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ASSOCIATION OF SINGLE-NUCLEOTIDE VARIANTS OF THE GLUCAGON-LIKE PEPTIDE-1 RECEPTOR GENE WITH SWEET TASTE PREFERENCES IN CHILDREN WITH OBESITY

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Background. *Single nucleotide variants (SNVs) of the glucagon-like peptide-1 receptor (GLP1R) gene determine impaired stimulation of anorexigenic neurons and inhibition of orexigenic neurons, which leads to a decrease in the feeling of satiety with an increase in the feeling of hunger and potential consumption of food.* ***The aim of the study:*** *to investigate the association level of SNVs of the GLP1R gene and sweet taste preference in the development of obesity among children.* ***Materials and methods.*** *316 children aged 6-18 years were examined. The main group (n=252) was represented by children with obesity. The control group (n=64) consisted of children with physiological body weight. Randomly selected 52 obese children underwent whole genome sequencing (CeGat, Germany). Taste preferences were assessed for all children using the Food and Beverage Preference Questionnaire. Static analysis included variation and correlation analysis, method for assessing the spread of data (estimation of dispersion). The critical value of the level of statistical significance (p) for all types of analysis was accepted at the level of p<0.05 (5%).* ***Results:*** *The average level of sweet taste preference among obese children was 3.38±0.06 points, while in the control group it was 3.74±0.05 points, p=0.000013. 14 SNVs of the GLP1R gene were found among obese children: rs761386, rs1042044, rs1126476, rs2235868, rs3765468, rs61754624, rs6918287, rs6923761, rs10305420, rs1030542 1, rs10305457, rs10305492, rs10305493, rs1472308929. SNVs of the GLP1R gene were highly associated with obesity, respectively in ascending order: rs1126476 (40.4%), rs2235868 (42.3%), rs1042044 (42.3%), rs6918287 (55.8%). The highest correlation between sweet taste preference was observed for SNV rs6918287 (r=0.61, p<0.05), when comparing 14 SNVs of the GLP1R gene.* ***Conclusions:*** *In children with obesity, a violation of sweet taste preferences is noted, which is highly associated with the presence of SNV rs6918287 of the GLP1R gene.*

Key words: *single nucleotide gene variants, sweet taste preferences, eating behavior, obesity, physiological body weight.*

АСОЦІАЦІЯ ОДНОНУКЛЕОТИДНИХ ВАРІАНТІВ ГЕНА РЕЦЕПТОРА ГЛЮКАГОНОПОДІБНОГО ПЕПТИДУ-1 ЗІ СМАКОВИМИ УПОДОБАННЯМИ ДО СОЛОДКОГО ПРИ ОЖИРІННІ У ДІТЕЙ

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Актуальність. *Однонуклеотидні варіанти (single nucleotide variants — SNV) гена рецептора глюкагоноподібного пептиду-1 (glucagon-like peptide-1 receptor — GLP1R) детермінують порушення стимуляції анорексигенних нейронів та інгібування орексигенних нейронів, що приводить до зменшення відчуття ситості зі збільшенням відчуття голоду та потенційного вживання їжі.* ***Мета дослідження:*** *дослідити рівень асоціації SNV гена GLP1R та уподобання солодкого смаку в розвитку ожиріння у дітей.* ***Матеріали і методи досліджень:*** *обстежено 316 дітей віком 6–18 років. Основну групу (n = 252) представили діти з ожирінням. Контрольну групу (n = 64) склали діти з фізіологічною масою тіла. Випадково обраним 52 дітям з ожирінням було проведено повне геномне секвенування (CeGat, Німеччина). Оцінку смакових уподобань проводили всім дітям за опитувальником Food and Beverage Preference Questionnaire. Статичний аналіз включав варіаційний та кореляційний аналіз, метод оцінки дисперсності даних. Критичне значення рівня статистичної значущості (p) для всіх видів аналізу приймалося на рівні p < 0,05 (5%).* ***Результати:*** *Середній рівень уподобання за солодким смаком серед дітей з ожирінням склав 3,38 ± 0,06 балів, тоді як в контрольній групі 3,74 ± 0,05 балів, p = 0,000013. Серед дітей з ожирінням виявлено 14 SNV гена GLP1R: rs761386, rs1042044, rs1126476, rs2235868, rs3765468, rs61754624, rs6918287, rs6923761, rs10305420, rs1030542 1, rs10305457, rs10305492, rs10305493, rs1472308929. Високо асоційованими з ожирінням виявилися SNV гена GLP1R, відповідно у порядку зростання: rs1126476 (40,4%), rs2235868 (42,3%), rs1042044 (42,3%), rs6918287 (55,8%). Найвищий кореляційний зв'язок між уподобанням солодкого смаку відмічався при SNV rs6918287 (r = 0,61, p < 0,05), при порівнянні 14 SNV гена GLP1R.* ***Висновки:*** *У дітей з ожирінням відмічається порушення уподобань до солодкого смаку, високо асоційоване із наявністю SNV rs6918287 гену GLP1R.*

