Leptin receptor gene polymorphisms are associated with adiposity and metabolic alterations in Brazilian individuals

Polimorfismos no gene do receptor de leptina são associados com adiposidade e alterações metabólicas em indivíduos brasileiros

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ABSTRACT

Objective: The aim of the study was to investigate whether adiposity and metabolic markers, such as leptin, glucose, and lipids, are influenced by leptin (*LEP*) and leptin receptor (*LEPR*) gene polymorphisms in a sample of our population. **Subjects and methods:** A group of 326 individuals of Caucasian-European descent, aged 30 to 80 years, 87 men and 239 women, 148 obese and 178 non-obese, was randomly selected at two clinical hospitals in the city of Sao Paulo, Brazil. All individuals declared their ethnic group as white during the initial interview. Anthropometric measurements, body mass index (BMI), and fat mass were evaluated. Blood samples were drawn for DNA extraction and measurements of leptin, soluble leptin receptor, glucose, and lipids. *LEP* -2548G>A and *LEPR* Lys109Arg (c.326A>G), Gln233Arg (c.668A>G) and Lys656Asn (c.1968G>C) polymorphisms were detected by PCR-RFLP. **Results:** Increased leptin and serum lipids, and *LEPR* Arg223Arg (GG genotype) were associated with higher risk for obesity ($p < 0.05$), while reduced risk was found in *LEPR* Arg109Arg (GG genotype) carriers (OR: 0.38, 95%CI: 0.19-0.77, $p = 0.007$). Multiple linear regression analysis showed a relationship between *LEPR* 223Arg, increased waist circumference, and leptinemia ($p < 0.05$), while *LEPR* 109Arg was associated with high total cholesterol and triglycerides ($p < 0.05$). *LEPR* haplotype 3 (AGG: 109Lys/233Arg/656Lys) carriers have increased risk for obesity (OR: 2.56, 95% CI: 1.19-5.49, $p = 0.017$). Moreover, this haplotype was associated with increased BMI, waist circumference, and leptinemia ($p < 0.05$). **Conclusions:** *LEPR* polymorphisms are associated with obesity, hyperleptinemia, and atherogenic lipid profile, suggesting their potential role for leptin resistance and cardiovascular risk. Moreover, *LEPR* haplotype 3 confers susceptibility to adiposity and hyperleptinemia in our population. Arq Bras Endocrinol Metab. 2013;57(9):677-84

Keywords

Leptin; leptin receptor; gene polymorphisms; adiposity; lipids

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RESUMO

Objetivo: O estudo teve por objetivo investigar a influência de polimorfismos nos genes da leptina (*LEP*) e do receptor de leptina (*LEPR*) na adiposidade e em marcadores metabólicos, como leptina, glicose e lipídeos, em uma amostra de nossa população. **Sujeitos e métodos:** Um grupo de 326 indivíduos com idade de 30 a 80 anos, 87 homens e 239 mulheres, 148 obesos e 178 não obesos, e de etnia branca foi selecionado aleatoriamente em dois hospitais clínicos da cidade de São Paulo, Brasil. Medidas antropométricas, índice de massa corporal (IMC) e gordura corporal foram avaliados. Amostras de sangue foram obtidas para extração de DNA e determinações de leptina, receptor de leptina solúvel, glicose e lipídeos. Os polimorfismos *LEP* -2548G>A e *LEPR* Lys109Arg (c.326A>G), Gln233Arg (c.668A>G) e Lys656Asn (c.1968G>C) foram detectados por PCR-RFLP. **Resultados:** Leptina e lipídeos séricos aumentados e *LEPR* Arg223Arg (genótipo GG) foram associados com maior risco de obesidade ($p < 0,05$), enquanto foi encontrado risco reduzido de obesidade, em portadores de *LEPR* Arg109Arg (genótipo GG) (OR: 0,38, 95%CI: 0,19-0,77, $p = 0,007$). A análise de regressão linear múltipla mostrou relação entre o alelo *LEPR* 223Arg e circunferência abdominal e leptinemia aumentadas ($p < 0,05$), enquanto o alelo *LEPR* 109Arg foi associado com aumento de colesterol total e triglicerídeos ($p < 0,05$). Os portadores do haplotipo 3 do *LEPR* (AGG: 109Lys/233Arg/656Lys) tiveram maior risco aumentado para obesidade (OR: 2.56, 95% CI: 1.19-5.49, $p = 0,017$). Além disso, esse haplótipo foi associado com IMC, circunferência abdominal e leptinemia aumentados ($p < 0,05$). **Conclusões:** Polimorfismos de *LEPR* são associados com obesidade, hiperleptinemia e perfil lipídico aterogênico sugerindo seu papel potencial para a resistência à leptina e risco cardiovascular. Arq Bras Endocrinol Metab. 2013;57(9):677-84

