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HUMAN GENETICS

Association of Polymorphisms in Neurotransmitter Genes and the *TMEM18* Gene with Eating Behavior in Obese Patients

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Abstract—Obesity and related complications are caused by eating disorders. The complex pathogenesis of the formation of food dependence has a genetic basis. This paper investigates the relationship between polymorphic variants of genes coding for glutamate receptors (*GRIK5* rs8099939, *GRIK3* rs534131, *GRIA1* rs2195450, *GRIN2B* rs7301328, rs2268132, rs1805476, *GRIN1* rs6293), (GABA) B receptor (*GABBR2* rs3750344), serotonin receptor (*HTR2A* rs6313, rs6311), brain-derived neurotrophic factor (*BDNF* rs925946, rs11030107), and transmembrane protein 18 (*TMEM18* rs2860323, rs6548238) with eating disorders in overweight and obese patients. BMI is shown to be associated with genotype GG of locus rs6293 of gene *GRIN1*, CC-AC of *GRIK5* rs8099939, CC-CT of *TMEM18* rs6548238, and CT-TT of *GRIA1* rs2195450. Disorder of restrained eating behavior was identified in AC-AA genotype carriers at locus rs1805476 of gene *GRIN2B* ($P = 0.04$) and GG genotype carriers at locus rs6293 of gene *GRIN1* ($P = 0.028$). Upset of emotional eating was characteristic of A allele carriers at locus rs1805476 of gene *GRIN2B* ($P = 0.005$) and AC-CC genotype carriers. Extern eating disorder was observed for carriers of genotypes AA and AC at the polymorphic locus rs1805476 of gene *GRIN2B* ($P = 0.0003$). Thus, polymorphisms of the glutamate and serotonin receptor genes, as well as the transmembrane protein 18 gene, are important factors in the development of eating disorders and obesity.

Keywords: obesity, eating disorder, serotonin receptors, glutamate receptors, association

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INTRODUCTION

Eating disorders, or food addiction, lead to overeating and overweight [1]. The complex pathogenesis of the formation of food addiction has a genetic basis [2]. A genome-wide association study (GWAS) identified loci that account for less than 10% of the inheritance of obesity; the probability of inheritance of obesity in twin studies reaches 85% [3], while eating behavior remains a poorly understood topic. One of the discussed obesity genes is the brain-derived neurotrophic factor (*BDNF*) gene, which according to the GWAS results is associated with body weight [4]. A large-scale study of genetic anthropometric traits (GIANT consortium) revealed additional loci that contribute to the development of obesity, for example, the gene for transmembrane protein 18 (*TMEM18*) [5].

Genes whose products participate in the formation of personality traits can cause addictive behavior in people, as well as depression, alcoholism, and eating disorders. It is known that polymorphic variants of the neurotransmitter system genes contribute to the formation of personality traits, including eating behavior [6].

Glutamate receptor genes play a central role in the development of synaptic plasticity and neurotoxicity [7]. Glutamate is the main excitatory neurotransmitter of the central nervous system (CNS) [8]. It was found that many neurodegenerative disorders have glutamatergic nature, and the neurotoxic effect of glutamate became the subject of highly intensive research after it was decided that neuropathological conditions caused by hyperactivity of the glutamatergic system should include not only classical neurodegenerative disorders (Alzheimer's disease, Huntington's chorea, Parkinson's disease, amyotrophic lateral sclerosis) but also ischemic brain lesions, various encephalopathies (including diabetic ones), cognitive and mnestic disorders, alcoholism, etc. [9–15].

Gamma-aminobutyric acid (GABA) is one of the main inhibitory neurotransmitters of the CNS. GABA plays a key role in the inhibition of the CNS. In this context, gamma-aminobutyric acid is a complete glutamine antagonist (which is an excitatory neurotransmitter).

It is known that serotonin affects mood, sleep and wakefulness, and also food intake and/or selective

consumption of foods containing simple carbohydrates [16]. The effect of this neurotransmitter on the energy balance in the body was described. Higher serotonin levels in the CNS contribute to a decrease in food intake and body weight. Overproduction of peripheral serotonin through interaction with adipocytokines leads to obesity [17]. Mental disorders most often share several common underlying neurobiological mechanisms, so we studied both genes associated with obesity (according to GWAS) and certain well-known genes and their association with the risk of developing eating disorders.

