ORIGINAL ARTICLE

The association between eating behavior and polymorphisms in *GRIN2B, GRIK3, GRIA1* and *GRIN1* genes in people with type 2 diabetes mellitus

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Abstract

Excess body weight is the main risk factor of type 2 diabetes. Recent studies have shown that psychological and behavioral factors affect weight. Additionally, emerging evidence indicates that polymorphisms of neurotransmitter genes can impact eating behavior. The aim of this study was to detect the associations between SNPs in glutamatergic system genes and type 2 diabetes in the ethnic group of Tatars origin living in the Republic of Bashkortostan (Russian Federation). In our case–control cross-sectional study, 501 patients with type 2 diabetes (170 men and 331 women, 60.9 ± 9.2 years old (mean $\pm$ SD), BMI 30.9 ± 3.9 kg/m^2 (mean $\pm$ SD) of Tatar ethnicity, and a control group of 420 Tatars (170 men and 250 women, 56.3 ± 11.6 years old (mean $\pm$ SD), BMI 24.4 ± 4.3 kg/m^2 (mean $\pm$ SD), were genotyped for five SNPs in four glutamatergic genes (*GRIN2B, GRIK3, GRIA1, GRIN1*). Three SNPs were associated with type 2 diabetes: rs7301328 in *GRIN2B* [odds ratio adjusted for age, sex and BMI (OR^{adj}) = 0.77 (95% CI 0.63–0.93), $p^{adj} = 0.0077$], rs1805476 in *GRIN2B* [OR^{adj} = 1.25 (95% CI 1.03–1.51), $p^{adj} = 0.0240$], and rs2195450 in *GRIA1* [OR^{adj} = 1.35 (95% CI 1.02–1.79), $p^{adj} = 0.0340$]. Regression analysis revealed that rs1805476 in *GRIN2B* was associated with LDL level, glomerular filtration rate, BMI ($p = 0.020$, $p = 0.012$ and $p = 0.018$, respectively). The SNP rs7301328 in *GRIN2B* was associated with triglyceride levels and HbA$_{1c}$ ($p = 0.040$, $p = 0.023$, respectively). These associations were not significant after Bonferroni correction. We found the association between rs534131 in *GRIK3*, rs2195450 in *GRIA1*, rs1805476 in *GRIN2B* and diabetic retinopathy ($p = 0.005$, $p = 0.007$, $p = 0.040$, respectively); rs7301328 in *GRIN2B* was associated with hypertension ($p = 0.025$) and cerebrovascular disease ($p = 0.013$). The association between rs534131 of *GRIK3*, rs2195450 of *GRIA1* genes and diabetic retinopathy remained significant after Bonferroni correction. The SNPs rs6293 in *GRIN1* was significantly associated with eating behavior in patients with type 2 diabetes (p = 0.01). Our results demonstrate that polymorphic variants of glutamatergic genes are associated with eating behavior and diabetic complications in Tatar ethnic group residing in the Republic of Bashkortostan. We detected novel associations of the polymorphic loci in *GRIN1* (rs6293) gene with external eating behavior in type 2 diabetes patients, *GRIK3* (rs534131) and *GRIA1* (rs2195450) genes with diabetic retinopathy.

Keywords Tatar population · Single nucleotide polymorphism · Susceptibility genes · Type 2 diabetes · Glutamatergic genes

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Abbreviations

AIC	Akaike information criterion
AMPA	D,L-Alpha-amino-3-hydroxy-5-methyl-isoxazole propionic acid
CKD-EPI	Chronic kidney disease epidemiology collaboration
CNS	Central nervous system
DEBQ	Dutch Eating Behavior Questionnaire
GRIA1	Gene of glutamate receptor, ionotropic, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate 1
GRIK3	Gene of glutamate receptor, ionotropic, kainate 3
GRIN1	Gene of glutamate receptor, ionotropic, N-methyl D-aspartate 1
GRIN2B	Gene of glutamate receptor, ionotropic, N-methyl D-aspartate 2B
NMDA	N-Methyl-D-aspartate
NMDAR	N-Methyl-D-aspartate receptor
TG	Triglyceride
T2D	Type 2 diabetes

Introduction

Type 2 diabetes (T2D) is a metabolic disorder characterized by chronic hyperglycemia and arising from both insulin resistance in obese people and impaired insulin secretion of pancreatic beta-cells [1]. It is associated with high frequency of complications (cardiovascular diseases, retinopathy, chronic kidney disease, neuropathy, and diabetic foot syndrome), which lead to early disability and death [2–5].

Even modest weight loss improves glycaemic control in patients with T2D and reduces the risk of cardiovascular diseases and mortality [6]. Excess weight and obesity result from the disturbances in eating behavior, particularly overeating. The combination of two specific eating peculiarities such as reduced cognitive control and low resistance to stress is linked with obesity risk [7].