Descritores

Leptina; receptor de leptina; polimorfismos nos genes; adiposidade; lipídeos

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INTRODUCTION

Obesity is caused by excessive fat accumulation that results from a positive balance between total energy intake and fat catabolism (1). Obesity arises from a complex interaction between genetic variance, environment, and lifestyle changes. Fat excess is an important predisposing factor for serious medical conditions such as type 2 diabetes, cardiovascular disease, stroke, cancer, psychiatric illness, and premature death (2).

Leptin, a hormone secreted mainly by the white adipose tissue, regulates food intake, body temperature and energy homeostasis through the interaction with leptin receptors (LEPR) expressed in the hypothalamus (3-5). The reduced ability of leptin to regulate appetite and weight gain is known as leptin resistance, which can lead to obesity-related phenotypes (6). Defects in leptin transport across the blood-brain barrier, in LEPR signaling and in the neural pathways involved in energy homeostasis regulation are some of the mechanisms involved in the resistance to leptin (6).

Single nucleotide polymorphisms (SNPs) in leptin (*LEP*) and leptin receptor (*LEPR*) have been shown to be linked to obesity-related metabolic markers and phenotype (7-10). Studies have suggested that *LEP* rs7799039 (-2548G>A) and rs2167270 (19A>G) SNPs are associated with either variation in increased leptinemia and/or obesity susceptibility in several populations (11-14).

Common SNPs in *LEPR*, such as Lys109Arg (rs1137100), Gln223Arg (rs1137101), and Lys656Asn (rs8179183) have also been related with adiposity, increased BMI, weight gain, hyperleptinemia or predisposition to leptin resistance in different populations (10). The *LEPR* Gln223Arg was associated with adiposity and increased leptinemia in middle-aged Caucasian individuals in Canada (15). This variant was also associated with obesity and predicted a small percentage of body weight and body composition variability in a genetically homogeneous population in Greece (16). The *LEPR* Q223R polymorphism is also one of the factors contributing to the high prevalence of obesity in the Pacific Island populations (17). In Brazil, the *LEPR* Gln223Arg variant was associated with BMI increase, especially in non-smokers, in a sample population of European descent in the South region of the country (18). In addition, Gln223Arg SNP was related to BMI increase in another Brazilian sample of different genetic backgrounds and ethnic origins settled in an urban area of the Rio de Janeiro (19). *LEPR* Lys109Arg variant was found to be associated with obesity and preference for sweets in individuals from Osaka, Japan (20). Moreover, this polymorphism was associated with quantitative measures of adiposity (weight, BMI, and waist and hip circumferences) in children from an urban region of India (21).

We have investigated the influence of the *LEP* and *LEPR* common variants on adiposity and metabolic markers, such as leptin, glucose, and lipids in a sample of obese and non-obese individuals in the city of Sao Paulo, Brazil.

