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SHORT REPORT

Association of the *5HTTLPR* Polymorphism with Obesity in Mexican Women with High Native American Ancestry

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Aims: The *5HTT* gene has been associated with obesity; this study aimed to determine the association between L- and S-alleles at the *5HTTLPR* polymorphism with obesity in indigenous Mexican populations.
Materials and Methods: A total of 362 individuals, 289 belonging to eight Native American (NA) groups; 40 Mexican mestizos; and 33 Caucasian Mennonites were enrolled in a cross-sectional study. High (≥90%) and low (<90%) NA ancestry was molecularly determined. A body mass index >30 kg/m^2 was considered as obese. The L- and S-alleles of the *5HTTLPR* locus were identified by PCR; the association between alleles and obesity was performed by logistic regression analysis.
Results: A significantly lower prevalence of obesity (35%) was observed in participants from communities with high NA ancestry ($p < 0.005$). Under a dominant heritance model the L-allele was associated with obesity in women with high NA ancestry (odds ratio [OR] 7.27; 95% confidence interval [CI] 1.6–32.5; $p = 0.009$) but not in women with low NA ancestry (OR 0.83; 95% CI 0.3–2.2; $p = 0.71$); no association was observed in men.
Conclusion: Our results suggest that the *5HTTLPR* L-allele is a risk factor for developing obesity in Mexican women with high NA ancestry (≥90%).

Keywords: serotonin, transporter proteins, genotype, obesity, ethnic groups

Introduction

Obesity has become a global public health issue. In 2018, the prevalence of overweight/obesity in Mexico was 74.4% and 69.3% for women and men, respectively (Shamah-Levy *et al.*, 2019). The Mexican Native American (NA) populations used to be lean because of their traditional lifestyles (Guerrero-Romero *et al.*, 1997); however, a progressive acculturation process (Rodríguez-Morán *et al.*, 2008) led to a rise in the total intake of calories in these populations (Rodríguez-Morán *et al.*, 2009).

Metabolism depends not only on customary diet and physical activity but is also influenced by genetic susceptibility (Yu and Zinman, 2007); to date, 127 obesity susceptibility genes have been described (Castillo *et al.*, 2017). Some genes, such as *SLC6A4* (*5HTT*) encoding a serotonin transporter, are involved in maintaining energy balance (Lan *et al.*, 2009). Brain serotonin transporters have a negative correlation with body mass index (BMI) in obese subjects (Erritzoe *et al.*, 2010).

The promoter of the gene *5HTT* contains the polymorphism *5HTTLPR* (serotonin transporter-linked polymorphic region), which has two variants, a 43 bp insertion (long allele, L) or a deletion (short allele, S), with the former having three times more basal activity than the latter (Lesch *et al.*, 1996). Paradoxically, both the S- (Sookoian *et al.*, 2007) and L- (Peralta-Leal *et al.*, 2012) alleles have been associated with obesity in Caucasian and Mexican mestizo populations,

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respectively. Therefore, this study aimed to evaluate whether this polymorphism is associated with obesity in 10 ethnic groups from Mexico with different degrees of NA ancestry.

Materials and Methods

Target population

The study was approved by the Ethics Committee of the Durango's General Hospital, at northern Mexico (031/007). Signed informed consent was obtained from all participants before the beginning of the study.

This study included 362 individuals, of them 289 belonging to eight NA groups, 40 Mexican mestizos, and 33 Mexican Mennonites.

Data related to demographic characteristics, medical condition, and customary diet were also collected.

Participants were recruited from their respective communities between 2010 and 2013. All seemingly healthy adult men and nonpregnant women were eligible for inclusion; persons with prior diagnoses of diabetes or hypertension were excluded.

Sampling strategy

Inhabitants from the eight NA groups were invited to join the study by community leaders. Initially, the participants themselves identified its NA ancestry by ratifying that their parents and grandparents belonged to the same ethnic group. Posteriorly, based on molecular characterization, the target population was divided into subjects with high (≥90%) or low NA ancestry (<90%) (Table 1).

Measurements

Weight and height were measured using a standard stadimeter. Obesity and nonobesity were defined by BMI ≥30 kg/m^2 and BMI <30 kg/m^2 (WHO, 2018). Blood pressure was measured using a mercurial sphygmomanometer according to standard recommendations (Jones and Hall, 2004).