This paper investigates the association of polymorphic variants of genes encoding glutamate receptors (*GRIK5* rs8099939, *GRIK3* rs534131, *GRIA1* rs2195450, *GRIN2B* rs7301328, rs2268132, rs1805476, *GRIN1* rs6293), gamma-aminobutyric acid (*GABBR2* rs3750344), serotonin receptor (*HTR2A* rs6313, rs6311), brain-derived neurotrophic factor (*BDNF* rs925946, rs11030107), and transmembrane protein 18 (*TMEM18* rs2860323, rs6548238) with eating disorders in overweight and obese individuals.

MATERIALS AND METHODS

DNA samples. We studied DNA of unrelated individuals, Tatars by ethnicity, living in the Republic of Bashkortostan. The number of surveyed individuals was 275. The average age of the subjects was 55.6 ± 7.68 years. The group included 172 women and 103 men. The average height was 171.56 ± 8.63 cm; the average body weight was 79.7 ± 13.23; the level of overweight (BMI) was 29.18 ± 3.30 kg/m^2. The subjects were differentiated according to their eating behavior; the first group included 60 people without eating disorders; that is, all parameters for three types of eating behavior were within reference values; and the second group consisted of subjects with at least one deviating parameter of eating behavior ($N = 215$). Obese patients (BMI ≥ 30 kg/m^2) were examined at the Center for the Correction of Weight and Associated Diseases based at the emergency care hospital, Hospital no. 21 in Ufa, at the Republican Blood Transfusion Station.

Eating behavior was assessed using the Dutch Eating Behavior Questionnaire (DEBQ) [18]. The questionnaire included 33 items to account for the following eating characteristics: restraint (ten questions), disinhibition (ten questions) measured as external nutrition, and emotional nutrition (13 questions). The questionnaire was adapted for Russia by Yu.L. Savchikova [19]. The following reference values of eating behavior were obtained: restrained eating, 2.41 points; emotional eating, 1.88 points; external eating, 3.22 points.

Genotyping. DNA was isolated from peripheral blood leukocytes using phenol-chloroform purification. Polymorphic variants *GRIK5* rs8099939 (g.42016956T>G), *GRIK3* rs534131 (g.20608A>G), *GRIN2B* rs2268132 (g.152108G>A), *GABBR2* rs3750344 (g.98578034T>C), *HTR2A* rs6313 (g.6230C>T), rs6311 (g.4692G>A), *BDNF* rs925946 (g.27645655T>G), rs11030107 (g.27673288A>G), and *TMEM18* rs2860323 (g.614210A>G), rs6548238 (g.634905T>C) were analyzed by real-time polymerase chain reaction using commercial kits with fluorescence detection (FLASH/RTAS) (http://test-gen.ru, Test-Gen LLC, Russia) and a BioRad CFX96 TM device (Bio-Rad Laboratories, Inc., United States). Endpoint fluorescence and genotype discrimination were measured according to the BioRad CFX96TM protocol using CFX Manager TM Software.

Polymorphic loci *GRIN2B* rs7301328 (c.366C>G), rs1805476 (1354C>A), *GRIK3* rs534131 (g.20608A>G), *GRIA1* rs2195450 (c.-750C>T), and *GRIN1* rs6293 (c.789A>G) were studied by PCR with subsequent cleavage of products using the corresponding restriction enzymes *Taq*I, *Hae*III, *Pst*I, *Taq*I, and *Msp*I. The PCR conditions and primer sequences are presented in Table 1.

Statistical processing of results. Allele and genotype frequencies and deviations of the frequency distribution of genotypes from the Hardy–Weinberg equilibrium were calculated; the statistical significance of differences between groups in the distribution of allele and genotype frequencies was estimated using the Pearson χ^2 test. In a number of cases, the significance of frequency differences was also estimated using the Cochran–Armitage test for trend [20]. The contribution of the genotypes of the studied loci to the variability of quantitative traits characterizing eating behavior (indicators of eating behavior for the three types of disorders), reflecting the level of food dependence and characteristics of obesity, was determined using the Kruskal–Wallis (in the case of three groups) or Mann–Whitney test (in the case of two groups). The calculations were carried out using Statistica v. 6.0 software (StatSoft Inc., United States) [21].

