# Association Analysis of the Leptin and Ghrelin Receptor Gene Polymorphism in the Human with BMI

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**Abstract**

The aim of this work was identification of *Leptin* and *Ghrelin receptor* gene polymorphism in the population. *Leptin* is a product of obese (ob) gene expression that plays a role in energy metabolism and body weight. The human leptin gene is located in the 17 chromosome. The restriction site is located at the position 2549 bp (C→A). Ghrelin, a peptide hormone predominantly produced by the stomach, was isolated as the endogenous ligand for the growth hormone secretagogue receptor. Ghrelin is a potent stimulator of growth hormone (GH) secretion and is the only circulatory hormone known to potently enhance feeding and weight gain and to regulate energy homeostasis following central and systemic administration. Therapeutic intervention with ghrelin in catabolic situations may induce a combination of enhanced food intake, increased gastric emptying and nutrient storage, coupled with an increase in GH thereby linking nutrient partitioning with growth and repair processes. The present study included 35 human samples. The average value of BMI was estimate on 24.45. The size of amplified PCR product is 242bp. Subsequently we used the specific restriction enzyme *HhaI* and length of fragments is 181+61 bp in the homozygote CC, 242+181+61 bp in the heterozygote AC and 242 bp in the homozygote AA. The restriction site is located at the position 171T/C. Examination of the polymorphism of the *GHSR* gene was accomplished used PCR-RFLP method. We used amplified the 593 bp product, which was subsequently digested with restriction enzyme *LweI* and length of fragmetnts is 593 bp in the homozygote TT, 593+567+26 bp in the heterozygote TC and 593+26 bp in the homozygote CC. We assume that this mutation has connection with human obesity level.

**Keywords**: BMI, Ghrelin, GHSR, Leptin, polymorphism.

## 1. Introduction

The biology of leptin has been studied most extensively in the rodents and in the humans. Leptin is involved in the regulation of food intake, energy homeostasis and immunity. Leptin is primarily produced in white adipose tissue and acts via a family of membrane bound receptors, including an isoform with a long intracellular domain (OB-Rb), and many isoforms with short role in energy metabolism and co-modulation of body weight [2]. The human Leptin gene is located in the 31.3 region of chromosome 17 [3].

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Ghrelin, a novel growth hormone- releasing peptide, was originally isolated from the rat and human stomachs [4]. Ghrelin is also known to enhance appetite and increase food intake in healthy men [5] and human ghrelin plasma levels are inversely correlated with body mass index (BMI) [6]. The human ghrelin gene is located at the chromosomal locus 3p26-p25, and the prepro-hormone is encoded by five exons [7].

Obesity is a global health problem with current status reaching epidemic level in all population. It is a multi-factorial physiological disorder, indicated by excessive accumulation of body fat which eventually predisposed the individual to serious medical condition such as diabetes, heart disease, stroke, high blood pressure and certain types of cancer. Obesity develops due to an imbalance in energy intake and expenditure

largely contributed by the individual's behavior and physiology which are affected by both environment and genetic factors [8]. Body mass index (BMI) is a simple index of weight-for-height that is commonly used in classifying overweight and obesity in adult populations and individuals. It is defined as the weight in kilograms divided by the square of the height in meters ($kg/m^2$) [9]. The World Health Organization (WHO) defines "overweight" as a BMI equal to or more than 25, and "obesity" as a BMI equal to or more than 30. These cut-off points provide a benchmark for individual assessment, but there is evidence that risk of chronic disease in populations increase progressively from a BMI 21[9]. This work was focused on the use of molecular genetic methods, PCR and PCR-RFLP used to identify polymorphisms of candidate genes. The aim of the study was to identify the LEP (HhaI) and GHSR (LweI) polymorphism, which can affect the body mass index (BMI).

## 2. Materials and methods

The present study included 35 human (21 men and 14 women). The average BMI is 24.45. For comparative analysis of genes, we used biological material derived from human blood samples. To isolate DNA, we used a classical salting-out method. The polymorphism of LEP gene was analyzed by PCR-RFLP. Amplification was carried out in a 25 µl volume containing 50ng DNA, 5 U/µl Taq polymerase (Fermentas), 10 mM dNTP, 25 mM $MgCl_2$, 1 x Reaction buffer and 1 µl primers. Primer pairs are shown in Table 1.

PCR fragments are visualized on 1% agarose gel at 130 V for 30 minutes.

After PCR was 10 µl of PCR product digested overnight by 37 ºC for at least 16 hour by *Hha*I.

