

## GHRL, LEP, LEPR genes polymorphism and their association with the metabolic syndrome in the Ukrainian population

Andrii PRODAN<sup>1</sup>, Ihor DZUBANOVSKY<sup>1</sup>, Oleksandr KAMYSHNYI<sup>2</sup>, Natalia MELNYK<sup>3</sup>,  
Svitlana PIDRUCHNA<sup>4</sup>, Stanislava VOLOSHYN<sup>5</sup>

<sup>1</sup>Department of Surgery of Postgraduate Faculty; <sup>2</sup>Department of Microbiology, Virology, and Immunology; <sup>3</sup>Department of General Hygiene and Ecology; <sup>4</sup>Department of Medical Biochemistry; <sup>5</sup>Department of Pediatrics No2, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

E-mail: [prodan@tdmu.edu.ua](mailto:prodan@tdmu.edu.ua)

**Objective.** Many conflicting results have been obtained in the study of leptin (LEP) and leptin receptor (LEPR) gene variants that are associated with the obesity and diabetes possibly due to differences in the study populations. The aim of this study was to evaluate changes in the metabolic hormones (leptin, ghrelin, adiponectin, resistin) levels in the blood of obese patients in relation to the GHRL (rs696217), LEP (rs7799039), LEPR (rs1137100, rs1137101, rs1805094) polymorphism in Ukrainian population.

**Methods.** The study involved 53 obesity cases and 48 non-obesity subjects (controls). The GHRL, LEP, and LEPR genes polymorphism (rs696217, rs7799039, rs1137100, rs1137101, rs1805094) was genotyped using a TaqMan real-time polymerase chain reaction method. Blood hormones (leptin, ghrelin, adiponectin, resistin) were determined with commercially available kits using a Multiskan FC analyzer.

**Results.** The study of the effect of genotypes of the GHRL (rs696217), LEP (rs7799039), and LEPR (rs1137100, rs1805094) polymorphisms on the level of metabolic hormones (leptin, ghrelin, adiponectin, resistin) in the blood of obese patients did not show reliably significant results. Thus, the presence of the LEPR genes (rs1137101) polymorphism in the Ukrainian population indicates an increased risk of the metabolic syndrome development regardless of the homozygous or heterozygous genotype (genotypes AA, AG, GG).

**Conclusions.** We established a significant effect of the presence of the A allele and G allele of the LEPR gene polymorphism (rs1137101) on the level of leptin, ghrelin, adiponectin, and resistin in the serum of patients diagnosed with the metabolic syndrome in the Ukrainian population.

**Key words:** metabolic syndrome, LEPR, LEP, GHRL gene polymorphism, adiponectin, resistin, ghrelin, leptin

Metabolic syndrome is a combination of heritable cardio-metabolic abnormalities that in final leads to cardio-vascular complications and diabetes mellitus. With the advent of modern molecular genetics, different approaches have been used to identify the genetic causes of metabolic syndrome. Only a few genes with large effects have been discovered that

can explain the association between main metabolic disorders. The heterogeneity and multifactorial heritability of the metabolic syndrome; however, has been a challenge towards understanding the factors underlying the association of these traits. Metabolic syndrome varies in prevalence by geographic, racial, and ethnic background. These features are caused

by genetic factors, and up to 50% of all metabolic syndrome cases have been reported to be inherited. However, a common shared genetic cause has not been established to clarify the coexistence of their components and further investigation is needed.

It is known that obesity is defined as the abnormal or excessive accumulation of fat over the acceptable limits. Leptin is a metabolic hormone present in the circulation in amounts proportional to fat mass. Leptin reduces food intake and increases energy expenditure, thus regulating body weight and homeostasis. Various polymorphisms are present in the leptin gene and its receptor. These polymorphisms may be associated with obesity and metabolic syndrome.

Leptin and ghrelin are two important appetite and energy balance-regulating peptides. Common polymorphisms in the genes coding these peptides and their related receptors are shown to be associated with body weight, different markers of obesity, and metabolic abnormalities. The leptin (LEP) and leptin receptor (LEPR) genes have been investigated in the search for gene variants that are potentially related to the pathophysiology of obesity, diabetes, and associated complications. There are some conflicting results regarding the correlation of the LEPR gene with obesity. Some studies have found significant associations between obesity and polymorphisms of the LEPR gene, but some other studies failed to find significant correlations. It is possible that these inconclusive results might be due to differences in the studied populations.

The aim of this study was to evaluate changes in the metabolic hormones (leptin, ghrelin, adiponectin, resistin) levels in the blood of obese patients in relation to the GHRL (rs696217), LEP (rs7799039), LEPR (rs1137100, rs1137101, rs1805094) polymorphisms in the Ukrainian population.

## Material and Methods

**Study population.** This case-control study consists of 96 Ukrainians population, which was categorized as 53 obesity cases and 43 non-obesity subjects (controls). All 96 participants were considered as 42 males and 54 females. In this study, no significant age and sex differences were found between the individual groups.

Sex, height, body weight, and body mass index (BMI) were analyzed. The initial criteria were to sign the patient consent form and to draw the blood samples to enroll in this study. The inclusion criteria of obesity case recruitment for this study were based

on BMI levels. Patients with BMI <30 kg/m<sup>2</sup> were confirmed as inclusion criteria for control of this study and exclusion is as per elevated BMI levels.

**Laboratory data.** Blood samples taken for the study were obtained after patients' fasting the night before the study. Plasma level of glycated hemoglobin (HbA1c) was measured using commercial kits (Roche Diagnostics) using Hitachi automatic analyzer. Determination of serum levels was performed using Leptin ELISA kit (LDN Labor Diagnostics Nord GmbH & Co. KG, Germany), Human Ghrelin ELISA Kit (Thermo Fisher Scientific, USA), Human Adiponectin ELISA Kit (Thermo Fisher Scientific, USA), OxLDL (Thermo Fisher Scientific, USA), Resistin Human ELISA Kit (Thermo Fisher Scientific, USA) on Multiskan FC analyzer (Skanlt Software version 4.1 for Microplate Readers RE, ver. 4.1.0.43) at a wavelength of 620 nm.