RESULTS

Before proceeding to the analysis of the association of candidate genes with the development of obesity and eating disorders, the frequency distribution of the genotypes of polymorphic gene variants was tested for the Hardy–Weinberg equilibrium; the comparison group included individuals without eating disorders; the data are presented in Table 2. The distribution of genotype frequencies for polymorphic loci did not deviate from the Hardy–Weinberg distribution in the comparison group.

On the basis of the results of analyzing the distribution of genotype and allele frequencies for 14 polymorphic gene variants, *GRIK5* rs8099939, *GRIK3* rs534131, *GRIA1* rs2195450, *GRIN2B* rs7301328,

Table 1. Polymorphic markers, localization, nucleotide sequences of primers, restriction enzymes, and alleles

Gene, locus	Polymorphism, localization	Primer, restriction enzyme	Allele, fragment size, bp
GRIN2B rs7301328	12:13865843 c.366C>G nsSNP	F 5'-TCAGCACAGACTCTCACCTC-3' R 5'-CCTCAGCACAAACCCGC-3' *Taq*I	C—112 G—93, 19
GRIN2B rs1805476	12:13561429 c.*1354C>A regSNP	F 5'-TTAAGAGAAATGAGCTTGGC-3' R 5'-TGTTAAGTGAAGGGAGCATC-3' *Hin*dIII	A—135, 181 C—19, 116, 181
GRIK3 rs534131	1:37018636 g.20608A>G	F 5'-GGCTGTGTGAGGGCAGAC-3' R 5'-CCCGATTCTACTGGGACCTT-3'	G—156 A—125, 31
GRIA1 rs2195450	5:153491449 c.-750C>T	F 5'-TCTAAGAGGAGGGGGCAAGG-3' R 5'-GCTTGGTAGATGGTGCTTGA-3' *Taq*I	T—122 C—94, 24
GRIN1 rs6293	9:137156786 c.789A>G	F 5'-CGTTCTTGCCGTTCATGA-3' R 5'-GTAAGAGCCAGCAGCAACGGAG-3' *Msp*I	G—138, 113, 59, 114 A—251, 173

rs2268132, rs1805476, *GRIN1* rs6293, *GABBR2* rs3750344, *HTR2A* rs6313, rs6311, *BDNF* rs11030107, rs925946, and *TMEM18* rs2860323, rs6548238, with the development of eating disorders, the association was revealed for loci rs2195450 of gene *GRIA1*, rs6293 of gene *GRIN1*, and rs8099939 of gene *GRIK5*.

Next, we analyzed quantitative parameters of obesity among all subjects, taking into account the body mass index (BMI). Statistically significant differences were found in BMI values in individuals with different genotypes for the polymorphic locus rs2195450 of gene *GRIA1* (Table 3). Individuals with heterozygous and homozygous genotypes for the rare allele at locus rs2195450 of gene *GRIA1* were characterized by higher BMI values (P = 0.0071). The association was also revealed for locus rs2268132 of gene *GRIN2B*; in this case, high BMI values are characteristic of genotypes AC and AA (P = 0.04). Another association was identified for locus rs6293 of gene *GRIN1*. AG and GG genotype carriers for this locus had high BMI values, 29.92 and 33.31 kg/m^2, respectively (P = 0.0068). CC and AC genotype carriers for the polymorphic locus rs8099939 of gene *GRIK5* also had higher BMI values, 30.16 and 30.06 kg/m^2, respectively (P = 0.0069). Statistically significant associations were also observed in the case of the polymorphic locus rs6548238 of gene *TMEM18*. Homozygous TT genotype carriers for gene *TMEM18* (rs6548238) had lower BMI values compared to CC and CT genotype carriers (P = 0.027).

The association was also found with other anthropometric and clinical parameters of obesity; the data are presented in Table 4. Polymorphic loci rs2195450 of gene *GRIA1*, rs2268132 of gene *GRIN2B*, rs6293 of gene *GRIN1*, and rs8099939 of gene *GRIK5* were associated with increased body weight. CT and CC genotype carriers for locus rs2195450 of gene *GRIA1* had higher body weight, 81.02 kg and 86.86 kg, respectively (P = 0.0071) (Table 4).