DNA fragments were separated on 2% agarose gel with the addition of Red Gel (Biotium) and visualized under UV transilluminator.

The polymorphism of GHSR gene was analyzed by PCR-RFLP. The reaction mixture for PCR was in 25 ml volume and contained 50ng DNA, 5 U / ml Taq polymerase (Fermentas), 10 mM dNTP, 25 mM$MgCl_2$, 1 x Reaction buffer and 0,5 µl primers. The PCR products (593 bp) were subsequently digested by restriction enzyme *Lwe*I for 5 min at 37 ºC. The digested samples were separated by electrophoresis throught a 3% agarose gel. Digestion of the 593 bp product with *LweI* produced fragment of the following sizes: 593 bp in the homozygote TT, 593, 567 and 26bp in the heterozygotes TC and 593, 26bp in the homozygote CC. Obtained fragments are visualized on 3% gel at 130 V for 35 min.

**Table 1.** Primer pairs for PCR amplification

| Gene | Primer sequence |
|---|---|
| LEP<br>Ren et al., 2004 | FOR<br>5´TTTCCTGATTTTCCCGTGAG 3´<br><br>REV 5´AAAGCAAAGACAGGCATAAAAA 3´ |
| GHSR<br>Wang et al., 2004 | FOR<br>5´ CGGGGTTCAACCTCACACT 3´<br><br>REV<br>5´ AGAGCGCACCGCAAACTC 3´ |

**Table 2.** Time and temperature profil PCR reakcie

| | LEP | | GHSR | |
|---|---|---|---|---|
| | Temperature | Time | Temperature | Time |
| Start | 94 ºC | 3 min | 95 ºC | 3 min |
| Denaturation | 94 ºC | 45 s | 95 ºC | 30 s |
| Annealing | 60 ºC | 30 s | 56 ºC | 20 s |
| Polymerisation | 72 ºC | 45 s | 72 ºC | 40 s |
| Elongation | 72 ºC | 5 min | 72 ºC | 5 min |
| Cooling | 15 ºC | forever | 15 ºC | forever |
| Cycle | 30 | | 30 | |

## 3. Results and discussion

We identified all three genotypes for LEP in our population: CC (9), AC (17) and AA (9). Allele and genotype frequencies are presented in Table 2.

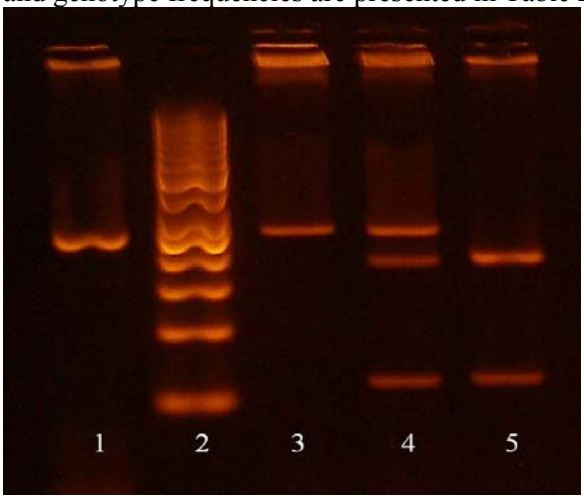


**Figure 1**.*HhaI* polymorphism of the LEP gene
1 – PCR product (242bp), 2 - DNA ladder 50 bp, 3 – genotyp AA ( 242 bp), 4 – genotyp AC (242bp, 181bp, 61bp), 5 – genotyp CC (181bp, 61bp)

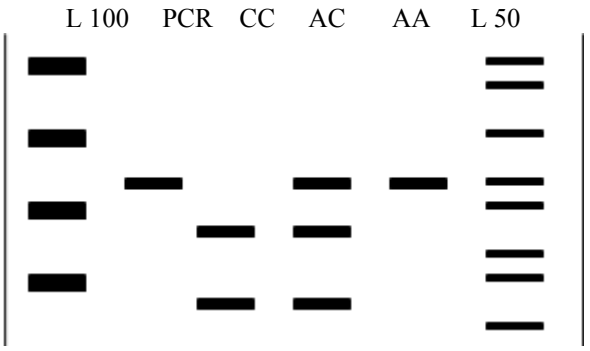


**Figure 2.** Schematic image of digested fragments LEP gene 242bp by *Hha*I
L100 – DNA ladder 100bp, PCR - PCR produkt (242bp), CC - (181bp, 61bp), AC - (242bp, 181bp, 61bp), AA - (242 bp), L50 – DNA ladder 50bp

PCR product was identified by the PCR –RFLP method of size 593bp, and was then digested by enzyme *Lwe*I and vizualized on 3% agarose gel. In the studied population of 35 individuals were detected all three genotypes and the TT genotype 593bp (16 subjects), TC genotype 593bp, 567bp, 26bp (11 subjects) and CC genotype 593bp and 26bp (8subjects).

