Clinical Study

Investigation of Associations between Obesity and \textit{LEP} G2548A and \textit{LEPR} 668A/G Polymorphisms in a Turkish Population

Server Şahin,1,2 Aydın Rüstemoğlu,1 Akın Tekcan,3 Türker Taşlıyurt,4 Hasan Güven,4 and Serbülent Yığın1

1 Department of Medical Biology, Faculty of Medicine, Gaziosmanpaşa University, Tokat, Turkey
2 Department of Medical Biology, Faculty of Medicine, Tokat and Dumlupinar University, Kutahya, Turkey
3 Blood and Therapeutic Apheresis Center, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey
4 Department of Internal Medicine, Faculty of Medicine, Gaziosmanpaşa University, Tokat, Turkey

Correspondence should be addressed to Akın Tekcan; akintekcan@hotmail.com

Received 13 July 2013; Revised 24 September 2013; Accepted 12 October 2013

Academic Editor: Robert Pichler

Copyright © 2013 Server Şahin et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Objective. Obesity is a complex heterogeneous disease that is caused by genes, environmental factors, and the interaction between the two [1]. Obesity is also a multifactorial condition, and many endocrine and inflammatory pathways are involved in its development and in obesity-related diseases [2]. Excess weight in obesity may come from muscles, bone, fat, and/or body water, but obesity specifically refers to having an abnormally high proportion of total body fat [3]. The World Health Organization defines “overweight” as a body mass index (BMI) of 25 or more and “obesity” as a BMI of 30 or more [4]. The prevalence of obesity has been stated as being near epidemic size [1–3, 5–7], and obesity has been associated with type II diabetes, hypertension, coronary artery disease, stroke, and many forms of cancer [8, 9]. Therefore, it is important that the underlying pathophysiology of obesity-related diseases is understood. Obesity results from the combined effects of genes, lifestyle, and the interactions of these factors [10], and both familial and nonfamilial factors play an important role in its development [1]. A genetic predisposition to obesity has been reported as a major risk factor for individuals [7].

With the increasing prevalence of obesity, studies on candidate genes for obesity have increased. Most obesity-predisposing genes encode the molecular components of physiological systems related to energy balance [11]. Leptin is a protein product of the \textit{ob} gene and is expressed and secreted by adipose tissue in amounts proportional to the body weight content; studies on its receptor have greatly advanced the comprehension of the mechanism for regulating body weight and energy homeostasis. The lipostat system,
mediated by leptin and its hypothalamic receptor, reduces food intake and increases thermogenesis [10, 12]. The leptin (LEP) and leptin receptor (LEPR) genes have been evaluated for polymorphisms that could potentially be related to the pathophysiology of obesity and its complications [11]. Although the polymorphisms in these genes have been evaluated [13–15], the association of these polymorphisms with obesity is still controversial. Therefore, we investigated whether the LEP gene G2548A polymorphism and LEPR gene 668A/G (Q223R) polymorphism might be involved in the pathogenesis of obesity.

2. Materials and Methods

2.1. Study Design. This study included 127 obesity patients (93 women, 34 men) and 105 controls (62 women, 43 men) provided from the department of Internal Medicine, Gazi Osmanpaşa University in Tokat, Turkey. Informed consent was in accordance with the study protocol, and all patients and controls signed a written consent form. All patients received a complete clinical evaluation, and all individuals in the control group were healthy and were selected by excluding the diagnosis of obesity. All participants, obesity patients and healthy controls, were of Turkish origin. The ethics committee of the Medical Faculty gave approval for this study.

2.2. DNA Extraction and Genotyping. Genomic DNA was isolated from white blood cells by a kit procedure (Invitrogen Life Technologies, Carlsbad, CA, USA) and stored isolated from white blood cells by a kit procedure (Invitrogen Life Technologies, Carlsbad, CA, USA) and stored at −20°C. LEP G2548A and LEPR 668A/G polymorphisms were analyzed by polymerase chain reaction based restriction fragment length polymorphism (PCR-based RFLP) methods. The PCR protocol consisted of an initial melting step of 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 55°C (for LEP), 30 s at 60°C (for LEPR), and 30 s at 72°C, and a final elongation step of 5 min at 72°C. Amplification was carried out using primers forward 5'-TTT CCT GTA ATT TTC CCG TGA G-3' and reverse 5'-AAA GCA AAG ACA GGC ATA AAA A-3' for the LEP gene and forward 5'-TCC TCT TTA AAG CCT ATC CAG TAT TT-3' and reverse 5'-AGC TAG CAA ATA TTT TTT TAA GCA AT-3' for the LEPR gene. PCR was performed with a 25 μL reaction mixture containing 25–50 ng/μL DNA, 1 μL of 10 pmol/μL of each primer, 1 μL of dNTP mixture (5 mM dNTP, 1 μL 2.5 mM MgCl2, 1 μL Taq DNA polymerase), 2.5 μL 10X PCR buffer (Mg free, Invitrogen Life Technologies, Carlsbad, CA, USA), and dH2O. The PCR products were visualized on a 2% agarose gel stained with ethidium bromide. Amplified products were digested with HhaI at 37°C for LEP and MspI at 37°C for LEPR, and the resulting fragments were separated by 2% agarose gel electrophoresis. The fragments were stained with ethidium bromide and visualized through a Vilber-Lourmat Gel Quantification and Documentation System (QUANTUM-ST4; Vilber Lourmat BP 66, Torcy, France).