The body weight in homozygous carriers of the rare allele at locus rs6293 of gene *GRIN1* was 88 kg compared to 78.43 and 81.02 kg in homozygous carriers of the frequent allele and heterozygotes (P = 0.048). Homozygous carriers of the rare allele at locus rs8099939 of gene *GRIK5*, on the other hand, had lower body weight (P = 0.035) (Table 4).

Waist size is an important characteristic of metabolic disorders. The analysis revealed associations of this parameter with the frequencies of the following loci: *GRIA1* rs2195450, *GRIK5* rs8099939, and *GRIN2B* rs2268132. CT and TT genotype carriers for locus rs2195450 of gene *GRIA1*, on average, had waist sizes of 100.81 and 104.71 cm, respectively (P = 0.006). AC and AA genotype carriers for locus rs2268132 of gene *GRIN2B* had waist sizes of more than 100.23 and 102.97 cm (P = 0.24). Homozygous C allele carriers at locus rs8099939 of gene *GRIK5* had average waist size of 100.95 cm, the value for heterozygotes was 98.97 cm, and homozygous A allele carriers had average waist size of 94.77 cm (P = 0.04). The association with elevated cholesterol levels was found for loci *GRIN2B* rs1805476 (P = 0.024) and *GRIA1* rs2195450 (P = 0.03).

Analysis of various eating disorders revealed associations with the following loci: *GRIN2B* rs1805476, *GRIN1* rs6293, *GRIK5* rs8099939, and *TMEM18* rs6548238. Disorder of restrained eating behavior was found for carriers of allele A at locus rs1805476 of gene *GRIN2B* in the homozygous and heterozygous states (P = 0.04) and in carriers of genotype GG at locus rs6293 of gene *GRIN1* (P = 0.028). AG and AA genotype carriers had a score corresponding to normal eating behavior (2.4 points), while GG genotype carriers

Table 2. Frequency distribution of genotypes and alleles of polymorphic loci of genes *GABBR2*, *GRIK3*, *GRIK5*, *HTR2A*, *GRIN2B*, *GRIN1*, *BDNF*, and *TMEM18* in groups of individuals with normal and impaired eating behavior

