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ORIGINAL PAPER

Genetic variants of the glucagon-like receptor-1 in obesity

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ABSTRACT

Introduction and aim. Dysfunction of the glucagon-like peptide 1 (GLP-1)/GLP-1 receptor (GLP-1R) axis promotes obesity and metabolic disorders. The aim was to study the associations of the single nucleotide variants (SNV) *GLP1R* gene with pro-inflammatory cytokines and metabolic disorders in children with various obesity phenotypes.

Material and methods. 252 children with obesity aged 6-18 years were examined. The first group (n=152) was represented by children with metabolically unhealthy obesity (MUO). The second group (n=100) consolidated of children with metabolically healthy obesity (MHO). Whole genome sequencing (CeGat, Germany) was performed in 52 children.

Results. An association with the development of obesity was noted for T alleles rs61754624 (t=3.33) and rs10305457 (t=2.06); with MUO – for C alleles rs1042044 (t=2.23), rs1126476 (t=2.63), rs2235868 (t=2.82); T alleles rs61754624 (t=3.33), rs10305457 (t=2.06) *GLP1R*, p<0.05. In the MHO group, a correlation was found with the levels of pro-inflammatory markers IL-1β, IL-6 in the presence of the GA genotype SNV rs3765468; with hyperglycemia - GA genotype SNV rs6923761, CC genotype SNV rs1042044, AA rs6918287; hyperinsulinemia - GA genotype SNV rs3765468, GG rs10305421; triglyceridemia - AA rs6918287 of *GLP1R*.

Conclusion. SNV rs1042044, rs3765468, rs6923761, s6918287, and rs rs10305421 *GLP1R* are associated with the development of MUO in individuals with MHO.

Keywords. analysis of single nucleotide gene variants, children, glucagon-like peptide-1 receptor, metabolically healthy obesity, metabolically unhealthy obesity

Introduction

The spread of obesity and associated metabolic disorders in populations of both adults and children in the last 50 years has reached epidemic levels throughout the world.[1-5]

Obesity significantly increases the risk of developing diseases such as type 2 diabetes mellitus, metabolically associated fatty liver disease, arterial hypertension, myocardial infarction, stroke, osteoarthritis, obstructive sleep apnea and some types of cancer, thereby contributing to a decrease in both quality and life expectancy.[6-8]

It has now been demonstrated that among the various molecular systems involved in the regulation of energy balance and eating behavior, the glucagon-like peptide-1 (GLP-1) and GLP-1 receptor (GLP-1R) axis plays one of the key roles. Dysfunction of the GLP-1/GLP-1R axis contributes to the development of obesity and metabolic disorders.[9-11]

A gastrointestinal GLP-1 peptide that, in response to direct food stimulation, is released from intestinal enteroendocrine cells and excites GLP-1R, which is expressed by various body cells. The *GLP1R* gene (HGNC:4324) is located on the short arm of chromosome 6 (6p21). The GLP-1R molecule consists of 463 amino acid residues and contains 7 transmembrane domains. The GLP-1R receptor belongs to the family of G protein-coupled receptors. Excitation of GLP-1R afferent vagal neurons leads to a decrease in appetite and

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a feeling of satiety; β-cells of the islets of Langerhans of the pancreas – to increase insulin secretion; α-cells of the islets of Langerhans of the pancreas – to inhibition of glucagon production; hepatocytes – to suppress the release of glucose and accumulation of glycogen; muscle cells – to increase the activity of glucose uptake and oxidation, adipocytes – to increase the activity of glucose uptake and suppression of lipolysis processes.[12-15]