Appetite is regulated by a coordinated interplay between gut, adipose tissue, and brain. A primary site for the regulation of appetite is the hypothalamus where interaction between orexigenic neurons, expressing Neuropeptide Y/Agouti-related protein (AgRP), and anorexigenic neurons controls energy homeostasis. Within the hypothalamus, several peripheral signals have been shown to modulate the activity of these neurons, including neuropeptides and amino acid neurotransmitters.

Glutamate is the dominant excitatory amino acid neurotransmitter in the central nervous system (CNS). Upon stimulation, glutamate is released in to the synaptic cleft to bind and elicit its effects on postsynaptic receptors, whether ionotropic [N-Methyl-D-aspartate (NMDA), D,L-Alpha-amino-3-hydroxy-5-methyl-isoxazole propionic acid (AMPA), kainic acid] or metabotropic receptors (mGluRs).

The intracerebroventricular injection, as well as the lateral hypothalamic injection of glutamate, or its excitatory amino acid agonists, kainic acid, AMPA, and NMDA, rapidly elicit an intense food intake in rats [8, 9]. Likewise, intracerebroventricularly injected mGluR5 agonists stimulate feeding in rodents whereas the mGluR5 antagonist inhibits food intake [10].

Neurons of the CNS and insulin-producing pancreatic β-cells share various common features, for example, similar hormone and neurotransmitter release mechanisms, oxidative stress defense mechanisms and expression of similar genes. In analogy to neurons of the CNS, NMDARs are also expressed in pancreatic tissue. It is generally accepted that glutamate modulates the function of pancreatic endocrine cells.

Thus, glutamate system is closely connected with key pathogenetic mechanisms of T2D which are obesity and decreased insulin secretion. The genes involved in synthesis, metabolism and regulation of both glutamate and its receptors could be, therefore, considered as potential candidates for causing/predisposing to the disorders of eating behavior and T2D when mutated.

There are only few studies of glutamate system genes, and none of them focuses on their association with eating behavior in T2D patients. A number of studies demonstrated the associations between glutamate system genes and mental diseases including schizophrenia, Alzheimer disease, alcoholism [11–14].

The study of Gast et al. suggests that rs2237781 within the glutamate receptor, metabotropic 8 gene (GRM8) may influence the regulation of human eating behavior and might potentially be involved in affecting human liability to addiction behavior [15].

Meanwhile, Liu et al. found that exactly ionotropic NMDA receptors on AgRP neurons play a critical role in controlling energy balance indicating that fasting-induced activation of AgRP-releasing neurons is associated with markedly increased glutamatergic input [16].

Therefore, our research objective was to analyze the contribution of polymorphic variants in ionotropic glutamatergic receptor genes (*GRIN2B* rs7301328 and rs1805476, *GRIK3* rs534131, *GRIA1* rs2195450, *GRIN1* rs6293) to the development of T2D in residents of the Republic of Bashkortostan.

Materials and methods

Study design

This is a cross-sectional case–control association study.

Participants

The study group consisted of 501 patients with T2D (affected group) and 420 nondiabetic persons (unaffected group). Both patients and controls were ethnic Tatars, living in the Republic of Bashkortostan (Russian Federation). The inclusion criteria for the affected group were the following: aged 40 years and older; T2D diagnosis established according to the WHO criteria (1999–2013); no clinical symptoms of other types of diabetes; residents of the Republic of Bashkortostan since birth; ethnic Tatars; not related to other study participants; written informed consent provided. Inclusion criteria for the unaffected group were as follows: aged 40 years and older; absence of any clinical or laboratory symptoms of carbohydrate metabolism disturbances and any type of diabetes; absence of the family history of diabetes; residents of the Republic of Bashkortostan since birth; ethnic Tatars; not related to other study participants; written informed consent provided. Ethnic origin (up to the third generation) of all participants was checked by direct interviews with persons undergoing examination. T2D patients and control group members were matched according to their age and sex. Clinical characteristics are described in Table 1.

The study was conducted in accordance with Helsinki Declaration. Study protocol was approved by the local Ethics Committee of the Institute of Biochemistry and Genetics of Ufa Federal Research Centre of Russian Academy of Sciences (IBG UFRC RAS), Ufa, Russia (Protocol No 17, December 7, 2010). All participants provided written informed consent. The patients and control group members were enrolled between 2012 and 2017 in the Department of Endocrinology of Ufa City Hospital No. 21 and the Department of General Therapy No. 1 of Bashkir State Medical University Clinic (Ufa, the Republic of Bashkortostan, Russian Federation). The experimental work was performed in the Department of Genomics of IBG UFRC RAS (Ufa, Russia). Blood samples (4 ml) were collected from individuals both in affected and unaffected groups.