Clinical assays

After 10–12 h overnight fasting, glucose, triglycerides, and high-density lipoprotein-cholesterol (HDL-c) plasma levels were determined enzymatically, according to the manufacturer's procedures (BioSystems, Reagents & Instruments; Analyzer A15 © Biosystems, Barcelona).

Genotyping

DNA was extracted from peripheral blood using the QIAamp® DNA blood kit (Qiagen, Hilden, Germany) and evaluated for integrity and concentration. *SLC6A4* allelic variants were detected as previously described (Peralta-Leal *et al.*, 2012). Genotyping was performed for 46 ancestry-informative markers in Mexican mestizos (Pereira *et al.*, 2012) and 15 short tandem repeats loci in the NA groups (Rangel-Villalobos *et al.*, 2013).

Statistical analyses

Data are presented as mean ± standard deviation or proportions. Differences between numerical variables were established with the Student's *t*-test for independent samples

Table 1. General Characteristics of the Target Population According to Ethnicity, $N = 362$

N	*Tepehuano* 92	*Huichol* 20	*Mexicanero* 13	*Cora* 55	*Tarahumara* 64	*Seri* 8	*Guarijio* 9	*Mayo* 28	*Mestizo* 40	*Mennonite* 33
Native American ancestry, %	96.4	96.3	94.5	93.9	92.1	88	81.6	65.6	47.2	0
Women, *n* (%)	62 (67.4)	10 (50)	8 (61.5)	38 (69.1)	43 (67.2)	7 (87.5)	7 (77.7)	22 (78.6)	29 72.5)	16 (48.5)
Age, years	36.6 ± 13.3	37.8 ± 19.3	43.5 ± 12.0	46.4 ± 20.1	42.8 ± 12.2	58.7 ± 15.5	60.0 ± 15.9	43.7 ± 17.6	44.8 ± 13.3	50.2 ± 12.3
BMI, kg/m^2	22.2 ± 3.4	22.7 ± 3.6	23.0 ± 1.9	25.2 ± 5.0	23.9 ± 4.9	27.0 ± 4.3	28.4 ± 7.5	27.9 ± 4.5	28.5 ± 4.6	29.8 ± 5.0
SBP, mmHg	104.8 ± 16.4	120.6 ± 22.6	116.9 ± 16.5	119.2 ± 13.6	120.4 ± 15.8	130.0 ± 15.1	141.0 ± 12.8	127.3 ± 19.8	128.5 ± 15.6	122.4 ± 14.6
DBP, mmHg	68.6 ± 11.5	77.2 ± 11.2	73.8 ± 8.7	77.2 ± 9.8	78.6 ± 9.3	87.5 ± 8.7	90.7 ± 8.0	83.2 ± 11.9	82.0 ± 10.2	85.3 ± 12.5
Glucose, mg/dL	79.6 ± 14.6	73.4 ± 13.2	79.4 ± 13.7	81.6 ± 16.8	82.2 ± 13.5	94.6 ± 6.9	96.6 ± 10.2	69.7 ± 11.7	90.7 ± 7.4	67.4 ± 13.5
Triglycerides, mg/dL	111.3 ± 62.0	121.9 ± 59.1	160.1 ± 67.8.0	181.1 ± 111.1	166.6 ± 93.7	125.3 ± 44	264.1 ± 151.1	131.0 ± 79.0	197.1 ± 115	103.4 ± 67.0
HDL-c, mg/dL	50.4 ± 12.7	43.7 ± 8.6	34.3 ± 9.4	38.0 ± 10.8	41.4 ± 11.1	46.7 ± 10.3	51.1 ± 8.0	50.9 ± 11.4	50.0 ± 10.6	59.8 ± 12.6

BMI, body mass index; DBP, diastolic blood pressure; HDL-c, high-density lipoprotein-cholesterol; SBP, systolic blood pressure.

