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THE ROLE OF LEPTIN GENES AND LEPTIN RECEPTOR GENES IN THE DEVELOPMENT OF CHILD OBESITY

The role of polymorphic variants of the leptin (*LEP rs2167270*) and leptin receptor (*LEPR rs1137100*) genes in the development of childhood obesity and eating behavior was assessed. Eating behavior was assessed using the CEBQ questionnaire. There was no association with the development of obesity when comparing children's groups with each other. At the same time, the association of the *LEP* (rs2167270) was established according to the following scales of the CEBQ questionnaire: “pleasure from eating, EF”, “slowness in eating, SE” and glucose level. For the *rs1137100* locus of the *LEPR* gene, associations are shown with such anthropometric parameters as birth weight, weight at present, Z-score, and percentile level.

Keywords: obesity in children, eating behavior, CEBQ, polymorphism, leptin, leptin receptor.

According to the WHO, the number of childhood obesity cases has reached alarming levels in many countries and continues to grow (https://www.who.int/end-childhood-obesity/facts/ru/). Pediatric obesity remains an ongoing serious in Russian, especially in boys aged 11 years [4]. It was found that only 60% of the school children had a normal weight, the prevalence of obesity and overweight reached 40% and 10% of children with underweight.

It is known that the main cause of childhood obesity is energy imbalance as result from excessive energy intake (WHO https://www.who.int/end-childhood-obesity/facts/ru/). However, only physical activity and frequent consumption of fatty foods cannot be explained cases of familial obesity. Besides more than 79 obesity-related syndromes are known. Identification of genes for morbid obesity will allow for corrective therapy starting from childhood [9].

One of the most well-known genes for obesity is the gene for the peptide hormone leptin, which is responsible for anorexigenic action or appetite suppression. Leptin, on the one hand, reduces the formation of insulin, and on the other, it increases the sensitivity of cells to insulin. In turn, this may contribute to the development of insulin resistance and the formation of type 2 diabetes mellitus (T2DM) in patients with high leptin levels. The *rs2167270* polymorphic marker of the *LEP* gene correlates with leptin levels and is also associated with metabolic syndrome, T2DM and is a risk factor for cardiovascular diseases [5, 14, 16]. The polymorphic marker causing the *A* to *G* substitution at position -2548 upstream of the ATG start site in the 5'-region of the leptin gene promoter is responsible for altered expression. Thus, in comparison with the *G* allele, the *A* allele is associated with a twofold increase in gene expression [18]. Obese people develop leptin resistance; they have both high concentrations of leptin in the blood plasma and very low. High concentration can be the cause of leptin resistance and responsible for activating the molecular mechanisms underlying leptin resistance. On the other hand, a well-known leptin defect leading to structural disruption and a decrease in leptin levels lead to a constant feeling of hunger in patients and leads to obesity. The manifestation of leptin is also mediated by binding to the leptin receptor (*LEPR*) located on the membrane of hypothalamic cells [12]. *LEPR* belongs to the family of cytokine class receptors [13]. There are functionally significant polymorphic variants of the leptin receptor gene with a possible biological effect on metabolic regulation. The polymorphic marker *rs1137100* of the *LEPR* gene is located in exon 4 and leads to an amino acid substitution in the protein sequence (K109R). In our study, Kochetova OV, 2019, an association of the *rs1137100* locus of this gene with the BMI level in the population of Tatars with type 2 diabetes mellitus was revealed [1].

Leptin leptin interacts with hypothalamic receptors to induce satiety, inhibiting the neuronal activity of orexigenic neuropeptide Y (NPY) / agouti-related peptide (AgRP), and stimulating anorexigenic neuronal activity. Children's eating behavior is provided by both genes and the environment [17]. Martín-Pérez C, et al., 2018 revealed a violation of the functional activity of the hypothalamic-pituitary system during overeating and obesity in adolescents; eating disorders were determined using the CEBQ questionnaire [11].

The aim of our work was to analyze the associations of polymorphic variants of the *LEP* and *LEPR* genes with childhood obesity and the assessment of eating behavior in children.

Material and methods. The study used DNA samples from 380 children living in the city of Ufa. Of these, 170 are obese and overweight patients and 270 children without signs of obesity. The description of the samples is given in table. 1. The average age of children in the obese group was 7.1 ± 2.3 years, in the control group 7.3 ± 2.5 years (the age ranged from 2 to 10 years). Anthropometric measurement was carried out according to standard methods. To assess anthropometric status, reference tables of the World Health Organization (WHO) for 2006 and 2007 were used, which are based on Z-scores for body mass index (BMI) depending on gender and age. For statistical analysis, overweight was determined as follows: for children under 5 years of age (z> +2 points) (http://who.int/childgrowth/standards/ru/), for children aged 5 to 10 years (z > +1) (http://who.int/growthref/who2007_bmi_for_age/en/index.html). The sample was formed on the basis of a multidisciplinary hospital (City Clinical Hospital No. 17, Ufa).