SUBJECTS AND METHODS

Study subjects

A group of 326 unrelated Brazilian individuals, 148 obese and 178 non-obese, 87 men and 239 women (110 post-menopause), aged 30 to 80 years old, was randomly selected from two Clinical Hospitals (Dante Pazzanese Institute of Cardiology and the University Hospital of the Univesity of Sao Paulo). All participants declared that they were of European-Caucasian descent, and lived in the urban area of the city of Sao Paulo, in the southeast of Brazil.

All individuals were informed about the study's protocol and then agreed to participate as volunteers by signing an informed consent form. The study protocol was approved by the ethics committees of the Dante Pazzanese Institute of Cardiology (Protocol # 3419), School of Pharmaceutical Sciences (Protocol # 471), and University Hospital of the University of Sao Paulo (Protocol # 812/08).

Anthropometric measurements, such as BMI, waist circumference (WC), waist-to-hip ratio (WHR), and body fat mass measured by bioimpedance were recorded for each participant. Systolic/diastolic blood pressure was measured in supine position after a 30-min rest period by a trained physician using a mercury column sphygmomanometer.

Based on World Health Organization (WHO) recommendations, subjects with BMI ≥ 30 kg/m^2 were classified as obese and those with systolic/diastolic blood pressure over to 140/90 mmHg or were under anti-hypertensive therapy were considered hypertensive. Individuals with fasting plasma glucose over 100 mg/dL were considered hyperglycemic. Current cigarette smoking was defined as a daily intake of one or more cigarettes. Alcohol consumption was considered

when daily intake of beer, wine, and distilled spirits was ≥ 1 g/day. Physical activity was considered practice of sports, for example, walking, running our swimming, for at least 2 hours a week. Family history of coronary artery disease (CAD) was also recorded.

Metabolic markers measurements

A 12-h fasting blood sample was collected from each participant for serum lipids, and plasma glucose, leptin, and soluble leptin receptor (sLEPR) determinations. Glucose, triglycerides, and total cholesterol and high--density lipoprotein (HDL) cholesterol were measured by enzymatic-colorimetric assays using a Roche-Hitachi 912 automated analyzer (Hitachi, Nakakojo, Japan). Low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) cholesterol values were calculated by Fridewald's formula. Plasma leptin and sLEPR were determined by an ELISA assay (Alexis Biochemical/ Vendor BioAgency, Sao Paulo, Brazil).

DNA isolation and genotyping

Genomic DNA was isolated from 1 mL EDTA-anticoagulated whole blood samples using the salting-out method (22). The *LEP* rs7799039 (-2548G>A) and *LEPR* rs1137100 (Lys109Arg, c.326A>G), rs1137101 (Gln223Arg, c.668A>G) and rs8179183 (Lys656Asn, c.1968G>C) SNPs were chosen based on literature reports.

LEP -2548G>A variant was detected by PCR-RFLP using a previously described protocol (23). *LEPR* Lys109Arg, Gln223Arg and Lys656Asn SNPs were also detected by PCR-RFLP. Primers and PCR conditions are shown in supplementary table 1. PCR assays contained 50 ng genomic DNA, 200 µmol/L primers (Invitrogen Corporation, CA, USA), 200 mmoles/L dNTPs (GE Healthcare, Buckinghamshire, England), and 1.0 U DNA polymerase and PCR buffer [50 mmol/L KCl, 20 mmol/L $(NH_4)_2 SO_4$, 2 mmol/L $MgCl_4$, 75 mmol/L Tris-HCl(pH 9.0)] (Biotools, Madrid, Spain). The amplification was carried out in a PTC-200 Thermal Cycler (MJ Research, Watertown/MA).

PCR products from *LEPR* Lys109Arg, Gln223Arg and Lys656Asn assays were digested with *Mbo*II (New England, BioLabs, USA), *Msp*I (Fermentas, Vilnius, Lithuania), and *BstU* I (Fermentas, Vilnius, Lithuania) endonucleases, respectively, at 37°C for 4 h. Restriction fragments were identified by 8% polyacrylamide gel electrophoresis.