**Genotyping of the GHRL, LEP and LEPR genes polymorphism (rs696217, rs7799039, rs1137100, rs1137101, rs1805094).** Venous blood samples for genomic study were collected in tubes with ethylenediaminetetraacetic acid (EDTA). The Thermo Scientific™ GeneJET™ Whole Blood Genomic DNA Purification Kit (Thermo Fisher Scientific, USA) was used for genomic DNA extraction according to the manufacturer's instructions. Pre-designed TaqMan™ SNP Genotyping Assays, Human, (Thermo Fisher Scientific, USA) were used for next SNPs: K109R (rs1137100), Q223R (rs1137101), K656N (rs1805094), G2548A (rs7799039), C214A (rs696217). TaqMan™ Universal Master Mix II, no UNG were used for DNA amplification using real-time polymerase chain reaction (PCR) technique of allele discrimination based on the magnitude of relative fluorescence units (RFU).

**Ethical details.** The ethical principles included in the Declaration of Human Rights adopted in Helsinki in 1975 and revised in 2008, were fully respected in this study. The enrolled subjects participated in this study voluntarily and with completed and signed written informed consent. Study protocol was approved by the Ethics Committee (Protocol No. 82) of I. Horbachevsky Ternopil National Medical University of October 04, 2023.

**Statistics.** Quantitative variables were assessed for normality using the Shapiro-Wilk test (when the number of subjects was less than 50) or the Kolmogorov-Smirnov test (when the number of subjects was more than 50). Quantitative variables following a normal distribution were described using mean (M) and standard deviation (SD), 95% confidence interval (95% CI) for the mean were estimated. Quantitative variables following

non-normal distribution were described using median (Me) and lower and upper quartiles (Q1–Q3). Comparison of frequencies in the analysis of 2 by 2 contingency tables was performed using Pearson's chi-square test (for expected values greater than 10), and Fisher's exact test (for expected values less than 10). ROC analysis was used to assess the diagnostic performance of quantitative variables in predicting a categorical outcome. The optimal cut-off value of the quantitative variable at was estimated using the Youden's J statistic. Comparisons of three or more groups on a quantitative variable whose distribution differed from normal were made using the Kruskal-Wallis test and Dunn's criterion with Holm correction as a *post-hoc* method. Mann-Whitney U-test was used to compare two groups on a quantitative variable whose distribution differed from the normal distribution. Comparison of frequencies in the analysis of 2 by 2 contingency tables was performed using Pearson's chi-square test (for expected values greater than 10), and Fisher's exact test (for expected values less than 10). Comparison of frequencies in the analysis of multifield contingency tables was performed using Pearson's chi-square test (for expected values greater than 10).

## Results

We performed analysis of allele conditioning on group (Table 1). We performed an analysis of allele G, T, and A of the GHRL (rs696217), LEP (rs7799039), LEPR (rs1137100, rs1137101, rs1805094) polymorphisms conditioning on the group in the Ukrainian population (Table 1).

According to the data obtained when comparing rs1137101 allele A and rs1137101 allele G, statistically significant differences were revealed depending on the group ( $p=0.009$ ,  $p=0.013$ , respectively). When comparing of rs1805094 allele C, rs1805094 allele G, rs7799039 allele A, rs7799039 allele G, rs1137100 allele A, rs1137100 allele G, rs696217 allele G, and rs696217 allele T, depending on the group, there were no statistically significant differences ( $p=0.658$ ,  $p=0.727$ ,  $p=0.257$ ,  $p=0.322$ ,  $p=0.460$ ,  $p=0.290$ ,  $p=0.448$ ,  $p=0.762$ , respectively). Analysis of genotype was performed conditioning on group (Table 1).

According to the presented Table 2, when comparing of rs1137101 genotype in the Ukrainian population, statistically significant differences were revealed depending on the group ( $p=0.008$ ) (applied method: Pearson's chi-square test). When comparing rs1805094 genotype, rs7799039 genotype, rs1137100 genotype, and rs696217 genotype, depending on

**Table 1**  
Analysis of allele conditioning on group

| Genotype           | Group     |            | p-value |
|--------------------|-----------|------------|---------|
|                    | Control   | Disease    |         |
| rs1805094 allele C | 31 (72.1) | 36 (67.9)  | 0.658   |
|                    | 12 (27.9) | 17 (32.1)  |         |
| rs1805094 allele G | 3 (7.0)   | 5 (9.4)    | 0.727   |
|                    | 40 (93.0) | 48 (90.6)  |         |
| rs7799039 allele A | 16 (37.2) | 14 (26.4)  | 0.257   |
|                    | 27 (62.8) | 39 (73.6)  |         |
| rs7799039 allele G | 7 (16.3)  | 13 (24.5)  | 0.322   |
|                    | 36 (83.7) | 40 (75.5)  |         |
| rs1137101 allele A | 3 (7.0)   | 15 (28.3)  | 0.009*  |
|                    | 40 (93.0) | 38 (71.7)  |         |
| rs1137101 allele G | 21 (48.8) | 13 (24.5)  | 0.013*  |
|                    | 22 (51.2) | 40 (75.5)  |         |
| rs1137100 allele A | 5 (11.6)  | 3 (5.7)    | 0.460   |
|                    | 38 (88.4) | 50 (94.3)  |         |
| rs1137100 allele G | 23 (53.5) | 34 (64.2)  | 0.290   |
|                    | 20 (46.5) | 19 (35.8)  |         |
| rs696217 allele G  | 1 (2.3)   | 0 (0.0)    | 0.448   |
|                    | 42 (97.7) | 53 (100.0) |         |
| rs696217 allele T  | 37 (86.0) | 47 (88.7)  | 0.762   |
|                    | 6 (14.0)  | 6 (11.3)   |         |

\*Differences are statistically significant ( $p<0.05$ ).

