

# Obesity in Children with Leptin Receptor Gene Polymorphisms

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## ABSTRACT

**Introduction:** The study of single nucleotide polymorphisms (SNPs) of the leptin receptor gene (*LEPR*) based on next generation genomic sequencing (NGS) data is becoming an increasingly important aspect of diagnosis, treatment and prevention of both metabolically healthy (MHO) and metabolically unhealthy obesity (MUO) phenotypes.

**Material and methods:** 35 obese children 6-18 years old were examined by the NGS method with bioinformatic analysis. The main group (n = 18) was formed by children with MUO, according to the recommendations of the expert group of the National Heart, Lung, and Blood Institute. The control group (n = 17) was represented by children with MHO. Statistical methods were used: analysis of variance, Wald's sequential analysis, Spearman's correlation analysis, analysis of nominal data and multiple discriminant analysis.

**Results:** 10 types of non-synonymous SNPs (rs3790435, rs1137100, rs2186248, rs70940803, rs79639154, rs1359482195, rs1137101, rs1805094, rs13306520, rs13306522) of the *LEPR* gene in obese children have been identified. Multiple discriminant analysis demonstrated that the following *LEPR* SNPs are of greatest importance in the development of MUO: rs3790435, rs13306522, rs13306520. Analysis of nominal data revealed significant differences in the groups for Copy number variation (CNV) rs3790435 of the *LEPR* gene. Wald's analysis allowed us to identify 6 important predictors of MUO ( $I \geq 0.5$ ): 2 CNV rs3790435 (Relative Risk, RR = 2, Prognostic coefficient, PC = +2.76); male gender of the child (RR = 1.3, PC = +1.35); rs3790435 (RR = 1.9, PC = +2.76); hyperleptinemia more than 40.56 ng/ml (RR = 2, PC = +3); CNV rs1359482195  $\geq 3$  (RR = 1.9, PC = +5.8); SNP of the *LEPR* gene  $\geq 4$  (RR = 3.8, PC = +5.8).

**Conclusion:** Children with the genotype rs3790435 gene *LEPR* had signs of MUO more often.

## KEYWORDS

obesity phenotypes; children; leptin receptor gene; single nucleotide polymorphisms; next generation genomic sequencing

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## INTRODUCTION

The key role in the regulation of the body's energy metabolism is played by leptin (LEP) produced by adipocytes, which activates the leptin receptor (LEPR) of hypothalamic neurons (1, 2). Activation of the leptin receptor leads to increased transcription of anorexigenic pro-opiomelanocortin (POMC) and inhibition of the transcription of orexigenic neuropeptides agouti-related protein (AgRP) and neuropeptide Y (NPY) (3). Polymorphisms of the *LEPR* gene, which is located on chromosome 1 (1p31.3), associated with the development of obesity, are inherited in an autosomal recessive manner. Biallelic nonsynonymous polymorphisms of the *LEPR* gene located in exons can lead to dysfunction of the LEP/LEPR system by altering the structure of the leptin receptor protein. LEPR anomalies may be accompanied by the disturbance interaction of the receptor with LEP, the lack of LEPR excitation, the appearance of soluble forms of LEPR that are not able to fix on the cell membrane, and the disturbance of interaction of the LEPR intradomain with components of intracellular signal conductivity cascades. The absence of the LEPR signal leads to the development of obesity, hyperphagia, and deficiency in the production of pituitary hormones already in the early period of childhood (4, 5). The prevalence of pathogenic *LEPR* mutations in a cohort of patients with severe monogenic early-onset obesity is 1.9% (6). There are currently 38 documented mutations in the *LEPR* gene associated with obesity. Most of the mutations are located in the region that encodes the extracellular domain, especially the leptin-binding site or activation domain of the receptor molecule (7). Missense mutations R612H, A409E, W664R, and H684P cause complete loss of leptin signaling, hyperphagia, severe obesity with early clinical manifestation, impairment of immune function and delay of puberty (8).

The detection of variants of leptin receptor gene polymorphisms based on next generation genomic sequencing (NGS) data is becoming an increasingly important aspect of diagnosis, treatment and prevention of both metabolically healthy (MHO) and metabolically unhealthy (MUO) obesity phenotypes associated with the formation of insulin resistance (9, 10). However, till now most studies have not examined the genetic effects of gene copies, including *LEPR*.

