

Effect of the GHRL gene (rs696217) polymorphism on the metabolic disorders in patients with obesity in the Ukrainian population

Andrii PRODAN¹, Ihor DZUBANOVSKY¹, Oleksandr KAMYSHNYI², Natalia MELNYK³,
Stepan GRYTSENKO⁴, Stanislava VOLOSHYN⁵

¹Department of Surgery of Postgraduate Faculty, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine; ²Department of Microbiology, Virology, and Immunology, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine; ³Department of General Hygiene and Ecology, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine; ⁴Department of Surgery No1, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine; ⁵Department of Pediatrics No2, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine
E-mail: prodan@tdmu.edu.ua

Objective. Over the past four decades, the prevalence of obesity has tripled and limited genetic studies with specific SNPs have been conducted, but no investigations using ghrelin and obestatin prepropeptide (GHRL) gene have been reported in the Ukrainians population. The aim of this study was to evaluate changes in the level of metabolic hormones in the blood of obese patients in relation to the GHRL (rs696217) polymorphism.

Methods. The study involved 53 obesity cases and 48 non-obesity subjects (controls). The GHRL (rs696217) polymorphism was genotyped using a TaqMan real-time polymerase chain reaction method. Blood hormones were determined with commercially available kits using a Multiskan FC analyzer.

Results. Carriers of the T allele of the GHRL (rs696217) polymorphism were statistically significantly more in patients diagnosed with obesity compared to controls indicating a genetically determined cause of obesity. We also established a significant effect of the presence of the T allele of the GHRL (rs696217) polymorphism on the decrease in the adiponectin level and the increase of resistin level in obese patients. The study of the effect of genotypes (TT, GT, GG) of the GHRL (rs696217) polymorphism on the metabolic hormone levels in the blood of obese patients did not show reliably significant differences.

Conclusions. The presence of the T allele of the GHRL (rs696217) polymorphism in Ukrainian population indicates an increased risk of the obesity development regardless on the homozygous or heterozygous genotype.

Key words: obesity, GHRL polymorphism, adiponectin, resistin, ghrelin, leptin

The prevalence of metabolic syndrome clearly increases with the age (from 6.7% among National Health and Nutrition Examination Survey III participants aged 20–29 years to 43.0% among participants aged 60 years and older), an important phenotypic feature in modulating genetic factors (Flanagan et al. 2021). The widespread prevalence of metabolic

syndrome and its role as a predictor primarily of cardiovascular diseases determines the intensity of scientific research in the key links of pathogenesis, issues of diagnosis, prevention, and treatment of this disease.

Over the past four decades, the prevalence of obesity has tripled. Polygenic (or common) obesity

and the rare severe early-onset monogenic obesity are often polarized as separate diseases. However, studies of the genes for both forms of obesity show that they share a common genetic and biological basis indicating a key role for the brain in controlling the body weight (Kuzuya et al. 2006; Kamyshna et al. 2022).

Although the environment changes have undoubtedly led to the rapid increase in the prevalence of metabolic syndrome, obesity is the result of an interaction between environmental and innate biological factors. Most importantly, there is a strong genetic component underlying the large interindividual variation in the body weight that determines how people respond to this “obesogenic” environment (Korbonits et al. 2002; Marushchak et al. 2023).

It is known that the ghrelin and obestatin prepropeptide (GHRL) gene is located on chromosome 3p25-26 and has five exons and four introns. The GHRL coding and noncoding areas contain at least 300 SNPs, four of which have been linked to pathogenicity. GHRL expression can be affected by high-fat diet, somatostatin, and parasympathetic stimulation. Limited genetic studies with specific SNPs have been conducted in the Ukrainians population and no GHRL gene investigations have been reported. Based on SNP rs696217, this study was designed to investigate the function of these SNP in the GHRL gene in obese Ukraine subjects (Dantas et al. 2011).

The aim of this study was to evaluate changes in the metabolic hormone levels (leptin, ghrelin, adiponectin, and resistin) in the blood of obese patients in relation to the GHRL (rs696217) polymorphism.

Materials and Methods

Study population. This case-control study consists of 101 Ukrainians categorized as 53 obesity and 48 non-obesity subjects (controls). From all 101 participants, 45 were males and 56 females. The obesity cases consisted of 28 females and 25 males, and controls of 23 males and 25 females. In this study, no significant age and sex differences were found between the individual groups.

Sex, age, 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 for the recruitment in this study was based on BMI levels. Patient with $<30 \text{ kg/m}^2$ was confirmed as inclusion criteria for control of this study and exclusion is as per elevated BMI levels.

Laboratory data. Blood samples were obtained after patients overnight fasting. Plasma level of glycated hemoglobin (HbA1c) was measured using commercial kits (Roche Diagnostics) using Hitachi automatic analyzer. Determination of serum metabolic hormone levels was performed using Leptin ELISA Kit (LDN Labor Diagnostics Nord GmbH and 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 620 nm wavelength.

Genotyping of the GHRL gene polymorphism (rs696217). DNA isolation from venous blood of patients was collected in a sterile vacutainer and stabilized with K2EDTA. Total DNA was isolated from peripheral blood using the GeneJET Whole Blood Genomic DNA Purification Mini Kit (K0781, Thermo Fisher Scientific, USA) following the manufacturer's instructions.

