, tranquilizers, and stimulants, 2) daily consumption or weekly alcohol drinkers, and 3) diagnosis of psychiatric or neurological disorders. All participants signed an informed consent, approved by the institutional ethics committee, in accordance with resolutions 8430 of 1993 and 2378 of 2008 of the Colombian legislation, and with the international declaration of Helsinki.

Anthropometric and physical activity

Body weight was measured with a digital scale to the nearest 0.1 kg, and height was measured using a stadiometer; BMI was calculated as weight in kilograms divided by the height in meters squared. BMI between 25 to 30 was considered overweight and BMI<18 was considered underweight. The International Physical Activity Questionnaire (IPAQ) was used to estimate the level of physical activity and to classify subjects as sedentary (low level) or active (moderate and high level).

Evaluation of anxiety and depression

To establish the level of anxiety and depression symptoms, a professional psychologist applied the Beck Depression Inventory (BDI) and the Beck Anxiety Inventory (BAI) respectively (Beck et al., 2011; Beck and Steer, 2011). BDI levels below 19 and BAI levels below 15 were the cut-off points to rule out depression and anxiety in the subjects.

DNA extraction and genotyping

DNA was isolated from peripheral blood leukocytes obtained in anticoagulated tubes with EDTA and stored at -80°C. DNA extraction was performed with the DNeasy Blood and Tissue Kit (Cat No. 69506 QIAGEN). The concentration was determined spectrophotometrically, measuring absorbance (A) at 260 nm, with the following formula: [DNA ng / µL] = A260nm x 50 ng / µL. The purity of the DNA was estimated with the ratio A260nm / A280nm. Then DNA was stored at -30°C.

To genotype the VNTR in exon 3 of the DDR4 gene, we used the primers F:5'-GCGACTACGTGGTCTACTCG-3', R:5'-AGGACCCTCATGGCCTTG-3' (Balcioglu and Wurtman, 1998). PCR reaction included 20–40 ng of genomic DNA, 0.5 M of each primer, and 1x GoTaq Green Master (Thermo Scientific, Catalog No. *M 7128).* The amplification program consists of initial denaturation at 95°C for 4 min, followed by 35 cycles of 95°C for 30 sec, 54°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 5 min. For the VNTR in region UTR 3' of the DAT-1 gene, the DNA was amplified using primers F:5'-TGTGGTGTAGGGAACGGCCTGAG-3' and R:5'CTTCCTGGAGGTCACGGCTCAAGG-3 (Waldman et al., 1998). The reaction for PCR contained 100 ng of genomic DNA, 1x amplification buffer, 0.2mM dNTPs, 0.1 - 0.15 µM of each primer, 1.5 - 2.5mM MgCl2 and 0.2 - 0.33 IU of DNA polymerase Taq Phusion (*Thermo Scientific,* Catalog No. F553L) The initial denaturation was at 98°C for 30 seg, followed by 35 cycles at 95° C for 30 seg, the annealing and extension step was performed in a single step at 72° C for 35s, and the final extension was at 72° C for 10 min. To evaluate the VNTRs, the PCR products were separated on a 2% agarose gel for 90 min at 70V and stained with Fluorescent Staining Dye (Smobio); then the bands were visualized to

260 nm on a photo-documenter. The size of VNTRs was established by comparison with a molecular weight standard of 50bp.

The SNPs of COMT rs4680 and BDNF rs6265 were genotyped by the Sanger sequencing method. DNA fragments of COMT were amplified by PCR using the following primers: F: 5'- CCAACCCTGCACAGGCAAGAT-3' and R: 5'-CAAGGGTGACCTGGAACAGCG- 3' (Vijayakumari et al., 2015). For BDNF rs6265, the DNA was amplified with the primers F: 3'-ACTCTGGAGAGCGTGAAT-5', R: 5'-ATACTGTCACACACGCTC-3' (Chen et al., 2011). For each reaction, we used 20 ng of genomic DNA, 0.2 mM of dNTPs, 1.5 mM of MgCl2, 1x Taq buffer HF, 0.02 U Taq DNA polymerase (Phusion™ High-Fidelity DNA Polymerase, Thermo Scientific, Catalog No. F553L) and 0.25 μM (COMT) – 0.2 μM (BDNF) of concentration each primer. The amplification for both genes was carried out in a thermo-cycler (BIORAD), with an initial denaturation at 98°C for 30 sec, followed by 30 cycles of 98°C for 10 sec., 66°C for the COMT gene or 59°C for the BDNF gene for 30 sec, and 72°C for 30 sec., and a final extension at 72°C for 10 min for both genes. After the amplification, the purity and size of the bands were confirmed by gel electrophoresis. The PCR products were sent to Macrogen Inc. (Seoul, South Korea) for the Sanger method sequencing. The sequences were aligned and analyzed with the program Sequencher *5.4.6 (Corperation, n.d.)*.