**Table 2**  
Analysis of genotype conditioning on group

| Genotype  | Categories | Group     |           | p-value |
|-----------|------------|-----------|-----------|---------|
|           |            | Control   | Disease   |         |
| rs1805094 | CC         | 3 (7.0)   | 5 (9.4)   | 0.877   |
|           | CG         | 9 (20.9)  | 12 (22.6) |         |
|           | GG         | 31 (72.1) | 36 (67.9) |         |
| rs7799039 | AA         | 7 (16.3)  | 13 (24.5) | 0.429   |
|           | AG         | 20 (46.5) | 26 (49.1) |         |
|           | GG         | 16 (37.2) | 14 (26.4) |         |
| rs1137101 | AA         | 21 (48.8) | 13 (24.5) | 0.008*  |
|           | AG         | 19 (44.2) | 25 (47.2) |         |
|           | GG         | 3 (7.0)   | 15 (28.3) |         |
| rs1137100 | AA         | 23 (53.5) | 34 (64.2) | 0.442   |
|           | AG         | 15 (34.9) | 16 (30.2) |         |
|           | GG         | 5 (11.6)  | 3 (5.7)   |         |
| rs696217  | GG         | 37 (86.0) | 47 (88.7) | 0.534   |
|           | GT         | 5 (11.6)  | 6 (11.3)  |         |
|           | TT         | 1 (2.3)   | 0 (0.0)   |         |

\*Differences are statistically significant ( $p<0.05$ ).

the group, no statistically significant differences were revealed ( $p=0.877$ ,  $p=0.429$ ,  $p=0.442$ ,  $p=0.534$ , respectively).

We performed an analysis of the genotype of the GHRL (rs696217), LEP (rs7799039), and LEPR (rs1137100, rs1137101, rs1805094) polymorphisms conditioning on obesity degree in the Ukrainian population (Table 3). In accordance with the presented table, when comparing of rs1137101 genotype, statistically significant differences were revealed depending on obesity degree ( $p=0.002$ ) (applied method: Pearson's chi-square test). When comparing rs1805094 genotype, rs7799039 genotype, rs1137100 genotype, and rs696217 genotype, depending on obesity degree, there were no statistically significant differences ( $p=0.284$ ,  $p=0.620$ ,  $p=0.499$ ,  $p=0.919$ , respectively).

We performed analysis of resistin conditioning on genotype of the GHRL (rs696217), LEP (rs7799039), LEPR (rs1137100, rs1137101, rs1805094) polymorphisms conditioning in the Ukrainian population (Table 4). According to the data obtained, when comparing of resistin, statistically significant differences were revealed depending on rs1137101 genotype ( $p=0.002$ ). When comparing of resistin, depending on rs1805094 genotype, rs7799039 genotype, rs1137100 genotype, and rs696217 genotype, no statistically significant differences

were revealed ( $p=0.619$ ,  $p=0.114$ ,  $p=0.207$ ,  $p=0.453$ , respectively).

When evaluating the dependence of the probability of yes rs1137101 allele A on the resistin using the ROC analysis, the following curve was obtained (Figure 1). The area under the ROC curve comprised  $0.755 \pm 0.070$  with 95%, CI: 0.618–0.893. The resulting model was statistically significant ( $p<0.001$ ). The cut-off value of resistin, corresponding to the highest Youden's J statistic, is 8.900. If resistin was less than this value, yes was predicted. The sensitivity and specificity of the method were 85.9% and 61.1%, respectively. Therefore, there is a relationship between the allele A of LEPR (rs1137101) polymorphism and the resistin level in the Ukrainian population.

When evaluating the dependence of the probability of yes rs1137101 allele G on the resistin using the ROC analysis, the following curve was obtained (Figure 2). The area under the ROC curve comprised  $0.643 \pm 0.057$  with 95%, CI: 0.531–0.755. The resulting model was statistically significant ( $p=0.021$ ). The cut-off value of resistin, corresponding to the highest Youden's J statistic, is 7.300. If resistin was greater than or equal to this value, yes was predicted. The sensitivity and specificity of the method were 59.7% and 70.6%, respectively. Therefore, there is a relationship between the allele G of LEPR (rs1137101) polymorphism and the resistin level in the Ukrainian population.

**Table 3**  
Analysis of genotype conditioning on obesity degree

| Genotype  | Categories | Obesity degree |            |            |           | p-value |
|-----------|------------|----------------|------------|------------|-----------|---------|
|           |            | no             | 1st degree | 2nd degree | 3d degree |         |
| rs1805094 | CC         | 3 (7.0)        | 0 (0.0)    | 2 (11.1)   | 3 (10.0)  | 0.284   |
|           | CG         | 9 (20.9)       | 2 (40.0)   | 7 (38.9)   | 3 (10.0)  |         |
|           | GG         | 31 (72.1)      | 3 (60.0)   | 9 (50.0)   | 24 (80.0) |         |
| rs7799039 | AA         | 7 (16.3)       | 2 (40.0)   | 3 (16.7)   | 8 (26.7)  | 0.620   |
|           | AG         | 20 (46.5)      | 3 (60.0)   | 9 (50.0)   | 14 (46.7) |         |
|           | GG         | 16 (37.2)      | 0 (0.0)    | 6 (33.3)   | 8 (26.7)  |         |
| rs1137101 | AA         | 21 (48.8)      | 2 (40.0)   | 5 (27.8)   | 6 (20.0)  | 0.002*  |
|           | AG         | 19 (44.2)      | 2 (40.0)   | 12 (66.7)  | 11 (36.7) |         |
|           | GG         | 3 (7.0)        | 1 (20.0)   | 1 (5.6)    | 13 (43.3) |         |
| rs1137100 | AA         | 23 (53.5)      | 3 (60.0)   | 14 (77.8)  | 17 (56.7) | 0.499   |
|           | AG         | 15 (34.9)      | 1 (20.0)   | 4 (22.2)   | 11 (36.7) |         |
|           | GG         | 5 (11.6)       | 1 (20.0)   | 0 (0.0)    | 2 (6.7)   |         |
| rs696217  | GG         | 37 (86.0)      | 5 (100.0)  | 16 (88.9)  | 26 (86.7) | 0.919   |
|           | GT         | 5 (11.6)       | 0 (0.0)    | 2 (11.1)   | 4 (13.3)  |         |
|           | TT         | 1 (2.3)        | 0 (0.0)    | 0 (0.0)    | 0 (0.0)   |         |

\*Differences are statistically significant ( $p<0.05$ ).

