Association between leptin G-2548A gene polymorphism, plasma leptin levels and lipid profiles in Turkish Cypriot obese subjects

Kıbrıslı Türk obez kişilerde leptin G-2548A gen polimorfizmi ile plazma leptin seviyeleri ve lipid profili arasındaki ilişki

Abstract: Objective: Leptin (LEP) is a metabolic and neuroendocrine hormone which is present in the circulation in amounts proportional to fat mass that acts to reduce food intake and increase energy expenditure thereby regulating body weight homeostasis. Various polymorphisms are shown to be present in LEP gene which play important roles in obesity and obesity-related metabolic biomarkers. The aim of this study was to investigate the association of one of these polymorphisms, leptin gene G-2548A polymorphism, on obesity in association with body mass index (BMI), lipid parameters, plasma leptin levels and homeostasis model assessment of insulin resistance (HOMA-IR).

Methods: The study included 110 obese and 90 non-obese subjects. The LEP G-2548A polymorphism was determined by polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP). Plasma leptin levels, serum lipid and antropometric parameters were measured.

Results: No association was found between LEP gene G-2548A polymorphism and BMI in both study and control groups. Strikingly study group with obese subjects and with the AA genotype had significantly higher serum total cholesterol (p<0.05) than GA and GG genotypes. In obese group, subjects with the AA genotypes had significantly higher leptin (p<0.05) levels than the GG and GA genotypes.

Conclusion: Our results suggest that the LEP gene G-2548A polymorphism may not be considered as a genetic risk factor for obesity in Turkish Cypriot population. However, the G-2548A polymorphism appear to be important in regulating leptin and total cholesterol levels in obese group through leptin gene expression and signaling.

Keywords: Leptin, body mass index, polymorphism, obesity

Özet: Amaç: Leptin (LEP), dolaşımındaki miktarı vücuttaki yağ kütleesi ile orantılı olan metabolik ve nöroendokrin bir hormondur. Leptin gıda alımı azaltarak ve enerji harcanmasını artırarak vücut ağırlığını düzenlemektedir. LEP geninde bulunan birçok polimorfizmin obezite ve obezite ile ilişkili metabolik biyobelirteçlerde önemli rol oynadığı gösterilmiştir. Bu çalışmanın amacı leptin gen G-2548A polymorfizmi ile obezite, vücut kütle indeksi (VKİ), lipit parametreleri, plazma leptin seviyesi ve homeostatik model değerlendirme indeksi (HOMA-IR) arasındaki ilişkinin belirlenmesidir.

Metod: Çalışma 110 obez ve 90 obez olmayan birey içermektedir. LEP G-2548A polimorfizmi belirlenmesi poli-
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meraz zincir reaksiyonu- restriksiyon parça uzunluğu polimorfizmi (PCR-RFLP) ile gerçekleştirilmiştir. Çalışma kapsamında plazma leptin ve serum lipit seviyeleri, antropometrik parametrelerin ölçümü yapılmıştır.

Bulgular: Çalışma ve kontrol gruplarında LEP gen G-2548A polimorfizmi ile V Kı arasında ilişki saptanmamıştır. Çalışma grubunda AA genotipine sahip obez kişilerin total kolesterol seviyesinin GA ve GG genotipine sahip kişilerle göre istatistiksel olarak analamlı derecede yüksek olduğu gözlemlenmiştir (p<0.05). Ayrıca AA genotipine sahip obez kişilerin leptin seviyelerinin GA ve GG genotiplerine göre daha düşük olduğu belirlenmiştir (p<0.05).

Sonuç: Sonuçlarımız LEP G-2548A gen polimorfizminin Kıbrıslı Türk popülasyonunda obezite için genetik bir risk faktörü olmadığını göstermektedir. Fakat obez kişilerde G-2548A gen polimorfizminin leptin gen ekspresyonu ve siniyalli üzerine olan etkisini ile bağlantılı olarak leptin ve total kolesterol seviyelerinin düzenlenmesinde önemli bir etkinin olduğunu düşündürmektedir.

Anahtar Kelimeler: Leptin, vücut kitle indeksi, polimorfizm, obezite

Introduction

Obesity is one of the most challenging health problems of the last century with a tremendous increase in the prevalence. It is associated with an increased risk for type 2 diabetes mellitus, hypertension and cardiovascular diseases [1]. Obesity arises from a complex interaction between environmental and genetic factors [2]. Although as yet the genetic determinants remain largely unkonown.

