

Leptin G-2548A Gene Polymorphism is Positively Associated with Increased Plasma Leptin and Glucose Levels in Obese Saudi Patients Irrespective Status of Blood Pressure

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Abstract

The association between LEP G-2548A gene polymorphism with increased plasma leptin and glucose levels and blood pressure in a sample of obese Saudi patients has been evaluated. This is a cross-sectional study involved 206 Saudi adult subjects (94 males and 112 females), randomly selected from the primary health care centers, Riyadh, Saudi Arabia. The study sample was categorized into three groups: 50 normotensive ND controls (age: 47.9 ± 5.4 yr.; BMI 22.9 ± 2.1 Kg/m²), 80 obese normotensive ND (age: 47.7 ± 6.0 yr.; BMI 34.1 ± 4.2 Kg/m²) and 76 obese hypertensive with T2D patients (age: 49.4 ± 5.9 yr.; BMI: 35.1 ± 4.7 Kg/m²). Analyses of LEP G-2548A gene polymorphism were determined using polymerase chain reaction (PCR), followed by restriction fragment length polymorphism (RFLP) with 2U of *HhaI* restriction enzyme. Plasma leptin and insulin levels were measured using the Luminex instrument. Fasting plasma glucose, total cholesterol, HDL-cholesterol, and triglycerides were measured using a chemical autoanalyzer Konelab machine. Also, blood pressure and anthropometric data were measured. The association analysis with metabolic parameters showed that homozygous AA of the LEP gene had significantly higher plasma glucose levels and HOMA-IR compared with homozygous GG (6.8 ± 0.55 vs. 5.8 ± 0.30 ; $p < 0.04$; 4.1 ± 0.84 vs. 2.6 ± 0.67 ; $p = 0.03$) respectively. Besides, heterozygous GA had significantly higher plasma leptin levels compared with homozygous GG (40.0 ± 2.6 vs. 29.6 ± 2.6 ; $P = 0.04$). GA, AA, GA+AA genotypes of the LEP G-2548A gene polymorphism are more prevalent among individuals with hyperglycemia (OR= 3.7, 95% CI= 1.6 to 8.4, $P = 0.001$; OR= 3.2, 95% CI= 1.2 to 8.6, $P = 0.03$; OR= 3.5, 95%CI= 1.6 to 7.7, $P = 0.001$) respectively. A allele of the LEP gene is more prevalent among subjects with hyperglycemia (OR= 1.9, 95%CI= 1.2 to 3.0, $P = 0.006$). G-2548A variant of the LEP gene may not be considered as a genetic risk factor for hypertension in Saudi obese patients. However, the genotypes (GA and AA) and -2548AA allele of this gene may represent important risk factors predisposing healthy subjects to develop T2DM irrespective of the status of blood pressure.

1. Introduction

Obesity is one of the most challenging health problems in the last century, associated with the risk of type 2 diabetes mellitus, hypertension, and cardiovascular disease [1-2]. Many patients with hypertension suffering from overweight or obesity [3] and 7.6 million early deaths are attributed to hypertension [4]. In Saudi Arabia, it is estimated that the prevalence of obesity and hypertension were 35% and 26.1% respectively [5-6]. Although the association between increased body weight and blood pressure has been documented [7-8], the pathophysiological bases of this association are not completely understood [9].

Adipose tissue is an active endocrine organ sensing metabolic signals and secreting hormones called adipokines that affect whole-body energy homeostasis [10-11]. Leptin is a polypeptide hormone produced predominately by white adipose tissue; one of the most important adipose-derived adipokines which play a critical role in the regulation of body weight by inhibiting food intake and stimulating energy expenditure [12]. Plasma leptin level is found to be increased in obese individuals and is proportional to body fat tissue [13]. Besides, leptin seems to be implicated in the pathogenesis of hypertension. It is conceivable that leptin-mediated sympathetic activation in the circulatory system and/or at the renal level may affect blood pressure control and contribute the occurrence of obesity-associated hypertension [14-15]