No.	Gene, polymorphism	Genotypes, alleles	Normal eating, abs. (%), $N = 60$	Eating disorder, abs. (%), $N = 215$	P^a	P^b	P^c
1	*GABBR2* rs3750344	TT/CT/CC T/C	36 (60.0)/21 (35.0)/3(5.0) 93 (78.0)/27 (22.0)	116(54.5)/80(37.6)/17(8.0) 312 (73)/114 (27)	1	0.62 0.41	0.35
2	*GRIK3* rs534131	GG/GA/AA G/A	22 (36.7)/34 (56.7)/4 (6.7) 78 (65.0)/42 (35.0)	82 (38.1)/114 (53.0)/19 (8.8) 278 (65.0)/152 (35.0)	0.09	0.81 0.97	0.93
3	*GRIA1* rs2195450	CC/CT/TT C/T	37 (61.7)/20 (33.3)/3 (5.0) 94 (78.0)/26 (22.0)	103 (47.9)/90 (41.9)/22 (12.0) 296 (69.0)/134 (31.0)	1	0.12 0.05	0.02
4	*GRIN2B* rs7301328	GG/GC/CC G/C	18 (30.0)/31 (51.7)/11 (18.3) 257 (60.0)/173 (40.0)	81 (37.7)/95 (44.2)/39 (18.1) 67 (56.0)/53 (44.0)	0.8	0.51 0.50	0.45
5	*GRIN2B* rs2268132	CC/AC/AA C/A	32 (53.3)/22 (36.7)/6 (10.0) 86 (72.0)/34 (28.0)	84 (39.1)/98 (45.6)/33 (15.3) 266 (62.0)/164 (38.0)	0.5	0.13 0.06	0.05
6	*GRIN2B* rs1805476	CC/ACAA C/A	12 (20.0)/29 (48.3)/19 (31.7) 53 (44.0)/67 (56.0)	63 (29.3)/98 (45.6)54 (25.1) 224 (52.0)/206 (48.0)	1	0.3 0.15	0.13
7	*GRIN1* rs6293	AA/AG/GG A/G	35 (58.3)/24 (40.0)/1 (1.7) 94 (78.0)/26 (22.0)	99 (46.0)/102 (47.4)/14 (6.5) 300 (70.0)/130 (30.0)	0.26	0.1 0.08	0.04
8	*GRIK5* rs8099939	CC/AC/AA C/A	18 (30.0)/26 (43.3)/16 (26.7) 62(52.0)/58 (48.0)	87 (45.0)/95 (44.2)/33 (15.3) 269 (63.0)/161 (37.0)	0.31	0.11 0.04	0.00
9	*HTR2A* rs6313	CC/CT/TT C/T	21 (35.0)/29 (48.3)/10 (16.7) 71 (59.0)/49 (41.0)	59 (27.4)/107 (49.8)/49 (22.8) 225 (52.0)/205 (48.0)	0.75	0.41 0.22	0.26
10	*HTR2A* rs6311	CC/CA/AA C/A	23 (38.3)/28 (46.7)/9 (15) 74 (62.0)/46 (38.0)	58 (27.0)/105 (48.8)/52(24.2) 221 (51.0)/209 (49.0)	0.66	0.14 0.05	0.15
11	*BDNF* rs11030107	AA/AG/GG A/G	39 (65.0)/20 (33.3)/1 (1.7) 98 (81.7)/22 (18.3)	159 (73.9)/54 (25.1)/2 (1.0) 372 (86.5)/58 (13.5)	0.34	0.38 0.23	0.16
12	*BDNF* rs925946	GG/GT/TT G/T	31 (51.7)/24 (40.0)/5 (8.3) 86 (71.6)/34 (28.4)	129 (60.0)/70 (32.6)/16 (7.4) 328 (76.3)/102 (23.7)	0.90	0.50 0.36	0.31
13	*TMEM18* rs2860323	CC/CT/TT C/T	40 (66.7)/18 (30.0)/2 (3.3) 98 (81.7)/22 (18.3)	155 (75.6)/49 (23.9)/1 (0.5) 359 (87.6)/51 (12.4)	0.98	0.10 0.13	0.08
14	*TMEM18* rs6548238	CC/CT/TT C/T	37 (61.7)/21 (35.0)/2 (3.3) 95 (79.2)/25 (20.8)	146 (67.9)/60 (27.9)/9 (4.2) 352 (81.9)/78 (18.1)	0.63	0.56 0.59	0.51

Significance levels: P^a, for the Hardy–Weinberg equilibrium; P^b, for the χ^2 test; P^c, for the Armitage trend test.

suffered from food self-restraint or unsystematic strict diets ($P = 0.03$). Upset of emotional eating behavior was found for A allele carriers for locus rs1805476 of gene *GRIN2B* ($P = 0.005$). Homozygous and heterozygous A allele carriers had indicators deviating from the norm, indicating hyperphagia. Homozygous carriers of the rare allele (genotype AA, locus rs8099939 of gene *GRIK5* ($P = 0.027$)) had emotional eating behavior corresponding to the norm, while AC and CC genotype carriers had elevated scores, that is, above the norm (Table 4).

Violation of external eating behavior was observed for carriers of genotypes AA and AC at the polymorphic locus rs1805476 of gene *GRIN2B* ($P = 0.0003$): they are able to eat whenever they see food.

There are data on gender differences in the analysis of the serotonin receptor gene [22]; in this regard, we analyzed the distribution of polymorphic variants *HTR2A* rs6313 and rs6311 separately in men and women. BMI in men was found to be associated with locus rs6311 of gene *HTR2A* ($P = 0.04$): BMI was 29 ± 0.87 kg/m^2 for CC genotype carriers, 23.38 ± 0.55 kg/m^2 for AC genotype carriers, and 27.52 ± 1.11 kg/m^2 for AA genotype carriers ($P = 0.04$). At the same time, such differences were not revealed for locus rs6313 of gene *HTR2A* ($P = 0.27$). Gender differences were analyzed for the rest of the SNPs as well. Associations were identified for the following polymorphic loci: *GRIA1* rs2195450 ($P = 0.008$), *GRIN1* rs6293 ($P = 0.022$), and *GRIK5* rs8099939 ($P = 0.017$). Thus, the