Obesity is defined as an imbalance between food intake and energy expenditure resulting in the storage of energy as fat, primarily in adipose tissue. Adipose tissue is an active endocrine organ sensing metabolic signals and secreting hormones called adipokines that affect whole-body energy homeostasis [3,4]. Thus, the search for adipokine gene mutations has become an obvious target in studies on molecular markers predisposing to obesity. However, single gene defects resulting in obesity are very rare; it is likely that a combination of various polymorphisms in candidate genes may contribute to its development [5].

Leptin is one of the most important adipose derived hormone which plays a critical role in the regulation of body weight by inhibiting food intake and stimulating energy expenditure [6]. Plasma leptin level is found to be increased in obese individuals and is proportional to body adiposity [7]. Leptin, an obese gene product, is a 16-kDa protein and consists 164-amino acids. The human leptin (LEP) gene is located on chromosome 7q31.3 and comprises 3 exons spanning about 20 kb [8].

Recent molecular studies revealed that leptin (LEP) and leptin receptor (LEPR) genes have been used in search for gene variants potentially related to the pathophysiology of human obesity and its' associated complications [9]. Several polymorphisms have been identified in these genes. One of these polymorphisms is a common G-2548A leptin promoter variant, which results from a G to A substitution at nucleotide -2548 upstream of the ATG start site [10]. The G-2548A polymorphism has previously been shown to be associated with either variations in serum leptin levels or the degree of obesity in obese and overweight subjects but available data are still limited and conflicting [11–13]. Therefore, in order to further evaluate this conflicting relationship between G-2548A polymorphism and obesity and obesity-related metabolic biomarkers (anthropometric variables, glucose, lipid profile, leptin level and HOMA-IR), additional research is still needed.

The aim of our study was to investigate the association between LEP gene G-2548A polymorphism, BMI, lipid parameters, plasma leptin levels and HOMA-IR in obese subjects compared to non-obese subjects.

Materials and Methods

Subjects

This prospective study examined patients who attended the outpatient clinic of the Endocrinology Department in Famagusta Government Hospital and was performed on two groups. One group was composed of 110 obese patients having a mean age of 40.38±8.71 years and BMI 35.75±6.88 kg/m². The second group was composed of 90 non-obese subjects. The mean age of subjects was 38.76±9.46 years and their mean BMI was 22.68±1.75 kg/m². None of the participants had hypertension, liver, kidney, thyroid, cardiovascular or any active inflammatory diseases and they were also questioned for any medical therapy that might effect the lipid and glucose metabolism. The participants neither received any medications nor participated in any dietary or exercise program. All subjects provided written informed consent before enrollment in the study and the
study was approved by the Near East University Research Ethics Committee (2011/3–15).

**Anthropometric measurements**

All the measurements were performed in the morning with the patients in a fasting state and anthropometric measurements, including weight (kg), height (m), hip circumference (cm) and waist circumference (cm) of each subject were measured barefoot and lightly clothed. Hip circumference was measured by placing a tape measure around the patient’s hips at the level of the prominences over the greater trochanters of both femurs. Waist circumference was taken midway between the lowest rip (laterally) and the iliacristale landmark by flexible tape. BMI was calculated as body weight (kg) divided by the square of height (m²) and obesity was defined as BMI ≥30 kg/m² [14].

**Biochemical parameters**

Blood samples were obtained after an overnight fasting. The levels of serum glucose, triglyceride (TG), total cholesterol, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels were measured by fully automated clinical chemistry analyzer (Abbott Architect C8000). Fasting insulin concentrations were measured by electrochemiluminescence kit (Ref. 12017547) (Elecsys Corporation, Lenexa, KS). Insulin resistance index was calculated using the homeostasis model assessment of insulin resistance (HOMA-IR), as the product of fasting insulin (µU/mL) and fasting glucose (mmol/L) divided by 22.5 [15].

**Leptin measurements by ELISA**

Plasma leptin levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) human leptin kit (EIA-2395) (DRG International, Inc, USA). Human leptin kit was used according to the protocol of the manufacturer. Results were expressed in ng/ml.

**LEP gene G-2548A polymorphism**

Genomic deoxyribonucleic acid (DNA) was extracted from whole blood by salting out procedure [16]. Genotyping of the LEP gene G-2548A polymorphism (rs7799039) was carried out using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay with previously described primer pairs (Mammés et al., 2000). PCR reactions were performed on a total volume of 50 µl using 1 µg of genomic DNA, 0.4 µM of each primer, 23.5 µl nuclease-free water (Fermentas International Inc, Canada) and 25 µl DreamTaq PCR Master Mix (Fermentas International Inc, Canada). PCR consisted of one cycle of initial denaturation for 2 min at 95°C, followed by 32 cycles denaturation for 30 sec at 95°C, annealing for 30 sec at 54°C and extension for 30 sec at 72°C and a final extension at 72°C for 10 min. PCR products were digested for 2 hours at 37°C with 5 U of HhaI restriction enzyme (Fermentas International Inc, Canada). In case of mutation HhaI site was naturally created in G-2548A variant allele and the resulting fragments were seperated by 2.5% agarose gels with ethidium bromide staining.