Ключові слова: *однонуклеотидні варіанти генів, уподобання солодкого смаку, харчова поведінка, ожиріння, фізіологічна маса тіла*

INTRODUCTION

Changes in metabolic status during obesity significantly affect taste perception and taste preferences. Obese individuals often have a decreased perception of sweet taste, which may lead to increased consumption of sugary foods [1; 2].

Glucagon-like peptide-1 (GLP-1), a 30 amino acid peptide hormone, is a typical peptide of the brain-gut axis and can influence the metabolism of various tissues and organs. GLP-1 not only stimulates insulin secretion, but also reduces glucagon release, slows gastric emptying, improves insulin sensitivity, and increases satiety. GLP-1 is secreted by intestinal enteroendocrine L-cells in response to nutrient intake. In addition to secreting GLP-1 in response to stimulation by sweet compounds, L-cells simultaneously express several taste transduction molecules, including the sweet taste receptor, taste 1 receptor member 2 (T1R2)/taste 1 receptor member 3 (T1R3) and taste G- protein gustducin [3].

Local GLP-1 production in taste bud cells, glucagon-like peptide-1 receptor (GLP1R) expression on adjacent nerves, a functional continuum in sweet agonist perception from the gut to the tongue, and the identification of GLP-1-induced signaling pathways in peripheral nerves as well as central taste coding all provide compelling evidence that GLP-1 is directly involved in the perception of sweet taste [4; 5].

Single-nucleotide variants (SNVs) of the GLP1R gene cause impaired stimulation of anorexigenic neurons and inhibition of orexigenic neurons, which leads to a decrease in satiety with an increase in hunger and potential food intake.

However, the role of GLP1R in taste coding and preferences for sweet tastes in obesity remains largely unexplored. Considering that pharmacological management of taste perception may represent a new potential strategy for combating metabolically associated diseases of civilization determined by SNVs of the GLP1R gene, the purpose of our study: to investigate the association level of SNVs of the GLP1R gene and sweet taste preference in the development of obesity among children.

MATERIALS AND METHODS

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 DSMU (ethical approval DSMU/EC/19/1107). Time of data collection: January 2020 - February 2023.

The main group (n = 252) was formed by obese children ((BMI ≥ 97th percentile) aged 6–18 years. The control group (n = 64) was represented by children with physiological body weight (BMI ≤ 85th percentile).

Anthropometric measurements were made in a child in underwear and without shoes. Height (cm) was measured using Heightronic Digital Stadiometer® to the nearest 0.1 cm. Weight (kg) and body fat (BF) percentage was measured using Tefal Bodysignal body composition analyzer (France).