Selection of SNPs genotyped in this study

SNPs of four genes involved in glutamate signaling pathway were selected according to the following criteria: (a) their suspected or proved functional significance; (b) previously detected association with mental disorder, and (c) minor allele frequency (MAF) of more than 5% in the Caucasian population (NCBI).

Regulatory scores of SNPs was assessed according to RegulomeDB (Version 1.1) (https://regulome.stanford.edu/) database. The polymorphisms *GRIN1* rs6293 and *GRIN2B* rs1805476 had regulatory scores 1 and 6 respectively which confirms the influence of these SNPs on gene expression. The data about impact of rs7301328, rs534131, rs2195450 on gene expression was not revealed.

The functional significance of silent SNPs was analyzed using HaploReg (v4.1) program. It was revealed that *GRIK3*

Table 1 Clinical characteristics of the studied cohorts

Characteristic	Control group members, n = 501	T2D patients, n = 420	p
Age, years	56.3 ± 11.6	60.9 ± 9.2	0.001
Sex, male/female (*n*)	170/250	170/331	0.05
BMI, kg/m^2	24.4 ± 4.3	30.9 ± 3.9	0.0005
Waist circumference, cm	92.0 ± 11.0	102.0 ± 11.2	0.0005
T2D duration, years	–	7.5 ± 5.9	–
Age at T2D onset, years	–	54.9 ± 9.3	–
HbA$_{1c}$, % (mmol/mol)	4.8 ± 0.6 (28.9 ± 3.6)	7.4 ± 1.3 (57.4 ± 7.8)	0.00001
Fasting blood glucose, mmol/l	4.9 ± 0.8	7.1 ± 1.9	0.000001
Systolic blood pressure, mm Hg	120.4	142.6	0.001
Diastolic blood pressure, mm Hg	80.2 ± 7.8	105.1 ± 11.6	0.0001
Cholesterol, mmol/l	5.01 ± 9.6	5.44 ± 1.13	0.000005
Triglycerides, mmol/l	1.4 ± 0.5	1.7 ± 1.3	0.003
LDL, mmol/l	2.9 ± 1.01	3.2 ± 1.4	0.04
HDL, mmol/l	1.08 ± 0.3	1.2 ± 0.5	0.001
C-peptide, ng/ml	2.26 ± 0.9	2.3 ± 1.2	0.81
Eating behavior characteristics (DEBQ)			
Emotional eating	1.99 ± 0.8	2.85 ± 1.12	0.0012
Restrained eating	1.42 ± 0.58	2.62 ± 1.27	0.00001
External eating	2.05 ± 0.5	3.32 ± 1.02	0.0005

The data are n, mean ± SD

DEBQ Dutch Eating Behavior Questionnaire

rs534131 is located in regulatory DNA motifs region which are binding sites of three transcriptional factors. *GRIN2B* rs7301328 is located in the region of regulatory DNA motifs which are binding sites for several transcription factors responsible for phenotypes including energy intake and T2D (https://www.genecards.org). *GRIA1* rs2195450 is located in the region of promoter and enhancer histone marks.

The novel aspects in this study are: (1) the analysis of associations with risk of T2D of glutamate system gene polymorphisms; (2) the analysis of associations of glutamate system gene polymorphisms which previously associated with mental disorders with eating behavior in T2D patients.

Genotyping SNPs

DNA was sampled from venous blood leukocytes by phenol–chloroform extraction [17]. For the current study, five SNPs (*GRIN2B* rs7301328 and rs1805476, *GRIK3* rs534131, *GRIA1* rs2195450, *GRIN1* rs6293) were examined by real-time PCR, using Taq-Man SNP discrimination assays (Applied Biosystems, Foster City, CA). Specific PCR-product accumulation by hybridization and cleavage of double-labeled fluorogenic probe during amplification was detected using BioRad CFX96 instrument (Bio-Rad Laboratories Inc., USA). End-point fluorescence and genotype discriminations were determined according to the BioRad CFX96 protocol, using CFX Manager software. For quality control, 5 per cent of dummy samples and blank control samples were also taken in each experiment. The genotyping was blind to case or control status of the samples. Quality control of genotyping data was assessed by subject and by marker. Subsequently, SNPs were analyzed according to their proportion of missing, MAF (5% threshold) or deviation from Hardy–Weinberg equilibrium (HWE) ($p \geq 0.05$).

Biological measurements

Body weight and height were measured in light indoor clothing barefoot. Blood samples were collected after a 12 h overnight fast and 2 h after meal. HbA$_{1c}$ level was measured by high-performance liquid chromatography. Plasma glucose was measured by glucose oxidase method.