DNA amplification and genotyping. All specimens were genotyped applying TaqMan probes and TaqMan Genotyping Master Mix (4371355) on CFX96™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., USA). Polymerase chain reaction (PCR) for TaqMan genotyping was conducted adhering to the requirements enclosed in the kit (Applied Biosystems, USA). TaqMan Genotyping Master Mix contains DNA polymerase AmpliTaq Gold®, dNTPs, reference dye ROX™, and the composition of buffers. TaqMan probes are target-specific oligonucleotides with reporter dyes labeled at the 5' end of each probe: (VIC® dye at the 5' end of the Allele 1 probe and 6 FAM™ dye at the 5' end of the Allele 2 probe), and a non-fluorescent quencher (NFQ) labeled at the 3' end of the probe. Genomic DNA was intensified in a 10 µl reaction mix comprising genomic DNA, forward and reverse primers, fluorescent probes, and TaqMan Genotyping Master Mix. Genotyping of the specimens was conducted on the CFX-Maestro™ software applying the 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.

72) of I. Horbachevsky Ternopil National Medical University of January 6, 2023.

Statistical analysis. 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). A receiver operating characteristic (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.

Results

According to the data obtained, when comparing of obesity degree, statistically significant differences were revealed depending on the group ($p < 0.001$), while patients with 1st degree of obesity were the least, the patients with 3rd degree of obesity the most (Figure 1).

Analysis of the quantitative indicators was performed conditioning on the group (Table 1). There were statistically significantly higher indicators of height, weight, BMI, and HbA1c levels compared to controls.

Table 1

Analysis of quantitative indicators conditioning on group

Variables	Categories	Group			p-value
		Median	Q1–Q3	n	
Height (m)	Control	2	2–2	48	0.042*
	Disease	2	2–2	53	
Weight (kg)	Control	68	60–70	48	<0.001*
	Disease	118	104–135	53	
BMI (kg/m ²)	Control	22	22–23	48	<0.001*
	Disease	41	37–45	53	
HbA1c (%)	Control	5	5–6	48	<0.001*
	Disease	6	6–6	53	

Abbreviations: BMI – body mass index; HbA1c – glycated hemoglobin; Q1–Q3 – lower and upper quartiles. * – differences are statistically significant ($p < 0.05$).

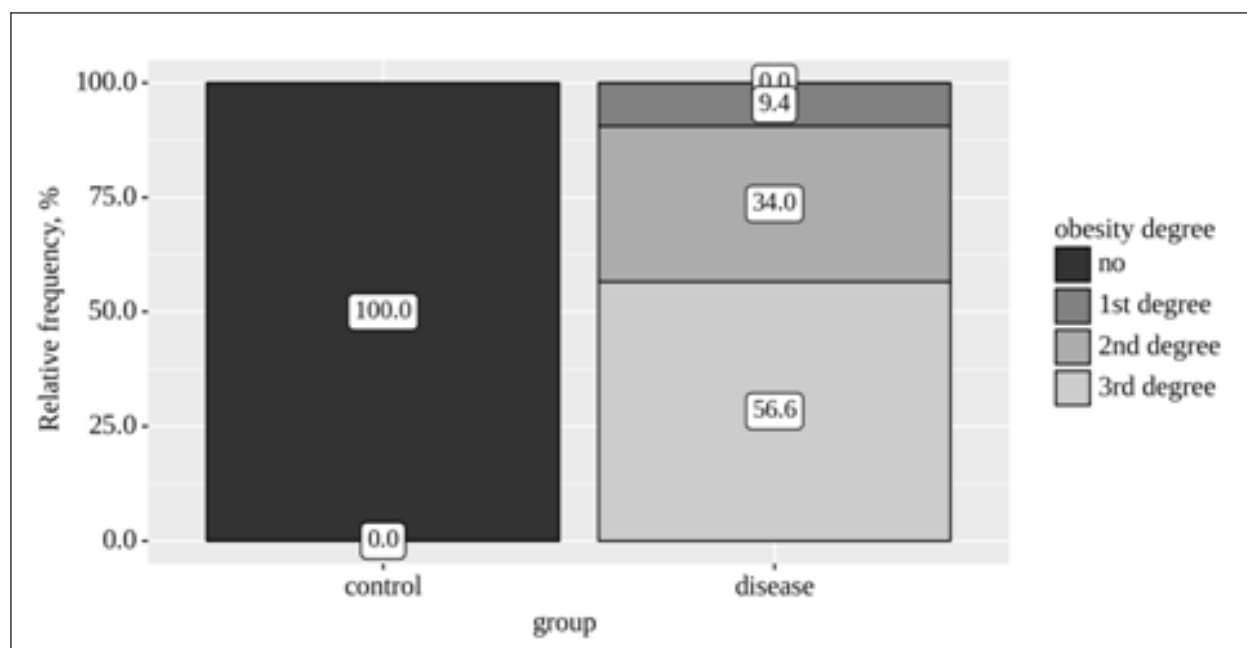


Figure 1. Analysis of obesity degree conditioning on group.

Data of the analysis of allele G and allele T of the GHRL (rs696217) polymorphism conditioning on the group in Ukrainian population are presented in Table 2. When comparing of allele G of the GHRL (rs696217) polymorphism depending on group, no statistically significant differences were revealed ($p=0.680$). Odds of «yes» were 1.878 times less in disease group than in control group. The relative difference in odds was not statistically significant ($OR=0.533$; 95% CI: 0.093–3.048).