Objective: to determine the contribution of various SNPs of the *LEPR* gene and the CNV of gene regions to the development of metabolically unhealthy obesity and their importance in the diagnosis of various phenotypes of obesity in children.

## MATERIAL AND METHODS

To accomplish the assigned tasks, a clinical and genetic examination was carried out in 35 children 6–18 years old with obesity, according to the recommendations of The American College of Medical Genetics and Genomics (ACMG) (11). The study was conducted among a Caucasian cohort of children with polygenic obesity.

Criteria for inclusion: all children enrolled in the study had a BMI greater than the 95th percentile or 2 SDS and

underwent inpatient treatment in the endocrinology department.

Exclusion criteria: patients with hereditary syndromes accompanied by obesity and diseases, the treatment of which requires the use of drugs that affect the metabolism of carbohydrates and lipids; pregnant.

The main group included of 18 children with metabolically unhealthy obesity (MUO), according to the recommendations of the expert group of the National Heart, Lung, and Blood Institute, USA – NHLBI (12). The control group was formed of 17 children with metabolically healthy obesity (MHO). For inclusion in the main observation group, the presence of abdominal obesity and two of the presented criteria were taken into account: 1). Fasting glycemia  $\geq 5.6$  mmol/L (13); 2). High-density lipoprotein (HDL)  $\leq 1.03$  mmol/L or less than 10th percentile of the age norm; 3). Triacylglyceride (TAG)  $\geq 1.7$  mmol/L or more than the 90th percentile of the age norm; 4) Systolic blood pressure (SBP) above the 90th percentile for a given age, gender and height (12). The abdominal type of obesity was determined according to the consensus of the International Diabetes Federation (IDF), based on the excess of the waist circumference over the 90th percentile for children 6–15 years old or more than 94 cm for boys aged 16–18 years and more than 80 cm for girls 16–18 years old (14–16).

Laboratory examination for the formation of observation groups for obesity phenotypes included general clinical methods. Blood samples were obtained after an overnight fast by venipuncture in vacutainer gel tubes, and serum was separated from cells by centrifugation in a certified laboratory “Synevo” (Dnipro, Ukraine) using an analyzer and a Cobas 6000 test system; Roche Diagnostics (Switzerland). The analysis of serum glucose was carried out by the hexokinase method; the determination of triglycerides and high-density lipoproteins of blood plasma was carried out by the enzymatic – colorimetric method. HbA1c was analyzed using the immunoturbidimetric method, certified according to the National Glycohemoglobin Standardization Program (NGSP) and standardized according to the reference values adopted in the Diabetes Control and Complications Trial (DCCT). The level of HbA1c 4.8–5.9% of total hemoglobin in venous blood was considered normal. Insulin was analyzed using the electrochemiluminescence immunoassay method (ECLIA). The level of basal insulin in venous blood of 2.6–24.9  $\mu$ IU/ml was considered normal. Insulin resistance was estimated using the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR):  $\text{Insulin } (\mu\text{IU/ml}) \times \text{Fasting blood glucose (mmol/L)} / 22.5$ . An increase in insulin resistance was observed at HOMA-IR > 95th percentile according to the percentile curves recommended by the IDEFICS Consortium for the European population according to the age and sex of the child (17, 18).

Determination of serum leptin level was performed using radioimmunoassay using an analyzer and LDN test system (Germany), reference values for girls were 3.7–11.1 ng/ml, for boys 2.0–5.6 ng/ml.

Height (cm) was measured using Heightronic Digital Stadiometer® to the nearest 0.1 cm. Weight (kg) and body fat percentage was measured using Tefal Bodysignal body

composition analyzer (France). The calculation of the percentage of fat or body fat (BF) in the body was performed automatically with a discreteness of 0.1%, according to the requirements of Tefal Bodysignal, with the evaluation of results according to the unified centile scales for children of this age (19, 20). Waist circumference (WC), hip circumference (HC) was measured using a standardized anthropometric tape, measuring the circumference at the midpoint between the top of the iliac crest and the lower part of the lateral rib cage to the nearest 0.1 cm. BMI was converted to SDS by means of the current WHO growth references (21).

