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Research Article

No Association of Leptin Receptor Gene Gln223Arg Polymorphism with Capillary Glucose Levels: A Preliminary Population Base Cross-Sectional Study

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The leptin receptor gene has been reported to associate with insulin and glucose metabolism and adiposity in different study settings and various populations. Therefore, the aim of the study was to investigate the associations of the leptin receptor gene Gln223Arg polymorphism (LEPR Gln223Arg) with high capillary glucose levels. Cross-sectional study with probabilistic sample was carried out in individuals aged ≥18 years in an urban area of Montes Claros, MG, Brazil. The capillary glucose was considered high when ≥140 mg/dL. The genotypes of LEPR Gln223Arg distribution were as the following: 10.43% GG ($n = 49$), 46.81% AG ($n = 220$), and 42.77% AA ($n = 201$), and there were no prevalence differences between genders, ($P = 0.57$). Multivariate-adjusted models showed that there is no association between the polymorphism LEPR Gln223Arg and capillary high levels of glucose even when adjusted for age, sex, smoking, schooling, and parental history of obesity. In conclusion, no association between the polymorphism LEPR Gln223Arg and elevated blood glucose levels was detected.

1. Introduction

The leptin hormone is an adipocyte-specific ob gene product that regulates the energy balance and multifaceted biological actions and performs its central effects through several neuroendocrine systems [1]. There are multiple lines of evidence with regard to the association between variants of the gene encoding the leptin receptor and the metabolism of the hormone, a certain degree affecting the biological function and serum leptin [2–5]. In this sense, specifically leptin receptor gene Gln223Arg polymorphism (LEPR Gln223Arg) has been mentioned as one of the factors of genetic predisposition to overweight and other cardiometabolic events [4, 6–10] and as suggesting that obesity that genetic variation plays a leptin-resistance [11]. Since the LEPR Gln223Arg has a functional importance for obesity, it could play a significant role in type 2 diabetes mellitus and pathophysiology of human obesity [12]. Furthermore, they may share a common genetic background; that is, the risk alleles for obesity may also be involved in the increased risk of developing type 2 diabetes [13, 14]. Studies with the leptin receptor, it will be important to clarify the functionality of different genetic variants, since several studies have found significant associations linking them to several traits of obesity, diabetes or the metabolic syndrome [10, 13]. For our knowledge, no data are yet available in Brazil on the association of the LEPR Gln223Arg genotypes and capillary glucose levels as a proxy of impaired metabolism glucose. In the present study, we investigated the association between LEPR Arg223Gln polymorphism and the glucose levels in a preliminary population-based study from urban population.

2. Methods

2.1. Study Population and Design. A cross-sectional population-based study was conducted with inhabitants aged ≥18 years in Montes Claros, MG, Brazil. Montes Claros now has about 361.915 inhabitants; 95.1% of them in the urban area of the municipality [15].

2.2. Sample Design. This research proposes the sample size procedure for estimating the prevalence of the LEPR Gln223Arg. To draw the sample, we use cluster sampling method in two stages with unequal selection probabilities form the city of Montes Claros, MG state, Brazil. The sample size was based on the expected prevalence of 10% of the less frequent polymorphism [16] with the standard deviation of 1.55 and variation coefficient of 15%. In the first stage, we used the database of census tracts (2010 Census, IBGE) [15] for the purpose of drawing the primary sampling units. On the second stage, we will use the address list for the purpose of drawing households, and all individuals found at the time of the survey were asked to participate in the study.

This study was approved by the Research Ethics Committee of Universidade Federal de MG (UFMG), and all participants gave written informed consent. In the first stage, we used the database of census tracts (2010 Census, IBGE) [15] for the purpose of drawing households.

Interview was conducted by answered a face-to-face survey questionnaire covering various aspects of their demographic (sex, age, skin color, marital status, and schoolarity) and lifestyle characteristics (physical activity, smoking habits, and alcohol consumption). At the conclusion of the interview, a clinical evaluation of participants was performed which included weight, height, waist circumference, and blood pressure measurements, carried out in triplicate by well-trained staff according to standard procedures [17, 18].

Weight and height were measured according to the recommendations of the World Health Organization, and it included all eligible participants in the selected households [18]. Capillary whole blood was obtained from a finger prick form all subjects in non-fasting status. This drop was placed on the tape reading in disposable and was immediately analyzed using Accucheck Roche (Mannheim, Germany) blood glucose analyzer. Participants were then divided into two categories considered those with high blood glucose values were ≥140 mg/dL with capillary whole blood glucose less than that value [19].

