Original Research Article

p.Q223R Leptin Receptor Polymorphism Associated with Obesity in Brazilian Multiethnic Subjects

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ABSTRACT Several genes play a major role in obese phenotypes, and studies suggest that genetic variations among individuals, as well as their lifestyles, may bring about different body compositions. Among these genes, LEP, which codifies leptin, and the LEPR gene encoding its receptor were extensively studied for variants that could explain the obese phenotype. The LEPR p.Q223R gene polymorphism was analyzed in a sample of obese and nonobese individuals from Brazil to evaluate the role of this polymorphism in the obese phenotype in the population. Two hundred obese patients (60 males, 140 females, body mass index (BMI) > 30 kg/m²) were screened, together with 150 lean or normal healthy individuals (63 males, 87 females, BMI < 24 kg/m²). Genomic DNA was extracted and amplified by polymerase chain reaction (PCR). PCR products were digested with the restriction of endonuclease MspI, and separated by electrophoresis through an 8% polyacrilamide gel stained with silver nitrate. There was a significant difference in LEPR p.Q223R polymorphism frequency when comparing obese and lean subjects, with an odds ratio of 1.92 and a 95% confidence interval of 1.15–3.22 (P = 0.013). There is a strong association of the LEPR p.Q223R gene polymorphism with obesity in Brazil. Am. J. Hum. Biol. 18:448–453, 2006.

Obesity is a complex disorder resulting from a net imbalance between energy intake and expenditure. Obesity results from the combined effects of genes, lifestyle, and the interactions of these factors, as found in several studies (Hebebrand et al., 2001; Barsh and Schwartz, 2002). The discovery of leptin, the protein product of the ob gene expressed and secreted by adipose tissue, and its receptor have greatly advanced the comprehension of the mechanism for regulating body weight and energy homeostasis (Zhang et al., 1994). The lipostat system, mediated by leptin and its hypothalamic receptor, reduces food intake and increases thermogenesis. The leptin receptor is a single-transmembrane protein with common extracellular and transmembrane sequences and several splice variations of the intracellular domain, including a putative soluble receptor form (Tar-taglia et al., 1995; Friedman and Halaas, 1998; Mantzoros, 1999).

Genetic variations of the leptin receptor may play a role in the pathophysiological mechanisms of human obesity. Single mutations of the leptin gene or leptin receptor (LEPR) gene were described in a few rare cases of morbid obesity in humans (Montague et al., 1997; Clement et al., 1998). In most overweight and obese individuals, a monogenic disorder has not been identified, and leptin levels are augmented with increasing amounts of fat mass (Considine et al., 1996). However, there are large differences in serum leptin levels between non-Westernized and Westernized populations (Lindeberg et al., 2001), as well as between leptin-level distribution and ethnic/cultural background in

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natives and Caucasian populations from South America (Perez-Bravo et al., 1998; Santos et al., 2000; Bribiescas, 2001; Lindegard et al., 2004). This suggests a leptin-sensitive state.

Several polymorphisms associated with the obese phenotype were identified in the LEPR gene. Of these, c.668A>G in the LEPR gene brings about a glutamine change to arginine at position 223 (p.Q223R) of the protein (Gotoda et al., 1997). This substitution occurs within the first of two duplicate cytokine motifs that represent two putative leptin-binding regions, and may be associated with an impaired LEPR signaling capacity (Yianakouris et al., 2001; Skibola et al., 2004).

There are significant associations between body composition and polymorphisms of the LEPR gene, as found in two recent studies: the Quebec Family Study (Chagnon et al., 1999), and the Heritage Family Study Cohort (Chagnon et al., 2000). Nevertheless, the results diverge when exploring potential associations between LEPR gene polymorphisms and obesity. On the other hand, a recent meta-analytic investigation, using pooled data from nine studies (a total of 3,263 related and unrelated subjects from diverse ethnic backgrounds), reported no significant association between LEPR polymorphisms, including p.Q223R, with body mass index (BMI) and waist circumference (Heo et al., 2001, 2002).

In the present study, we tested the possible association between the polymorphism LEPR p.Q223R and BMI in 200 obese subjects from a Brazilian multiethnic population, comparing the results with those obtained from a sample of lean or normal healthy subjects.