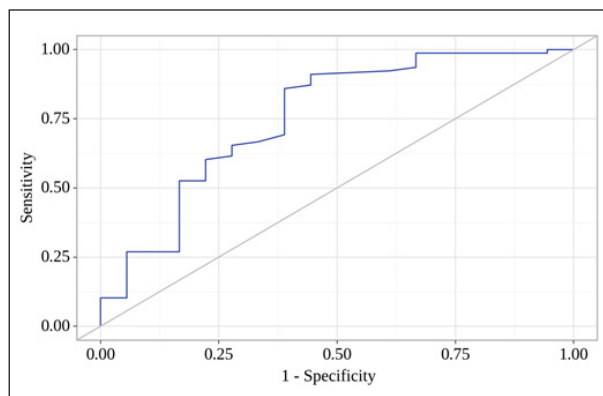


**Figure 1.** Receiver operating characteristic (ROC) curve characterizing the dependence of the probability rs1137101 allele A on resistin (ng/ml).

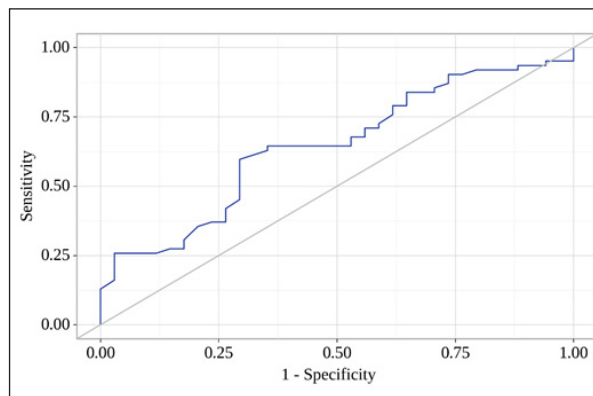


**Figure 2.** Receiver operating characteristic (ROC) curve characterizing the dependence of the probability rs1137101 allele G on resistin (ng/ml).

Analysis of adiponectin was performed conditioning on the GHRL (rs696217), LEP (rs7799039), and LEPR (rs1137100, rs1137101, rs1805094) polymorphisms conditioning in the Ukrainian population (Table 5). In accordance with the presented table, when comparing of adiponectin, statistically significant differences were revealed depending on rs1137101 genotype ( $p=0.004$ ). When comparing of adiponectin, depending on rs1805094 genotype, rs7799039 genotype, rs1137100 genotype, rs696217 genotype, there were no statistically significant differences ( $p=0.800$ ,  $p=0.649$ ,  $p=0.362$ ,  $p=0.235$ , respectively).

When evaluating the dependence of the probability of yes rs1137101 allele A on the adiponectin using the ROC analysis, the following curve was obtained (Figure 3). The area under the ROC curve comprised  $0.737 \pm 0.058$  with 95%, CI: 0.623–0.850. The resulting model was statistically significant ( $p=0.002$ ). The cut-off value of adiponectin, corresponding to the highest Youden's J statistic, is 6.400. If adiponectin was greater than or equal to this value, yes was predicted. The sensitivity and specificity of the method were 70.5% and 77.8%, respectively. Therefore, there is a relationship between the allele A of LEPR (rs1137101) polymorphism and the adiponectin level in the Ukrainian population.

When evaluating the dependence of the probability of yes rs1137101 allele G on the adiponectin using the ROC analysis, the following curve was obtained (Figure 4). The area under the ROC curve comprised  $0.637 \pm 0.061$  with 95%, CI: 0.518–0.756. The resulting model was statistically significant ( $p=0.027$ ). The cut-off value of adiponectin corresponding to the highest Youden's J statistic, is 8.200. If adiponectin

**Table 4**  
Analysis of resistin (ng/ml) conditioning on genotype

| Genotype  | Categories | Resistin (ng/ml) |                                |    | p-value                                          |
|-----------|------------|------------------|--------------------------------|----|--------------------------------------------------|
|           |            | Me               | Q <sub>1</sub> –Q <sub>3</sub> | n  |                                                  |
| rs1805094 | CC         | 8                | 5–9                            | 8  | 0.619                                            |
|           | CG         | 7                | 4–8                            | 21 |                                                  |
|           | GG         | 7                | 4–9                            | 67 |                                                  |
| rs7799039 | AA         | 8                | 5–9                            | 20 | 0.114                                            |
|           | AG         | 7                | 4–9                            | 46 |                                                  |
|           | GG         | 5                | 4–8                            | 30 |                                                  |
| rs1137101 | AA         | 5                | 4–8                            | 34 | 0.002*<br>$p_{GG-AA}=0.001$<br>$p_{GG-AG}=0.013$ |
|           | AG         | 7                | 4–8                            | 44 |                                                  |
|           | GG         | 9                | 8–10                           | 18 |                                                  |
| rs1137100 | AA         | 8                | 4–9                            | 57 | 0.207                                            |
|           | AG         | 7                | 4–8                            | 31 |                                                  |
|           | GG         | 4                | 3–8                            | 8  |                                                  |
| rs696217  | GG         | 7                | 4–9                            | 84 | 0.453                                            |
|           | GT         | 7                | 4–9                            | 11 |                                                  |
|           | TT         | 4                | 4–4                            | 1  |                                                  |

Abbreviations: Me – median; n – number of cases; Q<sub>1</sub>–Q<sub>3</sub> – lower and upper quartiles. \*Differences are statistically significant ( $p<0.05$ ).

was less than this value, yes was predicted. The sensitivity and specificity of the method were 64.5% and 64.7%, respectively. Therefore, there is a relationship between the allele G of LEPR (rs1137101) polymorphism and the adiponectin level in the Ukrainian population.