Table 2
Analysis of Allele G and Allele T conditioning on group

Variable	Categories	Group		p-value
		Control	Disease	
Allele G	no	2 (4.2)	4 (7.5)	0.680
	yes	46 (95.8)	49 (92.5)	
Allele T	no	42 (87.5)	37 (69.8)	0.031*
	yes	6 (12.5)	16 (30.2)	

* – differences are statistically significant ($p<0.05$).

According to the data obtained, when comparing of allele T of the GHRL (rs696217) polymorphism, statistically significant differences were revealed depending on the group ($p=0.031$). Odds of «yes» were 3.027 times greater in disease group comparing with control. The relative difference in odds was statistically significant (95% CI: 1.073–8.539). In the group with obesity, there was significantly more people with allele T of the GHRL (rs696217) polymorphism compared to the control group.

The analysis of allele G of the GHRL (rs696217) polymorphism conditioning on obesity degree is presented in Figure 2. When comparing allele G depending on obesity degree no statistically significant differences were revealed ($p=0.199$).

The analysis of allele T of the GHRL (rs696217) polymorphism conditioning on obesity degree is presented in Table 3. According to the data obtained when comparing the allele T, statistically significant differences were revealed depending on the obesity degree particularly in patients with no obesity and individuals with 2nd degree obesity ($p=0.048$).

Table 3
Analysis of Allele T conditioning on obesity degree

Variable	Categories	Obesity degree				p-value
		no	1st degree	2nd degree	3rd degree	
Allele T	no	42 (87.5)	4 (80.0)	10 (55.6)	23 (76.7)	0.048*
	yes	6 (12.5)	1 (20.0)	8 (44.4)	7 (23.3)	

* – differences are statistically significant ($p<0.05$).

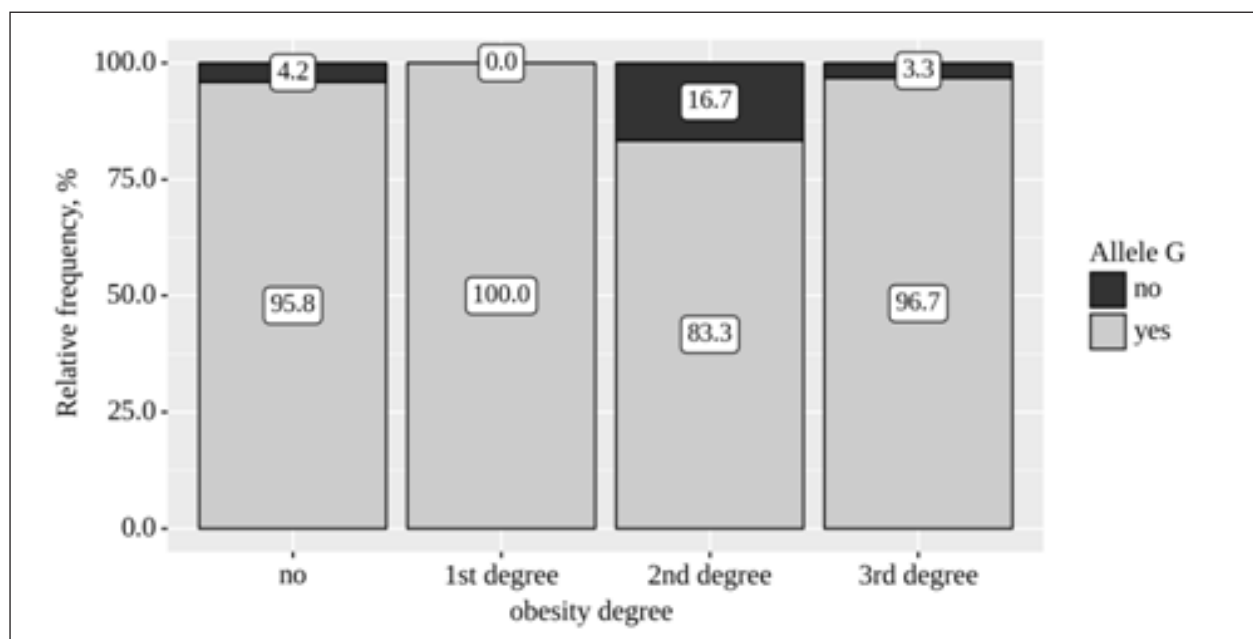


Figure 2. Analysis of the allele G conditioning on obesity degree.

The analysis of the dependence of the probability of «yes» in the presence of allele T of GHRL (rs696217) polymorphism on the BMI using the ROC analysis. The ROC curve obtained is demonstrated in Figure 3. The area under the ROC curve comprised 0.655 ± 0.070 with 95% CI: 0.518–0.791. The resulting model was statistically significant ($p=0.027$). The cut-off value of BMI, which corresponds to the highest Youden's J statistic, was 22.740. If BMI was greater or equal to this value, «yes» was predicted. The sensitivity and specificity of the method were 95.5% and 41.8%, respectively. There is a relationship between the allele T of GHRL (rs696217) polymorphism and an increase in BMI in the obesity patients.

Comparing leptin (ng/ml), statistically significant differences were revealed depending on the obesity degree ($p<0.001$), as it is shown in Figure 4. The concentration of leptin was statistically significantly higher in patients with the 3rd degree obesity.