Table 3. Results of the analysis of the association of polymorphic loci of candidate genes with BMI

No.	Gene, polymorphism	Genotype	M ($\pm SEM$), $N = 275$	P^a
1	*GABBR2* rs3750344	TT/CT/CC	29.51 (0.44) 29.46 (0.46) 31.39 (1.44)	0.3
2	*GRIK3* rs534131	GG/GA/AA	29.98 (0.53) 29.44 (0.42) 29.48 (1.17)	0.72
3	*GRIA1* rs2195450	CC/CTTT	28.75 (0.42) 30.34 (0.52) 31.66 (0.9)	**0.0071**
4	*GRIN2B* rs7301328	GG/GC/CC	30.01 (0.52) 29.73 (0.5) 28.73 (0.62)	0.36
5	*GRIN2B* rs2268132	CC/AC/AA	28.78 (0.44) 30.09 (0.48) 30.88 (1.01)	0.04
6	*GRIN2B* rs1805476	AA/AC/CC	29.77 (0.48) 29.77 (0.47) 29.3 (0.72)	0.8
7	*GRIN1* rs6293	AA/AG/GG	28.98 (0.45) 29.92 (0.46) 33.31 (1.31)	**0.0068**
8	*GRIK5* rs8099939	CC/AC/AA	30.16 (0.54) 30.06 (0.48) 27.53 (0.56)	**0.0069**
9	*HTR2A* rs6313	CC/CT/TT	29.42 (0.62) 29.66 (0.44) 29.92 (0.66)	0.85
10	*HTR2A* rs6311	CC/CA/AA	29.26 (0.62) 29.71 (0.45) 30.02 (0.63)	0.68
11	*BDNF* rs11030107	AA/AG/GG	29.99 (0.5) 30.67 (0.64) 28.98 (0.45)	0.7
12	*BDNF* rs925946	GG/GT/TT	31.63 (0.25) 31.5 (0.32) 31.94 (0.79)	0.88
13	*TMEM18* rs2860323	CC/CT/TT	31.7 (0.22) 31.25 (0.5) 29.78 (3.83)	0.42
14	*TMEM18* rs6548238	CC/CT/TT	31.64 (0.24) 31.88 (0.34) 29.56 (0.83)	**0.027**

M ($\pm SEM$), the mean and the standard error of the mean; P^a, significance for the Kruskal–Wallis H-test.

Table 4. Contribution of polymorphic variants of genes *GRIA1*, *GRIN1*, *GRIK5*, *GRIN2B*, and *TMEM18* to the variability of indicators characterizing obesity and eating behavior

Gene, polymorphism	Genotype	*M* (±*SEM*), *N* = 275	P^a
		Weight, kg	
GRIA1 rs2195450	CC/CT/TT	78.06 (1.19)/81.02 (1.5)/86.86 (3.07)	**0.01**
GRIN1 rs6293	AA/AG/GG	78.43 (1.33)/80.79 (1.3)/88 (3.42)	0.048
GRIK5 rs8099939	CC/CA/AA	80.65 (1.56)/81.15 (1.38)/75.9 (1.66)	0.11
	CC-CA/AA	80.92 (1.03)/75.9 (1.66)	0.035
		Waist size, cm	
GRIA1 rs2195450	CC/CT/TT	96.48 (1.17)/100.81 (1.31)/104.71(3.73)	0.006
GRIK5 rs8099939	CC/AC/AA	100.95 (1.64)/98.97 (1.24)/94.77 (1.54)	0.04
GRIN2B rs2268132	CC/AC/AA	96.29 (1.24)/100.23 (1.42)/102.97 (2.24)	0.024
		Total cholesterol, mmol/L	
GRIN2B rs1805476	AA/AC/CC	5.66 (0.16)/5.41 (0.11)/5.15 (0.16)	0.04
GRIA1 rs2195450	CC/CT/TT	5.39 (0.11)/5.37 (0.13)/5.95 (0.3)	0.093
	CC-CT/TT	5.38 (0.08)/5.95 (0.3)	0.03
		Restrained eating	
GRIN2B rs1805476	AA/AC/CC	2.67 (0.12)/2.47 (0.11)/2.22 (0.13)	0.04
GRIN1 rs6293	AA/AG/GG	2.34 (0.1)/2.47 (0.1)/ 3.22 (0.49)	0.028
	AA-AG/GG	2.4 (0.07)/3.22 (0.49)	0.012
TMEM18 rs6548238	CC/CT/TT	2.68 (0.14)/2.24 (0.14)/2.56 (0.29)	0.09
	CC/CT-TT	2.68 (0.14)/2.28 (0.12)	0.03
		Emotional eating	
GRIN2B rs1805476	AA/AC/CC	3.57 (0.26)/3.07 (0.21)/2.37 (0.24)	0.005
GRIK5 rs8099939	CC/AC/AA	3.05 (0.24)/3.2 (0.22)/2.36 (0.24)	0.08
	CC-AC/AA	3.13 (0.16)/2.36 (0.24)	0.027
		External eating	
GRIN2B rs1805476	AA /AC/CC	3.39 (0.15)/3.08 (0.1)/2.65 (0.11)	0.0003