The accuracy of genotyping was evaluated by performing duplicate analysis of 30% of the randomly selected samples, and no genotyping errors were detected. DNA samples with known genotypes confirmed by DNA sequencing were used as controls in PCR and RFLP assays.

Statistical methods

Chi-square test was used to compare categorical variables and the agreement of genotypes frequencies with Hardy-Weinberg equilibrium (HWE) expectations. The sample size to estimate the frequencies of polymorphisms in our population was calculated considering a confidence level of 95% (z = 1.96) and a 0.06 error for the estimated proportion. The minimum sample size for the study was 267 individuals. Continuous variables are presented as means ± SD, and the effects of the polymorphisms on metabolic markers were evaluated by *t*-test and one-way analysis of variance (ANOVA). Variables without normal distribution underwent log transformation for analysis. Multiple logistic regression analysis was performed to assess the influence of clinical variables and polymorphisms in the risk of obesity. The influence of polymorphisms in the risk for obesity was also tested using the SNPassoc software. The influence of polymorphisms on continuous variables was also evaluated by linear regression analysis adjusted by covariates. Statistical analyses were performed using the SAS Systems for Windows, version 8.02 (SAS Institute Inc., Carry, NC), assuming a significance level of $p < 0.05$. Haplotype frequencies and linkage disequilibrium coefficient (D') were assessed by the Expectation-Maximization (EM) algorithm using the Haploview Software. Comparison of haplotype frequencies between case and controls was performed by the chi-square test corrected for multiple tests using permutations testing. The haplo.glm function of the program Haplo.Stats (24) was used to evaluate the effect of haplotypes on the risk for obesity, and the influence of haplotypes on related continuous variables.

RESULTS

Data on anthropometric, clinical, and metabolic markers of the studied group are shown in table 1. As expected, obese individuals have higher mean age, BMI, waist circumference, waist-hip ratio and body fat mass than non-obese individuals ($p < 0.001$). Hypertension, hyperglycemia, family history of CAD, and menopause were more frequent in obese than in non-obese individuals ($p < 0.05$).

Table 1. Anthropometric, clinical, and metabolic data of obese and non-obese individuals

Variables	Obese (148)	Non-obese (178)	p-value
Age, years	51 ± 12	45 ± 12	< 0.001
Women, %	80.4 (119)	77.5 (138)	0.564
Menopause, %	42.6 (63)	26.4 (47)	0.033
Hypertension, %	50.0 (74)	14.0 (25)	< 0.001
Hyperglycemia, %	66.8 (99)	21.9 (39)	< 0.001
Family history of CAD, %	22.9 (34)	14.1 (25)	0.042
Cigarette smoking, %	39.2 (58)	33.7 (60)	0.107
Body mass index, kg/m^2	34.8 ± 4.8	23.5 ± 3.4	< 0.001
Waist circumference, cm	104 ± 16	86 ± 11	< 0.001
Waist-hip ratio	0.90 ± 0.08	0.85 ± 0.09	< 0.001
Body fat mass, %	39.9 ± 4.5	25.3 ± 5.2	< 0.001
Leptin, ng/mL[a]	35.0 ± 26.2	13.3 ± 11.7	< 0.001
sLEPR, ng/mL	23.2 ± 11.2	30.6 ± 23.2	< 0.001
Glucose, mg/dL	111 ± 34	93 ± 16	< 0.001
Lipid profile, mg/dL			
Total cholesterol[a]	217 ± 34	170 ± 39	< 0.001
HDL cholesterol	51 ± 11	58 ± 15	< 0.001
LDL cholesterol	134 ± 38	116 ± 32	< 0.001
VLDL cholesterol	31 ± 12	24 ± 12	< 0.001
Triglycerides	158 ± 66	108 ± 62	< 0.001
Apolipoprotein AI	131 ± 29	140 ± 32	0.363
Apolipoprotein B	107 ± 29	91 ± 25	< 0.001

Number of individuals is in parenthesis. Results are presented as mean ± SD and compared by *t*-test and Mann-Whitney test. Categorical variables were compared by chi-square. HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein; sLEPR: soluble leptin receptor. [a] Values of the variables were log transformed.