Systolic and diastolic blood pressure (SBP and DBP) were measured using a digital oscillimetric device, Dinamap ProCare (GE Healthcare).

Molecular genetic testing included complete genomic NGS with venous blood sampling in a certified CeXGat laboratory (Tubingen, Germany) using the Illumina CSeq Pro® Certified service provider platform. Average amount of DNA (µg) in samples – 0.875. Library Preparation: Quantity used 50 ng. Library Preparation Kit: Twist Human Core Exome plus Kit (Twist Bioscience). Sequencing parameters: NovaSeq 6000; 2 × 100 bp. QC values of sequencing, Q30 value: 96.07%.

Bioinformatic analysis – demultiplexing of the sequencing reads was performed with Illumina bcl2fastq (version 2.20). Adapters were trimmed with Skewer, version 0.2.2 (22). DNA-Seq: Trimmed raw reads were aligned to the human reference genome (hg19-cegat) using the Burrows-Wheeler Aligner, BWA – mem version 0.7.17-cegat (23). ABRA, version 2.18 (24) was used for local restructuring of readings in target regions to improve more accurate detection of indels in the genome during mutagenesis. Proprietary readout tools, alignment with more than one locus with the same alignment score, were used; duplicate reads were discarded.

Variant calling: additional proprietary software was used to detect variants of polymorphisms, including variants with low frequencies (Observed frequency of the alternative allele in the range, OFA up to 2% of sequenced readings). The mutation variants were annotated based on various publicly available databases (Ensembl v100, RefSeq Curated (20200723), CCDS r22, dbSNP154, GnomAD 2.1 (exonic) and 3.0 (genomic), Gencode 34). Copy number variations (CNV) were found by comparing the number of reads that overlap the target genome regions (“coverage”) with the expected number in the cohort of reference samples. All synonymous SNP types were excluded from the study.

The quality of FASTQ files was analyzed using FastQC, version 0.11.5-cegat (25). The plots were created with ggplot2 (26) in R version 3.6.1 (27). When interpreting the data of bioinformatic analysis, the combined annotated – dependent depletion (CADD) was calculated for each identified non-synonymous SNP of the *LEPR* gene (28, 29) and the software products FILTUS (30), SeqVISTA (31), Mutationassessor (32) were used.

Statistical processing of the results using parametric, nonparametric methods included: analysis of variance with the calculation of the Student's test (t); sequential Wald analysis with the calculation of the relative risk

(Relative Risk – RR) and the prognostic coefficient (PC); Spearman correlation analysis with calculation of Spearman's rank correlation coefficient ( $\rho$ ), analysis of nominal data with calculation of Chi-square test ( $\chi^2$ ),  $\chi^2$  test with Yates correction,  $\chi^2$  test with correction for likelihood, Fisher's exact test (p), Cramer test (V), Pearson's contingency coefficient (C), normalized value of Pearson's coefficient (C'); multiple discriminant analysis with the calculation of the coefficients of the standardized canonical discriminant function (C<sub>i</sub> MUO). The critical value of the level of statistical significance (p) for all types of analysis was taken at the level of  $p < 0.05$  (5%). To reduce the sensitivity of computational procedures to the size of the sample presented, we used special methods that reduce the magnitude of computation errors by multivariate statistical processing of biometric data to obtain verified confidence coefficients (33). Statistical processing of the results was performed using Microsoft Excel (Office Home Business 2KB4Y-6H9DB-BM47K-749PV-PG3KT) and STATISTICA 6.1 software (StatSoftInc, no. AGAR909E415822FA).

## RESULTS

The age distribution of polygenic obesity patients who took part in the survey was characterized by the following features. The proportion of children 6–10 years old (pre-pubertal period), in the main group, was 5.6% (1/18), 11–14 years old (early pubertal period) – 50% (9/18), 15–18 years old (late puberty period) – 44.4% (8/18). The proportion of children 6–10 years old in the control group was 5.8% (1/17), 11–14 years old – 47.1% (8/17), 15–18 years old – 47.1% (8/17),  $p > 0.5$ . The proportion of adolescents in the main group was 94.4% (17/18), in the control group – 94.2% (16/17),  $p > 0.5$ . The average age of patients in the observation groups was  $12.06 \pm 1.25$  years. The proportion of boys in the main group was  $61.1 \pm 5.5\%$  (11/18), while in the control group the proportion of boys in the study population was  $47.06 \pm 4.1\%$  (8/17),  $p = 0.049$ .