To highlight the prevailing modalities of taste preferences for five most important categories (sweet, sour, umami, salty and bitter), a questionnaire was carried out using an adapted version of IDEFICS (Identification and prevention of Dietary and lifestyle-induced health EFfects In Children and infantS Study) of the Food and Beverage Preference Questionnaire (FBPQ) on a 5-point scale with the calculation of the average value of the level of taste preference [6] and the analysis of food diaries.

The sample population examined by whole genome sequencing (NGS, Illumina CSPro®, CeGat, Germany) consisted of 52 children of the main group and was qualitatively homogeneous in relation to the general population. 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 [7]. 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 [8]. 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 [9; 10].

We used to annotate clinical and functional variants ClinVar Version 20200316 [11], InterVar gnomeAd Version 3.0 [12], dbnsfp Version 35c [13] and GWAS catalog database annotations [14].

Reference sequence obtained from the National Center for Biotechnology Information RefSeq database (http://www.ncbi.nlm.nih.gov/RefSeq/) [15].

Statistical analysis of the obtained results was carried out using a package of application programs: variational analysis, method for assessing the spread of data (estimation of dispersion), Spearman correlation analysis, method for testing statistical hypotheses Python version 3.8.10 in theintegrated development environment Visual Studio Code version 1.81.1. Only significant relationships ($p < 0.05$) were taken into account. Hardy-Weinberg equilibrium (HWE) was measured using the program SNPStats (http://bioinfo.iconcologia.net/SNPstats).

RESULTS AND ITS DISCUSSION

A total of 316 children aged 6-18 years were involved in the study of sweet taste preferences, who did not differ in age and gender in the main and control groups, $p > 0.05$. The average level of preference for sweet taste among obese children was 3.38 ± 0.06 points, while in the control group it was 3.74 ± 0.05

points (p = 0.000013; Student's t-test = 4.61; number of degrees of freedom f = 91; critical value = 1.99 at level significance a = 0.05). Currently, psychophysical studies have presented ambiguous data on the perception of taste among obese patients and persons with physiological body weight [16]. We found a decrease in the average level of similarity to licorice taste among children of the main group, equal to that of the control group, which is likely explained by the intermediate type of grub behavior and the presence of song SNVs for the GLP1R gene in children for obesity [17; 18].

The complete genome investigation using the NGS method was determined in 52 randomly selected obese children with small initial characteristics: exosome size 36.5 Mb; cross-sectional coverage as a whole (12 GB): ≥ 100x; for target indicators: ≥ 70%; more than 20X coverage – 96%; > 30X coverage – 95%.

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, rs10 305420, rs10305421, rs10305457, rs10305492, rs10305493, rs1472308929. The distribution of genotype frequencies was in Hardy-Weinberg equilibrium in both groups. One of the SNVs we identified in the GLP1R gene (rs61754624) was described in the ClinVar database as "likely benign" [19], which coincided with the results of our research. The clinical significance of the other 13 SNVs we identified in the GLP1R gene was not known at the moment and will be described by us later. The following SNVs rs1126476 (40.4%), rs2235868 (42.3%), rs1042044 (42.3%), rs6918287 (55.8%) of the GLP1R gene were found to be highly associated with obesity, respectively, in ascending order, the level of association of which exceeded the threshold accepted by 75% of the available data.

The highest correlation between sweet taste preference was observed for SNV rs6918287 ($r = 0.61$, $p < 0.05$), when comparing 14 SNVs of the GLP1R gene.

CONCLUSIONS

In children with obesity, a violation of sweet taste preferences is noted, which is highly associated with the presence of SNV rs6918287 of the GLP1R gene.

Determination of SNVs of the GLP1R gene makes it possible to predict the probability of obesity and to personalize the development trajectory of various metabolic disorders associated with obesity in children.

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

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REFERENCES

1. Sartor F, Donaldson LF, Markland DA, et al. Taste perception and implicit atti-tude toward sweet related to body mass index and soft drink supplementation. Appetite. 2011;57:237–246. doi: 10.1016/j.appet.2011.05.107.

