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Leptin levels and Q223R leptin receptor gene polymorphism in obese Mexican young adults

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ABSTRACT

Introduction

The Q223R polymorphism of the leptin receptor *(LEPR)* gene is one of the most common polymorphisms and it is believed to be associated with a damaged capacity of LEPR signaling and with high circulating leptin levels.

Methods

An observational, cross-sectional, analytical study was carried out in the Autonomous University of Ciudad Juarez, Mexico, where a sample of young adult participants (ranging from 18 to 30 years of age) was obtained. They were classified based on the results of body mass index: non-obese, and overweight/obese. The polymorphic variant was determined by Polymerase Chain Reaction (PCR) from the DNA sample and serum leptin levels were measured by Enzyme-Linked Immuno Sorbent Assay.

Results

A total of 159 participants were included (non-obese, n=103; overweight/obese, n=56). Leptin levels were 15.14±12.3 ng/mL in the non-obese group and 26.13±19.0 ng/mL in the overweight/obese group (p≤0.001). The allelic frequencies of the Q and R alleles of the *LEPR* gene in the studied subjects were as follows: non-obese, Q=0.56, R=0.44; overweight/obese, Q=0.62, R=0.38. The relative risk for the Q/Q genotype was 1.18 (CI 0.53-2.34), for Q/R was 1.14 (CI 0.59-2.18) and for R/R was 0.59 (CI 0.23-1.50).

Conclusions

This study shows that leptin levels are associated with overweight/obesity in Mexican young adults, but this is not related to the presence of the Q223R polymorphism in the *LEPR* gene, so the underlying mechanisms for a possible disturbance in leptin signaling in obese Mexican young adults await further studies.

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INTRODUCTION

Obesity plays a fundamental role in public health problems, which have reached epidemic scales. The prevalence of obesity increases year after year, and it has been related to a large number of risk factors for multiple diseases. Obesity is defined as an excessive amount of body fat or adipose tissue in relation to body mass. Overweight refers to the increase in weight in relation to height, which is later compared to an accepted weight standard. The body mass index is a common measure that expresses the relationship between height and weight. Adults with a body mass index (BMI) of 25 to 29.9 are considered to be overweight, while individuals with a BMI greater than 30 are considered obese individuals (1).

Obesity has become a problem affecting the health of millions of people around the world, in recent studies a prevalence of up to 39.6% has been established in adults (1), being an important comorbidity of many chronic degenerative diseases. However, it is also relevant for acute disorders like COVID-19 (2).

Obesity produces a significant cost in the global economy, being a major public health problem and a red flag for international health organizations. In different epidemiological studies, it has been shown that Mexico is one of the Latin American countries with a high obesity prevalence, data that goes hand in hand with the high numbers of type 2 diabetes mellitus, dyslipidemia, coronary heart diseases, sleep disturbances, cognitive dysfunction, cancer, kidney and liver diseases. BMI is considered an important measure to understand population trends; for individuals, it is one of many factors that can be considered to assess healthy weight, along with body fat composition, waist circumference, blood pressure, cholesterol levels and serum glucose levels (1).

Leptin is an anorexigenic hormone synthesized primarily in adipose tissue, its function is to regulate lipid metabolism by stimulating lipolysis and inhibiting lipogenesis (3). Zhang et al. identified leptin as a product of the obese (*ob*) gene via the positional cloning strategy (4). The leptin gene is located in the long arm of chromosome 7 (7q31.3) and contains three exons and two introns (5). Madej et al. predicted that leptin is a cytokine with a structure of four alpha helices and suggested a JAK/STAT-like signaling pathway for leptin action (6). Leptin deficiency is not the only factor involved in obesity, with a resistance to leptin being also involved, and as leptin reduces food intake and body weight, leptin resistance and high leptin levels are thus observed in obese people.

The leptin receptor (LEPR) can be classified as a class I cytokine receptor. It shows high similarity to interleukin 6, glycoprotein 130 signal-transducing chain, the receptor for the granulocyte colony stimulating factor and the receptor for the leukemia inhibitory factor. This family encompasses receptors marked by the presence of one or more cytokine receptors from homologous domains, all of which use JAK kinases for their intracellular signaling. The *LEPR* gene is located on chromosome 1 (1p31) which contains 20 exons (7).

Leptin function is mediated by the *LEPR*, and both the LEPR and leptin itself are involved in homeostatic control of appetite, weight, metabolism, and reproductive functions in women. A number of polymorphisms have been reported in the human *LEPR* gene. The Q223R polymorphism is one of the most common and is believed to be associated with impaired ability of leptin receptor signaling; this polymorphism has been associated with high leptin levels (8).