Leptin, the human obesity gene, has a 16-kDa protein of 164 amino acids. The human leptin (LEP) gene is located on chromosome 7q31.3 and comprises 3 exons and 2 introns, which span approximately 18 kb [16-17]. Among which, a common G-2548A leptin promoter variant, which results from a G to A substitution at nucleotide -2548 upstream of the ATG start site [18], was reported associated with increased plasma leptin levels, obesity, metabolic syndrome, and hypertension [19-25]. However, these available data are still conflicting. Therefore, our study was aimed to investigate the association between the LEP G-2548A gene polymorphism with increased plasma leptin and glucose levels and blood pressure in a sample of obese Saudi patients.

2. Subjects And Methods

2.1. Subjects

This is a cross-sectional study was conducted at the Chair for Biomarkers of Chronic Diseases, King Saud University, Riyadh, Saudi Arabia, during the period from January 2013 to March 2013. Two hundred and six Saudi adults (94 males and 112 females), randomly selected from over 17,000 consenting Saudi involved in the Biomarker Screening in Riyadh Project (RIYADH COHORT). All patients were ≥ 18 years old, diagnosed with diabetes mellitus according to the American Diabetes Association (ADA) criteria. Written consent was obtained from each participant entered into the study. The study was approved by the Ethics Committee of the Science College, King Saud University, Riyadh, Saudi Arabia.

2.2 Data Collection

A questionnaire form focusing on demographic information and past medical history was given to all participants. The patient's demographic information, clinical presentation, medical history, and physical findings were taken from each participant. This included the patient's age, sex, smoking status (never, current or past), hypertension status (yes or no), diabetes duration, diabetes medication, and diabetes complications. Patients were diagnosed with diabetes based on medical history, present intake of diabetes medications, or according to ADA criteria for the diagnosis of diabetes mellitus. Patients had a blood pressure of $\geq 140/90$ mmHg or were taking antihypertensive medications were diagnosed with hypertension. Classification of Body Mass Index (BMI) was based on the World Health Organization /International Association for the Study of Obesity/International Obesity Task Force (WHO/IASO/IOTF).

2.3. Anthropometric and Blood Pressure Measurements

Weight, height, hip circumference, and waist circumference were measured following measured according to WHO guidelines [26]. The Holtain Khan abdominal caliper by Holtain Ltd (Crymych, UK) was used to measure the sagittal abdominal diameter (SAD) as described by Al-Daghri et al. [27]. BMI was calculated as weight/height^2 (Kg/m^2) and obesity was defined as $\text{BMI} \geq 30 \text{ kg/m}^2$ [26]. The study sample was categorized into three groups: 50 normotensive Nondiabetes lean controls, 80 obese normotensive non-diabetes, and 76 obese hypertensives with T2DM patients. Obese subjects were defined as $\text{BMI} \geq 30 \text{ kg/m}^2$ and normal weight participants having BMI of 18–25 according to WHO guidelines. Hypertension was defined as systolic BP ≥ 140

mmHg and diastolic BP ≥ 90 mmHg [28]. Patients with T2DM were defined as fasting blood glucose level ≥ 126 mg/dl (≥ 7.1 mmol/L) or 2-hour postprandial plasma glucose level ≥ 200 mg/dl (≥ 11.1 mmol/L).

2.4. Exclusion Criteria

Patients with any acute co-morbidities that needed immediate medical attention were excluded.

2.5. Biochemical Data

Blood samples were obtained after overnight fasting (12 hours). Plasma fasting blood glucose, total cholesterol, triglycerides, and HDL-cholesterol were determined enzymatically using a chemical analyzer Konelab (Thermo Konelab 20i). Concentrations of LDL-cholesterol were calculated using Friedwald's formula [29]. Serum insulin and leptin levels were measured using Luminex Milliplex kits (Millipore Corporation, MA, USA). Insulin sensitivity was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR), which was calculated as fasting insulin ($\mu\text{U/mL}$) X fasting glucose (mmol/L)/22.5 [30].