Eating traits

Eating traits were assessed using the Dutch Eating Behavior Questionnaire (DEBQ) (van Strien, 1986) which allows differentiating types of eating behavior disorders [18]. This questionnaire consists of 33 items in two domains of eating traits, namely, restraint (10 items, including questions, e.g., "do you try to eat less at mealtimes than you would like to eat") and disinhibition, measured as external eating (10 items, including questions, e.g., "if food tasted good, do

you eat more than usual"), and emotional eating (13 items, including questions, e.g., "do you have desire to eat when you are irritated"). The items were scored on a five-point scale (1 = never, 3 = sometimes, and 5 = very often). Initial reliability and validity were provided by van Strien. The DEBQ was adapted for Russian speakers by Y.L. Savchikova (2005) with good factorial validity and reliability. The measures for internal consistency of Russian DEBQ subscales were 0.9 for external eating, 0.9 for emotional eating, and 0.9 for restrained eating subscales (Cronbach's alpha is 0.8).

Statistical analysis

Power analysis. The sample size was calculated by Quanto software (https://biostats.usc.edu/software). The sample size (n = 501 for the affected group and n = 420 for the unaffected group) was determined to be sufficient to detect the association between the five studied polymorphisms and T2D with more than 80% power (power: 95.53%, disease prevalence, 7%, error: 5%, OR, 2.0, and significance level < 0.05 for each model).

Power calculation for the study was performed based on the MAF of five candidate SNPs (rs7301328 and rs1805476 in *GRIN2B* gene, rs534131 in *GRIK3*, rs2195450 in *GRIA1*, rs6293 in *GRIN1*) in Caucasians (HapMapCEU) [19].

We examined the polymorphic structure of the candidate genes and selected SNPs with the most significant reported associations and MAF of over 5% in Caucasian population. Based on our calculations using the Power and Sample Size software program, our sample (N = 921) was considered adequate to study the selected SNPs.

Mean values and standard deviations of the quantitative traits were calculated, and the group comparison was performed using a non-parametric Mann–Whitney U-test, since our samples had an abnormal distribution and the Mann–Whitney U-test does not require normal distribution of the variables.

Categorical variables were compared using Pearson's chi-square test. Statistical analysis was performed using Statistica v. 6.0 software (StatSoft Inc., Tulsa, OK, USA). MAF calculation, the agreement of the observed genotype frequencies distribution with HWE (χ2), the association analysis using the basic allele test, the calculation of the OR for the minor allele and the Cochran–Armitage trend test were performed using PLINK v. 1.07 [20]. Bonferroni-adjusted *p*-value threshold of ≤ 0.01 was considered statistically significant. Logistic regression analysis was performed to detect associations under dominant and additive models. The significance of the obtained model accounting for all variables was verified by the significance of the likelihood ratio test (*p*). The best model was chosen using the Akaike's information criterion (AIC). For each significantly associated locus (*p* < 0.05), the model with the lowest AIC was

chosen. Linear regression analysis was performed to detect associations between the studied SNPs and quantitative phenotypes, such as body weight. The regression analysis was performed using PLINK software v. 1.07 [20].

Results

Prior to analyzing the association between candidate gene polymorphisms and T2D, we tested the compliance of the observed genotype frequencies distribution with HWE, and evaluated MAF in the total study group and in the affected and unaffected groups individually. For the control group, the following results were obtained: *GRIN2B* rs7301328 ($p = 0.69$, MAF = 0.45)*, rs1805476 ($p = 0.09$, MAF = 0.47)*, *GRIK3* rs534131 ($p = 0.15$, MAF = 0.40), *GRIA1* rs2195450 ($p = 0.08$, MAF = 0.21), *GRIN1* rs6293 ($p = 0.11$, MAF = 0.25). None of the study participants were excluded (Table 2).

We genotyped five SNPs in *GRIN2B* (rs7301328, rs1805476)*, GRIK3* (rs534131)*, GRIA1* (rs2195450)*, and *GRIN1* (rs6293) genes in T2D patients and in the control group. Table 3 shows the distribution of the genotypes and alleles of the studied polymorphic markers.

The frequency of the minor C allele of *GRIN2B* (c.366C > A, rs7301328) was significantly lower in T2D patients than in controls ($p = 0.02$, OR = 0.80). The more frequent G allele was associated with the disease (p = 0.02, OR = 1.37) (Table 3). A statistically significant association between *GRIN2B* (c.366C > A) polymorphism and T2D did not exist after Bonferroni's correction.

The proportion of CC homozygotes in the group of T2D patients was lower compared to the healthy subjects ($p^{ADJ} = 0.0089$, $OR^{ADJ} = 0.62$, in the recessive model). Association with T2D was found under the additive model ($p^{ADJ} = 0.0077$, $OR^{ADJ} = 0.77$) (Table 4).