Blood pressure was measured, using an ONRON HEM-742INT (SP, Brazil) automatic BP monitor, in the sitting position, using the right upper arm and an appropriately sized cuff after 5 minutes, according to The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure [17]. Measurements were accomplished three times in the participant right arm with a two-minute interval, after an at least five-minute rest. Hypertension was defined as a systolic blood pressure greater than or equal 140 mmHg and/or a diastolic blood pressure greater than or equal to 90 mmHg or reported use of medication for hypertension control [17].

2.3. DNA Extraction. DNA was extracted from oral mucosa scraping from study participants. DNA samples were isolated using silica particles, which are adsorbed to DNA. Then, DNA was washed to remove impurities, and it was eluted in TE buffer, as previously described [20].

Leptin receptor gene polymorphism LEPR Gln223Arg (A > G; rs1137101) was assessed by restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR). Polymerase chain reaction for LEPR gene was performed on 500 ng of genomic DNA, as template. Other reagents were used, including 4 μM of each primer (F: 5′-ACCCTTTAAGCTGGGTGTCCCAAATAG-3′; R: 5′-CAATATTTATGGGCTG AACTGACATT-3′; 330 bp), 0.1 mM of each DNTP (Amersham Biosciences, Pittsburg, PA, USA), 1X PCR buffer, 2.5 mM magnesium chloride, and 2.5 U of Platinum Taq DNA polymerase (Invitrogen Life Technologies, Carlsbad, CA, USA). The 330 bp PCR product was digested with M*spI* (HPAII) restriction endonuclease (Fermentas Life Sciences, Lithuania), that recognizes the restriction site (C/CGG). For this SNP, the A allele lacks M*spI* restriction site. Thus, individuals carrying A allele show only one PCR product (330 bp), while those who carry G allele show two bands (293 and 37 bp). Positive control for digestion reaction was used, and 10 μL amplified DNA was digested with 1.0 U of M*spI* for 16 h at 37°C. PCR and restriction reactions were performed into a thermocycler (Eppendorf AG, Hamburg, Germany). The products PCR were visualized by electrophoresis in 10% acrylamide gel stained with silver.

3. Statistical Analyses

The χ^2 test or Fisher's exact test have been performed to compare categorical variables, and Student's t-test has been performed to compare age as a continuous variable. All analyzes have been performed using Stata version 12.0 (Stata Corp, Texas, USA) and results with $P < 0.05$ have been considered statistically significant. Afterward variables with $P < 0.05$ have been entered into multivariate logistic regression analysis with the forward elimination method, and adjusted odds ratio (OR) and 95% confidence interval (CI) in order to explore the association between the Gln2234Arg polymorphism and capillary glucose levels.

4. Results

This cross-sectional study recruited 470 participants; the mean and standard deviation of age with average age of total population were 44.72 ± 17.99 years; 34.2% (161) of participants were males; and 65.7% (309) were females. Selected demographic characteristics and adiposity traits according to gender are shown in Table 1. The age group with the highest frequency was from 18 to 29 years 25% (119) followed by age greater or equal to 60 years 21% (101). Frequencies of schooling equal to or greater than 9 years of education and income were similar between the sexes. There were no statistically differences in sociodemographic characteristics between genders, except for the marital status. For the lifestyle variables, the consumption of alcohol and tobacco was higher

Table 1: Sociodemographic characteristics by sex.