2.6. Genetic Data

Genomic DNA was extracted from the peripheral blood using a blood genomic prep mini spin kit (GE Health Care, Buckinghamshire, UK) following the manufacturer's instruction. The -2548G/A of LEP gene polymorphism (rs7799039) were screened by polymerase chain reaction (PCR) - restriction enzyme length polymorphisms (RFLP). PCR was carried out using KAPA Taq ready mix DNA Polymerase (Kapa Biosystems, Cambridge, MA, USA), and primers 5'-TTTCCTGTAATTTTC CCGTGAG-3' (sense) and 5'-AAAGCAAAGACAGGCATA AAAA-3' (antisense) were employed to amplify the 242 bp DNA fragment. PCR reactions were performed in a thermal cycler as described by Mammés et al. [20]. 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 KAPA Taq ready mix DNA Polymerase (Kapa Biosystems, Cambridge, MA, USA). Initial denaturation for 5 min at 95°C followed by 35 cycles with each cycle containing denaturation at for 15 sec at 94 °C, annealing for 30 sec at 52 °C, an extension for 30 sec at 72 °C and followed by final extension for 5 min at 72 °C. PCR amplified DNA products were digested for 2 hours at 37 °C with 2 U of *Haemophilus haemolyticus* (HhaI) restriction enzyme (Fermentas International Inc, Canada) and separated by 2.5% agarose gels with ethidium bromide stain. The 242 bp PCR product (AA) was cleaved into 181 bp (GG) and 61 bp (GA) fragments in the presence of a G nucleotide (Polymorphic variant) but not in its absence.

2.7. Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences for Windows (SPSS version 16.0) and are expressed by mean \pm standard deviation (SD). Analysis of variance (ANOVA) was used to compare the parameters between the entire groups. Analysis of covariance (ANCOVA) is done controlling for the potential confounder age. Odds ratios (OR) and 95% confidence intervals were calculated by multinomial logistic

regression for the genotype and allele frequencies of LEP G-2548A gene polymorphism. P -value of <0.05 was considered statistically significant.

3. Results

Descriptive statistics of anthropometric and biochemical data of the study population are presented in table 1. Obese normotensive ND and obese hypertensive T2D patients had significantly increased BMI ($P<0.001$, $P<0.001$), waist ($P<0.001$, $P<0.001$), hips ($P=0.001$, $P<0.001$), SAD ($P<0.001$, $P<0.001$), systolic ($P=0.01$, $P<0.001$) and diastolic BP ($P=0.002$, $P<0.001$) respectively compared with normotensive ND control subjects. No significant difference was found in plasma total cholesterol ($P=0.60$), LDL-cholesterol ($P=0.99$), triglycerides ($P=0.08$), and insulin ($P=0.15$). Obese hypertensive T2D patients had significantly increased HDL-cholesterol ($P=0.04$) compared with the obese normotensive ND group. Obese hypertensive T2D patients had significantly increased plasma blood glucose ($P=0.009$) compared to obese normotensive ND and normotensive ND control groups. Obese normotensive ND and obese hypertensive T2D patients had significantly increased HOMA- IR ($P=0.01$) and plasma leptin levels ($P<0.001$, $P<0.001$) compared to normotensive ND controls.

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 mutant type. The genotype and allele distribution and frequencies for LEP G-2548A gene polymorphism are presented in table 2. Subjects with normotensive ND, the genotype frequencies of LEP G-2548A gene were 46.0% (GG), 40.0% (GA) and 14.0% (AA). In subjects with obese normotensive ND, the frequencies of GG, GA, and AA genotypes were 40.0%, 38.8%, and 21.2% respectively. In patients with obese hypertensive T2D, the frequencies of GG, GA, and AA were 33.0%, 48.7%, and 18.3% respectively. In contrast, the -2548G allele frequencies of the LEP variant was 66% in subjects with normotensive ND, 59.4% in subjects with obese normotensive ND, and 57.2% in obese hypertensive with T2D patients, whereas, frequencies of the -2548A allele was 34.0% in subjects with normotensive ND, 40.6% in subjects with obese normotensive ND and 42.8% in obese hypertensive with T2D patients. However, no significant differences in genotype and allele frequencies of the LEP G-2548A gene polymorphism detected between obese normotensive ND and obese hypertensive T2D patients compared with normotensive ND control subjects ($P=0.23$, $P=0.30$, $P=0.18$) respectively.