Supplementary Table 1. Primer sequences and PCR conditions for *LEPR* polymorphisms

Polymorphism	Primer sequences	Number of cycles	Annealing temperature	Amplicon size
Lys109Arg (rs1137100)	5'-TGCAGACAACATTGAAGGGA-3', 5'-CATACAGGTATCAAAGAATTAAAAAC-3'	35	57°C	128 bp
Gln223Arg (rs1137101)	5'-ATGTTGTGAATGTCTTGTGCCGG-3' 5'-CCATATTTATGGGCTGAACTGACATTAG-3	35	62°C	128 bp
Lys656Asn (rs8179183)	5´-ACAACTTGTCATTTTGCAGTTCCTA-3´ 5´-CCAAAGTAAAGTGACATTTTTCGC-3´	35	56°C	121 bp

Plasma leptin and glucose, serum lipids (total, LDL, and VLDL cholesterol, and triglycerides) and apoB were higher in obese individuals ($p < 0.001$), while plasma sLEPR and serum HDL cholesterol were higher in non-obese individuals ($p < 0.001$).

Genotype distributions of *LEP* and *LEPR* variants were expected from the HWE in both the obese and non-obese group ($p > 0.05$). Genotype and allele frequencies of *LEP* -2548G>A, *LEPR* Gln223Arg (c.668A>G) and *LEPR* Lys656Asn (c.1968G>C) polymorphisms were similar in the obese and non-obese group (Table 2). *LEPR* 109Arg (c.326G) allele, as well as GG genotype, was less frequent in obese (37.8%) than in non-obese individuals (47.2%, $p = 0.020$).

Multiple logistic regression analysis using clinically relevant covariates (age, gender, cigarette smoking and CAD) was performed (Table 3). After adjustment, an increased risk for obesity was observed for each unit increment (mg/dL) of total cholesterol (1%), LDL cholesterol (1%), VLDL cholesterol (4%) and triglycerides (1%). Similarly, each unit increment of glucose and leptin increased the risk for obesity (5% and 8%, respectively). Conversely, HDL cholesterol was related to a lower risk for obesity (OR: 0.95, 95%CI: 0.93-0.97).

Table 2. Frequencies of *LEP* and *LEPR* polymorphisms in obese and non-obese individuals

Polymorphisms	Genotypes/ Allele/ haplotypes	Obese (148)	Non-obese (178)	p-value
LEP -2548G>A	GG	12.2% (18)	19.7% (35)	
	GA	50.0% (74)	45.5% (81)	0.182
	AA	37.8% (56)	34.8% (62)	
	A allele	37.2%	42.4%	0.200
LEPR Lys109Arg	AA	35.8% (53)	31.5% (56)	
(c.326A>G)	AG	52.7% (78)	42.7% (76)	0.004
	GG	11.5% (17)	25.8% (46)	
	G allele	37.8%	47.2%	0.020
LEPR Gln223Arg	AA	41.9% (62)	47.2% (84)	
(c.668A>G)	AG	41.2% (61)	43.8% (78)	0.098
	GG	16.9% (25)	9.0% (16)	
	G allele	37.5%	30.9%	0.091
LEPR Lys656Asn	GG	43.9% (65)	46.1% (82)	
(c.1968G>C)	GC	46.6% (69)	48.9% (87)	0.304
	CC	9.5% (14)	5.0% (9)	
	C allele	30.7%	23.5%	0.321
LEPR haplotypes				
1	AAG	23.3%	25.3%	0.999
2	AAC	15.5%	15.4%	0.972
3	AGG	21.3%	10.9%	0.029
4	AGC	2.1%	1.2%	0.857
5	GAG	14.0%	19.6%	0.136
6	GAC	9.7%	8.8%	1.000
7	GGG	8.6%	14.7%	0.717
8	GGC	5.5%	4.1%	0.988

Comparison of frequencies between case and controls was performed by chi-square test. For haplotype analysis, the test was corrected for multiple tested using permutations testing using Haploview software.