Comparing ghrelin general (ng/ml), statistically significant differences were revealed depending on the obesity degree ($p<0.001$), as it is demonstrated in Figure 5. The concentration of general ghrelin was statistically significantly lowest in patients with the 3rd obesity degree and the highest in patients with the 1st degree obesity.

Comparing adiponectin ($\mu\text{g/ml}$), statistically significant differences were revealed depending on the obesity degree ($p<0.001$), as it is shown in Figure 6. The concentration of leptin was statistically significantly higher in not-obese patients.

Comparing resistin (ng/ml), statistically significant differences were revealed depending on the obesity degree ($p<0.001$), as it is demonstrated in Figure 7. The concentration of resistin

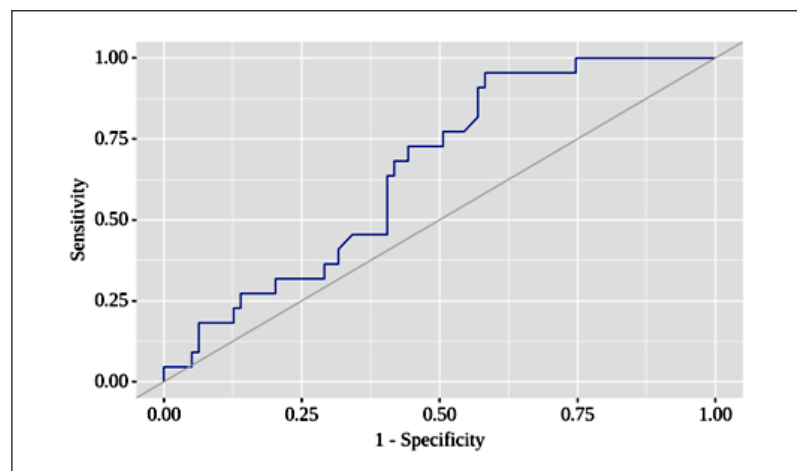


Figure 3. ROC curve characterizing the dependence of the probability the allele T on body mass index (BMI).

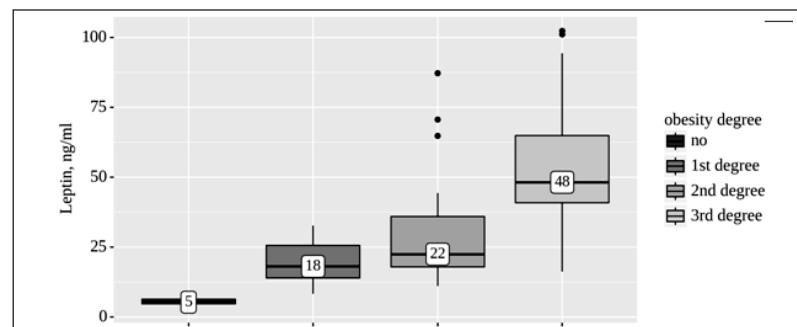


Figure 4. Analysis of leptin (ng/ml) conditioning on obesity degree.

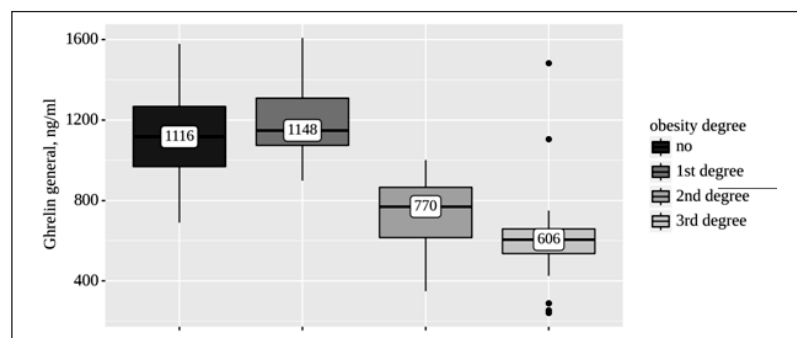


Figure 5. Analysis of ghrelin general (ng/ml) conditioning on obesity degree.

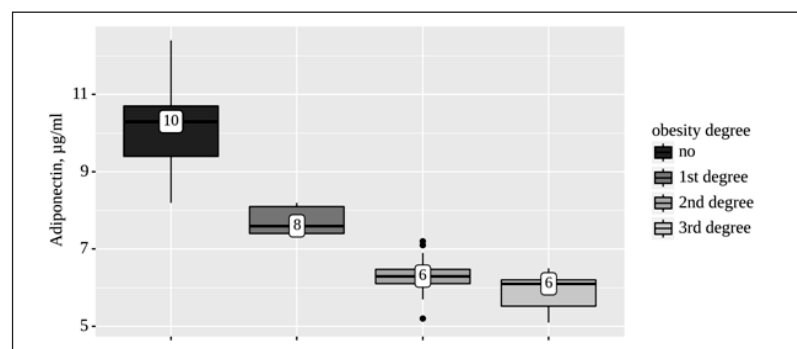


Figure 6. Analysis of adiponectin ($\mu\text{g/ml}$) conditioning on obesity degree.

was statistically significantly higher in not-obese patients.

Analysis of metabolic hormones was performed conditioning on allele G of the GHRL (rs696217) polymorphism demonstrated in Table 4. Comparing leptin (ng/ml), ghrelin general (ng/ml), adiponectin (μ g/ml), resistin (ng/ml) depending on allele G of the GHRL (rs696217) polymorphism in our patients, no statistically significant differences were revealed ($p=0.725$, $p=0.995$, $p=0.971$, $p=0.191$, respectively).