Genotyping. DNA was isolated from peripheral blood leukocytes using phenol-chloroform purification. Conditions for PCR, primer sequences are presented in the study of M. Krylov et al., 2010 [2]. The results of amplification and restriction were assessed using vertical electrophoresis in 6–8% polyacrylamide gel. The gel was stained with a solution of ethidium bromide (0.1 μg / ml) for 15 min and photographed in transmitted ultraviolet light. To determine the size of the product, a molecular weight marker with a step of 100 bp (SibEnzyme, Russia) was used.

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Eating behavior analysis (BP) was carried out using the Child Eating Behavior Questionnaire (CEBQ) [8]. The questionnaire consists of 8 scales, such as: Food responsiveness (FR); Enjoyment of food (EF), Satiety responsiveness, (SR); Slowness in eating (SE); Food fussiness (FF); Emotional over-eating (EOE); emotional under-eating (EUE); Desire to drink (DD). CEBQ has good psychometric properties: internal consistency, test reliability, and dynamic stability. Used to analyze the eating behavior of young children.

Statistical processing of results. Statistical processing of the data was performed using the SPSS Statistics 22 software packages. The association between polymorphic variants of the studied genes and obesity was assessed using the Pearson χ^2 test. The groups of obese patients and children of the control group were compared in pairs. The frequencies of alleles and genotypes, the correspondence of the distribution of genotype frequencies to the Hardy – Weinberg equilibrium (χ^2 and P) were calculated. Logistic regression was used to identify the association of polymorphic variants of the studied genes with the development of obesity and eating behavior; the exponent of the individual regression coefficient (beta) was interpreted as the odds ratio (OR) with the calculation of a 95% confidence interval. The contribution of allelic variants of the studied candidate genes to the variability of quantitative clinical and biochemical parameters (glucose, lipid levels, etc.) and CEBQ scores was determined using the Kruskal-Wallis test (in the case of three groups) or Mann-Whitney (in the case of two groups).

Results and discussion. An analysis was carried out for the correspondence of the frequency distribution of the genotypes of polymorphic loci to the Hardy – Weinberg equilibrium, and the frequency of a minor allele frequency (MAF) was tested in the patient and control samples. The following results were obtained in control group: for *LEPR rs1137101* gene (PX-B = 0.06, MAF=0.3019), for *LEP rs2167270* gene (P=0.53, MAF=0.3241), in the group of patients for *LEPR rs1137101* gene (PX -B = 0.23, MAF = 35.59) and for *LEP rs2167270* gene (PX-B=0.23, MAF=35.59).

N is the number of individuals in the group. P * is the significance level comparing the frequencies of alleles or genotypes of the control group and the group of patients, P ** is the significance level of the Armitage trend test, P *** is the significance level adjusted for gender, age of gestation, and feeding. Statistically significant differences (P<0.05) are marked in bold.

The analysis of the scales of the CEBQ questionnaire showed differences in the compared groups of children in terms of the indicators Food responsiveness (FR) (P=0.01) and Enjoyment of food, (EF) (P=0.03). These indicators determined low satiety, increased appetite and interest in food, that contributes to the development of obesity. Hirsch Ya. V. et al., 2018 confirmed the results [3]. Analysis of allele and genotype frequencies for polymorphic markers of *LEP* and *LEPR* genes between overweight children and the control group statistically significant differences were not obtained (Table 2).

Analysis of quantitative parameters of obesity and eating behavior (CEBQ) is presented in table 3. Statistically significant associations were also observed for gene *LEP* (*rs2167270*) with Enjoyment of food (EF) (P=0.03) and Slowness in eating (SE) (P=0.0096). Carriers of allele *A* had higher scores for the EF scale and low scores for the SE scale (Table 3). The association was also found with fasting glucose for the *rs2167270* locus of the *LEP* gene (P=0.032). Carriers of the *AA* genotype had high blood glucose levels, reaching 6.25 mmol/L. It can be assumed that allele *A* is an eating disorder risk allele in children, and also leads to the development of insulin resistance. Poitou C et al. (2005) showed a decrease in the leptin level in children with morbid obesity, carriers of the GG genotypes *LEP* (*rs2167270*) [15]. However, allele *G* was associated with decreased blood leptin levels according to other authors [6]. Our study confirms the absence of a relationship between *LEP rs2167270* gene polymorphic locus and the risk of obesity in children and indicates the ambiguity of the results obtained [7].

