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Original Article

Obesity is associated with the Arg389Gly *ADRB1* but not with the Trp64Arg *ADRB3* polymorphism in children from San Luis Potosí and León, México

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Abstract

This research was designed to analyze the possible associations of Arg389Gly ADRB1 and Trp64Arg ADRB3 polymorphisms in children with obesity. A cross-sectional study included 1,046 school-age Mexican participants (6-12 years old) from the cities of San Luis Potosí and León. Children were classified as non-obese or obese according to their body mass index (BMI) percentile; obese children had a BMI ⩾ 95th percentile for sex and age. Biochemical data were collected. Polymorphisms were detected using TaqMan qPCR assay. A logistic regression analysis was used to calculate the risk of obesity based on genotypes. Differences were found between groups where obese children had a significant increase in systolic and diastolic blood pressure, fasting plasma glucose, insulin, HOMA-IR, LDL-cholesterol, triglycerides, and lower HDL-cholesterol compared with the normal weight group ($P < 0.05$). The distribution of allele frequency in the population was Arg = 87.4 and Gly = 12.6 (Hardy Weinberg equilibrium $\chi^2 = 3.16$, $P = 0.07$); Trp = 81.5 and Arg = 18.5 (Hardy Weinberg equilibrium $\chi^2 = 2.2$, $P = 0.14$) for *ADRB1* and *ADRB3*, respectively. Even though no different frequencies of Arg389Gly polymorphism between groups were found ($P = 0.08$), children carriers of one Gly389 *ADRB1* allele had a risk for obesity of OR = 1.40 (95%CI, 1.03–1.90, $P = 0.03$) after adjustment for age and gender. No other association was found for Trp64Arg *ADRB3* polymorphism. Only the Arg389Gly *ADRB1* polymorphism was associated with risk for obesity in Mexican children.

Keywords: childhood obesity, β-adrenergic receptor (*ADRB*) gene, polymorphisms, Mexican children

Introduction

Obesity is a worldwide public health problem[1-2]. In México, the prevalence of overweight and obesity in school children was 42% in 2012[3], an important increase with respect to 1999 statistics. From 1990 to

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2007, the body mass index (BMI) increased in Tarahumara ethnic groups from 4% to 7% in boys and 9% to 13% in girls[4]. Obesity results from a chronic positive caloric balance and has strong interactions with genetic and environmental factors[5].

Central neural circuits regulating food intake and energy expenditure are closely interconnected[6]. These physiological functions have a close interaction with the sympathetic nervous system. Since the sympathetic tone given mainly by the β-adrenergic receptors (ADRB) participates in weight balance, several polymorphisms in the *ADRB* genes have been associated with obesity[7–9]. For instance, the Arg389 variant in the *ADRB1* gene has been associated with higher BMI as well as other related disorders such as insulin resistance, hypertension and acute myocardial infarction[10–13]. However, the allele frequency of this polymorphism is different among ethnic groups[14-15]. That is why in certain populations the Arg389Gly polymorphism does not seem to impact on the pathogenesis of obesity and traits correlated with it[16].

With regard to the Trp64Arg polymorphism of the *ADRB3* gene, the Arg64 variant was associated with higher 2-h post glucose insulin levels and BMI in Mexican Americans[17], as well as with abdominal obesity and insulin resistance, factors that may contribute to early onset type 2 diabetes mellitus[18-19]. However, Trp64Arg mutation is not a major determinant of this disease or for obesity in Dutch population[20]. Conversely, the Arg64Arg genotype was associated only with obesity and type 2 diabetes in a large Japanese sample[21]. Yet, other report from the same population did not show the ADRB3 polymorphism related with the metabolic syndrome[22]. The association of this polymorphism with risk factors for obesity and insulin resistance has given conflicting results, which might be explained by confounding variables such as age and ethnicity, as well as low statistical power[14,22].

Taking into consideration the discrepant reports about the polymorphisms of the *ADRB* genes and the role of ethnicity, the aim of this work was to calculate the allele and genotype frequencies of Arg389Gly *ADRB1* and Trp64Arg *ADRB3* polymorphisms and the possible association with obesity in children from two populated cities in the central region of México.