The analysis of metabolic hormones conditioning on allele T of the GHRL (rs696217) polymorphism is shown in Table 5. In accordance with the presented table, when comparing adiponectin (μ g/ml) and resistin (ng/ml), statistically significant differences

were revealed depending on allele T of the GHRL (rs696217) polymorphism ($p=0.045$ and $p=0.021$, respectively). When comparing leptin (ng/ml) and ghrelin general (ng/ml) depending on allele T of the GHRL (rs696217) polymorphism, there were no statistically significant differences found ($p=0.350$ and $p=0.340$, respectively).

Evaluation of the dependence of the probability of «yes» on the adiponectin (μ g/ml) using the ROC analysis, the following ROC curve was obtained, as it is indicated in Figure 8.

The area under the ROC curve comprised 0.640 ± 0.063 with 95% CI: 0.517–0.763. The resulting model was statistically significant ($p=0.045$). The cut-off value of adiponectin (μ g/ml), which

Table 4
Analysis of hormones conditioning on Allele G

Variables	Categories	Allele G			p-value
		Mean $\pm$ SD Median	95% CI Q1–Q3	n	
Leptin (ng/ml)	no	13	7–15	6	0.725
	yes	11	6–42	95	
Ghrelin general (ng/ml)	no	918 $\pm$ 342	559–1276	6	0.995
	yes	917 $\pm$ 340	847–986	95	
Adiponectin (μ g/ml)	no	7	6–9	6	0.971
	yes	8	6–10	95	
Resistin (ng/ml)	no	9	5–9	6	0.191
	yes	7	4–8	95	

Data are presented as mean (M) $\pm$ standard deviation (SD) or median (Me); Q1–Q3 – lower and upper quartiles.

Table 5
Analysis of hormones conditioning on Allele T

Variables	Categories	Allele T			p-value
		Mean $\pm$ SD Median	95% CI Q1–Q3	n	
Leptin (ng/ml)	no	7	5–40	79	0.350
	yes	20	7–43	22	
Ghrelin general (ng/ml)	no	934 $\pm$ 339	858–1010	79	0.340
	yes	856 $\pm$ 338	706–1005	22	
Adiponectin (μ g/ml)	no	9	6–10	79	0.045*
	yes	6	6–8	22	
Resistin (ng/ml)	no	5	4–8	79	0.021*
	yes	8	5–9	22	

Data are presented as mean $\pm$ standard deviation or median; Q1–Q3 – lower and upper quartiles. * – differences are statistically significant ($p<0.05$).

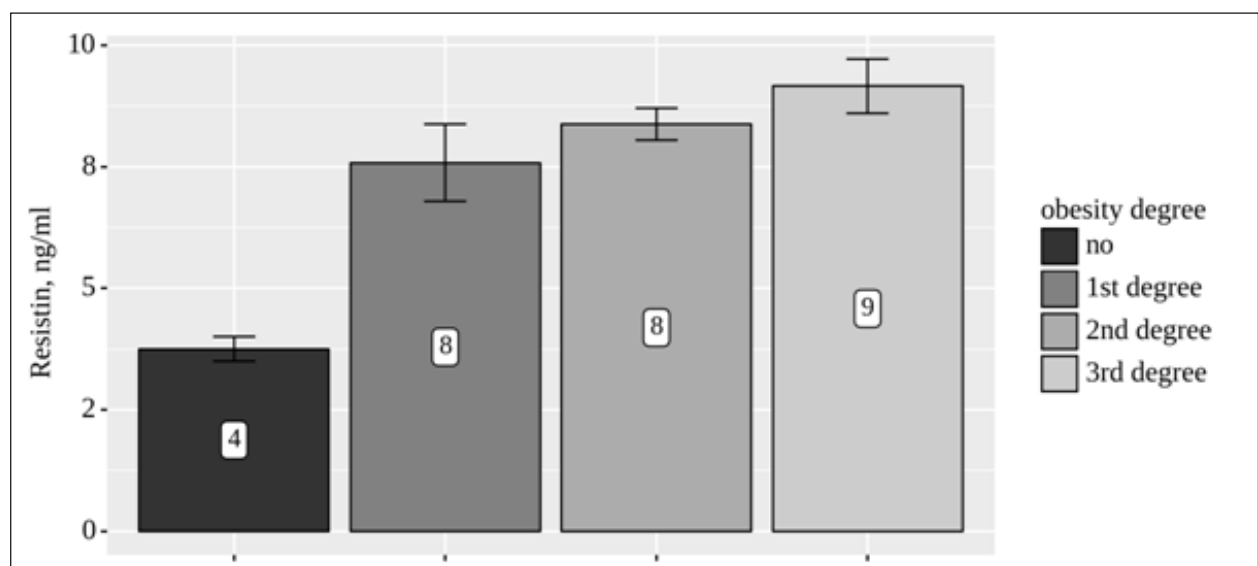


Figure 7. Analysis of resistin (ng/ml) conditioning on obesity degree.

corresponds to the highest Youden's J statistic is 9.100. If adiponectin ($\mu\text{g/ml}$) was less than this value, «yes» was predicted. The sensitivity and specificity of the method were 81.8% and 50.6%, respectively. There is a relationship between the allele T of GHRL (rs696217) polymorphism and the adiponectin level in Ukrainian population.