We performed analysis of ghrelin general conditioning on genotype of the GHRL (rs696217), LEP (rs7799039), and LEPR (rs1137100, rs1137101,

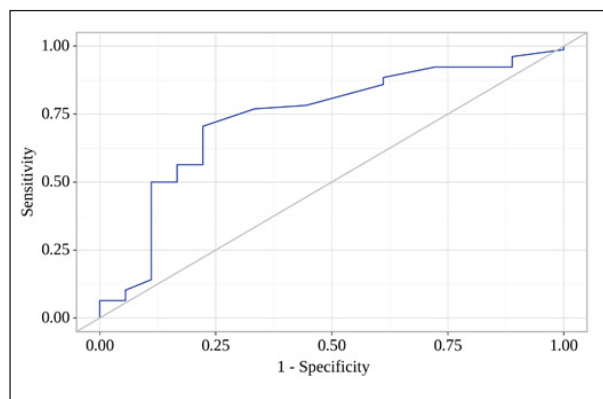


**Figure 3.** Receiver operating characteristic (ROC) curve characterizing the dependence of the probability rs1137101 allele A on adiponectin (µg/ml).

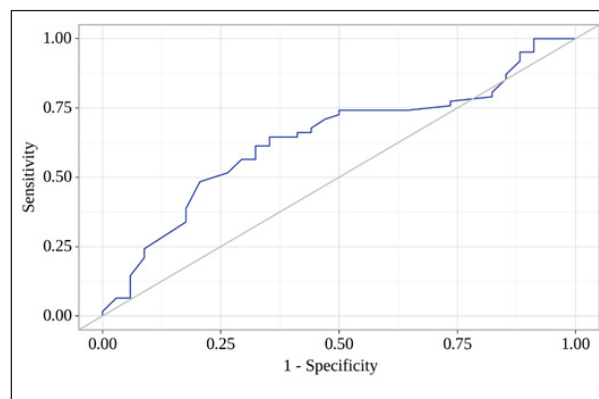


**Figure 4.** Receiver operating characteristic (ROC) curve characterizing the dependence of the probability rs1137101 allele G on adiponectin (µg/ml).

| Table 5<br>Analysis of adiponectin (µg/ml) conditioning on genotype |            |                     |                                |    |                                                                  |
|---------------------------------------------------------------------|------------|---------------------|--------------------------------|----|------------------------------------------------------------------|
| Genotype                                                            | Categories | Adiponectin (µg/ml) |                                |    | p-value                                                          |
|                                                                     |            | Me                  | Q <sub>1</sub> -Q <sub>3</sub> | n  |                                                                  |
| rs1805094                                                           | CC         | 7                   | 6-10                           | 8  | 0.800                                                            |
|                                                                     | CG         | 7                   | 6-10                           | 21 |                                                                  |
|                                                                     | GG         | 7                   | 6-10                           | 67 |                                                                  |
| rs7799039                                                           | AA         | 6                   | 6-9                            | 20 | 0.649                                                            |
|                                                                     | AG         | 7                   | 6-10                           | 46 |                                                                  |
|                                                                     | GG         | 9                   | 6-10                           | 30 |                                                                  |
| rs1137101                                                           | AA         | 9                   | 6-10                           | 34 | 0.004*<br>p <sub>GG-AA</sub> =0.003<br>p <sub>GG-AG</sub> =0.025 |
|                                                                     | AG         | 7                   | 6-10                           | 44 |                                                                  |
|                                                                     | GG         | 6                   | 6-6                            | 18 |                                                                  |
| rs1137100                                                           | AA         | 7                   | 6-10                           | 57 | 0.362                                                            |
|                                                                     | AG         | 8                   | 6-10                           | 31 |                                                                  |
|                                                                     | GG         | 10                  | 7-10                           | 8  |                                                                  |
| rs696217                                                            | GG         | 7                   | 6-10                           | 84 | 0.235                                                            |
|                                                                     | GT         | 6                   | 5-10                           | 11 |                                                                  |
|                                                                     | TT         | 10                  | 10-10                          | 1  |                                                                  |

Abbreviations: Me - median; n - number of cases; Q<sub>1</sub>-Q<sub>3</sub> - lower and upper quartiles. \*Differences are statistically significant (p<0.05).

rs1805094) polymorphisms conditioning in the Ukrainian population (Table 6). Statistically significant differences were revealed when comparing of ghrelin general depending on rs1137101 genotype (p=0.008). When comparing of ghrelin general depending on rs1805094 genotype, rs7799039 genotype, rs1137100 genotype, rs696217 genotype, no statistically significant differences were revealed (p=0.517, p=0.791, p=0.392, p=0.560, respectively).

When evaluating the dependence of the probability of yes rs1137101 allele A on the ghrelin general using the ROC analysis, the following curve was obtained (Figure 5). The area under the ROC curve comprised  $0.673 \pm 0.065$  with 95% CI: 0.546–0.800. The resulting model was statistically significant (p=0.022). The cut-off value of ghrelin general, corresponding to the highest Youden's J statistic, is 826.900. If ghrelin general was greater than or equal to this value, yes was predicted. The sensitivity and specificity of the method were 66.7% and 72.2%, respectively. Therefore, there is a relationship between the allele A of LEPR (rs1137101) polymorphism and the ghrelin level in the Ukrainian population.

When evaluating the dependence of the probability of yes rs1137101 allele G on the ghrelin general using the ROC analysis, the following curve was obtained (Figure 6). The area under the ROC curve comprised  $0.672 \pm 0.059$  with 95% CI: 0.556–0.788. The resulting model was statistically significant (p=0.005). The cut-off value of ghrelin general, corresponding to the highest Youden's J statistic, is 749.200. If ghrelin general was less than this value, yes was predicted. The sensitivity and specificity of the method were 48.4% and 85.3%, respectively. Therefore, there is a relationship between the allele G of LEPR (rs1137101) polymorphism and the ghrelin level in the Ukrainian population.