M (±*SEM*), the mean and the standard error of the mean; P^a, significance for the Kruskal–Wallis *H*-test.

identified associations were retained, but the significance level decreased.

DISCUSSION

Multiple large-scale GWAS and candidate-gene studies revealed genes that are potentially involved in both the development of obesity and the formation of eating disorders. Such potential candidate genes are neurotransmitter genes (genes for serotonin receptors, glutamate receptors, and gamma-aminobutyric acid) and genes associated with obesity according to the results of GWAS (brain-derived neurotrophic factor, transmembrane protein 18, melanocortin receptor 4, fat mass and obesity-associated protein (FTO), etc.). The analysis showed associations with the development of eating disorders when comparing the genotype and allele frequencies for loci *GRIN1* rs6293, *GRIA1* rs2195450, *GRIN2B* rs2268132, and *GRIK5* rs8099939 in the studied groups. Analysis of quantitative parameters revealed association of loci *GRIA1* rs2195450, *GRIN1* rs6293, *GRIK5* rs8099939, and *TMEM18* rs6548238 with BMI; association was also found for locus rs6311 of gene *HTR2A* with obesity only in men.

A separate analysis was carried out to examine the eating behavior in subjects using the results of the DEBQ questionnaire. Association with eating disorders was found for loci *GRIN2B* rs1805476, *GRIN1* rs6293, *GRIK5* rs8099939, and *TMEM18* rs6548238.

Previous studies showed the relationship of loci *GRIN1* rs6293 and *GRIN2B* rs1805476 with the eating disorder in type 2 diabetes mellitus [15]. The identified associations were confirmed in our sample of patients with obesity and disorder of restrained eating. Genotypes CC and CT of gene *TMEM18* (rs6548238) were more common in individuals with disorder of restrained eating and in individuals with increased body weight. Allele C is associated with eating disorders and obesity. In 2009, the Genetic Investigation of ANthropometric Traits (GIANT) consortium carried

out a large-scale meta-analysis of the data and revealed the association of allele C (a frequent allele of the polymorphic locus rs6548238 of gene *TMEM18*) with obesity [23]. It is known that gene *TMEM18* is expressed in some areas of the brain, especially in the hypothalamus, as well as in regions that play a decisive role in the regulation of energy homeostasis and in areas that control calorie intake. It was found that the caloric content of food and changes in the energy requirements of the body and body weight affect the expression of genes [24]. However, the molecular function of the protein is unknown. It is known though that *TMEM18* localizes on the nuclear membrane and binds DNA to its C-terminus, suppressing its transcription. This result was confirmed in both adults and children [25].

A number of studies identified association of polymorphic loci of genes of serotonergic receptors and the brain-derived neurotrophic factor with the risk of obesity [16, 26]. Nevertheless, this study revealed no such association, except for locus rs6311 of gene *HTR2A* in the group of men. Perhaps this is due to the different frequencies of these loci in different ethnic groups or because of gender differences [26]. Serotonin is a neurotransmitter that regulates basic physiological processes—sleep, appetite, and secretory function. The serotonin receptor HTR2A is involved in the regulation of cortisol secretion [27], which may play a pathogenetic role in abdominal obesity. It was shown that polymorphic variants of gene *HTR2A* are associated with obesity in Swedes [28] and with increased consumption of energy and fat in children from France [29]. Analysis of eating behavior and its association with polymorphic variants of gene *HTR2A* did not reveal statistically significant differences. The lack of association with eating behavior and obesity was reported in studies of T. Ando et al. in the Japanese and Spanish populations [30, 31]. On the other hand, in [32–34], the association with eating disorders was found for allele A. As part of eating behavior research, it seems relevant to continue the study of serotonin receptors.