The frequency of the AC-CC genotypes of *GRIN2B* (g.425584C > A, rs1805476) polymorphism was higher in T2D patients than in controls (74.3% vs 70.0%), and the association with T2D was detected under the dominant ($p^{ADJ} = 0.032$, $OR^{ADJ} = 1.41$) and additive ($p^{ADJ} = 0.024$, $OR^{ADJ} = 1.25$) models (Table 4).

The minor allele T of *GRIA1* (c.-750G > A, rs2195450) was also shown to be associated with T2D ($p = 0.015$, OR = 1.3) (Table 3). Regression analysis established the association between rs2195450 and T2D under the dominant ($p^{ADJ} = 0.034$, $OR^{ADJ} = 1.35$) and additive models ($p^{ADJ} = 0.018$, $OR^{ADJ} = 1.25$) (Table 4), but this associations in all models did not reach statistical significance after correction for multiple testing.

No association was detected between *GRIK3* rs534131 and *GRIN1* rs6293 loci and T2D.

Genes and the eating behavior variables

Tables 5 shows summary statistics for eating behavior scores calculated using the DEBQ and the genotype carrier status in the T2D group. We have found higher scores of the Emotional eating subscale in the carriers of the *GRIN2B* rs1805476 AC-AA genotypes compared to non-carriers (Table 5). For other loci, no association was detected. In the control group, we did not find any significant association either. The higher scores of the Restrained eating and External eating were observed for the carriers of the AG-GG genotypes of *GRIN1* (rs6293) polymorphism compared to the non-carriers (Table 5). After Bonferroni correction, there was a significant interaction between the *GRIN1* rs6293 SNP and External eating ($p = 0.01$).

Table 2 Chromosomal location, allele frequency and quality control information for the *GRIN2B, GRIK3, GRIA1, GRIN1* gene polymorphisms

Gene/SNP	Chromosome location	Alleles		MAF (T2D)	MAF (control)	HWE (control) *p*-value
		Minor	Major			
GRIN2B rs7301328	12:13865843 Exon 2 + 366 CG 122 Pro (synonymous)	C	G	0.39	0.45	0.69
GRIN2B rs1805476	12:13561429 3′UTR g.425584C > A	C	A	0.50	0.47	0.09
GRIK3 rs534131	1:37018636 Intron variant	A	G	0.36	0.40	0.15
GRIA1 rs2195450	5:153491449 Intron variant	T	C	0.26	0.21	0.08
GRIN1 rs6293	9:137,156,786 c.789A > G p.Pro263 Exon 6	G	A	0.22	0.25	0.11

Table 3 Genotypes and alleles frequency distribution by glutamate system gene loci in T2D patients and control group members

Gene SNP	Genotypes alleles	T2D (N = 501) N/%	Controls (N = 420) N/%	p^a	p^b	OR (95% CI)
GRIN2B rs7301328	GG	184/36.7	129/30.7	0.06	0.02	1.00
	GC	237/47.3	204/48.6			0.82 (0.61–1.11)
	CC	80/16.0	87/20.7			0.64 (0.44–0.93)
	G	605/60.4	462/55.0	0.02	–	0.80 (0.67–0.96)
	C	397/39.6	378/45.0			
GRIN2B rs1805476	AA	129/25.7	126/30.0	0.303	0.11	1.00
	AC	235/46.9	192/45.7			1.19 (0.87–1.63)
	CC	137/27.4	102/24.3			1.34 (0.93–1.91)
	A	493/49.2	444/52.9	0.12	–	1.15 (0.96–1.39)
	C	509/50.8	396/47.1			
GRIK3 rs534131	GG	190/37.9	143/34.1	0.252	0.11	1.00
	GA	255/50.9	217/51.7			0.89 (0.67–1.18)
	AA	56/11.2	60/14.3			0.70 (0.46–1.07)
	G	636/63.4	503/59.9	0.14	–	0.86 (0.71–1.04)
	A	368/36.6	337/40.1			
GRIA1 rs2195450	CC	283/56.5	267/63.6	0.06	0.017	1.00
	CT	174/34.7	128/30.5			1.28 (0.97–1.70)
	TT	44/8.8	25/5.9			1.66 (0.99–2.79)
	C	740/73.9	662/78.8	0.015	–	1.3 (1.06–1.63)
	T	262/26.2	178/21.2			
GRIN1 rs6293	AA	299/59.7	227/54.1	0.23	0.15	1.00
	AG	178/35.5	173/41.2			0.78 (0.59–1.04)
	GG	24/4.8	20/4.7			0.88 (0.46–1.68)
	A	776/77.4	627/74.6	0.17	–	0.85 (0.69–1.06)
	G	226/22.6	213/25.4			

p^a X^2 test for genotype frequency difference between T2D and control group

p^b Cochran–Armitage trend test, OR with 95% CI for minor allele in basic allele test

Association between anthropometric parameters, clinical parameters related to T2D and candidate gene polymorphisms

We investigated the relationship between the glutamate receptors gene polymorphisms and clinical parameters related to T2D in patients with T2D (Table 6).