Materials and methods

The study was conducted in two of the most populated cities in central México: San Luis Potosí and León. This project was previously approved by the institutional bioethics committee (IMSS-2004-3601-0020) in compliance with the Helsinki Declaration. Participants were randomly selected from school-age children and adolescents (6-12 years old) in ten public schools. A total of 1,046 individuals (500 boys and 546 girls) were included in the study after all participants and at least one of their parents signed informed consent.

Data collection and measurements

A family history questionnaire of diabetes and obesity was compiled. Individuals with evidence of hypothyroidism, chronic infections, congenital or metabolic diseases were excluded from the study. The weight and height of each participant was obtained using a digital scale (Torino, Italy) and a stadiometer (Tanita, Tokyo, Japan), respectively. BMI was calculated (kg/m^2) and the percentile of BMI was obtained according to the CDC tables[23]. All participants had a BMI greater than or equal to the 95th percentile for age and sex were considered obese. Normal weight was considered $\geqslant$ 10th and $<$ 85th percentiles. Overweight individuals were excluded from statistical analyses. Blood pressure was taken with a Tycos CE0050 mercury sphygmomanometer (Welch Allyn, NY, USA) in the morning as the mean of two readings at five minutes of interval, after five minutes in sitting position.

Laboratory measurements

Blood samples were obtained after twelve hour fasting to measure glucose, total cholesterol, LDL-C, HDL-C and triglycerides by spectrophotometric methods using Lab 350 Clinical Chemistry Systems (Instrumentation Laboratory, Barcelona, Spain). Insulin was measured by RIA (Diagnostic Products Corporation, Los Angeles CA, USA). Insulin resistance was calculated by homeostasis model assessment (HOMA-IR)[24].

Genotyping

Genomic DNA was extracted from a peripheral blood sample using a QIAamp (Qiagen, Germany) kit, and analyzed by electrophoresis in 0.8% agarose gel stained with ethidium bromide and visualized in a Gel Doc 2000 (BIORAD, CA, USA). DNA concentration was determined using a VICTOR3 1420 spectrophotometer (Perkin-Elmer, Germany). SNP analyses were made using real time PCR by TaqMan technology (7900HT Applied Biosystems, Foster City, California, USA), using probes *ADRB1* gene Arg389Gly (rs1801253), and *ADRB3* gene Trp64Arg (rs4994) according to the manufacturer (Applied Biosystems, Foster City, California, USA).

Statistical analysis

After analyzing the distribution of data, comparison among groups was made with t-test (parametric) or the Mann-Whitney U test (non-parametric) for continuous variables and chi-square (χ^2) test for categorical variables. The allelic and phenotypic frequencies were calculated and Hardy-Weinberg equilibrium was assessed for each polymorphism. A logistic regression analysis was used to calculate the risk of obesity in relation to the different genotypes in the three main inheritance models: co-dominant, dominant and recessive, with adjustment for age and gender taking into account the hormonal changes in adolescents ($\geqslant$10 years old). Statistical analysis was carried out with the Statistical Package STATA v11software (Stata Corporation, Texas).

Results

A total of 1,046 Mexican children and adolescents (500 boys and 546 girls) from San Luis Potosí and León were studied. ***Table 1*** compares the characteristics of children classified as non-obese and obese. The age was lower in the obese group than in the normal weight group. Systolic and diastolic blood pressure, fasting glucose, insulin, HOMA-IR, LDL-C and triglycerides were increased in the obese group compared with the normal group, while HDL-C levels were lower in this group (***Table 1***).

Genotype and allele frequencies for the Arg389Gly variants of *ADRB1* (rs1801253) and Trp64Arg of *ADRB3* (rs4994) are shown in ***Table 2*** according to the BMI groups. All calculated allelic frequencies were in Hardy-Weinberg equilibrium ($\chi^2 = 3.16$, $P = 0.07$; $\chi^2 = 2.2$, $P = 0.14$, respectively). No significant differences in genotype frequencies between the two groups were found for both polymorphisms (***Table 2***).