Evaluation of the dependence of the probability of «yes» on the resistin (ng/ml) using the ROC analysis, the ROC curve was obtained, as it is demonstrated in Figure 9.

The area under the ROC curve comprised 0.661 ± 0.069 with 95% CI: 0.525–0.797. The resulting model was statistically significant ($p=0.021$). The cut-off value of resistin (ng/ml), which corresponds to the highest Youden's J statistic is 7.300. If resistin (ng/ml) was greater or equal to this value, «yes» was predicted. The sensitivity and specificity of the method was 68.2% and 59.5%, respectively. There was a relationship between the allele T of GHRL (rs696217) polymorphism and the resistin level in Ukrainian population.

Comparing leptin (ng/ml), ghrelin general (ng/ml), adiponectin ($\mu\text{g/ml}$), and resistin (ng/ml) depending on genotype in obese patients, no statistically significant differences were revealed ($p=0.429$, $p=0.554$, $p=0.072$, $p=0.068$, respectively) (Table 6). The genotype (GG, GT, TT) of the GHRL polymorphism (rs696217) does not have a statistically significant effect on the level of leptin, ghrelin, adiponectin, and resistin in the serum of Ukrainian patients diagnosed with obesity.

Discussion

According to the research of Finnish scientists, it is known that the Leu72Met (C247A) polymorphism of the ghrelin gene was detected in 15.6% (12.5% heterozygotes and 3.1% homozygotes) of obese patients and in 12.5% (all heterozygotes) of control subjects;

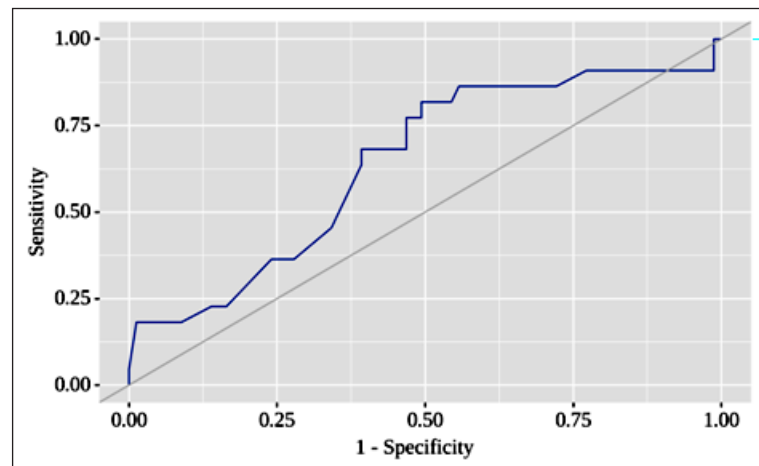


Figure 8. ROC curve characterizing the dependence of the probability the allele T on adiponectin ($\mu\text{g/ml}$).

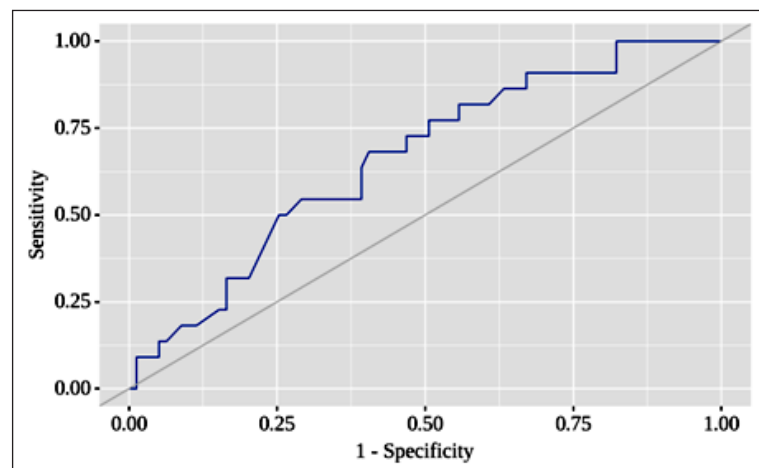


Figure 9. ROC curve characterizing the dependence of the probability the allele T on resistin (ng/ml).

Table 6

Analysis of the influence of GHRL (rs696217) polymorphism on the hormone levels (leptin, ghrelin, adiponectin, and resistin) in obese patients regarding their genotype

Genotype	Leptin (ng/ml)	95% CI Q1–Q3	Ghrelin (ng/ml)	95% CI Q1–Q3	Adiponectin ($\mu\text{g/ml}$)	95% CI Q1–Q3	Resistin (ng/ml)	95% CI Q1–Q3
GG	7	5–40	934	858–1010	9	6–10	6	4–8
GT	27	8–45	832	648–1016	6	6–8	8	6–9
TT	13	7–15	918	559–1276	7	6–9	9	5–9
p-value	0.429		0.554		0.072		0.068	

Data are presented as median; Q1–Q3 – lower and upper quartiles.

i.e., in subjects who did not suffer from obesity. This polymorphism was associated with an earlier onset of obesity (Li et al. 2019). There is also another study, in which the ghrelin gene was examined in obese French children and has been found a positive correlation between the Leu72Met heterozygous variant with higher BMI and early obesity (Rivera-Leon et al. 2020). Similar significant association between the Leu72Met polymorphism and early obesity has been observed in Italian children.