The association was found between *GRIN2B* rs1805476 and clinical parameters related to T2D. The carriers of the *GRIN2B* rs1805476 CC-AC genotypes had higher LDL ($p = 0.02$), lower Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) ($p = 0.012$) levels (Table 6). The carriers of the *GRIN2B* rs1805476 AC-AA genotypes had higher BMI ($p = 0.018$). The presence of the *GRIN2B* rs7301328 CC genotype was associated with increased triglyceride (TG) level ($p = 0.04$). Patients homozygous for the *GRIN2B* rs7301328 C allele had decreased HbA1c level ($p = 0.023$) (Table 6). None of the association between the SNPs and clinical parameters was statistically significant after correction for multiple testing.

GRIK3 rs534131, *GRIA1* rs2195450 and *GRIN1* rs6293 gene polymorphism were not associated with any clinical parameters related to T2D.

The analysis of diabetic complications has revealed the association of *GRIK3* rs534131, *GRIA1* rs2195450, and *GRIN2B* rs1805476 loci with diabetic nephropathy (Table 7). The carriers of *GRIN2B* rs1805476 CC-AC ($p = 0.04$), *GRIK3* rs534131 GG ($p = 0.005$), and *GRIA1* rs2195450 CC-TC genotypes ($p = 0.007$) had higher risk of this complication. The carriers of *GRIN2B* rs7301328 GC-CC genotype had higher risk of hypertension ($p = 0.025$) and cerebrovascular disease ($p = 0.013$). After correction for multiple testing was applied only the association of *GRIK3* rs534131 ($p = 0.005$) and *GRIA1* rs2195450 ($p = 0.007$) with diabetic retinopathy remained statistical significant.

Table 4 Analysis of the glutamate system genes polymorphisms association with T2D

Gene SNP	Test/model	T2D N (%)	Control N (%)	OR (95% CI)	p	p^{ADJ}	OR^{ADJ} (95% CI)
GRIN2B rs7301328	GG	184 (36.7)	129 (30.7)	1.00	0.062	0.02	1.00
	GC + CC dominant	317 (63.3)	291 (69.3)	0.77 (0.58–1.01)			0.76 (0.57–1.02)
	GG + GC	421 (84.1)	333 (79.3)	1.00	0.052	0.0089	1.00
	CC recessive	80 (15.9)	87 (20.7)	0.72 (0.51–1.00)			0.62 (0.43–0.89)
	Log-additive	–	–	0.80 (0.67–0.97)	0.02	0.0077	0.77 (0.63–0.93)
GRIN2B rs1805476	AA	129 (25.7)	126 (30)	1.00	0.15	0.032	1.00
	AC + CC dominant	372 (74.3)	294 (70)	1.24 (0.93–1.66)			1.41 (1.03–1.92)
	AA + AC	364 (72.6)	318 (75.7)	1.00	0.24	0.11	1.00
	CC recessive	137 (27.4)	102 (24.3)	1.20 (0.89–1.62)			1.29 (0.94–1.77)
	Log-additive	–	–	1.16 (0.97–1.38)	0.11	0.024	1.25 (1.03–1.51)
GRIA1 rs2195450	CC	283 (56.5)	267 (63.6)	1.00	0.029	0.034	1.00
	TC + TT dominant	218 (43.5)	153 (36.4)	1.34 (1.03–1.75)			1.35 (1.02–1.79)
	CC + TC	457 (91.2)	395 (94.0)	1.00	0.1	0.087	1.00
	TT recessive	44 (8.8)	25 (6.0)	1.52 (0.93–2.67)			1.57 (0.93–2.67)
	Log-additive	–	–	1.29 (1.04–1.61)	0.017	0.018	1.25 (1.00–1.55)

p^{ADJ} significance in the likelihood ratio test for the regression model adjusted for age, sex, BMI, OR^{ADJ} adjusted odds ratio

Discussion

In our study, we detected the association of glutamate system genes with eating disorder in T2D patients and specifically *GRIN1* rs6293 AG-GG genotype was associated with external eating. We also discovered the association of *GRIK3* rs534131 and *GRIA1* rs2195450 with diabetic retinopathy. The association of polymorphic variants in ionotropic glutamatergic receptor genes (*GRIN2B* rs7301328 and rs1805476, *GRIK3* rs534131, *GRIA1* rs2195450, *GRIN1* rs6293) with both T2D development and clinical parameters of the disease was not found in the Tatar population.