Anthropometric examination revealed that in children with different phenotypes of obesity in the percentage of BMI, taking into account the age and sex of the child, no statistically significant difference in the comparison groups was observed. The absence of statistically significant differences in the frequency of obesity ( $p > 0.05$ ) is due to the approach to the formation of survey groups, Table 1.

In order to take a part of children from BMI, but to surpass the 100th percentile in case of young variants of obesity phenotypes, and to the classification of extreme obesity in children, proposed by the American Heart Association and the Obesity Society (34). The MUO phenotype was detected in  $64.5 \pm 7.3\%$  of children, the MHO phenotype – in  $35.5 \pm 6.9\%$  of children with extreme obesity. The share of children with extreme obesity of class III (with body weight more than 140% of the 95th percentile) in those surveyed with the MUO phenotype was  $25 \pm 6.5\%$ , while among those surveyed with the MHO phenotype –  $7.3 \pm 4.1\%$ . Thus, children with the MUO phenotype have a higher genetic predisposition to develop severe obesity.

**Tab. 1** Average values of anthropometric and manometric examination of children with different phenotypes of obesity.

Indicator	Children with MUO (M ± m)	Children with MHO (M ± m)	Probability, p
BMI in percentiles, %	98.8 ± 0.34	98.0 ± 0.4	p > 0.05
Proportion of children with extreme obesity of the III class (with body weight more than 140% from 95 percentile), %	26 ± 6.5	7.3 ± 4.1	p < 0.05
Physical development in percentiles	68.8 ± 4.7	74.3 ± 6.5	p > 0.05
BF in girls, %	38.2 ± 2.3	28.9 ± 0.8	p < 0.05
BF in boys, %	35.5 ± 2.5	25.0 ± 2.1	p < 0.05
WC in girls, cm	92.6 ± 4.2	74.5 ± 3.1	p < 0.05
WC in boys, cm	111.6 ± 3.2	91.7 ± 4.8	p < 0.05
Correlation WC/HC in girls	0.92 ± 0.04	0.74 ± 0.1	p < 0.05
Correlation WC/HC in boys	0.98 ± 0.04	0.84 ± 0.02	p < 0.05
Proportion of children with SBP exceeding the 95th percentile, %	27.3 ± 6.7	9.8 ± 4.6	p < 0.05

Mean WC levels and WC/HC ratios in both boys and girls had statistically significant differences ( $p < 0.05$ ) in the comparison groups. In boys and girls with MUO phenotype, the average level of WC was  $111.63 \pm 3.21$  and  $92.55 \pm 4.18$  cm, respectively, the ratio of WC/HC was  $0.98 \pm 0.04$  and  $0.91 \pm 0.04$ , respectively, associated with abdominal obesity according to IDF Consensus 2007 (35).

The proportion of children in the comparison subgroups who had a SBP higher than the 95th percentile, which is associated with hypertension among children with the MUO phenotype was almost three times higher than among children with the MHO phenotype, namely –

$27.3 \pm 6.3\%$  and  $9.8 \pm 4.6\%$ ,  $p < 0.05$ . As a result of laboratory examination of carbohydrate metabolism in children with different phenotypes of obesity, the following results were obtained, Table 2.

**Tab. 2** Features of carbohydrate and fat metabolism in children with different obesity phenotypes.

Indicator	Children with MUO (M ± m)	Children with MHO (M ± m)	Probability, p
Fasting blood glucose, mmol/L	4.9 ± 0.1	3.9 ± 0.2	p < 0.05
HbA1c, %	5.7 ± 0.1	4.9 ± 0.1	p < 0.05
HOMA-IR	6.2 ± 1.6	3.6 ± 0.4	p < 0.05
Proportion of children with HOMA-IR exceeding the 95th percentile, %	88.2 ± 0.1	36.4 ± 0.4	p < 0.05
Leptin in boys, ng/ml	39.3 ± 8.9	26.0 ± 6.4	p < 0.05
Leptin in girls, ng/ml	47.8 ± 4.4	32.5 ± 4.3	p < 0.05

As a result of NGS, 10 types of nonsynonymous SNPs (rs3790435, rs1137100, rs2186248, rs70940803, rs79639154, rs1359482195, rs1137101, rs1805094, rs13306520, rs13306522) of the *LEPR* gene cohort were identified in 35 children.