According to the modern concept, obesity is considered as a disease that occurs with the development of a chronic inflammatory reaction with a low level of activity, called meta-inflammation. The molecular features of overweight-induced meta-inflammation are of particular practical interest in the context of the obesity pandemic in the human population. Adipose tissue, being in conditions of increased concentration of free fatty acids, which can induce a TLR-mediated inflammatory response, contains cellular anti-inflammatory mechanisms, the main component of which is a population of macrophages with the M_1 phenotype.[16] Of interest is the anti-inflammatory role of activation of the GLP-1/GLP-1R axis in the regulation of the immune response and the prevention of meta-inflammation. Both innate and innate-like cells express GLP-1R. The interaction of GLP-1R and its ligands activates several signaling pathways including PKA/STAT, PI3K/Akt, MAPK and NFκB.[17] GLP-1 and its analogs (GLP-1A) can directly polarize macrophages to the M_2 phenotype, also indirectly promote M_2 polarization by inhibiting M_1[18] and potentiating regulatory T cells (Treg).[19] Activation of the JNK/STAT3 signaling pathway by GLP-1 results in decreased c-Jun N-terminal kinase (JNK) phosphorylation and its signaling through the cyclic adenosine monophosphate/protein kinase A (PKA) signaling pathway, while STAT3 phosphorylation is increased, which additionally induces polarization of macrophages towards M_2.[20] Under the influence of GLP-1, activation of the MAPK/ERK and PKA signaling pathway suppresses fatty acid synthase (FASN), IL-6 production, and eliminates endothelial progenitor cell (EPC) dysfunction that is induced by high glucose.[21] Experimental administration of GLP-1A to rats inhibits the activation of nuclear factor kappa-B (NF-κB) and IL-1β, and thus reduces inflammation.[22] In addition, GLP-1RA may function via the phosphoinositide-3-kinase (PI3K)/Akt pathway to protect the microvascular endothelium from oxidative stress in cardiometabolic disorders.[23]

The meta-inflammation that drives the metabolically unhealthy obesity (MUO) phenotype is clearly associated with dysfunction of innate immune control, including the local intestinal intraepithelial lymphocyte (IEL)-GLP-1R signaling network. Therapy with GLP-1RA (liraglutide, semaglutide, and others) in patients with MUO leads to a decrease in body weight due to a decrease in visceral fat, and suppresses the activity of manifestations of metabolic disorders.[11, 24-28]

A decrease in the level of GLP-1 reception, which is caused by single nucleotide variants (SNVs) of the *GLP1R* gene, can induce the development of obesity and metabolic disorders.[29] However, the study of associations with MUO was carried out only for some SNVs of the *GLP1R* gene.

Aim

The research was aimed to study the associations of the SNV *GLP1R* gene with pro-inflammatory cytokines and metabolic disorders in children with various obesity phenotypes.

Material and methods

Ethical approval

Participants provided written informed consent, and research protocols and procedures were approved according to the ethical standards of the Helsinki Declaration 2013 and by the Human Research Ethics Committee of Dnipro State Medical University, Ukraine (meeting minutes No. 7 of December 11, 2019 and minutes from meeting No. 4 of September 2, 2020). We obtained formal written informed consent from the parents of the children to participate in the study. Time of data collection: January 2020–February 2023.

Study design

Study design: observational, analytical, longitudinal, cohort study.

Inclusion criteria: children with polygenic obesity (BMI≥97th percentiles) 6-18 years old. Exclusion criteria: children with monogenic and/or syndromic obesity, pregnancy.

252 children with obesity aged 6–18 years were examined. The first group (n=152) was represented by children with MUO. The second group (n=100) consolidated of children with metabolically healthy obesity (MHO). For inclusion in the first observation group, the presence of abdominal obesity and two of the presented criteria were taken into account: 1). Fasting glycemia ≥ 5.6 mmol/L and/or according to the recommendations of the IDEFICS Study, the level of basal insulinemia is more than 90 percentile; 2).[30,31] High density lipoprotein cholesterol (HDL-C) ≤ 1.03 mmol/L or less than 10th percentile of the age norm; 3).[32] Triglycerides (TG) ≥1.7 mmol/L or more than the 90th percentile of the age norm; 4) Systolic blood pressure (SBP) and Diastolic blood pressure (DBP) above the 90th percentile for a given age, gender and height.[33]

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.[34]

To study carbohydrate metabolism disorders, the level of basal glycemia and insulinemia was determined by the immunochemical testing method with electrochemiluminescent detection (ECLIA), in the certified Synevo Laboratory (Dnipro, Ukraine), followed by the calculation of the generally accepted marker of insulin resistance (HOMA-IR).[30,31]

To study lipid metabolism disorders, the level of HDL-C and TAG was determined by the enzymatic-colorimetric method using kits from Roche Diagnostics (Switzerland) on a Cobas 6000 analyzer in the certified Synevo Laboratory (Dnipro, Ukraine).