In addition, the Leu72Met polymorphism is thought to contribute to weight gain in middle-aged Japanese men (Huang et al. 2018). This statement has been confirmed by a meta-analysis in the Chinese population. The Leu72Met GHRL gene polymorphism has been shown to be significantly correlated with an increased risk of type 2 diabetes (T2DM). Individuals who carry the Met72 allele of the GHRL Leu72Met polymorphism, especially those of Chinese descent, may be more susceptible to developing the T2DM (Hamdy et al. 2018; Hutor et al. 2020). That is the occurrence of T2DM, which is often accompanied by metabolic syndrome and obesity and has a genetically determined cause in connection with the presence of the GHRL gene polymorphism, which is confirmed by the results of our study.

It is known that ghrelin is involved in glucose metabolism. It has also been confirmed that ghrelin secretion can be inhibited by insulin. For example, in obese people with elevated plasma glucose levels, ghrelin levels are reduced due to hyperinsulinemia (Chen et al. 2020; Kamyshna et al. 2021). Analyzing the data of the effect of the GHRL gene polymorphism (G/T rs696217) on the glucose metabolism (glucose level and HbA1c), the following data have been established. Kim et al. (2006) have found that the Leu72Met polymorphism of the preproghrelin gene is not associated with T2DM or its complications. Joatar et al. (2017) have found that the Leu72Met GHRL polymorphism was not associated with T2DM, insulin resistance or serum ghrelin levels in a Saudi population. No associations have been found between genotypes and serum ghrelin levels in the Mexican population (Ukkola et al. 2001). Bai et al. (2021) have found that the genotype and allele frequency of the GHRL gene polymorphism in obese participants did not show significant difference compared to non-obese controls in Chinese subjects. In our study, we did not establish any relationship between the genotype and frequency of alleles of the GHRL gene polymorphism and the levels of ghrelin and leptin in Ukrainian obese individuals. However, we found that the presence of the GHRL

polymorphism (rs696217) may affect the resistin and adiponectin levels.

There is also a statement that the consequence of the C214 G>T polymorphism is the amino acid substitution Leu72Met. This amino acid residue is outside of mature ghrelin. In a study of Becer and Ergoren (2021), it has been shown that the frequency of the minor allele rs696217 T (Met72) was significantly higher in obese individuals. At the same time, in the study of Yadegari et al. (2022) individuals with the TT genotype at the locus had the lowest prevalence of obesity compared to other genotypes among individuals. No significant relationship has been found between the two groups regarding the lipid profile and TT genotype. However, individuals with the TT or GT genotype had a higher risk of obesity compared to individuals with the GG genotype.

The work of Al-Nbaheen (2023) after analyzing the genotype in SNP rs696217 has confirmed the absence of association in alleles, genotypes or any other form of genetic patterns with no manifestations of the metabolic syndrome. Studies of Mager et al. (2006, 2008) and Berthold et al. (2009) have also been unable to demonstrate any effect of this variant on plasma ghrelin concentration, which is confirmed by the results of our study.

Conclusion

Carriers of the T allele of the GHRL (rs696217) polymorphism were statistically significantly higher in patients diagnosed with obesity compared to controls, indicating a genetically determined cause of obesity. It also was established a significant effect of the presence of the T allele of the GHRL (rs696217) polymorphism on the decrease of adiponectin level and the increase the resistin level in the obese patients. On the other hand, the study of the effect of genotypes (TT, GT, GG) of the GHRL (rs696217) polymorphism on the metabolic hormone levels (leptin, ghrelin, adiponectin, and resistin) in the blood of obese patients did not show reliably significant differences.

The presence of the T allele of the GHRL (rs696217) polymorphism in Ukrainian population indicates an increased risk of obesity developing regardless of the homozygous or heterozygous genotype.

Acknowledgements

The authors would like to thank all the 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