The interaction of leptin with its receptor in the hypothalamus stimulates a specific signaling cascade that results in the synthesis of anorectic and orexigenic peptides to regulate food intake and energy expenditure. Many polymorphisms in the leptin and the *LEPR* genes have been associated with body weight (9).

In addition to the environmental factors that have already been discussed, several genetic alterations that may play an important role in the etiology of obesity have been rigorously studied, based on the observation that not all individuals with a large amount of caloric intake and decreased physical activity are obese. There are several complex genetic interactions in obesity. In twin and family studies, it has been shown that more than 80% of the variation in BMI, 50% of the risk for type 2 diabetes mellitus, and 10-30% of the risk for metabolic syndrome is attributed to genetic factors. Among the factors that affect genetic variations, single nucleotide polymorphisms (SNPs) have been observed. Although SNPs are not usually enough to cause a disease, they can determine predisposition to special metabolic problems and, therefore, disease. Obesity is inherited mainly due to genetic factors. In rats, the gene that causes obesity was sequenced in 1994, with mutation of this gene resulting in increased food consumption, high insulin levels and obesity in non-insulin dependent diabetes mellitus (10).

In a literature review carried out in Iran in 2013, nine of the 17 articles that evaluated SNPs in obesity reported association or a possible risk factor; however, eight of those studies found no association (11). Hence the role of SNPs in this disorder awaits further studies.

The objective of this study was to evaluate the role of the Q223R polymorphism of the *LEPR* gene, leptin levels and its association with clinical characteristics of obese young Mexican adults.

METHODS

This is an observational, cross-sectional, analytical study. The recruitment of study participants was performed at the Clinic of Chronic Degenerative Diseases of the Institute of Biomedical Sciences of the Autonomous University of Ciudad Juárez (UACJ), in Chihuahua, Mexico. Young adults between 18 and 30 years were included. The sample size in this study (n=159) was higher compared to some association studies (12-14). The subjects were recruited consecutively as in previous studies (12). A randomized subset of subjects was extracted from this sample, as described below.

The selection criteria were A) Inclusion: subjects aged 18 to 30 years who agreed to participate in the study (who signed the informed consent letter); B) Exclusion: bacterial or parasitic infection 2 weeks prior to sampling, acute inflammatory process, coagulation disturbances; C) Elimination: subjects who had not agreed

to allow all necessary measurements, subjects who had decided to withdraw from the study.

During data collection, blood pressure, weight, height, circumferences (waist, hip, scapular, middle arm), folds (bicipital, tricipital, subscapular, suprailiac) and body fat percentage were recorded. The weight and height were measured on a hospital scale with a stadimeter (Torino-Oken, México). Height was measured, to the closest 0.5 cm, with the subject without shoes, heels together, and with the head in the Frankfurt plane position. Weight was measured to the closest 100 g registered by the scale, with the subject wearing light clothing. The body mass index was calculated by dividing the weight expressed in kilograms by the square of the height expressed in meters. Subsequently this value was used to classify participants according to the International classification of overweight and obesity in adults based on their BMI (kg/m^2), as follows: normal weight (18.5-24.99), overweight (≥25.0), and obesity (≥30.0) (15).

The body fat percentage was measured using a bioimpedance analyzer (Citizen Corporation, Japan), once programmed with the parameters required by the instrument (weight, height, age and sex) the individual placed his sweat-free palms on the electrodes of the equipment, until the record on the screen appeared.

The plicometry was carried out, to the closest millimeter, by using a Lange plicometer (Dynatronics Corporation, USA), on the right side of the subject, in a relaxed position. The bicipital, tricipital, subscapular and the suprailiac skinfolds were measured. Scapular, middle arm, waist and hip circumferences were established to the closest millimeter, by using a soft plastic measuring tape.

Two blood samples were taken: one for leptin levels and one more to obtain DNA. The laboratory procedures were: extraction of genomic DNA from peripheral blood, amplification of the polymorphic fragment by Polymerase Chain Reaction (PCR) and determination of genotypes.

Serum Leptin levels were quantified using a solid phase enzyme-linked immunosorbent assay (ELISA) according to the manufactured instructions (ALPCO, USA).