Analysis of leptin was performed conditioning on genotype of the GHRL (rs696217), LEP (rs7799039), LEPR (rs1137100, rs1137101, rs1805094) polymorphisms conditioning in the Ukrainian population (Table 7). According to the data obtained when comparing of leptin, statistically significant differences were revealed depending on rs1137101

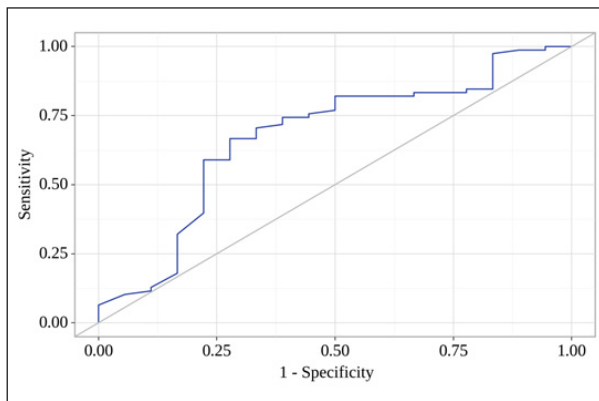


**Figure 5.** Receiver operating characteristic (ROC) curve characterizing the dependence of the probability rs1137101 allele A on ghrelin general (ng/ml).

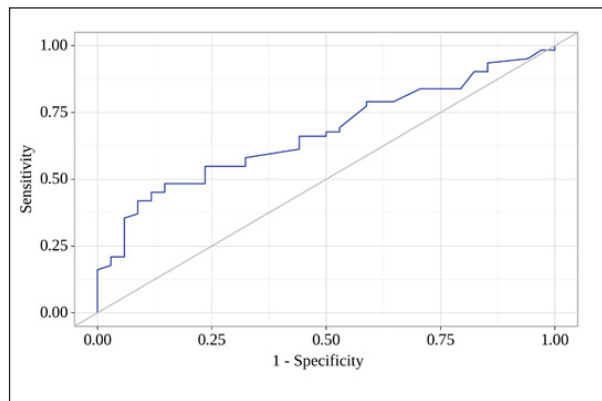


**Figure 6.** Receiver operating characteristic (ROC) curve characterizing the dependence of the probability rs1137101 allele G on ghrelin general (ng/ml).

**Table 6**  
Analysis of ghrelin general (ng/ml) conditioning on genotype

| Genotype  | Categories | Ghrelin general (ng/ml) |                                       |    | p-value                             |
|-----------|------------|-------------------------|---------------------------------------|----|-------------------------------------|
|           |            | M±SD/Me                 | 95% CI/Q <sub>1</sub> –Q <sub>3</sub> | n  |                                     |
| rs1805094 | CC         | 826±255                 | 613–1038                              | 8  | 0.517                               |
|           | CG         | 876±237                 | 769–984                               | 21 |                                     |
|           | GG         | 932±380                 | 840–1025                              | 67 |                                     |
| rs7799039 | AA         | 899±367                 | 727–1071                              | 20 | 0.791                               |
|           | AG         | 896±377                 | 784–1008                              | 46 |                                     |
|           | GG         | 943±275                 | 841–1046                              | 30 |                                     |
| rs1137101 | AA         | 998                     | 871–1241                              | 34 | 0.008*<br>p <sub>GG-AA</sub> =0.009 |
|           | AG         | 908                     | 606–1106                              | 44 |                                     |
|           | GG         | 634                     | 606–871                               | 18 |                                     |
| rs1137100 | AA         | 894±328                 | 807–982                               | 57 | 0.392                               |
|           | AG         | 901±386                 | 759–1042                              | 31 |                                     |
|           | GG         | 1071±261                | 852–1289                              | 8  |                                     |
| rs696217  | GG         | 909                     | 644–1153                              | 84 | 0.560                               |
|           | GT         | 786                     | 606–1195                              | 11 |                                     |
|           | TT         | 1168                    | 1168–1168                             | 1  |                                     |

Abbreviations: M – mean; Me – median; n – number of cases; SD – standard deviations; Q<sub>1</sub>–Q<sub>3</sub> – lower and upper quartiles. \*Differences are statistically significant (p<0.05).

genotype (p<0.001). When comparing of leptin, depending on rs1805094 genotype, rs7799039 genotype, rs1137100 genotype, rs696217 genotype, there were no statistically significant differences (p=0.644, p=0.657, p=0.738, p=0.411, respectively).

When evaluating the dependence of the probability of yes rs1137101 allele A on the leptin using the ROC analysis, the following curve was obtained (Figure 7). The area under the ROC curve comprised 0.781±0.068

with 95% CI: 0.648–0.914. The resulting model was statistically significant (p<0.001). The cut-off value of leptin, corresponding to the highest Youden's J statistic, is 23.610. If leptin was less than this value, yes was predicted. The sensitivity and specificity of the method were 69.2% and 83.3%, respectively. Therefore, there is a relationship between the allele A of LEPR (rs1137101) polymorphism and the leptin level in the Ukrainian population.

When evaluating the dependence of the probability of yes rs1137101 allele G on the leptin using the ROC analysis, the following curve was obtained (Figure 8). The area under the ROC curve comprised  $0.634 \pm 0.058$  with 95% CI: 0.522–0.747. The resulting model was statistically significant ( $p=0.030$ ). The cut-off value of leptin, corresponding to the highest Youden's J statistic is, 11.060. If leptin was greater than or equal to this value, yes was predicted. The sensitivity and specificity of the method were 64.5%

and 64.7%, respectively. Therefore, there is a relationship between the allele G of LEPR (rs1137101) polymorphism and the leptin level in the Ukrainian population.