Multiple discriminant analysis with the calculation of the coefficients of the standardized canonical discriminant function showed differences in the studied groups and the contribution of each of the 10 types of detected *LEPR* SNPs to the formation of MUO, Table 3.

The presented results of multiple discriminant analysis of 10 types of non-synonymous SNPs of the *LEPR* gene demonstrate that rs3790435, rs13306522, rs13306520, rs70940803, rs2186248, rs1359482195 make a certain contribution to the formation of MUO in children.

Analysis of nominal data on the criteria for assessing the significance of differences in outcomes depending

**Tab. 3** Characteristics of SNP types of the *LEPR* gene and their contribution to the formation of metabolically unhealthy obesity.

dbSNP	Ref	Alt	Consequence	Codon Change	CADD	C <sub>i</sub> MUO
rs3790435	T	C	5_prime_UTR	–/–	17.34	0.939
rs1137100 (exon-2) (K109R)	A	G	missense	aAgaca/aGgaca	17.74	–0.389
rs2186248	G	T	intronic	–/–	5.441	0.862
rs70940803	T	G	intronic	–/–	1.642	–0.894
rs79639154	T	G	intronic	–/–	2.884	–
rs1359482195	C	A	splice_region	–/–	21.70	–0.344
rs1137101 (exon-4) (Q223R)	A	G	missense	cAgta/cGgtca	18.44	0.002
rs1805094 (exon 14) (K656N)	G	C	missense	aaGgag/aaCgag	9.128	–
rs13306520	A	G	intronic	–/–	4.568	–0.870
rs13306522	G	A	intronic	–/–	0.326	0.914

Comment: dbSNP – database identifier (“rs” number) of this variant in dbSNP. Ref – reference allele. Alt – alternative allele. Consequence – functional consequence of the variation in relation to the transcript. The nucleotide change and position relative to the coding sequence of the affected transcript in HGVS nomenclature: c. CDS Position Reference Base > Alternative Base. Example: c.223A>T. Codon Change – the affected base is written as a capital letter in both codons. Example: aAt/aCt. This column is empty if the variant is intergenic. CADD – Combined Annotation Dependent Depletion (19, 20). C<sub>i</sub> MUO – coefficients of the standardized canonical discriminant function.



on the impact of a risk factor demonstrated significant differences in the observation groups in the CNV of the *LEPR* gene with rs3790435: Pearson Chi-Square ( $\chi^2$ ) = 6.59,  $p = 0.01$ ;  $\chi^2$ , Continuity Correction = 6.98,  $p = 0.009$ ; Yates's correction  $\chi^2$  (Likelihood Ratio) = 4.84,  $p = 0.03$ ; Fisher's Exact Test ( $p$ ) = 0.013,  $p < 0.05$ , with a minimum value of the expected phenomenon of 5.5. The assessment of the strength of the relationship between the risk factor and the outcome depending on the CNV of the *LEPR* gene c rs3790435 was relatively strong: Cramer's test ( $V$ ) = 0.44, Pearson's contingency coefficient ( $C$ ) = 0.4, normalized Pearson's coefficient ( $C'$ ) = 0.57,  $p < 0.05$  (Table 4).

Analysis of the prognostic load of factors involved in the development of MUO, according to Wald's sequential analysis, allowed them to be distributed in descending order as follows ( $I \geq 0.5$ ): 2 CNV rs3790435 (RR = 2, PC = +2.76); male gender of the child (RR = 1.3, PC = +1.35); rs3790435 (RR = 1.9, PC = +2.76); hyperleptinemia more than 40.56 ng/ml (RR = 2, PC = +3); 3 or more CNV rs1359482195 (RR = 1.9, PC = +5.8); more than 4 SNPs of the *LEPR* gene in an individual (RR = 3.8, PC = +5.8).