In the multiple logistic regression analysis, the co-dominant model for *ADRB1* gene adjusted for age and gender were associated with obesity (odds ratio 1.40, CI 95%, 1.03–1.90, $P = 0.03$). The *ADRB3* gene was not associated with increased BMI (***Table 3***).

Logistic regression was adjusted by age and gender with the *ADRB1* (rs1801253) and *ADRB3* (rs4994) polymorphism, respectively.

Discussion

Child obesity is becoming an epidemic and a public health problem, which can also lead to adulthood obesity and cardiovascular risks. In our study, 33.3% of the participants had obesity, and there was no significant differences between boys and girls ($P = 0.06$). In order to better elucidate the differences between groups, overweight individuals were excluded from statistical analysis. Biochemical parameters changed depending on the BMI (***Table 1***): systolic and diastolic blood pressure, fasting glucose, insulin, HOMA-IR, LDL-C and triglycerides were increased with the increase in BMI, while LDL-C levels were lower as expected. Total cholesterol did not significantly change between the groups. In a previous study, we found a strong and

Table 1 **Metabolic and anthropometric characteristics of the groups**

Parameters	Non-obese $n = 698$	Obese $n = 348$	*P*
Age (years)	10±2.2	9.8±2	0.02
Gender (n, %)			0.06
Girls	379 (54 %)	167 (48 %)	
Boys	319 (46 %)	181 (52 %)	
BMI (Kg/m^2)	17.4±3.3	25.1±4.9	< 0.00001
SBP (mm Hg)	102±12	112±14	0.003
DBP (mm Hg)	65±9	72±11	0.008
Fasting Glucose (mmol/L)	4.72±0.4	4.84±0.5	0.003
Insulin (pmol/L)	63.4±51.8	106.8±97	< 0.00001
HOMA-IR	2±1.7	3.5±4.5	< 0.00001
Total Cholesterol (mmol/L)	3.85±0.7	4.0±0.7	0.136
LDL-C (mmol/L)	2.23±0.6	2.4±0.7	0.01
HDL-C (mmol/L)	1.25±0.29	1.08±0.25	0.002
Triglycerides (mmol/L)	1.04±0.51	1.58±1.09	< 0.00001

Data are presented as mean±standard deviation. BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; LDL-C: low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol.

Table 2 **Genotypic and allelic frequencies of Arg389Gly *ADRB1* and Trp64Arg *ADRB3* polymorphisms**

Polymorphism	Non-obese *n* = 698	Obese *n* = 348	*P*
Arg389Gly *ADRB1* (rs1801253)			
Arg/Arg	549 (78.7%)	256 (73.6%)	0.08
Arg/Gly	132 (18.9%)	86 (24.7%)	
Gly/Gly	17 (2.4%)	6 (1.7%)	
Alleles			
Arg	88.3%	86.0%	0.33
Gly	11.7%	14.0%	
trp64Arg *ADRB3* (rs4994)			
Trp/Trp	467 (66.9%)	235 (67.5%)	0.98
Trp/Arg	202 (28.9%)	99 (28.5%)	
Arg/Arg	29 (4.2%)	14 (4.0%)	
Alleles			
Trp	82.1%	82.12%	0.99
Arg	17.9%	17.88%	

Table 3 **Multiple logistic regression analysis of the association of *ADRB1* and *ADRB3* polymorphisms with the risk of obesity**

	Genotype	OR (95% CI)	*P*
***ADRB1*(rs1801253**	Arg389Gly		
Co-dominant	Arg/Arg	1	-
	Arg/Gly	1.40 (1.03, 1.90)	0.03
	Gly/Gly	0.75 (0.27, 1.89)	0.58
Dominant	Arg/Arg	1	-
	Arg/Gly–Gly/Gly	1.32 (0.97, 1.78)	0.03
Recessive	Arg/Arg–Arg/Gly	1	-
	Gly/Gly	0.70 (0.25,1.75)	0.48
***ADRB3* (rs4994)**			
Co-dominant	Trp64Arg	1	-
	Trp/Trp	0.97 (0.72, 1.29)	0.85
	Trp/Arg	0.95 (0.48, 1.83)	0.91
	Arg/Arg		
Dominant	Trp/Trp	1	-
	Trp/Arg–Arg/Arg	0.97 (0.73, 1.27)	0.84
Recessive	Trp/Trp–Trp/Arg	1	-
	Arg/Arg	0.96 (0.49, 1.84)	0.93

independent association of diabetes family history with impaired fasting glucose in absence of obesity in Mexican children and adolescents[25].