Therefore, the genotype (GG, AG, AA) of the LEPR gene polymorphism (rs1137101) does have a statistically significant effect on the level of leptin, ghrelin, adiponectin, and resistin in the serum of patients diagnosed with the metabolic syndrome in the Ukrainian population.

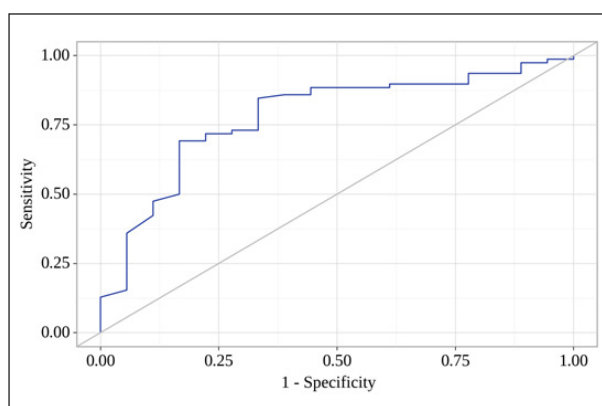


**Figure 7.** Receiver operating characteristic (ROC) curve characterizing the dependence of the probability rs1137101 allele A on leptin (ng/ml).

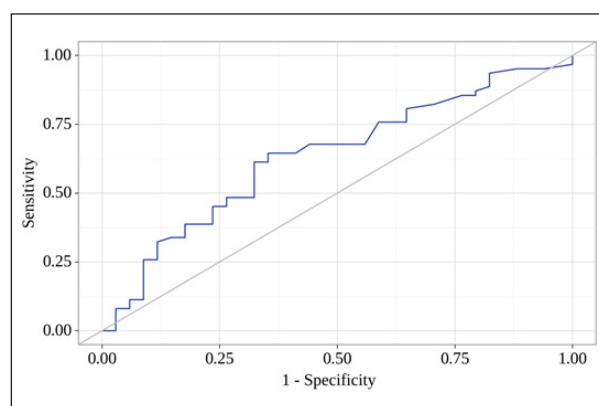


**Figure 8.** Receiver operating characteristic (ROC) curve characterizing the dependence of the probability rs1137101 allele G on leptin (ng/ml).

**Table 7**  
Analysis of leptin (ng/ml) conditioning on genotype

| Genotype  | Categories | Leptin (ng/ml) |                                |    | p-value                                                           |
|-----------|------------|----------------|--------------------------------|----|-------------------------------------------------------------------|
|           |            | Me             | Q <sub>1</sub> –Q <sub>3</sub> | n  |                                                                   |
| rs1805094 | CC         | 19             | 6–42                           | 8  | 0.644                                                             |
|           | CG         | 18             | 5–31                           | 21 |                                                                   |
|           | GG         | 12             | 6–43                           | 67 |                                                                   |
| rs7799039 | AA         | 20             | 6–42                           | 20 | 0.657                                                             |
|           | AG         | 14             | 6–39                           | 46 |                                                                   |
|           | GG         | 7              | 5–43                           | 30 |                                                                   |
| rs113710  | AA         | 7              | 5–25                           | 34 | <0.001*<br>p <sub>GG-AA</sub> <0.001<br>p <sub>GG-AG</sub> =0.003 |
|           | AG         | 15             | 6–37                           | 44 |                                                                   |
|           | GG         | 48             | 27–54                          | 18 |                                                                   |
| rs1137100 | AA         | 16             | 6–40                           | 57 | 0.738                                                             |
|           | AG         | 12             | 5–48                           | 31 |                                                                   |
|           | GG         | 7              | 5–30                           | 8  |                                                                   |
| rs696217  | GG         | 15             | 5–42                           | 84 | 0.411                                                             |
|           | GT         | 16             | 6–52                           | 11 |                                                                   |
|           | TT         | 5              | 5–5                            | 1  |                                                                   |

Abbreviations: Me – median; n – number of cases; Q<sub>1</sub>–Q<sub>3</sub> – lower and upper quartiles. \*Differences are statistically significant ( $p<0.05$ ).

## Discussion

Abdominal obesity coupled with polygenic hereditary defects is considered the initial event in the development of metabolic syndrome. Obesity is a global epidemic disease representing the fifth leading cause of death in the world. It was shown that it is caused by the interaction between environmental factors. It is known that leptin acts via its receptors. So, when fat cells increase, the leptin level increases proportionally and binds to leptin receptors in the hypothalamus. Several polymorphisms of both genes for leptin and leptin receptors have been studied in different populations for their potential association with obesity. For example, LEP G2548A, K109R, Q223R, K656N SNPs have been studied in detail by Aller *et al.* (2023), Mohanraj *et al.* (2022), Bilge *et al.* (2021), Hastuti *et al.* (2016).

In Indonesia population, polymorphisms of rs1137100 (K109R) and rs1137101 (Q223R) LEPR gene were associated with obesity. Foucan *et al.* (2019) genotypic analysis of three LEPR polymorphisms (K109R, Q223R, and K656N) population has indicated individuals carrying four variant alleles of the three SNPs had a significantly higher risk of obesity (OR 2.86;  $P=0.032$ ) than those carrying none variant allele. The results also suggest an influence of K656N polymorphism in the LEPR gene on obesity and abdominal obesity in this Afro-Caribbean.

Dagdan *et al.* (2018) have indicated that the LEP G2548A polymorphism was a relevant metabolic syndrome marker. Although the LEP/LEPR genotype combination was not observed to increase the risk of obesity. There is also a study aimed to show the association of leptin (+19) AG, leptin (2548) GA, and Gln223Arg leptin receptor polymorphisms with obesity and metabolic syndrome in Turkish children aged 6–17 years.

On the other hand, LEPR K109R, K656N, and LEP G2548A polymorphisms were not shown to be associated with obesity according to the results of Yarim *et al.* (2022). However, in the obese group, LEPR Q223R polymorphism has been found to have lower rate of AA genotype, higher rate of genotype and higher rate of G allele, suggesting that this polymorphism could be considered susceptible to obesity.