Associations with birth weight, current weight, Z-scores and percentiles were established for the *LEPR rs1137100* marker (P=0.02, P=0.032, P=0.028, P=0.04). Carriers of the genotypes *AA* and AG had a higher birth weight and currently weight, high Z-score and percentile levels. Obesity in adolescents was associated with both the *A* and *G* alleles [10]. *A* allele of the *LEPR rs1137100* locus is characterized by an increased level of leptin and impaired glucose tolerance in girls with an android fat mass; for the *G* allele, an association was found in girls with a gynoid fat mass[10]. Several studies have shown severe leptin resistance in obese that its level is much higher than in patients with obesity. The association is most likely associated with leptin resistance mediated by the leptin receptor.

Conclusion. These results indicate the association of polymorphic variants of the gene LEP with the nutritional characteristics of children (CEBQ) and glucose level and LEPR with anthropometric characteristics.

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Table 1

Clinical and biochemical characteristics of children

Parameters	Control group (N = 270)	Obesity (N=170)	P
Boys, N (%)	118 (56.2%)	92 (54.1%)	0.67
Girls, N (%)	92 (43.8%)	78 (45.9%)	0.71
Age, years	7.3±2.5	7.1±2.3	0.81
Weight, kg	19.7±3.5	22.4±2.6	**0.002**
Height, cm	113.1±8.9	106.8±7.3	0.08
Percentile	38.9±10.1	92.5±3.2	**0.0001**
BMI, kg / m^2	15.5±2.1	19.6±3.4	**0.0001**
Gestational age, weeks	39.1±1.1	39.0±1.4	0.78
Birth weight, g	3313±100	3376±110	0.69
CEBQ			
FR, Food responsiveness	1.9±0.47	2.2±0.77	**0.01**
EOE, Emotional over-eating	1.6±0.54	1.7±0.63	0.89
EF, Enjoyment of food	3.0±0.63	3.35±0.82	**0.03**
DD, Desire to drink,	2.9±0.84	2.8±0.81	0.13
SR, Satiety responsiveness	3.1±0.57	2.99±0.60	0.22
SE, Slowness in eating	2.5±0.58	2.6±0.70	0.94
EUE, Emotional Malnutrition	2.8±0.78	2.8±0.87	0.87
FF, Food fussiness	2.9±0.43	3.1±0.49	0.73

Note: - statistically significant differences are in bold, P - significance level.

Table 2

Frequency distribution of genotypes and alleles of LEP and LEPR genes

Genotypes and alleles	Obesity (N = 170) N (%)	Control (N = 270) N (%)	P*	P**	P***
	LEP rs2167270				
GG	73 (42.94)	126 (46.67)	0.63		
AG	73 (42.94)	113 (41.85)			
AA	24 (14.12)	31 (11.48)		0.07	0.66
G	219 (64.41)	365 (67.59)			
A	121 (35.59)	175 (32.41)	0.37		
	LEPR rs1137100				
AA	69 (40.59)	125 (46.30)	0.31		
AG	84 (49.41)	127 (47.04)			
GG	17 (10.00)	18 (6.67)		0.13	0.36
A	222 (65.29)	377 (69.81)			
G	118 (34.71)	163 (30.19)	0.19		

N is the number of individuals in the group. P * is the significance level comparing the frequencies of alleles or genotypes of the control group and the group of patients, P ** is the significance level of the Armitage trend test, P *** is the significance level adjusted for gender, age of gestation, and feeding. Statistically significant differences (P <0.05) are marked in bold.

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References

1. Анализ ассоциаций полиморфных вариантов генов LEPR (rs1137100), LRP5 (rs3736228) и LPL (rs320) с риском развития сахарного диабета 2-го типа / О. В. Кочетова, Д. Ш. Авзалетдинова, Л. Ф. Шарипова [и др.]. *Генетика*. 2019; 55(4); 458-467 [Analysis of associations of polymorphic variants of LEPR (rs1137100), LRP5 (rs3736228), and LPL (rs320) genes with the risk of developing type 2 diabetes mellitus / O. V. Kochetova, D. S. Avzaletdinova, L. F. Sharipova [et al.]. *Russian Journal of Genetics*. 2019; 55(4): 495-503 (in Russ.).]