The Miller method (16), was used to extract DNA from peripheral blood collected into a sterile tube with EDTA-anticoagulant. The DNA pellet was resuspended in 300 mL of sterile TE buffer and the concentration of the DNA obtained were calculated by spectrophotometry. The Q223R variants of the *LEPR* gene, were determined by PCR-RFLP technique, as described previously by Angel-Chávez et al. (17, 18)

Data were compared by Student's *t* test or Mann-Whitney *U* test, after checking for normality of data distribution. Categorical data were compared by the Fisher exact test. The sample size was calculated using G*Power 3.1, with a medium size effect (0.3), α=0.01, statistical power 1-β=0.8, and Df=2, resulting in 155 subjects. To confirm our results regarding the association between Q223R genotypes and obesity, we extracted a randomly selected subset (ca. 50%, n=79), which was analyzed as the original sample. Results are expressed as mean±SD and were considered significant at a bilateral $p<0.05$ value. All analyses were performed with the IBM SPSS Statistics version 25 (IBM Corporation, USA).

RESULTS

The clinical characteristics of the subjects are shown in Table 1. The total population that met the inclusion criteria were 159 individuals (non-obese n=103, 65%; overweight/obese n=56, 35%). Age was not different between the groups. There were significant differences in gender, weight, height, BMI, systolic and diastolic blood pressure, body fat percentage, waist-hip ratio, waist circumference, hip, scapula and arm,

Table 1 Clinical characteristics of the participants

	Non-obese	Overweight/obese[a]	*p*[b]
Gender (male/female)	34/69	31/25	*0.007*
Age (years)	20.96±2.0	21.02±1.9	0.863
Weight (kg)	58.47±9.2	81.05±11.3	<0.001
Height (m)	1.65±0.09	1.69±0.1	0.011
BMI (kg/m^2)	21.41±2.3	28.38±3.1	<0.001
Systolic pressure (mmHg)	115.40±11.1	123.31±12.8	<0.001
Diastolic pressure (mmHg)	78.60±6.6	83.64±9.0	<0.001
Body fat (%)	24.20±6.8	31.58±7.7	<0.001
ICC	0.80±0.1	0.86±0.1	<0.001
Circumferences (cm)			
Waist	74.83±6.9	91.25±8.5	<0.001
Hip	94.67±8.1	106.25±5.9	<0.001
Scapula	86.32±8.3	100.34±9.1	<0.001
Arm	26.59±3.3	32.44±3.6	<0.001
Skin Folds (mm)			
Biceps	5.69±3.7	6.88±4.4	0.070
Triceps	11.78±7.1	14.39±7.7	0.033
Subscapular	13.83±4.2	21.71±5.7	<0.001
Suprailiac	15.66±6.1	24.7±6.7	<0.001
Leptin (ng/mL)	15.14±12.3	26.13±19.0	<0.001

[a] *Overweight and obesity based on BMI values (>25 kg/m^2).*

[b] *Student's t-test except for age, waist circumference, and BMI, which were analyzed using the Mann-Whitney U test. Gender was analyzed with the Fisher exact test.*

Results are expressed as mean±SD, with the exception of gender, which was expressed in frequencies.

triceps, subscapular and supra-iliac skin folds, as well as in serum leptin levels.

The allelic frequencies of the Q223R polymorphism in both study groups (non-obese and overweight/obese) were in Hardy-Weinberg equilibrium (Table 2).

No statistical association between genotypes and overweight/obesity was observed. Table 3 shows that, in non-obese subjects, a total of 33 (32%) subjects were homozygous for the wild allele (Q/Q), 50 (49%) were heterozygous (Q/R) and 20 (19%) were homozygous for the Q223R (R/R) polymorphism. In the overweight/obese group, a total of 20 (36%) subjects showed Q/Q, 29 (52%) Q/R and 7 (12%) R/R.

There was no significant *p* value in any of the cases where the homozygote for the wild-type allele (Q/Q), the heterozygotes (Q/R) or the homozygotes for the polymorphism (R/R) were evaluated separately. Likewise, the relative risk obtained for the Q/Q genotype was 1.18 (IC 0.53-2.34), for the Q/R genotype was 1.14 (IC 0.59-2.18) and for the R/R genotype was 0.59 (IC 0.23-1.50).

Randomly selected subjects showed similar genotype frequencies as the original sample that were not significantly different between overweight/obese and non-obese groups (Q/Q nonobese: 32% randomized, 32% original; Q/Q obese: 37% randomized, 35% original; Q/R nonobese: 49% randomized, 48% original; Q/R obese: 54% randomized, 51% original; R/R nonobese: 18% randomized, 19% original; R/R obese: 8% randomized, 12% original; p>0.222).