The presence of the rs70940803 polymorphism was associated with hyperleptinemia in both children with MHO ( $p = +0.3$ ) and MUO ( $p = +0.4$ ),  $p < 0.05$ . At the same time, the rs1137101 polymorphism in children with MHO had an inverse correlation with hyperleptinemia ( $p = -0.62$ ),  $p < 0.05$ .

## DISCUSSION

In this work, we demonstrated the role of CNV of genes of different genotypes of single nucleotide polymorphisms of the *LEPR* gene in the formation of polygenic obesity and proved their association with the possibility of the formation of certain phenotypes of MUO and MHO among the Caucasian population. Insulin resistance is the main criterion that distinguishes MUO from MHO. The HOMA-IR index exceeding the 95th percentile was recorded 2.4 times more often among patients with MUO compared with chil-

dren with MHO, which was confirmed in a large number of previous studies that studied the effect of exposome on metabolic-associated diseases (10–17).

In our study, predictors of MUO were hyperleptinemia and male sex. A cross-sectional study by L. H. Barstad et al. (36) among Norwegian adolescents 12–18 years old seeking treatment for morbid obesity also showed similar gender differences. Namely, MUO signs were more often observed in boys (in 29% of cases) according to the following criteria: an increase in the level of triacylglycerides, systolic blood pressure, a decrease in the level of high-density lipoproteins compared with girls, in 19% of cases (17).

Our data are confirmed by the results of modern studies, which found that insulin resistance is associated with dysfunction of the LEP/LEPR system. Analysis of the scientometric PubMed database showed that the most studied association of obesity and type 2 diabetes with the following *LEPR* SNPs: rs3790435 and missense mutations rs1137100, rs1137101, rs1805094. The rs1137100 polymorphism is characterized by the replacement of Lys109Arg in the cytokine homology (CK) domain, rs1137101 – Gln223Arg in the loop of the CK-domain, rs1805094 – Lys656Asn in the domain of fibronectin type III of the *LEPR* molecule. It was shown that monogenic obesity is caused by homozygous mutations rs1137100, rs1137101, rs1805094, which are located in gene regions encoding functionally significant domains of the *LEPR* protein (38).

At the same time, there are conflicting data on the significance of mutations in the exons of the *LEPR* gene in the development of polygenic obesity. Thus, Aline Dos Santos Rocha et al. (39) demonstrated an association between rs1137100 (G) (OR = 1.92; 95% CI = 1.18–3.14) and overweight / obesity in children. Takuro Furusawa et al. (40) found no significant difference in the observation groups for this polymorphism. Ehab M.M. Ali (41) demonstrated that allelic frequencies of *LEPR* rs1137101 (Q223R) were significantly higher in obese subjects compared with non-obese ones in without obesity controls. At the same time, Malgorzata Roszkowska-Gancarz et al. (42) did not

**Tab. 4** Genotypes and Copy number variation of the *LEPR* gene in individuals with MHO and MUO.

SNP (genotypes: HOM <sup>p</sup> /HET/HOM <sup>n</sup> )	Genotype (%)						CNV (%)					
	MHO			MUO			MHO			MUO		
	HOM <sup>p</sup>	HET	HOM <sup>n</sup>	HOM <sup>p</sup>	HET	HOM <sup>n</sup>	1	2	≥3	1	2	≥3
rs3790435 (TT/TC/CC)	5.9	41.2	52.9	0	83.3	16.7	58.9	41.1	0	16.7	83.3	0
rs1137100 (AA/AG/GG)	0	52.9	47.1	0	50.0	50.0	47.1	52.9	0	50.0	44.4	5.6
rs2186248 (TT/TC/CC)	5.9	5.9	88.2	0	0	100.0	5.9	0	94.1	0	0	100.0
rs70940803 (GG/TG/TT)	0	17.6	82.4	0	13.5	86.5	82.4	0	17.6	76.5	23.5	0
rs79639154 (GG/TG/TT)	0	5.9	94.1	0	0	100.0	94.1	5.9	0	100.0	0	0
rs1359482195 (AA/CA/CC)	0	5.9	94.1	0	11.1	88.1	94.1	0	5.9	88.1	0	11.1
rs1137101 (GG/AG/AA)	29.4	52.9	17.6	27.8	50.0	22.2	17.6	0	82.4	22.2	0	77.8
rs1805094 (TT/GT/GG)	0	23.5	76.5	0	22.2	77.8	76.5	0	23.5	22.2	0	77.8
rs13306520 (GG/AG/AA)	0	5.9	94.1	0	5.6	94.4	94.1	0	5.9	77.8	0	22.2
rs13306522 (GG/AG/AA)	0	0	100.0		5.6	94.4	100.0	0	0	94.4	0	5.6