2. Крылов М. Ю. Полиморфизм A19G гена лептина и полиморфизмы Gln223Arg и Lys109Arg гена рецептора лептина при постменопаузальном остеопорозе / М.Ю. Крылов, Л.И. Беневоленская, В.А. Мякоткин // Научно-практическая ревматология. - 2010. - №.5. – С. 27-31. [Krylov M.Yu., Benevolenskaya L.I., Myakotkin V.A. Leptin A19G polymorphism and Leptin receptor Gln223Arg and Lys109Arg polymorphisms in postmenopausal osteoporosis. *Scientific-practical rheumatology*. 2010; 5: 27-31 (in Russ.).]

3. Особенности пищевого поведения детей младшей возрастной группы, проживающих в условиях урбанизированного города и районных центров / Я.В. Гирш, Т.А. Юдицкая, А.А. Тепляков [и др.]. *Вестник СурГУ. Медицина*. 2018; 2: 47-51 [Food behavior features of children of younger age group living in urbanized cities and district centers / Y.V. Girsh, T.A. Yuditskaya, A.A. Teplyakov [et al.]. *Bulletin of SurGU. Medicine*. 2018; 2: 47-51 (in Russ.).]

4. Оценка физического развития детей среднего и старшего школьного возраста:

Table 3

Analysis of associations of polymorphic loci of genes *LEP, LEPR*, clinical and anthropometric parameters and eating behavior scales (CEBQ) in children

Parameter	*LEP rs2167270*			*P**	*P***	*LEPR rs1137100*			P*	P**
	GG	*AG*	*AA*			*AA*	*AG*	*GG*		
FR	1.82 (0.09)	1.81 (0.1)	1.95 (0.45)	0.89	0.72	1.78 (0.08)	1.90 (0.24)	2 (0.14)	0.51	0.12
EOE	1.53 (0.12)	1.73 (0.14)	1.69 (0.53)	0.58	0.75	1.52 (0.1)	1.86 (0.21)	2 (0.49)	0.16	0.07
EF	2.62 (0.2)	3.04 (0.13)	3.62 (0.41)	**0.03**	**0.04**	2.9 (0.13)	3.08 (0.29)	0.86 (0.75)	0.84	0.12
DD	3.12 (0.22)	2.79 (0.19)	2.92 (0.25)	0.52	0.15	2.87 (0.15)	3.5 (0.25)	2.75 (0.21)	0.34	0.36
SR	3.25 (0.12)	3.12 (0.17)	3.05 (0.39)	0.75	0.79	3.19 (0.11)	3.1 (0.25)	2.95 (0.15)	0.75	0.37
SE	2.92 (0.15)	3.75 (0.37)	2.64 (0.14)	**0.0096**	**0.045**	2.88 (0.12)	2.96 (0.25)	3.19 (0.58)	0.43	0.29
EUE	2.84 (0.19)	2.62 (0.19)	2.81 (0.43)	0.72	0.83	2.76 (0.14)	2.71 (0.31)	2.94 (0.68)	0.80	0.73
FF	3.05 (0.12)	2.81 (0.09)	3.38 (0.22)	0.063	0.19	2.96 (0.09)	3.11 (0.09)	3.12 (0.38)	0.49	0.56
ИМТ, kg/m^2	15.86 (0.31)	20.68 (2.25)	18.24 (1.18)	0.17	0.19	18.35 (2.07)	19.05 (1.38)	18.18 (1.03)	0.96	0.80
Age, month	52.11 (7.53)	56.55 (7.91)	28.36 (9.42)	0.2	0.14	47.35 (6.41)	63.12 (9.52)	26.58 (7.14)	0.076	0.04
Birth weight, g	3226.32 (109.4)	3237.17 (112.93)	3282.5 (169.04)	0.97	0.96	3248.19 (110.19)	3264.92 (112.13)	2855.56 (226.15)	**0.02**	**0.03**
Weight, kg	18.16 (1.68)	20.92 (2.31)	14.84 (3.47)	0.34	0.32	17.37 (1.52)	23.3 (2.88)	12.38 (1.94)	**0.032**	**0.03**
Z-score	-0.29 (0.3)	0.67 (0.35)	1.01 (0.92)	0.13	0.05	-0.22 (0.31)	0.78 (0.41)	1.53 (0.79)	**0.028**	**0.015**
Precentiles	40.06 (5.02)	55.44 (4.73)	60.13 (10.47)	0.059	0.06	70.74 (9.95)	52.21 (5.43)	44.12 (4.56)	**0.041**	**0.015**
Glucose, mmol /l	5.32 (0.23)	5.18 (0.16)	6.25 (0.45)	**0.032**	**0.04**	5.35 (0.18)	5.46 (0.22)	5.1 (0.38)	0.73	0.46

P * significance for the Kruskal–Wallis H-test, P ** significance for the Kruskal–Wallis H-test adjusted for gender, age of gestation, and feeding. Statistically significant differences (P <0.05) are marked in bold.