Anthropometric data according to genotypes of LEP G-2548A polymorphism are presented in table 3. No significant association between genotypes of LEP G-2548A gene polymorphism with age, gender, BMI, hips, waist, systolic BP, and diastolic BP.

Biochemical data according to genotypes of LEP G-2548A polymorphism are presented in table 4. In the entire group, our study showed that AA genotype carriers were significantly associated with increased plasma glucose levels and HOMA-IR ($P<0.04$, $P=0.03$) respectively, whereas the GA genotype was significantly associated with increased plasma leptin levels ($P=0.04$). In normotensive ND subjects, the GA genotype was significantly associated with increased plasma leptin levels ($P=0.04$). In contrast, no significant association between genotypes of this gene and plasma total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, and insulin.

Genotype and allele distribution of LEP G-2548A gene polymorphism in individuals with normal BP vs. elevated BP (BP $\geq 140/90$) patients; and in normal glucose vs. elevated glucose levels (FBG ≥ 7.1 mmol/L) subjects; as

well as in normal leptin vs. elevated leptin levels are presented in figure 1. Logistic regression analysis revealed that GA, AA, GA+AA genotypes of LEP G-2548A gene polymorphism are more prevalent among individuals with hyperglycemia against those carrying GG genotype [OR 3.7(1.6 to 8.4), $P=0.001$; 3.2(1.2 to 8.6), $P=0.03$; 3.5(1.6 to 7.7), $P=0.001$, respectively]. Additionally, A allele of the LEP G-2548A gene was more prevalent among the subjects with hyperglycemia [OR 1.9 (1.2 to 3.0), $P=0.006$].

4. Discussion

The present study was performed to investigate the association of reported G-2548A polymorphism in the 5' promoter region of LEP gene with obesity-related biomarkers such as increased BMI, waist, hips, SAD, plasma leptin levels, plasma glucose levels, and blood pressure in obese Saudi patients. 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 mutant type. Subjects with normotensive ND had 46.0% (GG), 40.0% (GA) and 14.0% (AA). Obese normotensive ND subjects had 40.0% (GG), 38.8% (GA), and 21.2% (AA). Patients with obese hypertensive T2D had 33.0% (GG), 48.7% (GA), and 18.3% (AA).

Our data showed no association between LEP G-2548A variants with BMI and other anthropometric measurements such as waist, hips, and SAD, indicating that this genetic variant is not a relevant marker of obesity. Previous studies analyzing the association between the G-2548A LEP variant and increased BMI have been controversial. Indeed, a lack of association between the LEP G-2548A gene polymorphism and increased BMI was reported in different Caucasian populations including French [31], Spanish [32], Brazilian [33], Tunisian [25], and Romanian obese patients [34].

In contrast to these findings, other studies confirmed this relationship between the LEP G-2548A variant and increased BMI in overweight Europeans [20] and Taiwanese Aborigines with severe obesity [24]. A family heart study by Jiang et al. reported also a significant association between this variant of the LEP G-2548A gene and obesity [35]. Additionally, In the Taiwanese population, Wang et al. reported that the BMI of the GG genotype was significantly higher than that of GA and AA genotypes in extremely obese patients [24]. Since the LEP G-2548A gene polymorphism is not at a conserved region among humans, mice, and rats, therefore it is functional significance is uncertain [36]. On the other hand, this variant is located at the 5' end of the promoter region of this gene [19]. It has been postulated this region might contain inhibitory elements subcontinent [37].