**Statistical analysis**

Distribution of continuous variables in groups were expressed as mean±standard deviation (SD). Differences in baseline characteristics between groups were analyzed by Student’s t-test and χ² test for continuous variables and categorical variables, respectively. Analysis of variance (ANOVA) was used to compare means of continuous variables in the three genotype subgroups. The differences in the mean values of continuous variables in the three genotype subgroups were confirmed by post hoc Tukey test. A P value of less than 0.05 was considered statistically significant. All statistical analyses were performed with SPSS 15.0 statistical package (SPSS Inc, Chicago, IL).

**Results**

Descriptive statistics of anthropometric and metabolic characteristics of the study population are presented in Table 1. There was no significant differences between the mean age of both obese and non-obese subjects. Additionally, plasma glucose, total cholesterol, triglyceride, LDL-cholesterol and leptin levels were significantly higher in obese subjects (p<0.001) but had significantly lower mean HDL-cholesterol (p<0.001) levels. Non-obese subjects had significantly lower HOMA-IR compared to obese subjects (p<0.001).

Analysis of the LEP gene gave three different variants of the genotype: GG for the wild type, GA heterozygous and AA for the homozygous for the polymorphism. The LEP genotype frequencies were calculated and are
presented in Table 2. In obese subjects, the genotype frequencies were 39.1% for GG, 32.7% for GA and 28.2% for AA. The frequencies of GG, GA and AA genotypes were 35.5%, 39% and 25.5% in non-obese subjects, respectively. No significant differences in genotype frequencies of the LEP gene G-2548A polymorphism were detected between obese and non-obese subjects.

The LEP allele frequencies were calculated (Table 2). The -2548 G allele of LEP (found in 55% of non-obese subjects and 54.1% of obese subjects) and the -2548 A allele (found in 45% of non-obese subjects and 45.9% of obese subjects) did not differ between obese and non-obese subjects.

The LEP allele frequencies of female subjects were 36% for GG, 40% for GA, 24% for AA in non-obese group and were 39.7% for GG, 34.9% for GA and 25.4% for AA in obese group. No significant differences in genotype frequencies of the G-2548A polymorphism were detected between obese and non-obese female subjects (p=0.85). The frequencies of GG, GA and AA genotypes were 40.4%, 27.6% and 32% in obese male subjects and were 25%, 45% and 30% in non-obese male subjects, respectively. There were no significant differences in the frequencies between obese and non-obese male subjects (p=0.18).

LEP G-2548A polymorphism showed significant association with levels of total cholesterol in obese subjects (p=0.03). The homozygous AA subjects’ total cholesterol values were significantly higher than the heterozygote GA and homozygote GG obese subjects. Post hoc comparisons in obese subjects showed that AA homozygotes had significantly higher mean value compared to GG (p=0.039),

### Table 1: Baseline characteristics of studied populations.

| Parameter          | Non-obese subjects (n=90) | Obese subjects (n=110) | p     |
|--------------------|---------------------------|------------------------|-------|
| Age                | 38.76±9.46                | 40.38±8.71             | 0.06  |
| BMI (kg/m²)        | 22.68±1.75                | 35.75±6.88*            |       |
| Waist circumference | 85.57±7.52                | 112.25±12.83*          |       |
| Hip circumference  | 99.40±5.99                | 120.89±12.33*          |       |
| Fasting glucose (mg/dL) | 89.73±7.46               | 103.35±23.45*          |       |
| Total cholesterol (mg/dL) | 203.62±34.28            | 234.00±37.32*          |       |
| LDL-cholesterol (mg/dL) | 123.86±28.18             | 144.55±32.61*          | 0.06  |
| HDL-cholesterol (mg/dL) | 55.29±9.36               | 47.84±10.23*           |       |
| Triglycerides (mg/dL) | 105.41±40.18             | 166.97±83.39*          |       |
| Leptin (ng/ml)     | 8.27±4.08                 | 24.47±13.90*           |       |
| HOMA-IR            | 1.90±0.66                 | 4.58±3.95*             |       |

Data are expressed as means±SD and were compared by t-test. BMI: Body mass index; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; HOMA-IR: Homeostasis model assessment of insulin resistance. *p<0.001, between groups.