Statistical analysis

The continuous variables are described as mean ± standard deviation (SD) and the categorical variables as relative frequencies in percentage. The Kolmogorov-Smirnov test and Q-Q plots were used to test the normal distribution of the continuous variables. The mean differences and frequency by sex were estimated with the unpaired t-test and Chi-square ($\chi 2$) test respectively. The deviation of the polymorphisms from Hardy-Weinberg equilibrium was tested with a multi-allelic exact test (Graffelman and Weir, 2018). Multivariate linear regression was used to analyze the association between BMI with polymorphisms in *DRD4*, *DAT-1*, *BDNF*, and *COMT*. Due to the low frequency of certain polymorphisms, homozygotes of minor alleles and heterozygotes were grouped and compared with the major allele homozygotes. The analysis was performed with R programming language with R-studio platform version 4.0.2(Team, 2019). A P-value <0.05 was considered significant.

RESULTS

The anthropometric characteristics and physical activity of the subjects are presented in Table 1. The men had higher weight, height, and BMI, as well as overweight frequency of when compared to women ($P < 0.05$). There were no differences in the levels of physical activity by sex. The women and the men showed a mean of 9.2 ± 5.3 and 7.2 ± 3.7 in the BDI and 8.41 ± 4.85 and 6.50 ± 3.72 in the BAI respectively. Both sexes showed low levels of depression and anxiety as is expected for an apparently healthy population.

Table 1. Descriptive analysis of the anthropometric parameters and physical activity of study participants.

	Total population (n=62)	Men (n=30)	Women (n= 32)	p-value
Age (years)	20.1±1.4	20.4±1.5	20±1.3	0.203
Height (m)	1.67±0.87	1.73±0.66	1.56±0.29	3.5×10^{-11}
Weight (Kg)	64.1±11.5	71.4 ± 10	57.4 ± 8.5	1.9×10^{-6}
BMI (m^2/Kg)	22.6±4	23.8±2.6	22.3±2.7	0.025
BMI Classification				
Normal weight (n/%)	44 (36%)	18 (40%)	26 (60%)	0.07[a]
Overweight (n/%)	17 (27.4%)	12 (71%)	5 (29%)	0.03[a]
Underweight (n/%)	1 (1.6%)	0	1 (100%)	0.49[a]
Physical activity				
Sedentary (n/%)	13 (21%)	5 (17%)	8 (25%)	
Active (n/%)	49 (79%)	25 (83%)	24 (75%)	0.44[a]

[a]Chi-squared test.

The analysis of the distribution of polymorphism for *DRD4*, *DAT1*, *BDNF*, and *COMT* showed that the population was in Hardy-Weinberg equilibrium (Table 2). For the *DRD4* gene, we identified five *VTNR* alleles (2R, 3R, 4R, 6R, 7R), the 4R allele was the most frequent in the homozygous genotype, while the 3R allele was less frequent. Regarding the *DAT1* gene, four alleles were found (6R, 9R, 10R, 11R), with the 10R and 9R alleles being the most frequent, and the 11R and 6R alleles were rare variants in the population studied. The SNPs with the highest frequency for the rs6265 *BDNF* and rs4680 *COMT* genes were G/G and G/C, respectively.

Table 2. Distribution of DRD4, DAT-1, BDNF and COMT-1 genetic polymorphisms in study participants.