MATERIALS AND METHODS

Subjects

Our sample included 150 lean or normal individuals and 200 obese nondiabetic individuals from Rio de Janeiro, Brazil. The normal group comprised 63 men, aged 21–58 years (32.0 ± 9.96, mean ± SD throughout), and 87 women, aged 19–61 years (32.0 ± 9.51). The obese group comprised 60 men, aged 18–66 years (40.15 ± 11.66), and 140 women, aged 18–71 years (45.3 ± 12.21). The population was made up of European descent (mainly Portuguese, Spaniards, Italians, and other Western European countries) and African descent, but native Brazilian Indians also contributed to this multiethnicity. Obese subjects were selected from the Hypertension Clinic of the Rio de Janeiro State University. Lean individuals were recruited among blood donors from the Pedro Ernesto University Hospital Blood Bank. For all subjects, weight and height were measured to the nearest 0.5 kg and 0.5 cm, respectively. BMI was calculated as body weight (in kg) divided by height² (m²). Obese status was classified according to the World Health Organization (1995). Among the obese subjects, 49.5% were obese grade 1, 25.5% were obese grade 2, and 25% were obese grade 3. Lean individuals had a BMI less than 24 kg/m² during the previous 3 years. All subjects gave informed consent, and the Ethics Committee of Pedro Ernesto University approved the study protocol.

Molecular analysis

Genomic DNA was obtained from peripheral blood leukocytes, using the salting-out method (Miller et al., 1988). Genotyping of the LEPR p.Q223R polymorphism was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay, using primer pairs previously described (Gotoda et al., 1997). PCR reactions were performed on a total volume of 25 µl, using 100 µg of DNA, 1 × PCR buffer, 2.0 mM MgCl₂, 0.25 mM of each dNTP, 2.5 pmol of each primer, and 1.5 U Taq DNA polymerase. DNA was denatured for 5 min at 95°C, and amplified for 40 cycles with an annealing temperature of 52°C. Aliquots of 10 µl of the PCR products were digested with 2.5 U of the restriction endonuclease MspI, according to the manufacturer’s recommendations (New England Biolabs, Inc). The presence of the allelic variant G creates a site for restricting the MspI enzyme. In GG homozygotes, the 80-bp fragment generated by the PCR is cut into two fragments (58 bp and 22 bp). The resulting heterozygotes have, as well as the two fragments, an 80-bp fragment from the A allele which was not cut by the enzyme. Homozygotes AA have only a fragment with 80 bp. The digested samples were analyzed by electrophoresis in an 8% polyacrylamide gel stained by silver nitrate (Santos et al., 1993).

Statistical analysis

Allele frequencies were determined by gene-counting. The two-tailed chi-square test was used to test the significant association between obesity and LEPR genotypes. Odds ratios (ORs) were used to express the risk of obesity associated with a particular genotype. Adjusted ORs were estimated using stepwise logistic regression to allow for the potential imbalance of sex
and age. Statistical tests were based on 0.05 as significance level. The results are presented as means ± standard deviation (SD). All statistical analysis were performed using STATA 8.2 software (Stata Corp).

RESULTS

The genotype distribution and allele frequencies for the LEPR p.Q223R polymorphism are shown in Table 1.

The LEPR p.Q223R polymorphism had statistically different frequencies in obese compared to lean or normal individuals in the codominant and dominant models, but not in the recessive model (Table 2).

The genotype distribution of the p.Q233R polymorphism was different between lean and obese subjects, with a crude OR of 1.65 and a 95% confidence interval (CI) of 1.04–2.6 ($P = 0.0305$). The age of lean individuals, when compared with obese subjects, was significantly different ($P < 0.0001$). The higher risk of obesity in LEPR p.Q223R polymorphism subjects remained statistically significant when adjusted to sex and age covariants (OR, 1.92; 95% CI, 1.15–3.22; $P = 0.013$). Comparing the three subgroups of obese individuals, there were no significant differences in polymorphism frequencies and grade of obesity ($\chi^2 = 1.72; P = 0.42$) (Fig. 1).

DISCUSSION

In this case-control study of obese and lean Brazilian subjects, there is a significant association of LEPR p.Q223R with obesity and weight gain.