To study the role of pro-inflammatory markers in the development of meta-inflammation in children with obesity, the serum levels of interleukin-1β (IL-1β), interleukin-6 (IL-6) were determined in the certified Synevo Laboratory (Dnipro, Ukraine). IL-1β was detected by the immunochemical method with chemiluminescence immunoassay (CLIA). Analyzer and test – system: Immulite (Siemens AG), Germany. The reference value of IL-1β level was 0-5 pg/mL. IL-6 was determined by an enzyme-linked immunosorbent assay (ELISA) using a Cobas 6000/Cobas 8000 kit provided by Roche Diagnostics (Switzerland). The reference value of IL-6 level was 1.5–7 pg/mL.

From the first and second groups, 52 samples for WGS were selected by limited randomization for an unbalanced distribution with a distribution coefficient of 1.5 between baseline and selective subgroups with different obesity phenotypes.[35] The sample population examined by whole genome sequencing (NGS, Illumina CSPro®, CeGat, Germany) consisted of 31 children of the first and 21 children of the second group and was qualitatively homogeneous in relation to the general population).

Material for research: venous blood. Starting material: dried blood spot cards. For DNA extraction from blood cards, we use the following protocol: Sbeadex DNA Purification Kit, customized CeGaT version (Biosearch Technologies, LGC). 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 x 100 bp.

Bioinformatic analysis – demultiplexing of the sequencing reads was performed with Illumina bcl2fastq (version 2.20). Adapters were trimmed with Skewer, version 0.2.2. 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. [36,37] ABRA, version 2.18 and GenotypeHarmonizer v.1.4.20 were used for local restructuring of readings in target regions to improve more accurate detection of indels in the genome during mutagenesis.[38,39]

We used ClinVar Version 20200316, InterVar gnomeAd Version 3.0 and dbnsfp Version 35c for clinical and functional variant annotation and GWAS catalog database annotation.[33,34,40,41]

Reference sequence obtained from the National Center for Biotechnology Information RefSeq database (http://www.ncbi.nlm.nih.gov/RefSeq/).[44]

Statistical analysis

Statistical analysis of the obtained results was carried out using a package of application programs Statistica 6.1 (No AGAR909E415822FA) with help a personal computer based on an Intel processor Pentium 4.

For statistical processing of the materials studied, the normality of the distribution of signs was rechecked according to the Shapiro-Wilk test (SW-W), the evenness of the dispersions - according to the Fisher test (F). The arithmetic mean with the error of the mean value (M±m) was used to describe quantitative traits with a normal distribution; the standard deviation (SD) to describe the variation in the traits and the 95% confidence interval (95% CI), sequential Wald analysis with calculation of Relative Risk (RR), to define the range of the population means.

The relationship between indicators was determined using Spearman correlation analysis. The reliability assessment of the difference of means in multiple comparisons for quantitative traits with a normal distribution was carried out by one-way analysis of variance (ANOVA) with a posteriori pairwise comparisons according to the Tukey test. Intergroup comparisons of statistical characteristics were performed taking into account the law of distribution using parametric and non-parametric criteria: assessment of the probability of differences in means for unrelated samples – according to Student's criteria (t) in the Welch modification (R Studio, Version 1.0.136, 2016). Only statistically significant results were taken into account ($p<0.05$).

Results

The average age of children in the first observation group (n=152) was 13.64±0.43 years, while in the second group (n=100) it was 11.05±0.6, $p<0.005$. The number of children under 12 years old in the first group was 13%, in the second group – 48%. In this regard, the RR=4.67±0.46 (95% CI 1.87–11.16) of MUO in children over 12 years old was 4.67 times higher than in children under 12 years old, $p<0.05$. The number of boys in the first group – 42% (65/152), in the second group – 48% (48/100), $p>0.05$. RR of MUO in girls was 0.99±0.13 (95% CI 0.8–1.2), $p<0.05$.