### Table 2: Genotype and allele frequencies of the LEP G-2548A polymorphism compared between obese and non-obese subjects.

| Parameter          | Non-obese subjects (n=90) | Obese subjects (n=110) | p     |
|--------------------|---------------------------|------------------------|-------|
| Genotype           | GG                        | 41 (37.3%)             | 32 (35.5%) | 0.72 |
|                    | GA                        | 37 (33.6%)             | 35 (39%)  |       |
|                    | AA                        | 32 (29.1%)             | 23 (25.5%) |       |
| Allele frequency   | G                         | 119 (54.1%)            | 99 (55%)  | 0.91 |
|                    | A                         | 101 (45.9%)            | 81 (45%)  |       |

Results are given as n (%). X² analysis p-value.

### Table 3: Anthropometric and metabolic characteristics across LEP G-2548A genotypes of obese and non-obese subjects.

| Parameter          | Non-obese subjects | Obese subjects | p     |
|--------------------|--------------------|----------------|-------|
| Age                | 38.03±9.02         | 40.78±8.67     | 0.82  |
| BMI (kg/m²)        | 22.54±2.22         | 34.93±6.88     | 0.66  |
| Waist circumference | 87.16±6.64         | 111.37±11.73   | 0.83  |
| Hip circumference  | 99.75±6.21         | 119.51±12.18   | 0.81  |
| Fasting glucose (mg/dL) | 91.53±8.78        | 101.35±26.53   |        |
| Total cholesterol (mg/dL) | 197.7±36.1        | 103.5±22.1     | 0.49  |
| LDL-cholesterol (mg/dL) | 121.37±20.92      | 198.06±30.90   |        |
| HDL-cholesterol (mg/dL) | 118.8±31.9        | 226.67±28.74   | 0.03  |
| Triglycerides (mg/dL) | 53.6±5.64         | 143.5±28.94    | 0.54  |
| Leptin (ng/ml)     | 1.93±0.65          | 48.8±5.57      | 0.31  |
| HOMA-IR            | 8.60±5.01          | 50.4±11.29     | 0.48  |

Data are expressed as means±SD. For the comparison of subgroups, analysis of variance followed by ANOVA was performed. BMI: Body mass index; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; HOMA-IR: Homeostasis model assessment of insulin resistance. *Significant difference from GG (p=0.039) by post hoc Tukey test. **Significant difference from GG genotype (p=0.036) by post hoc Tukey test.
while no significant difference was found between the mean values of the AA and GA genotypes (p=0.92) and the GG and GA genotypes (p=0.95). Obese subjects carrying A allele showed significantly higher levels of leptin (p=0.03) than homozygous subjects for the G allele. Post hoc comparisons showed that AA homozygotes had higher mean leptin values compared to GG genotypes (p=0.036) and mean values of the AA and GA genotypes did not differ significantly (Table 3).

Discussion

In this study, we tested the association of previously reported G-2548A polymorphism in the 5’ promoter region of LEP gene with obesity and obesity-related metabolic biomarkers in both obese and non-obese groups. Our results showed no association of the LEP G-2548A polymorphism with BMI and other anthropometric measurements, whereas G-2548A polymorphism was found to be associated with plasma leptin levels in the obese group. Additionally, LEP gene polymorphism was also found to be associated with total cholesterol levels in the obese group.

The leptin (LEP) gene has been investigated in the search for gene variants that are potentially related to pathophysiology of obesity and its complications. Several single nucleotide polymorphisms have been described in LEP gene and one of these polymorphisms is G-2548A. The G-2548A polymorphism is located at the 5’ end of LEP gene with obesity and obesity-related metabolic biomarkers in both obese and non-obese groups. However the results are still controversial. Mammés et al [10] were the first to identify this polymorphism and they also showed that the G-2548A polymorphism was associated with BMI in overweight women.

It has also been reported that G-2548A LEP gene polymorphism was associated with BMI in various studies. However the results are still controversial. Mammés et al [10] were the first to identify this polymorphism and they also showed that the G-2548A polymorphism was associated with BMI in overweight women. Additionally, other studies reported that LEP G-2548A is associated with obesity in Finnish men and in Brazilian women [19,20]. Additionally, two other studies reported that LEP G-2548A is associated with BMI in French cohort, the AA genotype was found to be associated with increased plasma leptin levels [11]. This finding was confirmed by a Sweden study, Hoffstedt et al [13] who found that the -2548A allele was associated with increased messenger RNA (mRNA) levels and adipose tissue leptin secretion rate. In our results, obese subjects with -2548A allele had higher plasma leptin levels than homozygous for the -2548G allele [24].