Our data showed that the GA genotype of G-2548A LEP polymorphism had significantly higher plasma leptin levels compared with those carrying GG genotype in the study population and healthy ND control subjects. In contrast, the AA genotype of the LEP G-2548A gene polymorphism had significantly higher plasma glucose levels and HOMA-IR in the study population. However, conflicting reports have been shown. In the Taiwanese population, no association was found between this LEP variant and plasma leptin levels [24]. On the other hand, the French men cohort study found that the AA genotype of the LEP G-2548A gene was associated with increased plasma leptin levels [20]. This finding was confirmed by a Sweden study, which showed that the -2548A allele of this variant was associated with increased leptin messenger RNA (mRNA) levels and increased adipose tissue leptin secretion rate [21]. Conversely, a study carried out in a French population showed that the G-2548A LEP variant could potentially alter leptin expression, and female subjects with the AA homozygote had

lower mean leptin levels than girls with other genotypes [31]. The relationship between the AA genotype and lower plasma leptin concentrations was also described in obese Brazilian women [23].

Leptin inhibits the glucose-stimulated insulin secretion, and leptin receptors are present on β -cells as well as on fat cells, thus enabling leptin to modulate both insulin secretion and action [38]. Our study, therefore, found that the AA genotype of the LEP G-2548A gene was significantly associated with increased plasma glucose levels and HOMA-IR. Furthermore the -2458A allele of this gene was significantly associated with elevated plasma glucose levels. Our results are in agreement with data from Julie et al. [39], who suggested that the AA and AG genotype carriers have a significantly higher risk for gestational diabetes mellitus than those carrying the GG genotype. However, these findings are in disagreement with a previous study in which the LEP -2548G carriers were associated with higher plasma glucose in patients with T2DM [40]. Based on our data, it can be suggested that the AA and GA genotype carriers have a significantly higher risk for T2DM than those carrying the GG genotype, which supports the hypothesis that leptin has a role in the development of insulin resistance and subsequently diabetes.

The role of leptin in the pathogenesis of obesity-related hypertension seems through sympathetic activation in the circulatory system and/or at the renal level [14-15] and being prevented by adrenergic blockade drugs [14]. One of the main consequences of SNS activation in hypertension is the increased renal sympathetic nerve activity, which leads to sodium retention, volume expansion, and increased blood pressure [41]. In addition to SNS activation, leptin induces endothelin-1 synthesis in vascular endothelial cells, increases the expression of endothelin type A receptor in vascular smooth muscle cells [42] as well as smooth muscle cell proliferation [43], thus contributing to the increased peripheral vascular resistance. However, our data showed no association between genotypes and alleles of the LEP G-2548A polymorphism and increased blood pressure. However, previous studies found a positive association systolic and diastolic blood pressure with the LEP G-2548A gene. In Tunisian obese subjects, the AA genotype had significantly higher systolic and diastolic blood pressure [25], whereas, in obese Brazilian patients, the AA genotype of LEP G-2548A gene polymorphism had significantly lower levels of systolic, diastolic, and mean arterial blood pressure [44].

Finally, conflicting in results between our data and previous studies may be due to interactions of LEP G-2548A polymorphism with other variants in leptin and/or leptin receptor genes, as well as other variables such as gender, characteristics of subjects, sample size and population, or the model used in genetics analyses. In conclusion, the present study showed that the genotypes distribution of G-2548A variant of the LEP gene (GA and AA) are associated with increased plasma leptin and glucose levels in a set of Saudi individuals. Moreover, AA and GA genotypes and A allele might be an important risk factor predisposing healthy subjects to T2DM. A larger clinical study should be undertaken with a larger population sample to investigate the real meaning of correlations between the LEP gene polymorphism and diabetes mellitus, supporting evidence for leptin gene polymorphism as a genetic factor on diabetes mellitus risk.

Declarations

Conflicts of Interest

The authors declare no conflicts of interest.