	Total population (n=62)	Men (n=30)	Women (n= 32)	P-value Hardy-Weinberg
VTNR DRD-4				
2R/2R	1.6%	3.3%	0%	0.0958
4R/4R	41.9%	33.3%	50%	
4R/2R	8.1%	13.3%	3.1%	
4R/6R	4.8%	6.6%	3.1%	
4R/7R	35.5%	36.7%	34.4%	
6R/7R	3.2%	6.6%	0%	
7R/2R	3.2%	0%	6.3%	
6R/3R	1.6%	0%	3.1%	
VTNR DAT-1				
10R/10R	56.5%	50%	63%	0.844
10R/9R	33.9%	38%	30%	
9R/9R	6.5%	9%	3.5%	
10R/6R	1.6%	0%	3.5%	
10R/11R	1.6%	3%	0%	
SNPs BDNF (rs6265)				
G/G	68.5%	70.4%	66.7%	0.887
A/A	3.7%	3.7%	3.7%	
G/A	27.8%	25.9%	29.6%	
SNPs COMT-1 (rs4680)				
G/G	6.8%	8.7%	4.4%	0.112
C/C	34.1%	21.7%	43.5%	
G/C	59.1%	61.6%	52.2%	

The regression analysis showed an association between BMI and sex (β=1.54, 95% CI: 0.19; 2.89; P = 0.025) but did not show a relationship with the level of physical activity (β=0.22, 95% CI: -1.44; 1.88) After adjusting the model for sex, no association was found between BMI and genotypes 7R/7R of the *DRD4* gene, 9R/10R of *DAT1*, and the SNPs rs4680 of *COMT* and rs6265 *BDNF* (Table 3).

Table 3. Multiple regression to BMI for sex, age, and genotype DAT1, DRD4, BDNF, and COMT-1 in study participants.

	Non-adjusted models				Sex adjusted models			
	β	**95% CI**	**Adjusted r^2**	**P-value**	**β**	**95% CI**	**Adjusted r^2**	**P-value**
Sex (masculine)	1.54	0.19; 2.89	0.065	0.02	-	-	-	-
Age (years)	0.30	-0.17; 0.79	0.009	0.208	0.30	-0.25; 0.70	0.009	0.348
Physical Activity	0.56	-1.14; 2.28	-0.009	0.51	0.37	-1.29; 2.05	0.05	0.659
VTNR DDR4 (7R)	0.46	-1.11; 2.03	-0.012	0.559	0.32	-1.18; 1.81	0.089	0.672
VNTR DAT1 (9R/10R)	-0.56	-2.07; 0.93	-0.007	0.45	-0.24	-1.70; 1.22	0.078	0.743
COMT-1 (rs4680)	-0.18	-1.77; 1.42	-0.021	0.822	0.11	-1.35;1.58	0.146	0.877
BNDF (rs6265)	0.22	-1.44; 1.88	-0.017	0.791	0.29	-1.30;1.89	0.179	0.714

DISCUSSION

The purpose of this study was to investigate the relationship between polymorphisms of *DRD4*, *DAT1*, *BDNF*, and *COMT1* genes with BMI in young subjects without depression and anxiety disorders. We found several allelic variants, which, when adjusted for sex and age, were not associated with BMI.

Among the allelic variants studied, we observed that VNTR 9R and 10R in the *DAT1* gene were the most frequent, which is in agreement with the results of studies in the Colombian population (Fonseca et al., 2015; Ortega-Rojas et al., 2017; González-Giraldo et al., 2018), and other Latin populations such as Mexico (Martínez-Levy et al., 2013), and Chile (Vieyra et al., 2003). The VNTR 9R of *DAT1* has been associated with higher BMI (43.6 ± 6.8) in Caucasian women (n=506) aged 18-84 years old (Sikora et al., 2013) and has also been associated with low BMI (22.9 ± 0.4) in young Colombian university students aged 18-30 years old (n=232) (González-Giraldo et al., 2018). However, our results for *DAT1* agree with what was reported in a sample of young healthy people from Turkey (n=382), where no association of BMI was found with any of the VNTRs of the *DAT1* gene (Uzun et al., 2015).

The most frequent VNTR for the *DRD4* gene was 4R, a finding that is similar to that reported for Latin America (Vieyra et al., 2003; Fonseca et al., 2015; González-Giraldo et al., 2015;) and the European population (Ariza et al., 2012; Uzun et al., 2015). The VNTR homozygous 4R has been related to low BMI (24.7 ± 2.8) (Sikora et al., 2013; González-Giraldo et al., 2018). In contrast, the 7R allele has been associated with higher BMI in adolescent girls (n= 44, age= 15.6 ± 0.96 years old)(Stice et al., 2010), adult women with bulimia nervosa (n=163, age= 19-40 years old) (Ariza et al., 2012), and women with the seasonal affective disorder (n=108, age= 20- 65 years old) (Levitan et al., 2004). However, our results indicate that VNTRs 7R is not associated with BMI in the same way as was reported in Hispanic-American adolescents between 12 and 18 years old, diagnosed

with psychiatric disorders (n=2600) (Guo et al., 2007; Levitan et al., 2004), and by (Uzun et al., 2015) in Turkish obese young people.