The results of clinical and paraclinical examinations (Table 1) of children with various obesity phenotypes revealed the most frequent clinical associations of markers of the complicated course of obesity (dyslipidemia,

hyperglycemia, pro-inflammatory orientation of the immune response).

Table 1. Data of clinical and paraclinical examination of children with different obesity phenotypes

Significative	MUO (n=152), M±m	MHO (n=100), M±m	p
BMI in percentiles, %	99.54±0.21	98.74±0.29	0.12
Presence of extreme obesity 2nd stage (120-139% over the 95th percentile), %	19±3.92	16.1±3.68	0.06
Presence of extreme obesity 3rd stage (over 140% out of 95th percentile), %	32.3±4.66	0	0.00001
WC in percentiles	96.65±0.42	93.38±0.82	0.0004
SBP in percentiles	83.77±3.05	71.38±3.96	0.014
DBP in percentiles	87.48±2.75	66.33±4.09	0.0006
HDL in percentiles	30.83±4.04	32.81±2.79	0.68
TAG in percentiles	87.7±2.28	83.33±3.63	0.3
Fasting blood glucose, mmol/L	4.15± 0.37	3.36±0.48	0.2
Basal insulin, mcU/mL	29.47±1.14	12.53±1.44	0.00001
HOMA-IR	5.32±0.3	2.13±0.12	0.00001
IL-6, pg/mL	3.4±0.82	1.04±0.22	0.007
IL-1β, pg/mL	3.6±0.63	1.78±0.17	0.008

In children with obesity examined by whole genome sequencing, 14 SNVs of the *GLP1R* gene were identified: rs761386, rs1042044, rs1126476, rs2235868, rs3765468, rs61754624, rs6918287, rs6923761, rs10305420, rs10305421, rs10305457, rs10305492, rs10305493, rs1472308929. The distribution of genotype frequencies was in Hardy-Weinberg equilibrium in both groups.

Molecular genetic characteristics of the identified SNVs of the *GLP1R* gene are presented in Table 2.
Among the identified SNV of the *GLP1R* gene, the highest CADD was observed in three nonsynonymous variants rs10305493, rs10305421, rs10305492 (26.1; 25; 22.5, respectively).

Associations of SNV GLP1R gene with obesity phenotypes in children

The frequency of occurrence of SNV of the *GLP1R* gene in children with different obesity phenotypes is presented in Table 3.

In obesity, the AF of the minor T alleles for SNV rs61754624 (t=3.33) and rs10305457 (t=2.06) of the *GLP1R* gene was significantly higher than the allele frequency of these polymorphisms among healthy Europeans of non-Finnish origin, p<0.05.

In individuals with MUO, the AF of the minor C alleles of SNV rs1042044 (t=2.23, p<0.05), rs1126476 (t=2.63, p<0.05), rs2235868 (t=2.82, p<0.05); T alleles of SNV rs61754624 (t=3.33, p<0.05) and rs10305457 (t=2.06, p<0.05) of the *GLP1R* gene were significantly higher than the allelic frequency of these polymorphisms among healthy non-Finnish Europeans.

Among probands with MUO, the AF of the minor T allele rs761386 and A allele rs10305492 (t=2.29, p<0.05) was significantly higher compared to the allele frequency of these SNV gene *GLP1R* among children with MHO.

Table 2. Characteristics of SNV types of the *GLP1R* gene[a]