The influence of genetic variants of *ADRB1* on obesity in children is a matter insufficiently studied. Tafel *et al*.[26] did not find association between Arg389Gly *ADRB1* with early onset obesity; however, they reported higher frequencies of Gly389 allele in obese and lean children (31.9 and 32.8, respectively) with respect to our study (obese 14.0, and normal weight group 11.7). Other studies also found higher frequencies of Gly389 allele (25.0) in Caucasian women and white subjects[10,15]. In Afro-American subjects, a frequency of 39 has been reported for the Gly389 allele, associated with lower BMI[15]. In contrast, the frequency of Arg389 allele is higher in our group (87.4), a value similar to a previous report in Mexican Mestizos and Teenek (Huasteca indigenous)[14], European, and Asian populations[26-27].

In our work, the Arg389Gly *ADRB1* showed

association with risk of obesity according to the co-dominant model: OR = 1.40, (95% confidence interval 1.03- 1.90; $P = 0.03$), (***Table 3***). These data agree with the previously reported association of Arg389Gly polymorphism with BMI in Caucasian women[10]. Moreover, insulin concentration and HOMA-IR showed association with Arg389Gly polymorphism in adult and obese women[7,11]. When the β1 adrenergic receptor is stimulated, there is a decrease in circulating levels of leptin having a positive correlation between weight loss and reduction of β1 adrenergic receptor expression[28]. Although this polymorphism does not change *ADRB1* gene mRNA expression, the amino acid variation at 389 position of ADRB1 modifies G-protein coupling and subsequently the stimulation of adenylyl cyclase[29].

In our work, Arg389Gly *ADRB1* polymorphism was associated with obesity, which agrees with a role of this polymorphism on obesity in Mexican Mestizos. However, functional studies are necessary to support this role. It is also necessary to evaluate the obese groups of different ages in order to ascertain if this polymorphism is associated with hypertension, cardiopathy or another metabolic disorder in adult life.

In this study, we were unable to detect any significant association between obesity and Trp64Arg *ADRB3* polymorphism, which is consistent with several studies in children and adolescents where no association was found between the Trp64Arg polymorphism and BMI, glucose and insulin concentrations, accumulation of body fat, and morbid obesity including different populations such as Polish, Japanese, and Germans[27,30-31]. However, a meta-analysis showed the association of Trp64Arg *ADRB3* genetics variants with BMI in Japanese adult population[32]. The 64Arg allele has been found associated with long-term changes in body weight in Japanese obese subjects[33], and with the prevalence of metabolic syndrome in Chinese men[34]. Nevertheless, other studies did not report association of the Trp64Arg *ADRB3* polymorphism with the incidence of overweight or type 2 diabetes mellitus in Polish population[35]. The allelic frequencies of Trp64Arg polymorphism show ample variation among races: the 64Arg allele frequency 38.0 for the Alaskan Eskimos[36], 10.0 for African-Americans[37], 7.5 for Caucasian subjects[38], and 18.0 to 19.0 in Japanese groups[22,33]. The latter frequencies are very similar to that found in our study (18.5) that included 1046 children from two Mexican cities. Probably the heterogeneity in the genetic background of the Mexican Mestizo population may contribute to the lack of association of this polymorphism with obesity[39]. In addition, it should be noted that this age might be more influenced by parental life style and environmental factors.

We concluded that in children from our population, the Arg389Gly *ADRB1* polymorphism shows a risk of obesity after adjustment for age and gender. The Trp64Arg *ADRB3* polymorphism is not associated with increased BMI. Unlike other recent studies where ADRB polymorphisms have been explored in adults with certain diseases[40-41], this work could add another piece of evidence that ADRB1 polymorphism may relate to the onset of obesity. However, a limitation is that the study group will not necessarily represent the children population in Central México. Further studies will be required with the precise mechanisms of ADRB1-induced obesity.

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