The SNP rs6525 for *BDNF* was the most representative allele, which has been reported with similar frequency in Latin American (Fonseca et al., 2015; Ortega-Rojas et al., 2017; González-Giraldo et al., 2018); German (Kalenda et al., 2018) and Pakistani populations (Zamani et al., 2019). The association of BMI with the *BDNF* SNP rs6525 is controversial, some studies report a positive relationship such as (Beckers et al., 2008) in women between 18-55 years old (n=729), (Martínez-Ezquerro et al., 2017) in Mexican children (n=498) and (Skledar et al., 2012) in Croatian children (n=300). In contrast, they did not observe an association in German children (n=370) with attention deficit hyperactivity disorder and eating disorders (Friedel et al., 2005). On the other hand, (Zamani et al., 2019) reported a positive relationship between the plasma level of *BDNF* and BMI of Iranian adults (n=208, age = 28-40 years old), however, they found no association with the rs6525 polymorphism. Goldfield et al (2021) reported in obese youth (14-18 years) that SNP rs6525 (G/A, A/A) is associated with increased intake of both calories, proteins and lipids, along with alterations in cardiometabolic markers, this could be related to the location of *BDNF* in areas of the brain involved in appetite regulation and (Lebrun et al., 2006) lipid and carbohydrate metabolism (Matthews et al., 2009). In addition, other SNPs in the *BDNF* gene region have been linked to psychiatric disorders, eating behaviors and BMI (Thorleifsson et al., 2008). Studies that have not found an association with this SNP suggest that environmental factors and interactions with other variants of the *BDNF* gene (rs925946 and rs7481311) could modulate the effect of this polymorphism on BMI (Marti et al., 2009; Rana et al., 2018).

For the SNP rs4680 of the *COMT* gene, we found that the G allele is more frequent, similar to those reports in the Colombian (Ortega-Rojas et al., 2017) and Caucasian populations (Need et al., 2006). However, the relationship between this SNP and BMI has not been widely studied. (Kring et al., 2009) found an association of the GG genotype of this SNP with higher BMI in adult men (n=1562, age = 46-49 years old). However, in UK women (n=1150) between 21-71 years, this relationship was not observed (Need et al., 2006).

Behavioral conditions such as physical activity and food intake have been suggested as factors related to the variation of BMI and with the development of overweight in Mexicans young (n= 1028)(Saucedo-Molina et al., 2015). BMI has also been associated with sociodemographic characteristics, such as ethnicity, sex, marital status, and salary (Guo et al., 2007; Fuemmeler et al., 2008; García & Katheryne, 2021). Several genes expressed in the central nervous system (*SLC6A4, MAOA, TMEM18, INSIG2, FAIM2/ BCDIN3, GNPDA2,* and *MC4R)* have been associated with BMI, suggesting that multiple gene variants may be determinants of BMI in humans (Fuemmeler et al., 2008). Therefore, we suggest that future studies consider behavioral, and sociodemographic characteristics as well as other genetic variants that may influence BMI.

A limitation of our study was the lack of characterization of lifestyle factors such as nutrition and medication consumption; the sample size was also small; however, other studies with fewer participants (n= 44), found an association between BMI and polymorphisms as *DRD4* (Stice et al., 2010). The strengths of our study were the simultaneous analysis of four polymorphism genes related to the dopaminergic system in samples of healthy young Latin Americans without depression and anxiety disorders, which

ensured that the participants did not present behavioral deviations that could be related to BMI.

The relationship between body composition and the polymorphisms of *DRD4, DAT1*, *COMT*, and *BDNF* could be mediated by mental disorders such as anxiety and depression, as previous research suggests (Alfimova et al., 2014; Hosang et al., 2014; Gafarov et al., 2021) as well as with BMI (Luppino et al., 2010). Hence, we recommend new studies that evaluate the relationship between BMI and these genes study subjects with anxiety and depression and without these disorders, which would allow knowing if the effect of *VNTR* of *DRD4*, *DAT1*, and SNP of *COMT*, and *BDNF* on BMI can be mediated by the mental states, such as anxiety and depression.