- Al-Nbaheen MS. Relationship between single nucleotide polymorphism studies in ghrelin gene with obesity subjects. *Journal of King Saud University - Science* 35, 102393, 2023.
- Bai R, Liu Y, Zhao C, Gao J, Liu R. Distribution and effect of ghrelin genotype on plasma lipid and apolipoprotein profiles in obese and nonobese Chinese subjects. *Hormones (Athens)* 20, 527–535, 2021.
- Becer E, Ergoren MC. Dual effect of the GHRL gene variant in the molecular pathogenesis of obesity. *Balkan J Med Genet* 24, 27–34, 2021.
- Berthold HK, Giannakidou E, Krone W, Mantzoros CS, Gouni-Berthold I. The Leu72Met polymorphism of the ghrelin gene is associated with a decreased risk for type 2 diabetes. *Clin Chim Acta* 399, 112–116, 2009.
- Chen B, Xiao W, Zou Z, Zhu J, Li D, Yu J, Yang H. Ghrelin gene single nucleotide polymorphisms and their effects on Nile tilapia (*Oreochromis niloticus*) growth. *Aquacult Rep* 18, 100469, 2020.
- Dantas VGL, Furtado-Alle L, Souza RLR, Chautard-Freire-Maia EA. Obesity and variants of the GHRL (ghrelin) and BCHE (butyrylcholinesterase) genes. *Genet Mol Biol* 34, 205–207, 2011.
- Flanagan S, Rowe A, Duggan V, Markle E, O'Brien M, Barry G. Development of a real-time PCR assay to detect the single nucleotide polymorphism causing Warmblood Fragile Foal Syndrome. *PLoS ONE* 16, e0259316, 2021.
- Hamdy M, Kassim SK, Khairy E, Maher M, Mansour KA, Albreedy AM. Ghrelin gene polymorphism as a genetic biomarker for prediction of therapy induced clearance in Egyptian chronic HCV patients. *Gene* 649, 74–79, 2018.
- Huang R, Tian S, Cai R, Sun J, Shen Y, Wang S. Ethnicity-specific association between ghrelin Leu72Met polymorphism and type 2 diabetes mellitus susceptibility: An updated meta-analysis. *Front Genet* 9, 541, 2018.
- Hutor NS, Pidruchna SR, Melnyk NA, Avdeev OV, Boykiv AB, Kovtun NY, Skochylo OV, Tverdokhlib NO, Goncharuk-Khomyn MY. The role of prooxidant-antioxidant system in the development of alveolitis after teeth extraction. *J Int Dent Med Res* 13, 561–565, 2020.
- Joatar FE, Al Qarni AA, Ali ME, Masaud AA, Shire AM, Das N, Gumaa K, Giha HA. Leu72Met and other intronic polymorphisms in the GHRL and GHSR genes are not associated with type 2 diabetes mellitus, insulin resistance, or serum ghrelin levels in a Saudi population. *Endocrinol Metab (Seoul)* 32, 360–369, 2017.
- Kamyshna II, Pavlovych LB, Sydorchuk LP, Malyk IV, Kamyshnyi AM. BDNF blood serum linkage with BDNF gene polymorphism (rs6265) in thyroid pathology patients in the West-Ukrainian population. *Endocr Regul* 55, 193–203, 2021.
- Kamyshna II, Pavlovych LB, Kamyshnyi AM. Association between NMDA gene polymorphism (rs4880213) and GRIN2B blood serum levels in thyroid pathology patients. *J Med Life* 15, 109–116, 2022.
- Kim SY, Jo DS, Hwang PH, Park JH, Park SK, Yi HK, Lee DY. Preproghrelin Leu72Met polymorphism is not associated with type 2 diabetes mellitus. *Metabolism* 55, 366–370, 2006.
- Korbonits M, Gueorguiev M, O'Grady E, Lecoer C, Swan DC, Mein CA, Weill J, Grossman AB, Froguel P. A variation in the ghrelin gene increases weight and decreases insulin secretion in tall, obese children. *J Clin Endocrinol Metab* 87, 4005–4008, 2002.
- Kuzuya M, Masuda Y, Hirakawa Y, Iwata M, Enoki H, Hasegawa J, Izawa S, Iguchi A. Falls of the elderly are associated with burden of caregivers in the community. *Int J Geriatr Psychiatry* 21, 740–745, 2006.
- Mager U, Lindi V, Lindstrom, Eriksson JJG, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukkaanniemi S, Tuomilehto J, Laakso M, Pulkkinen L, Uusitupa M. Association of the Leu72Met polymorphism of the ghrelin gene with the risk of type 2 diabetes in subjects with impaired glucose tolerance in the Finnish Diabetes Prevention Study. *Diabet Med* 23, 685–689, 2006.
- Mager U, Kolehmainen M, de Mello VD, Schwab U, Laaksonen DE, Rauramaa R, Gylling H, Atalay M, Pulkkinen L, Uusitupa M. Expression of ghrelin gene in peripheral blood mononuclear cells and plasma ghrelin concentrations in patients with metabolic syndrome. *Eur J Endocrinol* 158, 499–510, 2008.
- Li YY, Lu XZ, Yang XX, Wang H, Geng HY, Gong G, Zhan YY, Kim HJ, Yang ZJ. GHRL gene Leu72Met polymorphism and type 2 diabetes mellitus: A meta-analysis involving 8,194 participants. *Front Endocrinol (Lausanne)* 10, 5592019, 2019.
- Marushchak M, Mazur L, Krynytska I. Insulin receptor substrate-1 gene polymorphism and lipid panel data in type 2 diabetic patients with comorbid obesity and/or essential hypertension. *Endocr Regul* 57, 1–11, 2023.

- Rivera-Leon EA, Llamas-Covarrubias MA, Sanchez-Enriquez S, Martinez-Lopez E, Gonzalez-Hita M, Llamas-Covarrubias IM. Leu72Met polymorphism of GHRL gene decreases susceptibility to type 2 diabetes mellitus in a Mexican population. *BMC Endocr Disord* 20, 109, 2020.
- Ukkola O, Ravussin E, Jacobson P, Snyder EE, Chagnon M, Sjostrom L, Bouchard C. Mutations in the preproghrelin/ghrelin gene associated with obesity in humans. *J Clin Endocrinol Metab* 86, 3996–3999, 2001.
- Yadegari M, Zare-Feyzabadi R, Zakariaeiseraji M, Sahebi R, Shabani N, Khedmatgozar H, Ferns GA, Ghazizadeh H, Mohammadi-Bajgiran M, Jalalian M, Zoghi M, Darban RA, Mohammadian-Ghosooni M, Esmaily H, Avan A, Ghayour-Mobarhan M. Interaction between the genetic variant of rs696217-ghrelin and food intake and obesity and dyslipidemia. *Ann Hum Genet* 86, 14–23, 2022.