The pathogenic mechanism of T2D is still enigmatic, and there is currently no cure for the disease. According to the recent data, *N*-methyl-D-aspartate receptor (NMDAR) antagonists may be beneficial for the patients with T2D [21].

We chose to analyse the genes in the glutamatergic signaling pathway due to its role in NMDAR-dependent regulation of insulin secretion that can explain the anti-diabetic effect of NMDAR-antagonists and their therapeutic potential for T2D treatment.

NMDARs are heterotetrameric cationpermeable glutamate receptors widely expressed in CNS, also found in vascular endothelial cells, airway smooth muscle cells, osteoblasts and osteoclasts, and pancreatic beta-cells. They contribute to Ca^{2+} and calcium/calmodulin-dependent protein kinase kinase (CaMKK) trafficking of K_{ATP} and Kv2.1channels to plasma membrane. NMDARs play an important role in CNS regulating cell communication, synaptic plasticity (formation of memory and learning processes), and neuronal survival or death [22].

It has been shown that glucose stimulation causes intracellular increase of glutamate in pancreatic beta-cells, while glutamate and its metabolites in turn contribute to insulin release. Extracellular glutamate activates glutamate receptors and results in insulin and glucagon release. Extracellular glutamate is secreted by alpha-cells or is formed in blood plasma and transported into pancreatic islets through the fenestrated endothelium of their vascular walls. Thus, it has been shown that glutamate modulates the function of endocrine pancreatic cells [21].

The highest number of associations was detected for *GRIN2B* gene, located on chromosome 12 (12p12). This gene consists of 13 exons and encodes for glutamate ionotropic receptor NMDA type subunit 2B that contributes to the long-term potentiation dependent on the increase of the synaptic transmission efficiency [23]. Polymorphism rs7301328 is a synonymous substitution (c.366C > G) in the exon 2 of the *N*-methyl-D-aspartate receptor gene (*GRIN2B*) [13].

Table 5 Analysis of the glutamate system genes polymorphisms association with eating behavior in T2D patients

Gene, SNP	Genotype, model	Mean (SD)	Beta (CI 95%)	p^b
Restrained eating				
GRIN2B	GG	2.89 (0.12)	0.00	0.096
rs7301328	GC	3.04 (0.13)	0.15 (− 0.20 to 0.50)	
	CC	2.52 (0.2)	− 0.37 (− 0.84 to 0.10)	
GRIN2B	CC	2.9 (0.11)	0.00	0.62
rs1805476	AC	2.74 (0.14)	− 0.16 (− 0.50 to 0.18)	
	AA	2.89 (0.13)	− 0.01 (− 0.44 to 0.41)	
GRIK3	GG	2.9 (0.15)	0.00	0.56
rs534131	GA	2.82 (0.12)	− 0.09 (− 0.44 to 0.27)	
	AA	3.14 (0.17)	0.24 (− 0.39 to 0.87)	
GRIA1	CC	2.97 (0.12)	0.00	0.42
rs2195450	TC	2.73 (0.14)	− 0.24 (− 0.59 to 0.11)	
	TT	2.89 (0.19)	− 0.09 (− 0.69 to 0.52)	
GRIN1	AA	3.22 (0.11)	0.00	0.093
rs6293	AG	3.53 (0.13)	0.31 (− 0.03 to 0.65)	
	GG	3.97 (0.42)	0.76 (− 0.20 to 1.72)	
	AA	3.22 (0.11)	0.00	0.047
	AG-GG	3.52 (0.12)	0.34 (0.01 to 0.67)	
Emotional eating				
GRIN2B	GG	4.48 (0.2)	0.00	0.5
rs7301328	GC	4.29 (0.19)	− 0.19 (− 0.71 to 0.34)	
	CC	4.7 (0.28)	0.22 (− 0.48 to 0.93)	
GRIN2B	AA	4.83 (0.16)	0.00	0.08
rs1805476	AC	4.63 (0.2)	0.50 (− 0.05 to 1.05)	
	CC	4.13 (0.22)	0.70 (0.02 to 1.39)	
	CC	4.13 (0.22)	0.00	0.03
	AC-AA	4.69 (0.15)	0.56 (0.06 to 1.06)	
GRIK3	GG	4.41 (0.2)	0.00	0.65
rs534131	GA	4.54 (0.17)	0.14 (− 0.38 to 0.65)	
	AA	4.14 (0.41)	− 0.26 (− 1.18 to 0.65)	
GRIA1	CC	4.44 (0.17)	0.00	0.59
rs2195450	TC	4.57 (0.18)	0.12 (− 0.40 to 0.64)	
	TT	4.09 (0.54)	− 0.36 (− 1.25 to 0.53)	
GRIN1	AA	3.36 (0.12)	0.00	0.5
rs6293	AG	3.35 (0.14)	− 0.01 (− 0.36 to 0.35)	
	GG	2.75 (0.33)	− 0.61 (− 1.62 to 0.41)	
External eating				
GRIN2B	GG	3.57 (0.13)	0.00	0.28
rs7301328	GC	3.37 (0.11)	− 0.20 (− 0.53 to 0.13)	
	CC	3.69 (0.2)	0.12 (− 0.32 to 0.57)	
GRIN2B	CC	3.32 (0.17)	0.00	0.11
rs1805476	AC	3.55 (0.1)	− 0.06 (− 0.42 to 0.30)	
	AA	3.80 (0.12)	0.01 (− 0.44 to 0.46)	
GRIK3	GG	3.49 (0.14)	0.00	0.58
rs534131	GA	3.58 (0.11)	0.09 (− 0.24 to 0.42)	
	AA	3.29 (0.16)	− 0.20 (− 0.79 to 0.39)	
GRIA1	CC	3.55 (0.09)	0.00	0.22
rs2195450	TC	3.58 (0.15)	0.03 (− 0.30 to 0.36)	
	TT	3.07 (0.29)	− 0.48 (− 1.05 to 0.08)	
GRIN1	AA	3.64 (0.1)	0.00	0.037
rs6293	AG	4.02 (0.11)	0.39 (0.09 to 0.69)	
	GG	4.03 (0.28)	0.39 (− 0.47 to 1.25)	
	AA	3.64 (0.1)	0.00	0.01
	AG-GG	4.02 (0.11)	0.39 (0.10 to 0.68)	