2.3. Statistical Analysis. Analysis of the data was performed using SPSS 16.0 (SPSS, Chicago, IL, USA) and OpenEpi Info (http://www.openepi.com). Continuous data were given as mean ± standard deviation. The frequencies of the alleles and genotypes (Hardy-Weinberg equilibrium) in patients and controls were compared with χ² analysis, and 95% confidence intervals were calculated. A P value less than 0.05 (two-tailed) was regarded as statistically significant. The Bonferroni method was applied so that the corrected P value could be calculated.

3. Results

Table 1 shows the demographic variables and baseline characteristics of the patients and controls. The mean age and BMI were 44.86 ± 1.51, 35.45 ± 4.56, and BMI < 35 kg/m² was 45.01 ± 1.38, while the mean BMI of these patients was 38.68 ± 3.11 (BMI > 35 kg/m² and BMI < 35 kg/m² was 45.01 ± 1.38 and 44.7 ± 1.65, respectively, while the mean BMI of these patients was 38.68 ± 3.11 (BMI > 35 kg/m²) and 32.06 ± 1.56 (BMI < 35 kg/m²). Patients and controls were genotyped for both the G2548A polymorphism in the LEP gene promoter and the 668A/G (Q223R) polymorphism might be involved in the pathogenesis of obesity.

Table 2: Distribution of LEP gene G2548A polymorphism and allele frequencies between obesity patients and controls.

| Genotype | Patients (n=127) (%) | Controls (n=105) (%) | P |
|----------|---------------------|---------------------|---|
| AA       | 37 (29)             | 32 (30)             |   |
| GA       | 61 (48)             | 52 (49.5)           | 0.87 |
| GG       | 29 (22)             | 21 (20)             |   |
| GA+GG    | 98:29               | 84:21               | 0.60 |
| GG+AA    | 90:37               | 73:32               | 0.82 |
| Allele frequency |   |                    |   |
| A        | 135 (53)            | 116 (55)            | 0.65 |
| G        | 119 (46.8)          | 94 (44.7)           |   |

Table 1: Demographic variables and baseline characteristics of the patients and controls.

| Characteristic | Mean       |
|---------------|------------|
| Age of patients (n: 127) | 44.86 ± 1.51 |
| Age of controls (n: 105) | 35.45 ± 4.56 |
| Body mass index (BMI) of patients | 45.01 ± 1.38 |
| BMI of controls | 21.57 ± 1.89 |
| Age of BMI > 35 kg/m² patients | 47.7 ± 1.65 |
| Age of BMI < 35 kg/m² patients | 38.68 ± 3.11 |
| BMI of BMI > 35 kg/m² patients | 32.06 ± 1.56 |
| BMI of BMI < 35 kg/m² patients | 32.06 ± 1.56 |
patients and controls, but this was not statistically significant after the Bonferroni correction ($P = 0.05$).

Allele frequencies in the \textit{LEPR} gene showed no statistically significant association ($P > 0.05$) (Table 3). The \textit{LEPR} gene A allele was 61.4% in patients and 69.5% in the control group, while the G allele frequency was 38.5% in patients and 30.4% in the control group. In the combined analysis of the \textit{LEP} and \textit{LEPR} genes, the \textit{LEP/LEPR} GG/GG combined genotype was found to increase the risk of obesity compared to the controls ($P < 0.05$) (Table 4). In the combined genotype analysis based on the mean BMI of obesity patients, there was no association of the \textit{LEP/LEPR} combined genotype and obesity between patients with a BMI $\geq 35$ kg/m$^2$ and patients with a BMI near $30$ kg/m$^2$ ($P > 0.05$) (Table 4).