SNV	Variant name and GRCh38 reference sequence file identifier (HGVS)	GnomAD _maxPOP	Ref	Alt	Consequence	Base Change	CADD	RawScore	Clinical significanse (ClinVar)
rs6918287	6:39065826A>G (NM_002062.5: c.399A>G)	EAS	A	G	synonymous	c.399A>G	9.35	0.49	not reported
rs6923761	6:39066296G>A (NM_002062.5: c.502G>A	NFE	G	A	missense	c.502G>A	16.12	1.47	not reported
rs761386	6:39079095C>T (NM_002062.5: c.955-17C>T)	AMR	C	T	intronic	c.955-17C>T	4.32	0.10	not reported
rs1042044*	6:39073726A>C (NM_002062.5: c.526A>C)	AMR	A	C	missense	c.526A>C	14.9	1.25	not reported
rs1126476*	6:39080715A>C (NM_002062.5: c.1200A>C)	AMR	A	C	synonymous	c.1200A>C	11.53	0.75	not reported
rs2235868*	6:39072878A>C (NM_002062.5: c.526A>C)	AMR	A	C	synonymous	c.526A>C	12.35	0.85	not reported
rs3765468	6:39065817G>A (NM_002062.5: c.390G>A)	EAS	G	A	synonymous	c.390G>A	8.41	0.40	not reported
rs61754624*	6:39066295C>T (NM_002062.5: c.501C>T)	AMR	C	T	synonymous	c.501C>T	0.11	-0.47	likely benign
rs10305420	6:39048860C>T (NM_002062.5:c.20C>T)	NFE	C	T	missense	c.20C>T	13.38	0.99	not reported
rs10305421	6:39048899G>A (NM_002062.5: c.59G>A)	NFE	G	A	missense	c.59G>A	22.5	2.49	not reported
rs10305457*	6:39066319C>T (NM_002062.5: c.509+16C>T)	AMR	C	T	intronic	c.509+16C>T	0.43	-0.28	not reported
rs10305492	6:39079018G>A (NM_002062.5: c.946G>A)	NFE	G	A	missense	c.946G>A	25	3.51	not reported
rs10305493	6:39079155C>G (NM_002062.5: c.998C>G)	OTH	C	G	missense	c.998C>G	26.1	3.77	not reported
rs1472308929	6:39066202C>T (NM_002062.5: c.408C>T)	NFE	C	T	synonymous	c.408C>T	9.32	0.49	not reported

[a] HGVS – Human Genome Variation Society;[45] GnomAD_maxPOP – the frequency distribution of *GLP1R* mutations. AFR, NFE represent African, Non-Finnish European; 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 (c. - interpretation for DNA coding sequence: first nucleotide of the translation start codon of the coding DNA reference sequence).[46] This column is empty if the variant is intergenic; CADD – combined annotation dependent depletion; *- SNV *GLP1R* associated with MUO

Table 3. The frequency of occurrence of SNV *GLP1R* gene in children with different obesity phenotypes[a]

SNV	gnomAD browser		The frequency of occurrence of major and minor options (%)				The value of Student's t-test in Welch's modification		
			MHO (n=21)		MUO (n=31)				
	Popmax AF (HET/HOM^P), %	AF NFE, (HET/HOM^P), %±m	(HOM^N), % (n)	(HET/HOM^P), %±m (n)	(HOM^N), % (n)	(HET/HOM^P), %±m (n)	t_1	t_2	t_3
rs6918287	98	99±2.18	5 (1)	95±2.18 (20)	6 (2)	94±2.37 (29)	0.31	1.67	1.94
rs6923761	32	33±4.7	57 (12)	43±4.95 (9)	55 (17)	45±4.97 (14)	0.28	1.46	1.75
rs761386	19	3±2.18	95 (20)	5±2.18 (1)	100 (31)	0	2.29*	0.72	1.76
rs1042044	56	56±4.96	38 (8)	62±4.85 (13)	29 (9)	71±4.54 (22)	1.35	0.86	2.23*
rs1126476	50	50±5	52 (11)	48±5 (10)	32 (10)	68±4.66 (21)	2.93*	0.28	2.63*
rs2235868	46	52±5	48 (10)	52±5 (11)	29 (9)	71±4.54 (22)	2.82*	0	2.82*
rs3765468	8	7±2.55	90 (19)	10±3 (2)	90 (28)	10±3 (3)	0	0.76	0.76
rs61754624	0.7	0.6±0.77	90 (19)	10±3 (2)	100 (31)	0	3.33*	3.33*	3.33*
rs10305420	37	39±4.88	48 (10)	52±5 (11)	48 (15)	52±5 (16)	0	1.86	1.86
rs10305421	0.2	0.5±0.71	100 (21)	0	97 (30)	3±1.71 (1)	1.76	0	0
rs10305457	18	9±2.86	81 (17)	19±3.92 (4)	77 (24)	23±4.21 (7)	0.7	2.06*	2.06*
rs10305492	1	1±0.99	95 (20)	5±2.18 (1)	100 (31)	0	2.29*	1.67	1.67
rs10305493	0	0.01±0.1	100 (21)	0	97 (30)	3±1.71 (1)	1.76	0	0
rs1472308929	-	-	100 (21)	0	97 (30)	3±1.71 (1)	1.76	-	-