We found that BMI was not associated with polymorphisms of *DRD4*, *DAT1*, *BDNF*, and *COMT1* genes in young subjects without depression and anxiety disorders. Other factors such as lifestyle, environmental factors, behavioral variables, sociodemographic and ethnic origins, and interactions with other genetic variants could modulate the influence of these polymorphisms on BMI; therefore, future studies should simultaneously consider biological, social, and psychological factors to understand the role of polymorphisms on BMI.

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AUTHORS' CONTRIBUTIONS

Karen Dayana Losada – Casallas: Conducted sample processing, molecular experiments, statistical analysis, and writing- original draft preparation. Natalia Cadavid Ruiz, Iván Leonardo Cepeda Leal, and Beatriz Elena Muñoz Ospina participated in the selection of subjects, Funding acquisition, Writing – review, and editing. José Guillermo Ortega-Avila: Performed conceptualization, methodology design, statistical analysis, writing-reviewing, and editing manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Alfimova M, Korovaitseva G, Lezheiko T and Golimbet V (2014). Interaction effects of the COMT and DRD4 genes with anxiety-related traits on selective attention. *Span. J. Psychol.* 17: E44. doi:10.1017/sjp.2014.46.

Ariza M, Garolera M, Jurado MA, Garcia-Garcia I, et al. (2012). Dopamine genes (DRD2/ANKK1-TaqA1 and DRD4-7R) and executive function: their interaction with obesity. *PLoS One.* 7: e41482. doi:10.1371/journal.pone.0041482.

Balcioglu A and Wurtman RJ (1998). Effects of phentermine on striatal dopamine and serotonin release in conscious rats: in vivo microdialysis study. *Int J Obes Relat Metab Disord.* 22: 325-328. doi:10.1038/sj.ijo.0800589.

Beck AT, Brown GK and Steer RA (2011). BDI-II: inventario de depresión de Beck-II : manual (Adaptación española: Sanz, J., y Vázquez, C.). Madrid: *Pearson Education*.

Beck AT and Steer RA (2011). BAI: inventario de ansiedad de Beck. Madrid: *Pearson Education*.

Beckers S, Peeters A, Zegers D, Mertens I, et al. (2008). Association of the BDNF Val66Met variation with obesity in women. *Mol. Genet. Metab*. 95: 110-112. doi:10.1016/j.ymgme.2008.06.008.

Chen S-P, Fuh J-L, Wang S-J, Tsai S-J, et al. (2011). Brain-derived neurotrophic factor gene Val66Met polymorphism modulates reversible cerebral vasoconstriction syndromes. *PLoS One*. 6: e18024. doi:10.1371/journal.pone.0018024.

Corperation GC. Sequencher® version 5.4. 6 DNA sequence analysis software. Ann Arbor, MI USA. Available at [http://www genecodes com] Accessed June 4, 2023.

Fonseca DJ, Mateus HE, Gálvez JM, Forero DA, et al. (2015). Lack of association of polymorphisms in six candidate genes in colombian adhd patients. *Ann. Neurosci*. 22: 217-221. doi:10.5214/ans.0972.7531.220405.

Friedel S, Horro FF, Wermter AK, Geller F, et al. (2005). Mutation screen of the brain derived neurotrophic factor gene (BDNF): identification of several genetic variants and association studies in patients with obesity, eating disorders, and attention-deficit/hyperactivity disorder. *Am. J. Med. Genet. B. Neuropsychiatr. Genet*. 132B: 96-99. doi:10.1002/ajmg.b.30090.

Fuemmeler BF, Agurs-Collins TD, McClernon FJ, Kollins SH, et al. (2008). Genes implicated in serotonergic and dopaminergic functioning predict BMI categories. *Obesity*. 16: 348-355. doi:10.1038/oby.2007.65.

García B and Katheryne B (2021). Determinación de porcentaje de grasa corporal en etnia indígena, mestiza, y afroecuatoriana de 20 a 45 años y su relación con el IMC y la índice cintura estatura. Universidad Peruana Cayetano Heredia. Available at https://repositorio.upch.edu.pe/handle/20.500.12866/9543.

Goldfield GS, Walsh J, Sigal RJ, Kenny, GP, et al. (2021). Associations of the BDNF Val66Met Polymorphism With Body Composition, Cardiometabolic Risk Factors, and Energy Intake in Youth With Obesity: Findings From the HEARTY Study. *Front Neurosci*. 15: 715330. https://doi.org/10.3389/fnins.2021.715330.