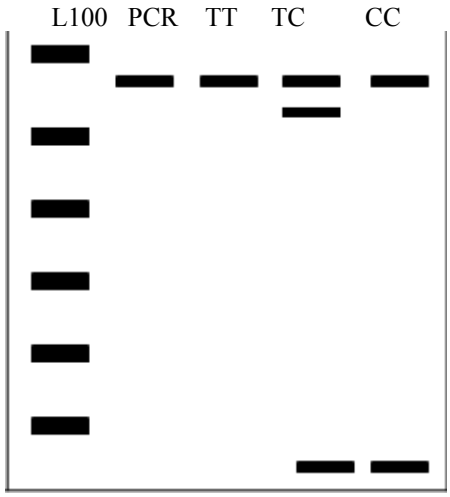


**Figure 3.** Schematic image of digested fragments GHSR gene 593bp by *Lwe*I
L100 – DNA ladder 100bp, PCR – PCR product (593bp), TT – (593bp), TC – (593bp, 567bp, 26bp), CC – (593bp, 26bp)

**Table 2.** Genetic structure - genotypes and allele frequency

| Gene | Genotypes | | | Alelles | |
|---|---|---|---|---|---|
| **LEP** | **AA** | **AC** | **CC** | **A** | **C** |
| | 9 | 17 | 9 | 0.50 | 0.50 |
| | (0.257) | (0.486) | (0.257) | ±0.05 | ±0.05 |
| **GHSR** | **TT** | **TC** | **CC** | **T** | **C** |
| | 16 | 11 | 8 | 0.61 | 0.38 |
| | (0.457) | (0.314) | (0.228) | ±0.02 | ±0.02 |

Observed results for the LEP gene corresponds to [10] datas, which also indicated the occurrence of alleles $Lep^A$ (242bp) and $Lep^C$ (181 and 61 bp). Our observations have been different in the frequencies of alleles and genotypes. While in our evaluated population was distribution of genotypes balanced, [10] show that the East Asian population allele C was predominates.
The results for the GHSR gene 171T/C corresponds with data [11]. His study demonstrates the association of GHSR gene 171T/C polymorphism with Bulimia nervosa, but not with Anorexia nervosa. [12]. It showed towards association between 171T and obesity.

**Table 3.** Average BMI values for LEP and GHSR genotypes

| | **AA** | **AC** | **CC** |
|---|---|---|---|
| **LEP** | | | |
| **MAN (21)** | 25.82 | 24.00 | 24.60 |
| | ±1.79 | ±2.42 | ±1.75 |
| **WOMEN (14)** | 24.71 | 23.26 | 26.10 |
| | ±2.98 | ±3.22 | ±3.65 |
| **GHSR** | **TT** | **TC** | **CC** |
| **MAN (21)** | 25.03 | 28.43 | 22.36 |
| | ± 2.21 | ±1.68 | ±1.38 |
| **WOMEN (14)** | 26.10 | 21.25 | 24.55 |
| | ±2.77 | ±1.82 | ±1.48 |

In LEP gene tested population was confirmed largest average BMI in women with CC genotype, in this genotype was also detected the low standard deviation. Similar findings indicate [13] and [14] for another type of mutation in the LEP gene in position 2548 A / G.
In GHSR gene was confirmed largest average BMI in man with TC genotype. In [15], the study showed that ghrelin stimulates appetite, and food intake in humans is growing, what we value as associated with increased BMI in men. The biological function of the GHSR gene is a candidate gene that may contribute to the risk of obesity.

## 4. Conclusions

LEP and GHSR gene shows polymorphism using restriction endonuclease *Hha*I and *Lwe*I, and thus it is possible to identify genetic variants of alleles A, C and T, C, respectively. In human populations there are three combinations of LEP gene genotypes AA, AC, CC and three combinations of GHSR gene genotypes TT, TC and CC. On basis of their distribution can be analyzed LEP and GHSR genes as markers for metabolic disorders. We assume that the CC genotype in LEP gene and the TC genotype in GHSR gene are susceptible to weight gain and may lead to obesity.

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