[a] HOM^P – homozygous variant (biallelic single nucleotide substitution), HET – heterozygous variant (single allelic single nucleotide substitution), HOM^N – homozygous variant (absence of nucleotide substitutions); Popmax AF – Maximum population allele frequency in the genome (gnomAD browser); AF NFE – Allele frequency for Non-Finnish Europeans in the genome (gnomAD browser); * – Critical value of Student's t-test modified by Welch >1.97, number of degrees of freedom f=198, at which the differences in the compared groups are significant, $p<0.05$; t_1 – Student's test of significance in the MUO and MHO comparison groups; t_2 – Student's test of significance in the comparison groups MHO and healthy Non-Finnish Europeans; t_3 – Student's test of significance in the comparison groups MUO and healthy Non-Finnish Europeans; m – relative indicator mean error

Associations of SNV GLP1R gene with inflammatory activity

Correlations of heterozygous SNV phenotypes rs6923761, rs3765468, rs10305420 of the *GLP1R* gene with the level of pro-inflammatory cytokines in blood serum were observed in children with MHO. Thus, rs6923761 and rs10305420 of the *GLP1R* gene were inversely proportional to the levels of IL-1β, IL-6 (r=-0.38 rs6923761 – IL-1β); r=-0.33; -0.48 (rs10305420 – IL-1β, IL-6), respectively), and rs3765468 of the *GLP1R* gene is directly proportional to the level of IL-1β, IL-6 concentration in blood serum (r=0.30; 0.72, respectively), $p<0.05$.

One-way analysis of variance (ANOVA) revealed the influence of SNV rs3765468 genotype of the *GLP1R* gene in children with MHO on the pro-inflammatory variant of the immune response in the form of increased IL-6 (F=5.77; p=0.05). A pairwise comparison of indicators depending on the genotype revealed the formation of a pro-inflammatory immune response in the form of an increase in IL-6 with the AA rs3765468 genotype ($p<0.05$ for pairwise comparisons of AA rs3765468 genotypes with others according to the Tukey test).

While in patients with MUO, there was no association of the level of pro-inflammatory cytokines in the blood serum with any SNV of the *GLP1R* gene.

Associations of SNV GLP1R gene with disorders of carbohydrate metabolism

It has been established that SNVs rs6923761, rs1042044, rs1126476, rs2235868, rs3765468 of the *GLP1R* gene are associated with the mechanisms of regulation of carbohydrate metabolism in children with MHO. It was shown that the GA genotype SNV rs6923761 (RR=1.39) and the CC genotype rs1042044 (RR=1.35) of the *GLP1R* gene are associated with the level of glycemia (r=0.35; 0.33, respectively), $p<0.05$. Whereas the CC SNV genotypes rs1126476 and rs2235868, as well as the GA genotype rs3765468 of the *GLP1R* gene, are associated with basal serum insulin levels (r=0.48; 0.51; 0.56, respectively), $p<0.05$.

In contrast to children with MHO, in children with MUO carbohydrate metabolism disorders were not associated with SNV of the *GLP1R* gene. In addition, the presence of the GG genotype SNV rs6918287 of the *GLP1R* gene prevented a decrease in carbohydrate tolerance (r=-0.43), and the presence of the GA genotype SNV rs10305421 of the *GLP1R* gene prevented the development of insulin resistance (r=-0.72), $p<0.05$. The results of ANOVA also showed that the genotype SNV rs6918287 and rs10305421 of the *GLP1R* gene affects the level of basal glycemia and insulin resistance in children with obesity (respectively F=6.26 and F=5.62; $p<0.05$). A pairwise comparison of the indicators of the formation of basal hy-

perglycemia and insulin resistance among themselves, depending on the genotype, revealed statistically significant higher levels of indicators in AA rs6918287 and GG rs10305421 genotypes ($p<0.05$ for pairwise comparisons of AA rs6918287 and GG rs10305421 genotypes with others according to the Tukey test).