González-Giraldo Y, Camargo A, López-León S and Forero DA (2015). No Association of BDNF, COMT, MAOA, SLC6A3, and SLC6A4 Genes and Depressive Symptoms in a Sample of Healthy Colombian Subjects. *Depress Res. Treat*. 2015: 145483. doi:10.1155/2015/145483.

González-Giraldo Y, Trujillo ML and Forero DA (2018). Two dopaminergic genes, DRD4 and SLC6A3, are associated with body mass index in a Colombian sample of young adults. *Arch. Physiol. Biochem*. 124 (4): 330-334. doi:10.1080/13813455.2017.1401643.

Graffelman J and Weir BS (2018). Multi-allelic exact tests for Hardy-Weinberg equilibrium that account for gender. *Mol. Ecol. Resour*. 18: 461-473. doi:10.1111/1755-0998.12748.

Guo G, North KE, Gorden-Larsen P, Bulik CM, et al. (2007). Body Mass, DRD4, Physical Activity, Sedentary Behavior, and Family Socioeconomic Status: The Add Health Study*. *Obesity*. 15 (5): 1199-1206.doi:10.1038/oby.2007.640.

Herrera BM and Lindgren CM (2010). The genetics of obesity. *Curr Diab. Rep*. 10: 498-505. doi:10.1007/s11892-010-0153-z.

Hosang GM, Shiles C, Tansey KE, McGuffin P, et al. (2014). Interaction between stress and the BDNFVal66Met polymorphism in depression: a systematic review and meta-analysis. *BMC Med*. 12: 7 (2014). doi:10.1186/1741-7015-12-7.

Kalenda A, Landgraf K, Löffler D, Kovacs P, et al. (2018). The BDNF Val66Met polymorphism is associated with lower BMI, lower postprandial glucose levels and elevated carbohydrate intake in children and adolescents. *Pediatr. Obes*. 13: 159-167. doi:10.1111/ijpo.12238.

Kring SII, Werge T, Holst C, Toubro S, et al. (2009). Polymorphisms of serotonin receptor 2A and 2C genes and COMT in relation to obesity and type 2 diabetes. *PLoS One*. 4(8): e6696. doi:10.1371/journal.pone.0006696.

Lebrun B, Bariohay B, Moyse E and Jean A (2006). Brain-derived neurotrophic factor (BDNF) and food intake regulation: a minireview. *Auton. Neurosci*. 126-127: 30-38. doi: 10.1016/j.autneu.2006.02.027.

Levitan RD, Masellis M, Lam RW, Muglia P, et al. (2004). Childhood inattention and dysphoria and adult obesity associated with the dopamine D4 receptor gene in overeating women with seasonal affective disorder. *Neuropsychopharmacology*. 29: 179-186. Doi: 10.1038/sj.npp.1300314.

Luppino FS, de Wit LM, Bouvy PF, Stijnen T, et al. (2010). Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies. *Arch. Gen. Psychiatry*. 67: 220-229. doi:10.1001/archgenpsychiatry.2010.2.

Marti A, Santos JL, Gratacos M, Moreno-Aliaga MJ, et al. (2009). Association between leptin receptor (LEPR) and brain-derived neurotrophic factor (BDNF) gene variants and obesity: a case-control study. *Nutr. Neurosci*. 12: 183-188. doi:10.1179/147683009X423355.

Martínez-Ezquerro JD, Rendón-Macías ME, Zamora-Mendoza G, Serrano-Meneses J, et al. (2017). Association Between the Brain-derived Neurotrophic Factor Val66Met Polymorphism and Overweight/Obesity in Pediatric Population. *Arch. Med. Res*. 48: 599-608. doi:10.1016/j.arcmed.2018.02.005.

Martínez-Levy GA, Benjet C, Briones-Velasco M, Pérez-Molina A, et al. (2013). Estudio de la variabilidad genética del DRD4 y del DAT1 en individuos habitantes de la Ciudad de México. *Salud Ment*. 36: 189-192. ISSN 0185-3325.

Matthews VB, Astrom MB, Chan MH, Bruce CR, et al. (2009). Brain-derived neurotrophic factor is produced by skeletal muscle cells in response to contraction and enhances fat oxidation via activation of AMP-activated protein kinase. *Diabetologia*. 52: 1409-1418. doi.org/10.1007/s00125-009-1364-1.