On the contrary, some group of investigators from different geographies have reported no association between G-2548A LEP polymorphism and BMI. In a research carried out with Turkish population, Şahin et al [22] showed no significant association between the G-2548A polymorphism and BMI. Constantin et al [23] and Ben Ali et al [24] have also reported that this LEP variant was not associated with BMI in Romanian and Tunisian populations, respectively. Additionally, a lack of association between the G-2548A LEP polymorphism and obesity has been reported in different populations, including, French, Spanish and Brazilian groups [12,25,26]. In accordance with these findings, our results also showed no association between the leptin gene polymorphism and BMI in Tunisian Cypriots. Thus, our results may suggest that it is not the leptin gene alone that is involved; other potential intervening factors such as ethnicity, pleiotropic genotype effects, life style and nutritional factors which altogether may effect the energy homeostasis.

The G-2548A polymorphism located at the 5’ end of the promoter region of LEP, and it has been suggested that this variant may influence the LEP gene expression and the leptin secretion by adipose tissue [27]. LEP G-2548A polymorphism is close to an Sp1 transcription factor binding site, as well as two repetitive sequence MER11 and Alu that may regulate LEP transcription [28]. Moreno-Aliaga et al [29] reported that the Sp1 binding site has a key role in the transcriptional activation of the LEP promoter by insulin-mediated glucose metabolism. The potential effects of G-2548A LEP variant on leptin expression has been assessed but the reported data are still conflicting. Moreover, the -2548A allele had lower plasma leptin levels than homozygous for the -2548G allele [24]. Yiannakouris et al [30] showed significant association between LEP G allele and increased leptin levels in healthy Greek individuals. Additionally, the relationship between LEP -2548GG variant and increased serum leptin levels were described for obese European, Brazilians and Romanian individuals [12,20,23]. On the other hand, in men from French cohort, the AA genotype was found to be associated with increased plasma leptin levels [11]. This finding was confirmed by a Sweden study, Hoffstedt et al [13] who found that the -2548A allele was associated with increased messenger RNA (mRNA) levels and adipose tissue leptin secretion rate. In our results, obese subjects with -2548A allele had higher plasma leptin levels than homozygous for the -2548G allele. Moreover, the same genotypic effect was also reported in the study by Şahin et al [31] in Turkish population. To our knowledge collected from the literature, these conflicting results can be said to arise from interaction of G-2548A LEP polymorphism with other polymorphisms in leptin, leptin receptor or others, the model used in statistical analysis, ethnic background and also characteristics of subjects.

To date, as for the total cholesterol levels, one unique study demonstrated that the G-2548A polymorphism in Tunisian volunteers was associated with significant elevation of serum total cholesterol levels in obese subjects [17]. In agreement with this study, our results showed that...
subjects with AA genotype had significantly higher serum total cholesterol levels than the GA heterozygotes and GG homozygotes genotypes in obese subjects. Minokoshi et al [32] discovered that leptin phosphorylate and activate AMP-activated protein kinase (AMPK) via central and periperal mechanisms. Additionally, AMPK was iniatially identified as a protein kinase which inhibits HMG-CoA reductase [33]. Nevertheless, later evidances indicated that most of the obese patients and animals have elevated circulating leptin levels but this sufficient leptin fails to suppress feeding and decrease body weight, showing that most obese subjects exist a phenomenon of leptin resistance, and peripheral administration of leptin cannot develop its proper physiological effects [34,35]. Based on the obovevementioned information, we suggest that the G-2548A polymorphism may cause impairment in cholesterol metabolism through elevated leptin levels in obese subjects with leptin resistance. Thus our results indicate that by disturbing the lipid metabolism, leptin gene G-2548A polymophism may lead to metabolic syndrome and consequent obesity.

The strength of the current study is that it was performed in a well characterized cohort of individuals, with or without obesity. The main limitations of this study were the lifestyle characteristics which have an influence on the relationships between gene variants and phenotype.

In conclusion, based on the literature and our findings, leptin G-2548A polymorphism appears to have a role in leptin gene expression and secretion and also on lipid metabolism but has no direct association with obesity. In order to resolve this contradiction, further research is required with a larger cohort which will include all possible polymorphisms that might affect BMI, plasma leptin levels and lipid metabolism.

**Conflict of Interest:** The authors have no conflict of interest.

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