This finding is in accordance with other groups. For example, in a genetically homogeneous population from Greece, the polymorphism LEPR p.Q223R was not only associated with obesity, but also predicted small variations in body-weight composition (Yiannakouris et al., 2001). On the other hand, in young white Dutch adults, genetic variation in the leptin receptor gene (p.K109R or p.Q223R polymorphisms) was associated with high leptin levels and predicted weight gain, suggesting that these polymorphisms lead to leptin

<table>
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<tr>
<th>Genotypes</th>
<th>Allele frequency</th>
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<tr>
<td>Genotypes (%)</td>
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<tr>
<td><strong>Codominant model</strong></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>53 (26.50)</td>
</tr>
<tr>
<td>AG</td>
<td>120 (60.00)</td>
</tr>
<tr>
<td>GG</td>
<td>27 (13.50)</td>
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<tr>
<td><strong>Dominant model</strong></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>53 (26.50)</td>
</tr>
<tr>
<td>AG + GG</td>
<td>147 (73.50)</td>
</tr>
<tr>
<td><strong>Recessive model</strong></td>
<td></td>
</tr>
<tr>
<td>AA + AG</td>
<td>173 (86.50)</td>
</tr>
<tr>
<td>GG</td>
<td>27 (13.50)</td>
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<tr>
<td><strong>Alleles</strong></td>
<td></td>
</tr>
<tr>
<td>A (%)</td>
<td>56.5</td>
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<tr>
<td>G (%)</td>
<td>43.5</td>
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resistance (van Rossum et al., 2002). A Caucasian North American population study also demonstrated a greater BMI and fat-mass accumulation in males carrying the G allele (Chagnon et al., 2000). In postmenopausal Caucasian women, there was an association of a single-nucleotide polymorphism in the leptin receptor with BMI, fat mass, and leptin levels (Quinton et al., 2001). Also, the Quebec Family Study (Chagnon et al., 1999) and Heritage Family Study Cohort (Chagnon et al., 2000) found a significant association between polymorphisms of the LEPR gene and body composition.

Nevertheless, there has been a lack of association of this polymorphism in other Caucasian populations, including British male subjects (Gotoda et al., 1997) and Danes with juvenile-onset obesity (Echwald et al., 1997), and in several American obesity trials (Silver et al., 1997). In other non-Caucasian populations such as Pima Indians and Japanese, an association of the leptin receptor gene and obesity were not found (Thompson et al., 1997; Matsuoka et al., 1997). A meta-analytic investigation of linkage and association of common leptin polymorphisms with BMI and waist circumference failed to demonstrate statistically compelling evidence that any of the three alleles is associated with obesity in the overall population (Heo et al., 2001, 2002).

The frequencies of the LEPR p.Q223R polymorphism reported in 18 studies conducted in Rio de Janeiro, located in southeastern Brazil. The general population of Rio de Janeiro constitutes an admixture of populations arising from different ethnic groups, including Negroid (52%), Caucasian (40%) from Europe (predominantly from Portugal), and Amerindian descendants (8%) (Lopes-Camelo et al., 1996). This population differs, with respect to other populations of previous studies (Heo et al., 2002; Paracchini et al., 2005), not only in genetic background, but also in culture, traditions, climate, type of diet, lifestyle, and prevalence of exposure to common environmental risk factors for obesity and related disorders. On the other hand, approximately 65% were female, and the subjects included in this study were unrelated. Interestingly, our results, showing that LEPR p.Q223R and obesity are correlated, coincide with those of another Brazilian sample, from southern Brazil, and composed exclusively of European descent (Mattevi et al., 2002). Although the ages of lean and obese individuals were significantly different, the potential contribution of this difference may be irrelevant, as 98% of our male and female subjects of both categories were older than 20 years, and the age-associated changes in circulating levels of soluble leptin receptor were correlated only during childhood and early adolescence (Mann et al., 2003).

It should be noted in the more prevalent heterozygous state AG among Brazilian obese subjects that, firstly, no other means or additional genotypic factors compensated for the amino-acid substitution, allowing for the obese phenotype to manifest itself. Secondly, the potential downstream effects of this heterozygous state may influence intermediate traits or phenotypes, which are currently being further examined as an ongoing part of this study.

In conclusion, our findings suggest that genetic variability in the leptin receptor gene is involved in body-weight homeostasis. The

![Fig. 1. Genotype distribution of c.668 A>G (p.Q223R) in LEPR gene among obese classes.](image-url)
LEPR p.Q223R variant is associated with BMI increase in a multiethnic population.

ACKNOWLEDGMENTS

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LITERATURE CITED