Associations of SNV GLP1R gene with lipid metabolism disorders

Correlation analysis made it possible to establish that in children with MHO, the presence of CT genotype SNV rs10305420, GA genotype rs10305421 of the *GLP1R* gene is accompanied by a lower level of atherogenicity of the blood serum lipid spectrum (r=-0.43, -0.35, respectively), $p<0.05$. Whereas in children with MUO, the presence of the GG genotype SNV rs6918287 of the *GLP1R* gene is associated with a lower level of triglyceridemia (r=-0.49), $p<0.05$.

According to the results of ANOVA, significant differences in the influence of genotypes of SNV rs6918287 of the *GLP1R* gene in children with MHO on the level of triglyceridemia were found (respectively, F=51.34; p=0.05). A pairwise comparison of indicators of the formation of hypertriglyceridemia among themselves, depending on the genotype, revealed statistically significant higher levels of indicators with the AA rs6918287 genotype ($p<0.05$ for pairwise comparisons of the AA rs6918287 genotype with others according to the Tukey test).

Discussion

Considering the key role of the GLP-1/GLP-1R axis in maintaining the body's energy balance and regulation of carbohydrate metabolism, it is assumed that impaired GLP-1 reception or GLP-1R functioning will contribute to the development of obesity and metabolic disorders.[26] Currently, rs1042044 (Leu260Phe), rs10305420 (Pro7Leu), rs6923761 (Gly168Ser), and rs3765467 (Arg131Gln) are considered the most common nonsynonymous SNVs of the *GLP1R* gene.[28] We did not find the rs3765467 variant from the SNV data group of the *GLP1R* gene in obese individuals.

This study demonstrates that healthy children in the European population with at least one copy of the minor allele T rs61754624, rs10305457 have a higher risk of obesity, and with the presence of one or two copies of the minor allele C rs1042044, rs1126476, rs2235868 or minor T alleles rs61754624, rs10305457 *GLP1R* genes have a higher risk of developing MUO than children with null copies of the above alleles.

We have shown for the first time that SNV rs3765468 of the *GLP1R* gene in children with the MHO obesity phenotype is associated with pro-inflammatory status, and SNV rs6923761, rs10305420 of the *GLP1R* gene with anti-inflammatory status. In all likelihood, given that GLP-1R is expressed by various immune cells, such as monocytes, macrophages, and T cells, SNV-mediated changes in GLP-1R activity may predetermine the level of production of pro-inflammatory cytokines.[47]

The GLP-1/GLP-1R axis is known to be a key regulator of carbohydrate metabolism. We have shown that the SNVs rs6923761, rs1042044, rs1126476, rs2235868, rs3765468 of the *GLP1R* gene introduce specific features into the functioning of carbohydrate metabolism in children with MHO.

It was found that two nonsynonymous SNVs rs6923761, rs1042044 of the *GLP1R* gene contribute to the development of glycemia. The most common polymorphism of the *GLP1R* gene is the genetic variant rs6923761, which, according to our data and the results of other researchers, is moderately associated with the level of glycemia.

The rs6923761 (G>A/C) variant is a missense variant, which is accompanied by the substitution of a glycine for a serine residue at position 168 (Gly168Ser) of the GLP-1R molecule. According to the ACMG classification, SNV rs6923761 was classified as a benign variant. At the same time, Michałowska et al. revealed a tendency to hyperglycemia in carriers of the AA rs6923761 genotype, compared with carriers of the AG rs6923761 genotype, but found no association with the metabolic syndrome, which also coincided with the results of our studies.[48] Sathananthan et al.[49], also Daniel Antonio de Luis et al. demonstrated that GLP-1R in heterozygotes for SNV rs6923761 of the *GLP1R* gene (GA genotype) has a low receptor affinity for GLP-1, which leads to relatively reduced insulin secretion in response to GLP-1 infusion and, as a result, promotes the development of hyperglycemia. Individuals with the A allele of SNV rs6923761 of the *GLP1R* gene and morbid obesity have higher levels of triglycerides, insulin, and insulin resistance.[50] Interestingly, carriers of the GG SNV rs6923761 genotype show a weaker response to treatment with liraglutide than carriers of the non-wild-type allele.[51] Individuals with the genotype AA rs6923761 are at higher risk of becoming overweight.[48]