### Table 3: Distribution of \textit{LEPR} gene 668 A/G polymorphism and allele frequencies between obesity patients and controls.

| Genotype       | Patients (n: 127) (%) | Controls (n: 105) (%) | $P$  |
|----------------|-----------------------|-----------------------|------|
| AA            | 50 (39)               | 50 (47.6)             |      |
| GA            | 56 (44)               | 46 (43.8)             |      |
| GG            | 21 (16.5)             | 9 (8.5)               |      |
| AA + GA : GG  | 106 : 21              | 96 : 9                | 0.10 |
| GA + GG : AA  | 77 : 50               | 55 : 50               | 0.20 |

Allele frequency

| Allele | Patients (n: 127) (%) | Controls (n: 105) (%) | $P$  |
|--------|-----------------------|-----------------------|------|
| A      | 156 (61.4)            | 146 (69.5)            | 0.08 |
| G      | 98 (38.5)             | 64 (30.4)             |      |

* According to Bonferroni correction is not significant.

### Table 4: The distribution of combined genotypes \textit{LEP} and \textit{LEPR} gene polymorphism between patients and control groups.

| Combined genotypes | Patients (n: 127) (%) | Controls (n: 105) (%) | $P$  |
|--------------------|-----------------------|-----------------------|------|
| AA-AA              | 17 (13.39)            | 19 (18.10)            |      |
| AA-GA              | 15 (11.81)            | 10 (9.52)             |      |
| AA-GG              | 5 (3.94)              | 3 (2.86)              |      |
| GA-AA              | 23 (18.11)            | 22 (20.95)            |      |
| GA-GA              | 27 (21.26)            | 24 (22.86)            |      |
| GA-GG              | 11 (8.66)             | 6 (5.71)              | 0.047* |
| GG-AA              | 10 (7.87)             | 9 (8.57)              |      |
| GG-GA              | 14 (11.02)            | 12 (11.43)            |      |
| GG-GG              | 5 (3.94)              | 0                     |      |

* According to Bonferroni correction is not significant.

| Combined genotypes | Patients (n: 127) (%) | Controls (n: 105) (%) | $P$  |
|--------------------|-----------------------|-----------------------|------|
| AA-AA              | 8 (12.31)             | 9 (14.52)             |      |
| AA-GA              | 6 (9.23)              | 9 (14.52)             |      |
| AA-GG              | 3 (4.62)              | 2 (3.23)              |      |
| GA-AA              | 13 (20.00)            | 10 (16.13)            |      |
| GA-GA              | 11 (16.92)            | 16 (25.81)            |      |
| GA-GG              | 5 (7.69)              | 6 (9.68)              |      |
| GG-AA              | 6 (9.23)              | 4 (6.45)              |      |
| GG-GA              | 9 (13.85)             | 5 (8.06)              |      |
| GG-GG              | 4 (6.15)              | 1 (1.61)              |      |

* The results that are statistically significant.

4. Discussion

Human obesity is a complex trait determined by the interaction of multiple genes and environmental factors [1]. Obesity may arise as a result of increased energy intake, decreased energy expenditure, or increased partitioning of nutrients into fat, either alone or in combination [16]. The prevalence of obesity and being overweight continues to increase worldwide, not only causing serious personal health problems but also imposing a substantial economic burden on societies [17]. Although the development of obesity has a genetic component, the mechanism is unknown. Genetic influences are difficult to elucidate, and identification of the involved genes is not easily achieved [3].

In the present study, we analyzed the frequencies of \textit{LEP} G2548A and \textit{LEPR} 668A/G polymorphisms in obesity
patients in a Turkish population. There was no statistically significant difference between the groups with respect to the LEP genotype distribution (P > 0.05) and allele frequencies (P > 0.05). Hoffstedt et al. suggested that the LEP G2548A variant may influence gene expression of leptin and leptin secretion by adipose tissue [18]. Mammi et al. noted that the LEP G2548A polymorphism may influence a BMI increase by means of its effects on leptin secretion [19]; however, they identified a significant and independent association between the LEP 2548GG carrier status and higher leptin levels. An association of the LEP G2548A polymorphism and increased BMI was reported in overweight Europeans and in Taiwanese subjects with obesity [15] and the combined LEP 759C/T and LEP G2548A genotype may be a determinant of obesity [20]. The results of our study do not support the results of these studies but do support those of other studies that showed no association between the LEP G2548A polymorphism and obesity-related phenotypes [11, 14, 15, 21]. We found that LEP genotypes show a difference, but not statistically significant, between obesity patients and controls. We attribute this lack of significance to the low number of patients included in our study, but finding obese patients that have no other disease is difficult. Some researchers have proposed that the polymorphisms of the leptin receptor gene (especially LEPR 668A/G polymorphism) may contribute to common forms of human obesity [11, 14, 22–24]. Our results with respect to the LEPR polymorphism are in agreement with the results of these studies.