Variable	Sex						P value*
	Male		Female		Total		
	n	%	n	%	n	%	
Age groups (years)							
18–29	40	24.84	79	25.57	119	25.32	0.472
30–39	30	18.63	52	16.83	82	17.45	
40–49	20	12.42	58	18.77	78	16.60	
50–59	34	21.12	56	18.12	90	19.15	
≥60	37	22.98	64	20.71	101	21.49	
Skin color							
White	26	16.25	73	23.70	99	21.15	0.061
Nonwhite	134	83.75	235	76.30	369	78.85	
Marital status							
With spouse	106	65.84	140	45.31	246	52.34	<0.001
Without spouse	55	34.16	169	54.69	224	47.66	
Education (years)							
<5	19	11.88	52	16.88	71	15.71	0.410
≥5 and <8	68	42.50	118	38.31	186	39.74	
≥8 e <12	24	15.00	38	12.34	62	13.25	
≥12	49	30.62	100	32.47	149	31.84	
Income (minimum wages)							
<2	25	15.53	44	14.29	69	14.71	0.251
≥2 a <4	74	45.96	121	39.29	195	41.58	
≥4	62	38.51	143	46.42	205	43.71	
Physical activity							
Yes	113	70.19	239	77.35	352	74.89	0.089
Inactive	48	29.81	70	22.65	118	25.11	
Smoking							
Yes	32	20.00	16	5.23	48	10.30	<0.001
Ex-smoking	43	26.87	35	11.44	78	16.74	
No	85	53.13	255	83.33	340	72.96	
Alcohol intake							
Yes	60	37.27	81	26.21	141	30.00	0.013
No	101	62.73	228	73.79	329	70.00	
Nutritional status							
Underweight	08	4.97	13	4.23	21	4.49	0.099
Normal weight	73	45.34	123	40.07	196	41.88	
Overweight	54	33.54	90	29.32	144	30.77	
Obese	26	16.15	81	26.38	107	22.86	
Abdominal obesity							
No	135	83.85	178	57.79	313	66.74	<0.001
Yes	26	16.15	130	42.21	156	33.26	
Hypertension							
Yes	96	59.63	176	57.33	272	58.12	0.632
No	65	40.37	131	42.67	196	41.88	
History of parent's obesity							
Yes	144	89.44	262	85.06	406	86.57	0.187
No	161	10.56	46	14.94	63	13.43	
Glucose level							
Normal (<140 mg/dL)	137	85.09	267	86.97	404	86.32	0.574
High (≥140 mg/dL)	24	14.91	40	13.03	64	13.68	

*Chi-square test.

Table 2: Sociodemographic characteristics by glucose level.

Variable	Glucose level						P value*
	Normal < 140 mg/dL		High ≥ 140 mg/dL		Total		
	n	%	n	%	n	%	
Age groups (years)							
18–29	117	28.96	2	3.13	119	25.43	<0.001
30–39	78	19.31	4	6.25	82	17.52	
40–49	68	16.83	9	14.06	78	16.42	
50–59	71	79.78	18	28.14	90	19.02	
≥60	70	69.31	31	48.44	101	21.60	
Skin color							
White	79	19.65	20	31.25	99	21.24	0.035
Nonwhite	323	80.35	244	68.75	367	78.76	
Marital status							
With spouse	205	50.74	39	60.94	244	52.14	0.129
Without spouse	199	49.26	25	39.06	224	47.86	
Education (years)							
<5	65	16.17	4	6.25	69	15.17	0.001
≥5 and <8	168	41.79	18	28.13	186	39.74	
≥8 e <12	54	13.43	8	12.50	62	13.30	
≥12	115	28.61	34	53.13	149	31.97	
Income (minimum wages)							
<2	60	14.89	9	14.06	69	14.78	0.981
≥2 a <4	166	41.19	27	42.19	193	41.33	
≥4	177	43.92	28	43.75	205	43.90	
Physical activity							
Yes	313	77.48	37	57.81	350	74.79	0.001
Inactive	91	22.52	27	42.19	118	25.21	
Smoking							
Yes	42	10.45	6	12.50	48	10.34	0.002
Ex-smoking	57	14.18	20	25.97	77	16.59	
No	303	75.37	36	58.06	339	73.06	
Alcohol intake							
Yes	128	31.68	13	20.31	141	30.13	0.065
No	276	68.32	51	79.69	327	69.87	
Nutritional status							
Underweight	19	4.73	2	3.13	21	4.51	0.012
Normal weight	174	43.28	21	32.81	195	41.85	
Overweight	127	31.59	16	25.00	16	30.69	
Obese	82	20.40	25	39.06	25	22.96	
Abdominal obesity							
No	279	69.06	33	52.38	312	66.81	0.009
Yes	125	30.94	30	47.62	155	33.19	
Hypertension							
Yes	254	63.18	16	25.00	270	57.94	<0.001
No	148	36.82	48	75.00	196	42.06	

*Chi-square test.

in the male group ($P < 0.05$). The genotypes of Gln223Arg polymorphism in the LEPR gene distribution were as the following: GG genotype was 10.43% ($n = 49$), 46.81% AG ($n = 220$), and 42.77% AA ($n = 201$) (data not shown).

The percentage of individuals having fasting blood glucose levels greater than or equal to 140 g/L in our sample was 13.68% ($n = 64$, 95% CI: 0.12–0.20). When the sample was distributed by glucose level was observed that age group,

Table 3: Multivariate logistic odds ratio and confidence interval (95% CI) of association between genotype and high capillary glucose levels (≥140 mg/dL).