Need AC, Ahmadi KR, Spector TD and Goldstein DB (2006). Obesity is associated with genetic variants that alter dopamine availability. *Ann. Hum. Genet*. 70: 293-303. doi:10.1111/j.1529-8817.2005.00228.x.

OMS (2021). Obesidad y sobrepeso. Organización Mundial de La Salud. 9 June 2021. Available at [https://www.who.int/es/news-room/fact-sheets/detail/obesity-and-overweight]. Accessed 6 July 2022.

Ortega-Rojas J, Arboleda-Bustos CE, Morales L, Benítez BA, et al. (2017). Estudio de variantes de los genes BDNF, COMT, DAT1 y SERT en niños colombianos con déficit de atención. *Rev. Colomb. Psiquiatr*. 46: 222-228. doi: 10.1016/j.rcp.2016.08.006.

Rana S, Mirza S and Rahmani S (2018). The BDNF rs6265 variant may interact with overweight and obesity to influence obesity-related physical, metabolic and behavioral traits in Pakistani individuals. *Ann. Hum. Biol*. 45(6-8): 496-505. doi:10.1080/03014460.2018.1561947.

Vieyra SG, Moraga MV, Henríquez BH, Aboitiz F, et al. (2003). Distribución de alelos de los genes DRD4 y DAT1 del sistema dopaminérgico en la población mixta de Santiago de Chile. *Rev. Med. Chil*. 131: 135-143. doi:10.4067/s0034-98872003000200002.

Saucedo-Molina TJ, Rodríguez J, Oliva LA, Villarreal M, et al. (2015). Relationship between body mass index, physical activity and meal times in Mexican adolescents. *Nutr. Hosp*. 32: 1082-1090. doi:10.3305/nh.2015.32.3.9331.

Sikora M, Gese A, Czypicki R, Gąsior M, et al. (2013). Correlations between polymorphisms in genes coding elements of dopaminergic pathways and body mass index in overweight and obese women. *Endokrynol. Pol*. 64(2): 101-107.

Skledar M, Nikolac M, Dodig-Curkovic K, Curkovic M, et al. (2012). Association between brain-derived neurotrophic factor Val66Met and obesity in children and adolescents. *Prog Neuropsychopharmacol Biol Psychiatry*. 36: 136-140. doi:10.1016/j.pnpbp.2011.08.003.

Stice E, Yokum S, Bohon C, Marti N, et al. (2010). Reward circuitry responsivity to food predicts future increases in body mass: moderating effects of DRD2 and DRD4. *Neuroimage*. 50: 1618-1625. doi:10.1016/j.neuroimage.2010.01.081.

Team RC (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at [https://www.R-project.org/.] Accessed 15 December 2022.

Thorleifsson G, Walters GB, Gudbjartsson DF, Steinthorsdottir V, et al. (2009). Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. *Nat. Genet*. 41: 18-24. doi: 10.1038/ng.274.

Uzun M, Saglar E, Kucukyildirim S, Erdem B, et al. (2015). Association of VNTR polymorphisms in DRD4, 5-HTT and DAT1 genes with obesity. *Arch. Physiol. Biochem*. 121: 75-79. doi:10.3109/13813455.2014.985686.

Gafarov VV, Gromova EA, Maksimov NV, Gagulin IV, et al. (2021). Association of personal anxiety with dopamine receptor D4 (DRD4), DAT genes polymorphism. Anxiety Disorders - The New Achievements. *IntechOpen*. doi:10.5772/intechopen.94386.

Vijayakumari AA, John JP, Halahalli HN, Paul P, et al. (2015). Effect of polymorphisms of three genes mediating monoamine signalling on brain morphometry in schizophrenia and healthy subjects. *Clin. Psychopharmacol. Neurosci*. 13: 68-82. doi:10.9758/cpn.2015.13.1.68.

Waldman ID, Rowe DC, Abramowitz A, Kozel ST, et al. (1998). Association and Linkage of the Dopamine Transporter Gene and Attention-Deficit Hyperactivity Disorder in Children: Heterogeneity owing to Diagnostic Subtype and Severity. *Am. J. Hum. Genet*. doi:10.1086/302132.

Zamani M, Hosseini SV, Behrouj H, Erfani M, et al. (2019). BDNF Val66Met genetic variation and its plasma level in patients with morbid obesity: A case-control study. *Gene*. 705: 51-54. doi:10.1016/j.gene.2019.04.045.