The missense mutation rs1042044 (A>C,G,T) of the *GLP1R* gene is a variant that is accompanied by the substitution of a leucine for a phenylalanine residue at position 260 (Leu260Phe), which is accompanied by a decrease in receptor excitation and the development of glycemia.[52-55]

Li et al.demonstrated that the nonsynonymous SNV rs10305492, which leads to the replacement of an alanine by a tryptophan residue at position 318 (Ala316Thr) of the GLP-1R molecule, is also accompanied by a decrease in insulin secretion by β-cells.[56] However, in our study, we did not find any correlation between the presence of the minor allele and the level of glycemia or insulin in the blood serum. Wessel et al. also found no association

of SNV rs10305492 with fasting insulin levels or incretin response. [57] According to our data, synonymous SNVs rs1126476 (A>C), rs2235868 (A>C,G,T), rs3765468 (G>A) of the *GLP1R* gene are associated with a greater ability of basal insulin secretion by β-cells in children with MHO, which is consistent with the data other researchers.[58] The absence of associations of these SNV data of the *GLP1R* gene in children with MUO with hyperglycemia is probably due to the fact that the development of hyperglycemia in children with MUO is mainly due to insulin resistance induced by meta-inflammatory factors.

We have shown for the first time that in children with MHO, the presence of the missense mutation rs10305420 (C>T), which is accompanied by the replacement of proline with a leucine residue in position 7 (Pro7Leu), the missense mutation rs10305421 (G>A), which is accompanied by the replacement of arginine by lysine the residue at position 20 (Arg20Lys) of the GLP-1R receptor molecule prevents the occurrence of atherogenic disorders of lipid metabolism.

While in children with MUO, the GG SNV rs6918287 genotype of the *GLP1R* gene protects against the development of triglyceridemia. We did not find any relationship between SNV rs6923761 and lipid metabolism disorders. At the same time, de Luis et al.[12] demonstrated that individuals with the wild GG genotype, compared with individuals with the AA SNV rs6923761 genotype of the *GLP1R* gene, had a significantly lower HDL-C level and a higher serum triglyceride level.

In this work, we demonstrated the role of SNV *GLP1R* in the formation of a pro-inflammatory immune response and metabolic disorders with the possibility of the formation of certain MUO and MHO phenotypes among the European population. However, to determine the significance of SNV *GLP1R* gene polymorphisms and taking into account the limitation of their influence in the development of MUO, further study of their clinical associations in large cohorts of individuals with different obesity phenotypes is required.

Conclusion

Variants of the *GLP1R* gene in children with the MHO phenotype determine the level of inflammatory status (GA/AA SNV rs3765468), carbohydrate tolerance (GA rs6923761, CC rs1042044 and AA rs6918287), insulin resistance (GA/AA SNV rs3765468, CC rs10305421), and serum lipid spectrum atherogenicity blood (AA rs6918287) and thus predetermine its transformation into MUO.

From a practical point of view, the determination of the SNV genotype of the *GLP1R* gene will make it possible to predict the likelihood of MUO and personalize the trajectory of the development of various metabolic disorders associated with obesity in children.

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Declarations

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Author contributions

Conceptualization, A.N.; Methodology, A.N.; Software, A.N.; Validation, A.N.; Formal Analysis, A.N.; Investigation, A.N.; Resources, A.N.; Data Curation, A.N.; Writing – Original Draft Preparation, A.N.; Writing – Review & Editing, A.N.; Visualization, A.N.; Supervision, A.N.; Project Administration, A.N.; Funding Acquisition, A.N.

Conflicts of interest

The author declare no competing interests.

Data availability

The datasets used and/or analyzed during the current study are open from the corresponding author on reasonable request.

Ethics approval

Human Research Ethics Committee of Dnipro State Medical University, Ukraine (meeting minutes No. 7 of December 11, 2019 and minutes from meeting No. 4 of September 2, 2020).

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