In the analysis of the genotype characteristics of the Q223R polymorphism of each study group (non-obese, overweight/obese, table 4), two subgroups were separated based on the

Table 2 Frequency of alleles of the *LEPR* Q223R gene polymorphism

Allele	*LEPR* Q223R		*p* [a]	RR (IC 95%)
	Non-obese	Overweight/obese		
Q	0.56	0.62	0.633	1.25 (0.78-1.99)
R	0.44	0.38		

[a] *Evaluation with Fisher exact test for Hardy-Weinberg equilibrium.*

Table 3 Genotype frequency of the *LEPR* Q223R gene polymorphism

Genotype	*LEPR* Q223R		*p* [a]	RR (IC 95%)
	Non-obese	Overweight/obese		
Q/Q	33	20	0.320	1.18 (0.53-2.34)
Q/R	50	29	0.350	1.14 (0.59-2.18)
R/R	20	7	0.139	0.59 (0.23-1.50)

[a] *Evaluated with the Fisher exact test.*

respective genotypes: in the first group, the homozygous for the wild-type allele (Q/Q); and in the second group, the heterozygotes (Q/R) and homozygous for the Q223R polymorphism (R/R) combined. No significant association was observed in either group, in any of the parameters evaluated (gender, age, weight, height, BMI, systolic and diastolic blood pressure, percentage of body fat, CHF; circumferences in the waist, hip, scapula and arm, biceps, triceps, subscapularis; suprailiac skinfolds, and serum leptin levels) between genotype groups.

Table 4 **Characteristics of non-obese and overweight/obese individuals, based on the genotype of the *LEPR* gene, with the Q223R polymorphism**

	Non-obese			Overweight/obese		
	Q/Q (n=33)	Q/R and R/R (n=70)	*p* [a]	Q/Q (n=20)	Q/R and R/R (n=36)	*p* [a]
Gender (male/female)	(14/19)	(20/50)	0.163	(10/10)	(21/15)	0.548
Age (years)	20.94±1.9	20.97±2.1	0.940	21.40± .8	20.81±2.0	0.266
Weight (kg)	58.52±9.3	58.45±9.2	0.972	81.59±14.1	80.75±9.7	0.795
Height (m)	1.65±0.1	1.65±0.1	0.927	1.70±0.1	1.68±0.1	0.635
BMI (kg/m^2)	21.44±2.1	21.40±2.3	0.929	28.23±3.6	28.46±2.8	0.789
Systolic pressure (mmHg)	115.48±10.5	115.36±11.4	0.959	122.00±13.8	124.00±12.4	0.587
Diastolic pressure (mmHg)	79.52±7.5	78.18±6.2	0.355	84.05±10.0	83.42±8.5	0.805
Body fat (%)	23.73±6.7	24.41±6.9	0.639	30.81±7.2	32.02±8.0	0.576
ICC	0.79±0.1	0.80±0.1	0.776	0.87±0.1	0.85±0.1	0.235
Circumferences (cm)						
Waist	74.59±7.5	74.94±6.6	0.809	92.08±11.00	90.79±6.9	0.640
Hip	94.26±6.5	94.86±8.8	0.729	105.36±7.3	106.75±4.9	0.400
Scapula	86.71±7.0	86.13±8.9	0.742	100.50±12.5	100.25±6.8	0.923
Arm	27.20±2.2	26.30±3.7	0.129	32.48±3.5	32.42±3.7	0.950

Skin Folds (mm)						
Biceps	5.52±3.5	5.77±3.8	0.746	6.80±4.7	6.92±4.3	0.926
Triceps	10.79±7.3	12.24±7.0	0.335	13.65±6.3	14.81±8.5	0.596
Subscapular	12.82±3.9	14.30±4.3	0.096	23.00±5.9	21.00±5.6	0.213
Suprailiac	14.94±6.1	16.00±6.2	0.414	25.35±7.2	24.33±6.4	0.588
Leptin (ng/mL)	13.73±14.94	16.00±11.94	0.408	24.96±18.2	26.79±19.7	0.734

[a] Student's t-test with the exception of gender, which was analyzed with the Fisher exact test.

Results are expressed as mean±SD, with the exception of gender, which was expressed in frequencies.