Table 3. Multiple logistic regression analysis of variables associated with obesity

Independent variables		Odds ratio	95% CI	p-value
Leptin		1.08	1.06 – 1.10	< 0.001
sLEPR		1.00	0.98 – 1.01	0.742
Glucose		1.05	1.03 – 1.08	< 0.001
Total cholesterol		1.01	1.00 – 1.01	0.004
HDL cholesterol		0.95	0.93 – 0.97	< 0.001
LDL cholesterol		1.01	1.01 – 1.02	0.001
VLDL cholesterol		1.04	1.02 – 1.06	< 0.001
Triglycerides		1.01	1.01 – 1.02	< 0.001
ApoB		1.02	1.01 – 1.03	< 0.001
ApoAI		1.00	0.99 – 1.01	0.847
Polymorphisms[a]				
LEP -2548G>A	GG (ref)	1.00	--	--
	GA	0.97	0.58 – 1.60	0.891
	AA	0.56	0.27 – 1.15	0.113
LEPR Lys109Arg	AA (ref)	1.00	--	--
(c.326A>G)	AG	0.85	0.50 – 1.45	0.549
	GG	0.38	0.19 – 0.77	0.007
LEPR Gln223Arg	AA (ref)	1.00	--	--
(c.668A>G)	AG	0.85	0.51 – 1.40	0.513
	GG	2.14	1.01 – 4.52	0.047
LEPR Lys656Asn	GG (ref)	1.00	--	--
(c.1968G>C)	GC	0.96	0.59 – 1.55	0.870
	CC	1.78	0.69 – 4.59	0.234

Age, gender, cigarette smoking and CAD were selected as covariates. ApoAI: apolipoprotein AI; ApoB: apolipoprotein B; HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein; sLEPR: soluble leptin receptor. [a] Data were also analyzed and confirmed by SNPassoc software.

Assessment of the *LEPR* variants, after adjustment for these covariates, showed that individuals carrying the c.326GG genotype have lower risk for obesity (OR: 0.38, 95%CI: 0.19-0.77, p = 0.007) than c.326AA+AG genotype carriers (Table 3). On the other hand, the *LEPR* c.668GG genotype was associated with increased risk for obesity (OR: 2.14, 95%CI: 1.01-4.52, p = 0.047). *LEP* -2548G>A and *LEPR* c.1968G>C were not related to obesity in this sample. These results were confirmed when we used the SNPassoc software analysis.

We also evaluated the influence of polymorphisms on anthropometric and biochemical variables related to obesity. Multiple linear regression analysis demonstrated that the *LEPR* 109Arg (c.326G) allele contributed with 0.027 to the increase in total cholesterol, and with 0.049 to the increase in triglyceride log transformed concentrations. Moreover *LEPR* 233Arg (c.668G) allele (c.668AG/GG genotypes) contributed with 4.86 cm to the increase in waist circumference, and with 0.136 ng/mL to the increase in plasma leptin ($p < 0.05$) (Table 4). Altogether, this allele and the selected covariates (age, gender, cigarette smoking, CAD, and obesity) accounted for 55% and 41% of the variability in waist circumference and leptinemia, respectively.

The involvement of *LEPR* haplotypes in the susceptibility to obesity was also evaluated in this study. As shown in table 2, the haplotype 3 (AGG) comprised by *LEPR* 109Lys (c.326A), *LEPR* 223Arg (c.668G) and *LEPR* 656Lys (c.1968G) was more frequent in the obese (21.3%) than in the non-obese group (10.9%, p = 0.029). Analysis using haplo.glm function from Haplo.Stats showed that the presence of at least one copy of this haplotype increased the risk for obesity even when adjusted by age (OR: 2.56; 95%IC: 1.19-5.49, p = 0.017) (Table 5). Moreover, haplotype 3 contributed with a 4.32 kg/cm^2 increase in BMI, 9.45 cm in waist circumference, and 7.90 ng/mL in leptinemia ($p < 0.05$, Table 5).