2. Nishihara T, Nozaki T, Sawamoto R, et al. Effects of Weight Loss on Sweet Taste Preference and Palatability following Cognitive Behavioral Therapy for Women with Obesity. Obes. Facts. 2019;12:529–542. doi: 10.1159/000502236.

3. Jensterle M, Rizzo M, Janez A. Glucagon-Like Peptide 1 and Taste Perception: From Molecular Mechanisms to Potential Clinical Implications. Int J Mol Sci. 2021;22(2):902. Published 2021 Jan 18. doi:10.3390/ijms22020902.

4. Jensterle M, Rizzo M, Janez A. Glucagon-Like Peptide 1 and Taste Perception: From Molecular Mechanisms to Potential Clinical Implications. Int J Mol Sci. 2021 Jan 18;22(2):902. doi: 10.3390/ijms22020902.

5. Takai S, Yasumatsu K, Inoue M, et al. Glucagon-like peptide-1 is specifically involved in sweet taste transmission. FASEB J. 2015 Jun;29(6):2268-80. doi: 10.1096/fj.14-265355.

6. Jilani HS, Intemann T, Bogl LH, et al. I.Family consortium. Familial aggregation and socio-demographic correlates of taste preferences in European children. BMC Nutr. 2017 Dec 6;3:87. doi: 10.1186/s40795-017-0206-7.

7. Hongshan J, Rong L, Shou-Wei D, et al. Skewer: a fast and accurate adapter trimmer for next-generation sequencing paired-end reads. In BMC Bioinformatics. 2014;15:182. doi: 10.1186/1471-2105-15-182.

8. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25(14):1754–1760. doi: 10.1093/bioinformatics/btp324.

9. Mose LE, Wilkerson MD, Hayes DN, et al. ABRA: improved coding indel detection via assembly-based realignment. Bioinformatics. 2014;30(19):2813-2815. doi: 10.1093/bioinformatics/btu376.

10. Deelen P, Bonder MJ, van der Velde KJ, et al. Genotype harmonizer: automatic strand alignment and format conversion for genotype data integration. BMC Res Notes. 2014;7:901. doi: 10.1186/1756-0500-7-901.

11. Landrum MJ, Lee JM, Benson M, et al. ClinVar: improving access to variant interpretations and supporting evidence. Nucleic Acids Res. 2018 Jan 4;46(D1):D1062-D1067. doi: 10.1093/nar/gkx1153.

12. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature. 2020;581:434–43.

13. Ulintz PJ, Wu W, Gates CM. Bioinformatics Analysis of Whole Exome Sequencing Data. Methods Mol Biol. 2019;1881:277-318. doi:10.1007/978-1-4939-8876-1_21.

14. Buniello A, MacArthur JAL, et al. The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted

arrays and summary statistics 2019. Nucleic Acids Res. 2019 Jan 8;47(D1):D1005-D1012. doi: 10.1093/nar/gky1120.

15. RefSeq: NCBI Reference Sequence Database. Available at: https:www.ncbi.nlm.nih.govrefseq. Accessed 2 November 2023.

16. Archer NS, Shaw J, Cochet-Broch M, et al. Obesity is associated with altered gene expression in human tastebuds. Int. J. Obes. 2019;43:1475–1484. doi: 10.1038/s41366-018-0303-y.

17. Abaturov A, Nikulina A. Disoders of eating behavior at different phenotypes of obesity in children. Child's health. 2020;5 (15):55-59. doi: 10.22141/2224-0551.15.5.2020.211441.

18. Abaturov A, Nikulina A. Taste preferences and obesity. Pediatria Polska - Polish Journal of Paediatrics. 2022;97(1):1-6. doi:10.5114/polp.2022.115139.

19. Wessel J, Chu AY, Willems SM, et al. Low-frequency and rare exome chip variants associate with fasting glucose and type 2 diabetes susceptibility. Nat Commun. 2015 Jan 29;6:5897. doi: 10.1038/ncomms6897.