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Tables

Tables 1-4 are available in the Supplementary Files

Figures

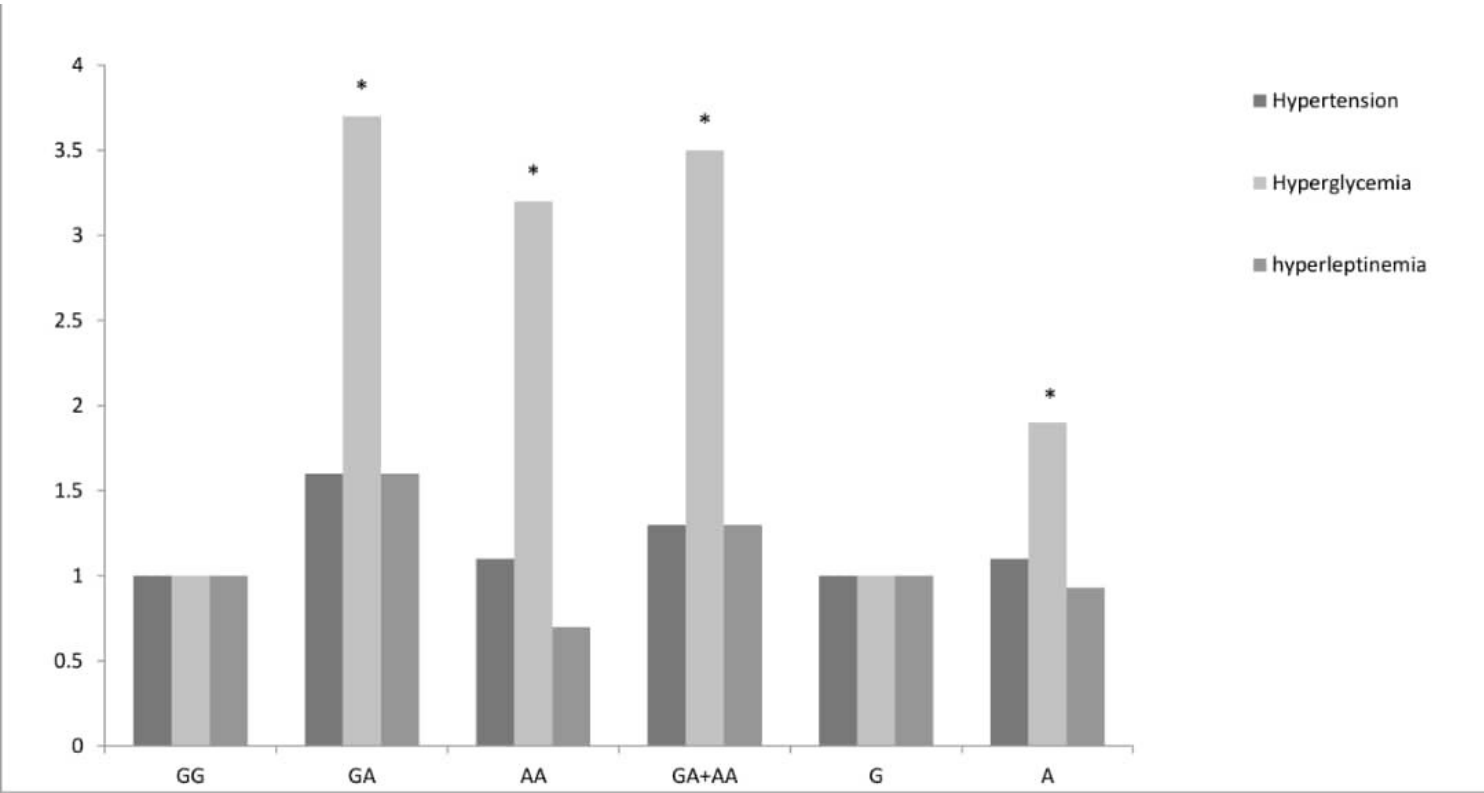


Figure 1

Genotype and allele distribution of the LEP G-2548A gene polymorphism in individuals with normal vs. elevated blood pressure (blood pressure $\geq 140/90$) and normal vs. elevated blood glucose (≥ 7.1 mmol/L) participants; as well as normal vs. hyperleptinemia Subjects (9 ng/ml males and 27 ng/ml females).

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