Table 4. Multiple linear regression analysis: influence of *LEP* and *LEPR* polymorphisms on variables associated with obesity

Variables	LEP -2548G>A		*LEPR* Lys109Arg (c.326A>G)		*LEPR* Gln233Arg (c.668A>G)		*LEPR* Lys656Asn (c.1968G>C)		$R^{2\#}$
	B (SE)	p-value	B (SE)	p-value	B (SE)	p-value	B (SE)	p-value	
Waist circumference	0.36 (1.24)	0.772	1.78 (1.29)	0.168	4.86 (1.22)	< 0.001	-1.75 (1.21)	0.150	55%
Leptin*	-0.032 (0.041)	0.433	-0.054 (0.043)	0.212	0.136 (0.041)	0.001	-0.021 (0.041)	0.601	41%
Total cholesterol*	0.008 (0.009)	0.428	0.027 (0.01)	0.009	-0.002 (0.01)	0.862	0.002 (0.01)	0.864	11%
Triglycerides*	-0.007 (0.022)	0.739	0.049 (0.02)	0.036	0.025 (0.022)	0.261	-0.017(0.02)	0.429	20%

Variables were adjusted for covariates age, gender, cigarette smoking, CAD and obesity. Polymorphisms were introduced as dummy variables for absence or presence of the rare allele. * Dependent variables were Log-transformed to achieve normality; # Coefficient of determination for all the four polymorphisms in the model.

Table 5. Relationship of *LEPR* haplotypes and variables associated with obesity

Variables	Obesity			Body mass index		Waist circumference		Leptin	
	B (SE)	OR (95%CI)	p-value	B (SE)	p-value	B (SE)	p-value	B (SE)	p-value
Haplotype 2	0.34 (0.38)	1.41 (0.67-2.96)	0.365	0.59 (0.99)	0.553	1.22 (2.28)	0.594	2.40 (3.21)	0.454
Haplotype 3	0.94 (0.39)	2.56 (1.19-5.49)	0.017	4.32 (0.96)	< 0.001	9.45 (2.18)	< 0.001	7.90 (3.17)	0.013
Haplotype 4	0.05 (1.13)	1.05 (0.11-9.62)	0.967	1.49 (3.08)	0.629	3.94 (7.21)	0.586	3.48 (8.98)	0.699
Haplotype 5	-0.02 (0.34)	0.98 (0.52-1.99)	0.943	0.84 (0.88)	0.340	2.26 (2.01)	0.261	-0.71 (2.92)	0.808
Haplotype 6	0.02 (0.42)	1.02 (0.45-2.32	0.968	0.60 (1.12)	0.593	3.36 (2.60)	0.199	-1.81 (3.72)	0.627
Haplotype 7	-0.72 (0.44)	0.49 (0.21-1.15)	0.101	0.38 (1.03)	0.712	1.57 (2.45)	0.521	-1.66 (3.52)	0.638
Haplotype 8	0.72 (0.80)	2.05 (0.43-9.85)	0.366	2.79 (1.74)	0.112	4.03 (3.83)	0.293	4.70 (5.39)	0.384

Analysis was carried out by Haplo.Stats using haplotype 1 as reference. Variables were adjusted for the covariate age. Odds Ratio (OR) and 95% confidence interval (95%CI) were calculated using the antilog function (e^x) from regression coefficients (B). *LEPR* genotypes were included together and B coefficients for haplotypes are calculated by the haplo.glm function for each model (Obesity, BMI, WC and leptin).

DISCUSSION

In this sample, obesity was associated with hypertension, hyperglycemia and an atherogenic lipid profile, suggesting that obese individuals have increased susceptibility to metabolic dysfunction and atherosclerosis, as previously proposed (25).