DISCUSSION

Regarding the weight status of the individuals studied, it was observed that 35% of the subjects have some degree of overweight/obesity; taking into account that their age was 18 to 30 years, with an average of 20.9 in the non-obese group, and 21.0 in the overweight/obese group, the percentage of subjects with overweight/obesity in our study population is consistent with national and international values.

Leptin levels were significantly higher in overweight/obese subjects compared to non-obese subjects. It has been suggested that in pro-inflammatory states these values may be altered (19). Research has shown that the increase in leptin causes obesity in laboratory animals, due to the effect of the hormone in the inhibition of appetite. On the other hand, it has been described, that in obese subjects, leptin rises in a parallel way to BMI. Since there is a greater amount of adipose tissue, an increase in body fat will consequently increase the serum leptin concentration (10).

Allelic frequencies of the Q and R alleles of the *LEPR* gene in the individuals studied were as follows: in non-obese subjects Q = 0.56, R = 0.44; and those with overweight/obesity Q=0.62, R=0.38; which are consistent with other studies carried out in Mexican population (17).

No significant association was found between the Q223R polymorphism of the leptin receptor with overweight/obesity in our study population. The results from the randomized subset of subjects suggests that the absence of a random selection in the original sample does not bias our results. However, the absence of significant association in the present study does not rule out that it could be found in another sample.

Around the world, the Q223R polymorphism has been studied in different populations showing contrasting results (11). In India, Tabassum et al. reported an association of this polymorphism with overweight/obesity in children (20). In other study, carried out by Boumaiza et al., a significant association was found between this polymorphism, BMI and other variables in obese people (21). Another study was carried out in Indonesia, where the *LEPR* K109R and Q223R gene polymorphisms were examined, BMI and waist circumference were analyzed and it was found that, the K109R and Q223R polymorphisms of the *LEPR* gene are associated to obesity (22).

In Japan, Furusawa et al. also reported an association of this polymorphism with BMI and obesity (23). The relationship between obesity and the Q223R polymorphism was sought in a Brazilian population, and it was found that the

polymorphism has statistically different frequencies in the obese compared to normal individuals in the dominant and codominant models, but not in the recessive model. It showed a significant relationship between the *LEPR* Q223R polymorphism with obesity and weight gain in the Brazilian population (24).

In the Mexican population, the presence of the polymorphism was associated with less accumulation of body fat in obese subjects (25). An investigation was conducted in obese children where the association between obesity and leptin receptor polymorphisms K109R, Q223R and K656N was evaluated, arguing the changes that may occur based on an alteration in leptin metabolism. In this study, no specific association was found with obesity and these polymorphisms (17). Other studies have found no association in Mexican populations (9,26) or in other different populations (27-34).

It is noteworthy that a large majority of studies carried out in Asia show a significant association between the Q223R polymorphism and overweight/obesity, while in studies carried out in the Caucasian and Latino population, there is a trend towards no association between these variables. Studies comparing the genotype of the Asian, Caucasian and Latino population could be carried out in the future.

It is possible that the lack of association is due to the sex or age of the participants, since only young adults were included in this study. Also, in the current study, the gender distribution was different between the groups: in non-obese patients, 67% were female, while of the overweight/obese group 44.64% were female. However, a randomized subset of subjects did confirm that there was no significant association with the Q223R polymorphism. Hormonal factors can alter the metabolism of leptin and other proteins involved in the pathophysiology of obesity.

CONCLUSIONS

In this study, 35% of the participants (18-30 years old) showed some degree of overweight/obesity. The allelic frequencies in the studied subjects were: non-obese, Q=0.56, R=0.44; overweight/obese, Q=0.62, R=0.38. No significant association was found between overweight/obesity and the presence of the *LEPR* Q223R polymorphism. Leptin levels were significantly elevated in overweigh/obese subjects compared to non-obese subjects. This study shows that leptin levels are associated with overweight/obesity in Mexican young adults but this is not related to the presence of the Q223R polymorphism in the *LEPR* gene, so the underlying mechanisms for a possible disturbance in leptin signaling in obese Mexican young adults await further studies.

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Ethical concerns

This study was performed according to Mexican regulations and the Declaration of Helsinki. In order to avoid contamination of the environment with biological material, these were handled as stipulated in NOM-087-ECOL-SSA1-2002.

The study subjects were recruited after a detailed explanation of the risks and benefits of their participation, as well as their signing an informed consent letter in which the confidentiality and handling of their data was also assured, based on the provisions of the Declaration of Helsinki. The research was approved by the Institutional Committee of Ethics and Bioethics of the UACJ.

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