Our results showed a statistically significant difference between groups with respect to the distribution of the LEP/LEPR GG/GG combined genotype. Obesity results from both gene-gene and gene-environment interactions [1], and in our study we examined the gene-gene interactions of the LEP/LEPR genes and their link to obesity. Duarte et al. demonstrated that the haplotype association of the LEP G2548A and LEPR Q223R variants was related to a 58% increase in obesity risk, and they considered the interactions between LEP and LEPR gene polymorphisms to intensively influence modulation of energy homeostasis [11]. In agreement with the findings of our study, Boumaiza et al. reported that the LEP G2548A and LEPR Q223R polymorphisms and haplotype combination were associated with a metabolic syndrome and obesity risk in Tunisian subjects [25]. The G2548A and 3′HVR variants of the LEP gene have been noted as being in linkage disequilibrium, and I/G combined genotypes are associated with obesity [26]. In addition, the interactions between the polymorphisms of the LEP and LEPR genes have been shown to increase the risk of non-Hodgkin’s lymphoma and influence insulin plasma concentrations and blood pressure levels [11].

Our findings indicate that the LEP G2548A polymorphism is not a relevant obesity marker and that the LEPR 668A/G polymorphism may be related to obesity in a Turkish population. Additionally, the LEPR/LEPR GG/GG combined genotype was found to increase the risk of obesity in patients compared to controls. However, the association of these polymorphisms with obesity is still controversial, and further research with larger patient populations is necessary.

Acknowledgment

This study was funded by Gaziosmanpaşa University Research Fund (2010/97).

References

[1] A. Nirmala, B. M. Reddy, and P. P. Reddy, "Genetics of human obesity: an overview," International Journal of Human Genetics, vol. 8, no. 1-2, pp. 217–226, 2008.
[2] R. de Mutsert, M. den Heijer, T. J. Rabelink et al., "The Netherlands Epidemiology of Obesity (NEO) study: study design and data collection," European Journal of Epidemiology, vol. 28, no. 6, pp. 513–523, 2013.
[3] A. K. Afridi and A. Khan, "Prevalence and etiology of obesity—an overview," Pakistan Journal of Nutrition, vol. 3, no. 1, pp. 14–25, 2004.
[4] S. F. Noria and T. Granitcharov, "Biological effects of bariatric surgery on obesity-related comorbidities," Canadian Journal of Surgery, vol. 56, no. 1, pp. 47–57, 2013.
[5] Y. S. Lee, "The role of genes in the current obesity epidemic," Annals of the Academy of Medicine Singapore, vol. 38, no. 1, pp. 45–47, 2009.
[6] B. Rokholm, C. S. Andersen, and T. I. A. Sørensen, "Developmental origins of obesity—genetic and epigenetic determinants," The Open Obesity Journal, vol. 3, pp. 27–33, 2011.
[7] L. Brunkwall, U. Ericson, S. Hellstrand, B. Gullberg, M. Orholm, and E. Sonestedt, "Genetic variation in the fat mass and obesity-associated gene (FTO) in association with food preferences in healthy adults," Food & Nutrition Research, vol. 12, p. 57, 2013.
[8] N. S. Wellman and B. Friedberg, "Causes and consequences of adult obesity: health, social and economic impacts in the United States," Asia Pacific Journal of Clinical Nutrition, vol. II, supplement 8, pp. S705–S709, 2002.
[9] C. G. Bell, A. J. Walley, and P. Froguel, "The genetics of human obesity," Nature Reviews Genetics, vol. 6, no. 3, pp. 221–234, 2005.
[10] S. F. Pimentel Duarte, E. A. Francischetti, V. Genelhu-Abreu et al., "p.Q223R leptin receptor polymorphism associated with obesity in Brazilian multiethnic subjects," American Journal of Human Biology, vol. 18, no. 4, pp. 448–453, 2006.
[11] S. F. P. Duarte, E. A. Francischetti, V. A. Genelhu, P. H. Cabello, and M. M. G. Pimentel, "LEPR p.Q223R, β3-AR p.W64R and LEP c.-2548G>A gene variants in obese Brazilian subjects," Genetics and Molecular Research, vol. 6, no. 4, pp. 1035–1043, 2007.
[12] I. Bircan, "Genetics of obesity," Journal of Clinical Research in Pediatric Endocrinology, supplement 1, pp. 54–57, 2009.
[13] A. Nieters, N. Becker, and J. Linseisen, "Polymorphisms in candidate obesity genes and their interaction with dietary intake of n-6 polyunsaturated fatty acids affect obesity risk in a subsample of the EPIC-Heidelberg cohort," European Journal of Nutrition, vol. 41, no. 5, pp. 210–221, 2002.
[14] O. Portolés, J. V. Sorlí, F. Francés et al., "Effect of genetic variation in the leptin gene promotor and the leptin receptor gene on obesity risk in a population-based case-control study in Spain," European Journal of Epidemiology, vol. 21, no. 8, pp. 605–612, 2006.
[15] A. Constantin, G. Costache, A. V. Sima, C. S. Glavce, M. Vladica, and D. L. Popov, "Leptin G-2548A and leptin receptor
Q223R gene polymorphisms are not associated with obesity in Romanian subjects," *Biochemical and Biophysical Research Communications*, vol. 391, no. 1, pp. 282–286, 2010.