Table 2. Anthropometric and Biochemical Characteristics of the Target Population, According to Gender and Native American Ancestry

	Women			*Men*		
Native American ancestry, %	*≥90*	*<90*		*≥90*	*<90*	
N	*161*	*81*	p*	*83*	*37*	p*
Age, years	39.0 ± 15.0	47.1 ± 14.9	<0.0005	44.9 ± 16.5	49.6 ± 15.8	0.14
BMI, kg/m^2	24.1 ± 4.5	28.7 ± 5.2	<0.0005	22.5 ± 4.0	28.1 ± 4.3	<0.0005
SBP, mmHg	113.0 ± 16.9	127.8 ± 16.7	<0.0005	116.5 ± 19.4	126.8 ± 16.7	0.004
DBP, mmHg	73.6 ± 11.0	83.5 ± 10.6	<0.0005	75.4 ± 11.8	85.8 ± 12.6	<0.0005
Glucose, mg/dL	79.6 ± 13.5	80.0 ± 16.6	0.84	82.5 ± 16.9	79.1 ± 14.6	0.27
Triglycerides, mg/dL	147.1 ± 94.0	155.8 ± 107.5	0.54	138.9 ± 75.6	149.1 ± 105.5	0.60
HDL-c	43.2 ± 12.8	53.2 ± 12.7	<0.0005	45.0 ± 12.4	50.6 ± 9.6	0.008

*Student's *t*-test for independent samples.

(Mann–Whitney *U* test for nonparametric data) and chi-squared test for categorical variables. Analysis of variance with a Games–Howell *post hoc* test was used to compare more than two groups. Genotype frequencies were obtained by direct counts and Hardy–Weinberg equilibrium (HWE) was determined by chi square. Admixture proportions were estimated with STRUCTURE v.2.2 (Falush *et al.*, 2003), and included 327 individuals from three parental populations (Africa, Europe, and America). The analysis was performed using the correlated allele frequencies and admixture model and the number of clusters was determined (Evanno *et al.*, 2005). A logistic regression analysis (LRA) adjusted by gender, age, and biochemical parameters was used to evaluate the association between *5HTTLPR* polymorphisms

Table 3. Association between *5HTTLPR* Polymorphisms with Obesity in Mexican Women

Native American ancestry ≥90

		Obesity, n *(%)*				
Model	*Genotype*	*No* (n = *141*)	*Yes* (n = *20*)	*OR (95% CI)**	p	*AIC*
Codominant	S/S	63 (44.7)	2 (10.0)	1.0		
	L/S	53 (37.6)	13 (65.0)	7.73 (1.7–35.8)	0.009	116.4
	L/L	25 (17.7)	5 (25.0)	6.30 (1.1–34.6)		
Dominant	S/S	63 (44.7)	2 (10.0)	1.0		
	L/S–L/L	78 (55.3)	18 (90.0)	7.27 (1.6–32.5)	0.009	114.5
Recessive	S/S–L/S	116 (82.3)	15 (75.0)	1.0		
	L/L	25 (17.7)	5 (25.0)	1.55 (0.5–4.6)	0.43	124.3
	Alleles				p	
	S	179 (58)	17 (42)		0.011	
	L	103 (42)	23 (58)			

Native American ancestry <90

		Obesity, n *(%)*				
Model	*Genotype*	*No* (n = *51*)	*Yes* (n = *30*)	*OR (95% CI)**	p	*AIC*
Codominant	S/S	15 (29.4)	10 (33.3)	1.0		
	L/S	23 (45.1)	14 (46.7)	0.91 (0.3–2.6)	0.86	112.4
	L/L	13 (25.5)	6 (20.0)	0.69 (0.2–2.4)		
Dominant	S/S	15 (29.4)	10 (33.3)	1.0		
	L/S–L/L	36 (70.6)	20 (66.7)	0.83 (0.3–2.2)	0.71	110.6
Recessive	S/S–L/S	38 (74.5)	24 (80.0)	1.0		
	L/L	13 (25.5)	6 (20.0)	0.73 (0.2–2.2)	0.57	110.5
	Alleles				p	
	S	53 (52)	34 (56)		0.484	
	L	49 (48)	26 (44)			

Nonobesity, BMI <30 kg/m^2. Obesity, BMI ≥30 kg/m^2.
*Model adjusted by age and biochemical parameters.
AIC, Akaike Information Criterion; CI, confidence interval; OR, odds ratio.

(independent variables) with obesity (dependent variable). Statistical significance was defined as a p-value <0.05. Data were analyzed using the statistical package SPSS for Windows 25.0 (SPSS Inc., Chicago, IL).

Results

A total of 362 volunteers were enrolled in this study; of whom 244 (67.4%) had high NA ancestry. Most participants were women ($n=242$; 66.8%), among them 161 (66.5%) had high NA ancestry ($p=0.38$). Table 1 shows the anthropometric and biochemical variables of all participants. In the entire cohort, 60 individuals (16.6%) were identified as obese, 20 (33.3%) of whom had high NA ancestry ($p<0.005$).