Comment: HOM<sup>p</sup> – homozygous variant (biallelic single nucleotide substitution), HET – heterozygous variant (single allelic single nucleotide substitution), HOM<sup>n</sup> – homozygous variant (absence of nucleotide substitutions).

find a significant effect of rs1137100 on the probability of development of obesity.

According to our data, heterozygous missense mutations rs1137100, rs1137101, rs1805094 are not accompanied by an increase in the risk of developing MUO during childhood.

According to the results of our study, the CT genotype rs3790435 is more common in children with MUO (83.3%) and less often in children with MHO (41.2%).

It was found that a mutation located in the 5'-UTR may be associated with the development of some features in the course of obesity. Thus, Juan Li et al. (43), having examined 205 patients with physiological body weight and 117 obese patients, found that the presence of TT/CT rs3790435 genotypes in obese individuals was accompanied by an increased risk of obstructive sleep apnea syndrome, compared with individuals with the CC genotype (44).

It should be emphasized that in the absence of a difference in the frequency of occurrence of various exon and intron SNPs of the *LEPR* gene in groups of children with MHO and with MUO, however, according to the results of multiple discriminant analysis, these groups significantly differed from each other in terms of the set of studied polymorphisms. According to ENCODE (ENCyclopedia Of DNA Elements), not only exome, but also intronic or intergenic SNPs can represent functional significant variants (45). Thus, we can assume that the presence of several heterozygous SNPs of the *LEPR* gene is a significant pathogenic factor that determines the risk of developing MUO in children.

In contrast to the described studies, for the first time we obtained data on the presence of an association of two copies of the *LEPR* rs3790435 gene with the presence of MUO traits. It is believed that CNVs affect gene expression and are involved in the formation of the phenotype (46, 47). Maria Pettersson et al. (48) demonstrated an increase in CNV up to 19% in obese patients. It also presents evidence of the effect of changing the CNV of one gene on the likelihood of developing obesity. In particular, it was shown that a decrease in the CNV of the *AMY1* gene is associated with the development of overweight and low expression of IL-10 (49). In all likelihood, an increase in the CNV of the *LEPR* gene rs3790435 is associated with an increase in the activity of insulin resistance.

To determine the significance of *LEPR* gene polymorphisms in the development of MUO, it is necessary to further study their clinical associations in large cohorts of individuals with different obesity phenotypes.

## CONCLUSIONS

Predictors of MUO formation in children are hyperleptinemia and male sex. The cumulative effect of the simultaneously present four or more SNPs of the *LEPR* gene pre-determines the development of MUO. The greatest risk of MUO formation is associated with an increase in the CNV of the *LEPR* gene from rs3790435. The number of concurrently present different SNPs of the *LEPR* gene in one patient is associated with the level of the likelihood of MUO occurrence in childhood.

## AUTHORS' CONTRIBUTIONS

AA was responsible for the idea and study design, looked over the articles, extracted the data, and interpreted bioinformatics analysis data. AN provided the collection of biological material using dried blood spot shipping kit, analyzed the data and interpreted it. Both authors reviewed the paper and approved the final manuscript.

## COMPLIANCE WITH ETHICAL STANDARDS

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

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**Ethical approval:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 2000 Helsinki declaration (52nd WMA General Assembly, Edinburgh, Scotland) and its later amendments or comparable ethical standards. The submissions were reviewed by the Ethics Committee of the Dnipro State Medical University (meeting minutes No. 7 of December 11, 2019 and minutes from meeting No. 5 of September 3, 2020).

**Informed consent:** Informed consent was obtained from all individual participants included in the study.

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