[16] I. S. Farooqi and S. O’Rahilly, "Genetic factors in human obesity," *Obesity Reviews*, vol. 8, no. 1, pp. 37–40, 2007.

[17] R. J. F. Loos, "Recent progress in the genetics of common obesity," *British Journal of Clinical Pharmacology*, vol. 68, no. 6, pp. 811–829, 2009.

[18] J. Hoffstedt, P. Eriksson, S. Mottagui-Tabar, and P. Arner, "A polymorphism in the leptin promoter region (-2548 G/A) influences gene expression and adipose tissue secretion of leptin," *Hormone and Metabolic Research*, vol. 34, no. 7, pp. 355–359, 2002.

[19] O. Mammès, D. Betoulle, R. Aubert, B. Herbeth, G. Siest, and F. Fumeron, "Association of the G-2548A polymorphism in the 5′ region of the LEP gene with overweight," *Annals of Human Genetics*, vol. 64, no. 5, pp. 391–394, 2000.

[20] J. G. Gregoor, H. Mulder, D. Cohen et al., "Combined HTR2C-LEP genotype as a determinant of obesity in patients using antipsychotic medication," *Journal of Clinical Psychopharmacology*, vol. 30, no. 6, pp. 702–705, 2010.

[21] Z. Yu, S. Han, X. Cao, C. Zhu, X. Wang, and X. Guo, "Genetic polymorphisms in adipokine genes and the risk of obesity: a systematic review and meta-analysis," *Obesity*, vol. 20, no. 2, pp. 396–406, 2012.

[22] Y. C. Chagnon, J. H. Wilmore, I. B. Borecki et al., "Associations between the leptin receptor gene and adiposity in middle-aged caucasian males from the HERITAGE family study," *Journal of Clinical Endocrinology and Metabolism*, vol. 85, no. 1, pp. 29–34, 2000.

[23] N. Yiannakouris, M. Yannakoulia, L. Melistas, J. L. Chan, D. Klimis-Zacas, and C. S. Mantzoros, "The Q223R polymorphism of the leptin receptor gene is significantly associated with obesity and predicts a small percentage of body weight and body composition variability," *Journal of Clinical Endocrinology and Metabolism*, vol. 86, no. 9, pp. 4434–4439, 2001.

[24] V. S. Mattevi, V. M. Zembrzuski, and M. H. Hutz, "Association analysis of genes involved in the leptin-signaling pathway with obesity in Brazil," *International Journal of Obesity*, vol. 26, no. 9, pp. 1179–1185, 2002.

[25] I. Boumaiza, A. Omezzine, J. Rejeb et al., "Relationship between leptin G2548A and leptin receptor Q223R gene polymorphisms and obesity and metabolic syndrome risk in Tunisian volunteers," *Genetic Testing and Molecular Biomarkers*, vol. 16, no. 7, pp. 726–733, 2012.

[26] H. M. Hinuy, M. H. Hirata, M. F. Sampaio et al., "Relationship between variants of the leptin gene and obesity and metabolic biomarkers in Brazilian individuals," *Arquivos Brasileiros de Endocrinologia e Metabologia*, vol. 54, no. 3, pp. 282–288, 2010.