Participants with low NA ancestry were older and exhibited higher BMI, systolic blood pressure, diastolic blood pressure, and HDL-c levels than those with high NA ancestry (Table 2). The allele distribution in the whole population was in HWE ($p>0.05$).

In the whole population, the adjusted LRA model showed that L-allele was associated with obesity (odds ratio [OR] 2.21; 95% confidence interval [CI] 1.1–4.4; $p=0.02$). After stratification by NA, the adjusted LRA model showed that L-allele was significantly associated with obesity in the high NA ancestry group (OR 5.88; 95% CI 1.3–27.1; $p=0.02$), but not in the low NA ancestry group (OR 1.25; 95% CI 0.5–3.0; $p=0.61$).

Stratified according to gender, men in the group of low NA showed no association between L-allele and obesity, whereas in the group with high NA no obese men were identified. Under a dominant inheritance model, women with high NA, but not women with low NA ancestry, showed a strong association of L-allele with obesity (Table 3). Furthermore, the adjusted LRA showed that L-allele was associated with BMI (OR 1.57; 95% CI 1.1–2.9; $p=0.02$) in women with high NA ancestry.

Finally, individuals with high NA ancestry had a significantly higher intake of alcoholic beverages and lower intake of canned foods and meat than individuals with low NA ancestry.

Discussion

Our results suggest that the L-allele of *5HTTLPR* is a risk factor for developing obesity in Mexican women with high NA ancestry (≥90%). To our knowledge, this is the first study to evaluate the effects of indigenous and mestizo Mexican ethnicity on the association of this polymorphism with obesity.

We observed a high frequency of obesity in participants with low NA ancestry, which agrees with an earlier report from Stoddard *et al.* (2011) who found significantly higher rates of obesity in nonindigenous populations. This finding could be explained by geographical isolation and low exposure to industrialized diets in communities with high NA ancestry (Rodríguez-Morán *et al.*, 2008).

The S-allele was linked with overweight/obesity in French (Fumeron *et al.*, 2000) and Caucasian Argentinian (Sookoian *et al.*, 2007, 2008) populations; in Hispanic men, the S-allele and SS genotype have been associated with obesity (Fuemmeler *et al.*, 2008). Conversely, the L-allele was associated with obesity in the Turkish population (Mergen *et al.*, 2007). In 2002, Camarena *et al.* (2002) found in Mexican obese women with impulsivity a preferential transmission of L-allele from the heterozygous parents, providing significant evidence for allelic association. These findings suggest a gender difference in the function of serotonin system, which could be a risk factor for obesity in women.

In male and female *5HTT* knockout mice, both *Cyp19a1* messenger RNA expression and 17β-estradiol serum levels are reduced, which is associated with an increase of fat mass (Zha *et al.*, 2017). The effects of *5HTT* $^{-/-}$ would be functionally equivalent to those of the *5HTT* S/S genotype. Supporting this hypothesis, our results did not find any association between obesity and the S-allele.

Administration of estrogens to castrated male Wistar rats induces *5HTT* expression (McQueen *et al.*, 1999), and a similar induction of expression is also exerted by the L-allele. Therefore, the synergistic effect of estrogens and the L-allele could induce overexpression of *5HTT*, explaining the association between the L-allele and obesity that we observed in women with high NA ancestry.

Mexican NAs have developed adaptive mechanisms to preserve energy during famine periods, with the thrifty genes playing an important role (Neel *et al.*, 1998). The observation of the association between L-allele and obesity, exclusively in women with high NA ancestry, suggests inherited differences in gene action and its environmental adaptations.

Finally, we did not find significant difference in the customary diet between groups with high and low NA ancestry. These findings suggest that the former have adopted westernized diets, which, in the presence of the L-allele, may elicit a ''saving phenotype'' leading to obesity in women.

Some limitations of this study deserve to be mentioned: (1) an accurate recording of physical activity was not possible; (2) there were not obese men in the group with high NA; so, we cannot discard with certainty the gender-dependent differential functionality for the L- and S-alleles of *5HTTLPR*; (3) we evaluated the influence of the L- and S-alleles in a sample of individuals from northern Mexico with different NA ancestry. So, these findings must be considered as tentative until its replication in larger samples and other ethnic groups.

Author Disclosure Statement

No competing financial interests exist.

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