Dieguez-Campa *et al.* (2020) study has shown that leptin levels were associated with obesity in Mexican young adults, but this was not related to the presence of the Q223R polymorphism in the LEPR gene. Leptin and leptin receptor polymorphisms were not associated with BMI, plasma leptin, sleep,

eating behavior, or psychological state in Malaysian population.

The results of Taghizadeh *et al.* (2017) have shown that SNPs in LEP gene and LEPR gene including A19G and K109R were unlikely related to obesity and diabetes in Iranian people. The same results correspond with Than *et al.* (2022) data, in which the K109R, Q223R, and K656N polymorphisms of the LEPR gene were not linked to obesity in the population of Myanmar.

There is a study of Bouafi *et al.* (2023) indicating an association between the LEP gene polymorphisms rs7799039 and rs11761556 with obesity in Moroccan individuals. No significant association was found between the polymorphisms rs7799039 and rs11761556 and the risk of obesity. The comparison of biochemical and clinical parameters between the genotypes of the rs7799039 polymorphism, showed a significant increased triglycerides levels in carriers of AA or GA genotypes ( $P=0.040$ ). The meta-analysis showed no significant association between the rs7799039 polymorphism and obesity under all genetic models. Finally, the case-control study and meta-analysis demonstrated that the LEP gene polymorphisms rs7799039 and rs11761556 cannot be considered as genetic risk factors for obesity.

Thus, the association of these polymorphisms with metabolic syndrome is still controversial. In addition, there have been contradictory findings regarding an association of metabolic syndrome with the G2548A polymorphism of the LEP gene and with the A223G polymorphism of the LEPR gene.

## Conclusions

We established a significant effect of the presence of the A allele and G allele of the LEPR gene polymorphism (rs1137101) on the level of leptin, ghrelin, adiponectin, and resistin in the serum of patients diagnosed with the metabolic syndrome in the Ukrainian population. On the other hand, the study of the influence of genotypes of the GHRL (rs696217), LEP (rs7799039), and LEPR (rs1137100, rs1805094) polymorphisms on the level of metabolic hormones (leptin, ghrelin, adiponectin, resistin) in the blood of obese patients did not show reliably significant results.

Thus, the presence of the LEPR genes (rs1137101) polymorphism in the Ukrainian population indicates an increased risk of developing the metabolic syndrome regardless of the homozygous or heterozygous genotype (genotypes AA, AG, GG).

### Acknowledgements

The authors would like to thank all our participants and technical staff of hospitals for their cooperation. This work was supported by the state budget of Ukraine as part of the scientific work on

the topic “Newest methods of surgical treatment of metabolic syndrome”, No. 0123U100300.

**Conflict of interest:** The authors declare no conflict of interest.

### References

- Aller R, Primo Martin D, Izaola O, Martinez JA, de Luis Roman D. Association of the leptin receptor rs 1805134 polymorphism with obesity parameters, dietary intakes, and metabolic syndrome in Caucasian obese subjects. *Nutr Hosp* 40, 35–40, 2023.
- Bilge S, Yilmaz R, Karaslan E, Ozer S, Ates O, Ensari E, Demir O. The relationship of leptin (+19) AG, leptin (2548) GA, and leptin receptor Gln223Arg gene polymorphisms with obesity and metabolic syndrome in obese children and adolescents. *Pediatr Gastroenterol Hepatol Nutr* 24, 306–315, 2021.
- Bouafi H, Krami AM, Morjane I, Slaoui K, Harmak H, Charoute H, Saile R, Barakat A. Genetic association of LEP gene polymorphisms with obesity in Moroccan individuals: Case-control study and updated meta-analysis. *Biochem Genet* 61, 1758–1774, 2023.
- Dagdan B, Chuluun-Erdene A, Sengeragchaa O, Malchinkhuu M, Janlav M. Leptin gene G2548A polymorphism among Mongolians with metabolic syndrome. *Med Sci (Basel)* 7, 3, 2018.
- Dieguez-Campa CE, Angel-Chavez LI, Reyes-Ruvalcaba D, Talavera-Zermeno MJ, Armendariz-Cabral DA, Torres-Muro D, Perez-Neri I. Leptin levels and Q223R leptin receptor gene polymorphism in obese Mexican young adults. *EJIFCC* 31, 197–207, 2020.
- Foucan L, Bassien-Capsa V, Rambhojan C, Lacorte JM, Larifla L. Influence of K656N polymorphism of the leptin receptor gene on obesity-related traits in nondiabetic Afro-Caribbean individuals. *Metab Syndr Relat Disord* 17, 197–203, 2019.
- Hastuti P, Zukhrufia I, Padwaswari MH, Nuraini A, Sadewa AH. Polymorphism in leptin receptor gene was associated with obesity in Yogyakarta, Indonesia. *Egypt J Med Hum Gen* 17, 271–276, 2016.
- Mohanraj J, D’Souza UJ, Fong, SY, Karkada IR, Jaiprakash H. Association between leptin (G2548A) and leptin receptor (Q223R) polymorphisms with plasma leptin, BMI, stress, sleep and eating patterns among the multi-ethnic young Malaysian adult population from a healthcare university. *Int J Environ Res Public Health* 19, 8862, 2022.
- Taghizadeh H, Abdolkarimi H, Bazireh H, Houshmand R, Shahbazi Z, Mohammadi S, Taghizadeh E. Association study of leptin and leptin receptor gene polymorphisms with diabetes type 2 and obesity. *Health Biotechnol Biopharma* 1, 61–69, 2017.
- Than MM, Soe YN, Lwin ZM, Kyaw YW, Paing KT, Oo KK, Htut ZM. Association of leptin receptor gene polymorphisms with blood glucose concentration and obesity. *Biochem Mol Biol* 7, 54–60, 2022.
- Yarim AK, Rasulova K, Catli G, Kume T, Abaci A, Kizildag S. Leptin and leptin receptor gene polymorphisms in obese and healthy children. *Cukurova Med J* 47, 71–78, 2022.