анализ результатов одномоментного исследования / Л.С. Намазова-Баранова, К.А. Елецкая, Е.В. Кайтукова [и др.]. *Педиатрическая фармакология.* 2018; 15(4): 333–342 [Assessment of the physical development of children of middle and senior school age: analysis of the results of a cross-sectional study. L.S. Namazova-Baranova, K.A. Eletskaya, E.V. Kaitukova [et al.]. *Pediatric Pharmacology*. 2018; 15(4): 333–342.

5. Adiponectin and leptin gene polymorphisms in patients with post-transplant diabetes mellitus / M. Romanowski, V. Dziedziejko, A Maciejewska-Karlowska [et al.]. *Pharmacogenomics.* – 2015; 16(11): 1243-51.

6. A polymorphism in the 5' untranslated region of the human ob gene is associated with low Leptin levels / J. Hager, K. Clement, S. Francke [et al.]. *Int J Obes*. – 1998. – V. 22. – P. 200–5.

7. Associations of maternal diet and placenta leptin methylation / T.E. Daniels, A.I. Sadovnikoff, K.K. Ridout [et al.]. *Mol Cell Endocrinol*. 2020; 505: 110739.

8. Carnell S. Measuring behavioural susceptibility to obesity: Validation of the child eating behaviour questionnaire / S. Carnell, J. Wardle. *Appetite.* 2007; 48(1): 104-113.

9. Comprehensive identification of pleiotropic loci for body fat distribution using the NHGRI-EBI Catalog of published genome-wide association studies / Y. Kaur, D.X. Wang, H.Y. Liu [et al]. *Obes Rev*. 2019; 20 (3): 385-406.

10. GP222 Clinical and metabolic parameters in girls-carriers of LEPR rs1137100 with android and gynoid obesity / E. Sheneman, T. Bairova, K. Ievleva, L. Rychkova [et al.]. *ADC*. 2019; 104. Suppl 3: A1–A428.

11. Hypothalamic Networks in Adolescents With Excess Weight: Stress-Related Connectivity and Associations With Emotional Eating/ C. Martín-Pérez, O. Contreras-Rodríguez, R. Vilar-López [et al.]. *J. Am. Acad. Child. Adolesc. Psychiatry*. 2019; 58(2): 211-220.e5.

12. Lavens D. Review: negative regulation of leptin receptor signaling/ D. Lavens, J. Piessevaux, J. Tavernier. *Eur Cytokine Netw.* 2006; 17: 211-219.

13. Leptin activation of Stat3 in the hypothalamus of wild-type and ob/ob mice but not db/db mice / C. Vaisse, J.L. Halaas, C.M. Horvath [et al.]. *Nat Genet*. 1996; 14: 95-97.

14. Leptin and incident cardiovascular disease: The Multi-Ethnic Study of Atherosclerosis (MESA) / S.S. Martin, M.J. Blaha, E.D. Muse [et al.]. *Atherosclerosis.* 2014; 239(1): 67–72.

15. Relationship between single nucleotide polymorphisms in Leptin, IL6 and Adiponectin genes and their circulating product in morbidly obese subjects before and after gastric banding surgery / C. Poitou, J.M. Lacorte, M. Coupaye [et al.]. *Obes Surg.* 2005; 15(1): 11-23.

16. The effect of leptin promoter and leptin receptor gene polymorphisms on lipid profile among the diabetic population: modulations by atorvastatin treatment and environmental factors / S.I. Al-Azzam, O.F. Khabour, K.H. Alzoubi [et al.] *J. Endocrinol. Invest*. 2014; 37(9): 835–842.

17. M.B. Van den Bree. Genetic and environmental influences on eating patterns of twins aged >50 y/ M.B. Van den Bree, L.J. Eaves, J.T. Dwyer. *Am J Clin Nutr.* 1999; 70: 456 - 465.

18. Wu L. Leptin Receptor Gene Polymorphism and the Risk of Cardiovascular Disease: A Systemic Review and Meta-Analysis. *Int J Environ Res Public Health*. 2017; 14(4): 375.