Capillary glucose (≥140 mg/dL)	OR	95% CI	*P* value
Model 1 (not adjusted)			
Genotype GG	1	—	—
Genotype AG	0.83	0.34–2.05	0.702
Genotype AA	1.06	0.43–2.59	0.889
Model 2 (adjust: age)			
Genotype AG	0.84	0.32–2.16	0.721
Genotype AA	0.88	0.79–2.26	0.798
Model 3 (adjust: age and gender)			
Genotype AG	0.84	0.72–2.13	0.724
Genotype AA	0.89	0.34–2.28	0.809
Model 4 (adjust: age, gender, and education)			
Genotype AG	0.87	0.33–2.27	0.790
Genotype AA	0.89	0.34–2.31	0.824
Model 5 (adjust: age, gender, education, and smoke)			
Genotype AG	0.99	0.36–2.75	0.999
Genotype AA	1.01	0.37–2.75	0.981
Model 6 (adjust: age, gender, education, smoke, and alcohol intake)			
Genotype AG	1.05	0.38–2.90	0.917
Genotype AA	1.02	0.37–2.80	0.962
Model 7 (adjust: age, gender, education, smoke, alcohol intake, and WC)			
Genotype AG	1.00	0.36–2.77	0.995
Genotype AA	1.01	0.37–2.78	0.976
Model 8 (adjust: age, gender, education, smoke, alcohol intake, WC, and parental history of obesity)			
Genotype AG	0.97	0.35–2.71	0.968
Genotype AA	1.00	0.36–2.76	0.988

WC: waist circumference.

skin color, schooling, physical activity, nutritional status, waist circumference and hypertension were associated with glucose levels excepted marital status, income and alcohol intake (Table 2). Neither in the univariate analysis nor in the multivariate analysis for the models adjusted by the potentially confounders (age, sex, schooling, smoking, alcohol intake and waist circumference) an association between the polymorphism LEPR Gln223Arg and elevated blood glucose levels was detected (Table 3).

5. Discussion

In this population-based study, we estimate the associations between genotype frequencies determined using PCR-RFLP analysis of the Gln223Arg polymorphism and the high capillary glucose levels. Several studies have described the associations with obesity, hypertension, or other chronic diseases [9, 21–23], but the conclusions about the glucose levels are not yet clear. The Gln223Arg polymorphism is within the region encoding the extracellular domain of the leptin receptor, and, therefore, the amino acid change affects all forms of the receptor. It has been shown that LEPR Gln223Arg polymorphism is associated with the variation in ligand binding; higher levels of ligand binding activity have been demonstrated in individuals homozygous for the G (LEPR Arg223Arg) allele than in carriers of the A (LEPR 223Gln) allele, and the leptin receptors in the hypothalamus and the pancreatic beta-cells could be where they mediate leptin-induced inhibition of insulin secretion [8]. Despite some studies have been found to be associated with diabetes and insulin homeostasis [13, 24]. However, in this study, no association between the polymorphism LEPR Gln223Arg and elevated blood glucose levels was detected. This is similar to a previous study conducted in other populations in which the Gln223Arg in the leptin receptor was not associated with body weight, leptin concentration, and metabolic parameters [13, 25]. Furthermore, the knowledge on the complex signaling pathways involved the insulin resistance and diabetes and could provide the foundation for improved clinical management of patients with metabolic diseases. The lack of association could be due to this complex pathogenesis of hyperglycemia which involves a numerous of genetic and environmental factors which other studies should attempt to control.

6. Conclusions

In summary, in this study, we report no association between Gln223Arg polymorphism and capillary whole glucose levels. This association remained insignificant after controlling

many potentially confounders, and we did not measure leptin levels.

Conflict of Interests

The authors declared that there is no conflict of interests.

Authors' Contribution

Gustavo Velasquez-Melendez, João Felício Rodrigues Neto, Geórgia das Graças Pena, and Andre Luiz Sena Guimarães designed research; Gustavo Velasquez-Melendez, João Felício Rodrigues Neto, Geórgia das Graças Pena, Rosângela Ramos Veloso, and Tatiana Carvalho Reis conducted research; Gustavo Velasquez-Melendez, Geórgia das Graças Pena, and Andre Luiz Sena Guimarães analyzed data; Gustavo Velasquez-Melendez, Geórgia das Graças Pena, and Andre Luiz Sena Guimarães wrote the paper. Gustavo Velasquez-Melendez and Geórgia das Graças Pena had primary responsibility for final content. All authors read and approved the final paper. All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

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