Hyperleptinemia and reduced sLEPR found in obese individuals indicate leptin resistance status, which is probably caused by disruption of the negative feedback loop, a classical mechanism of hormone resistance (26). Leptin resistance has been suggested to produce metabolic and inflammatory injury in several tissues and organs, including the liver, pancreas and heart. Therefore, it may increase the risk for obesity-related cardiovascular disease (27).

The gene candidate approach revealed that *LEPR* Arg223Arg (c.668GG) increased the susceptibility to adiposity in our sample, as demonstrated by the positive relationship of *LEPR* 223Arg allele with increased waist circumference and leptinemia. The effect of this variant was even greater when it was combined with the non-protective *LEPR* 109Lys (c.326A) allele, as shown by haplotype 3.

A relationship between the *LEPR* Gln223Arg variant and increased BMI was previously reported in a sample of Brazilian subjects (150 lean and 200 obese) of different genetic backgrounds and ethnic origins (European-Caucasians, mulattoes, and autochthonous Amerindians) from the urban area of the city of Rio de Janeiro. Moreover, the combination between *LEPR* Gln223Arg and *LEP* -2548G>A polymorphisms was related to a 58% increase in obesity risk (19). More recently, an interaction between *LEPR* Gln223Arg and *ADRB2* Arg16Gly variants was associated with overweight/obesity in Brazilian individuals, highlighting the relevance of multilocus effects in the molecular basis of the obesity (28).

Data analysis from two large community-based cohort studies in North America that analyzed polymorphisms in eight obesity-related genes also demonstrated significant association between *LEPR* Gln223Arg variant and BMI, and change in BMI over time (29). *LEPR* Gln223Arg polymorphism was also found to be related to increased BMI, leptin, and insulin in diabetic individuals in India (30), as well as to hyperleptinemia after adjusting for BMI in a Micronesian population (31).

A genome-wide association study has suggested a role of *LEPR* Lys109Arg and Gln223Arg polymorphisms in modulating plasma levels of the soluble leptin receptor (32). However, we did not found an effect of *LEP* and *LEPR* variants on sLEPR, probably due to the limited sample size, influence of a number of covariates, or even to ethnic complexity.

Taking altogether, these results are suggestive of a potential role of *LEPR* Gln223Arg on leptin and insulin resistance and other metabolic alterations that predispose to adiposity in different populations. However, no effects were detected on body weight, composition, or energy expenditure of 129P3/J mice expressing this variant and fed fat diets up to seven months (33). *In vitro* experiments also demonstrated that Gln223Arg did not affect activation of STAT3, which reflects leptin signaling, in 293 transfected cells treated with various doses of leptin (33). Therefore, the metabolic effects associated with this variant in humans may result from its interaction with other genetic, epigenetic or environmental factors, as previously suggested (34). As for genetic interactions, it is noteworthy that the combination of 109Lys and 223Arg alleles in *LEPR* (haplotype 3) was more strongly associated with adiposity and hyperleptinemia in our study. Nevertheless, this result may be influenced by the limited sample size.

Interestingly, *LEPR* Lys109Arg (c.A326G) variant was associated with increase in total cholesterol and triglycerides, suggesting its contribution to the development of a more atherogenic lipid profile in obese individuals. This result corroborates the findings of the OPERA (Oulu Project Elucidating Risk of Atherosclerosis) study, which reported an increase in total cholesterol in Arg109Arg carriers, when analysis was adjusted for sex and age (35). The authors suggested that this and other *LEPR* polymorphisms are independently associated with early atherosclerosis and some of its risk factors.

In conclusion, the *LEPR* Lys109Arg and Gln223Arg variants are associated with obesity, hyperleptinemia, and an atherogenic lipid profile. Moreover, *LEPR* haplotype 3 confers susceptibility to adiposity and insulin resistance in our population.

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