The highest number of statistically significant associations in this study was found for polymorphic loci of glutamate receptor genes. There are only a few studies on the glutamatergic system in relation to the problem of alimentary obesity. So, the work of J. Wright et al. reports that the systemic administration of glutamate receptor antagonists of the *N*-methyl-D-aspartate type (NMDAr-type antagonists) increases appetite and the amount of food eaten [35]. It was found that cholecystokinin (CCK), by activating gastrointestinal afferents of the vagus nerve and NMDA receptor antagonists, promotes rapid satiation and the formation of a state of satiety. This may indicate a role of the glutamatergic system in the control of food intake. It was found that the genes of glutamate receptors play a central role in the development of synaptic plasticity (formation of memory and learning processes) and neurotoxicity, as well as neuronal survival or neuronal death [7]. For example, the neuronal *N*-methyl-D-aspartate receptor (NMDAR) plays a key role in the pathophysiology of schizophrenia, bipolar disorder, depression, and other psycho-emotional disorders [6]. It was found that patients with depressive syndrome have reduced expression of NMDAR receptors.

The *GRIN2B* gene of the *N*-methyl-D-aspartate receptor (NMDA), located at 12p12, contains 13 exons. It encodes glutamate ionotropic receptor subunit NR2 and participates in long-term potentiation that depends on the activity of increasing the efficiency of synaptic transmission [6]. In this work, allele C of locus rs1805476 was found to be associated with eating disorders on the three scales. The polymorphic locus rs1805476 is a substitution in the 3' region of gene *GRIN2B*. Using the RegulomeDB database (Version 1.1) (http://regulome.stanford.edu/), the regulatory index of single nucleotide polymorphism (SNP) was estimated. SNP rs1805476 is located in the region that is the site of binding to transcription factors. This polymorphic variant corresponds to a coefficient of 5 and a scoro level of 0.00143, although these values indicate low regulatory potential. At the same time, it is known that SNP rs1805476 and rs1805502 of gene *GRIN2B* are in linkage disequilibrium $D' = 0.9$. The work of S.C. Weickert et al. [37] showed that the polymorphic locus rs1805502 is associated with reduced mRNA expression and protein levels. The variability of the gene encoding the NR2B subunit of the glutamate NMDA receptor can negatively affect the expression of other NMDAR subunits.

The polymorphic variant of gene *GRIN2B* (rs2268132) is associated with smoking and quantitative indicators of smoking severity [38]. A polymorphic variant of gene *GRIN1* (rs6293) is associated with restrained eating. Polymorphism rs6293 was considered in the study of brain diseases, and the association was revealed with the age at onset in patients with Huntington's disease in the Turkish population [39]. Intronic polymorphism rs2195450 of gene *GRIA1* is associated with the risk of migraine, especially in Asian populations. Its association with diseases is probably explained by close linkage with another functionally significant polymorphism, or this polymorphism may cause variable post-transcriptional editing of the protein, which changes the rate of receptor desensitization [40].

The risk of obesity is associated with allele C of the polymorphic locus rs809993 of gene *GRIK5*. According to the RegulomeDB database, this trait has a coefficient of 5, which indicates that this locus is a binding site for transcription factors.

These results indicate the association of polymorphic variants of the genes coding for glutamate and serotonin receptors and transmembrane protein 18 with eating disorders and obesity formation.

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COMPLIANCE WITH ETHICAL STANDARDS

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

Statement of compliance with standards of research involving humans as subjects. All procedures performed in studies involving human participants comply with the ethical standards of the institutional and/or national committee on research ethics and the 1964 Declaration of Helsinki and its subsequent amendments or comparable standards of ethics.

The study was approved by the Ethics Committee of the Institute of Biochemistry of the Ufa Research Centre, Russian Academy of Sciences. Informed voluntary consent for the use of biological material in planned studies was obtained from all participants.

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