Beta coefficient of regression

p^b significance of regression coefficient

Table 6 Analysis of the glutamate system genes polymorphisms association with clinical characteristics in T2D patients

Characteristics	Gene SNP	Genotype model	Mean (SD)	Beta (CI 95%)	p^b
BMI, kg/m^2	*GRIN2B* rs1805476	AA	29.60 (0.39)	1.92	0.018
		AC-CC	31.62 (0.96)	(0.34 to 3.83)	
CKD-EPI, ml/min/m^2	*GRIN2B* rs1805476	AA	78.78 (5.45)	9.95	0.012
		AC-CC	68.83 (1.41)	(2.30 to 17.60)	
LDL, mmol/l	*GRIN2B* rs1805476	AA	2.63 (0.16)	− 0.58	0.020
		AC-CC	3.23 (0.12)	(− 1.08 to − 0.07)	
TG, mmol/l	*GRIN2B* rs7301328	CC	1.60 (0.08)	0.54	0.040
		GG-GC	2.40 (0.24)	(0.02 to 1.06)	
HbA1c, %	*GRIN2B* rs7301328	GG	7.69 (0.15)	− 0.33	0.023
		GC-CC	7.36 (0.06)	(− 0.61 to − 0.05)	

Beta coefficient of regression

p^b significance of regression coefficient

Table 7 Analysis of the glutamate system genes polymorphisms association with diabetic complications

Complication (co-morbidity)	Gene SNP	Model	Yes N (%)	No N (%)	Beta (CI 95%)	p^b
Retinopathy	*GRIN2B* rs1805476	CC-AC	71 (86.6)	73 (74.5)	0.45	0.040
		AA	11 (13.4)	25 (25.5)	(0.21–0.99)	
Retinopathy	*GRIK3* rs534131	GG	42 (48.3)	32 (28.8)	0.43	0.005
		GA-AA	45 (51.7)	79 (71.2)	(0.24–0.78)	
Retinopathy	*GRIA1* rs2195450	CC-TC	82 (96.5)	94 (85.5)	0.21	0.007
		TT	3 (3.5)	16 (14.6)	(0.06–0.76)	
Hypertension	*GRIN2B* rs7301328	GG	60 (36.4)	18 (58.1)	2.42	0.025
		GC-CC	105 (63.6)	13 (41.9)	(1.15–5.29)	
Cerebrovascular disease	*GRIN2B* rs7301328	GG	44 (74.6)	122 (89.0)	2.77	0.013
		GC-CC	15 (25.4)	15 (10.9)	(1.25–6.14)	

Beta coefficient of regression

p^b significance of regression coefficient

We did not detected the association of the *GRIN2B* rs7301328 and rs1805476 polymorphisms with eating behavior. According the literature data, the *GRIN2B* rs7301328 polymorphism is associated with bipolar disorder, schizophrenia and other psychoneurological disorders [24]. Polymorphic locus rs7301328 in *GRIN2B* gene is associated with impulsive personality disorder in Malaysians and deviant behavior in Chinese [25, 26].

Gareeva et al. have shown the association of *GRIN2B* rs1805476 C allele with the risk of paranoid schizophrenia in Russians and Tatars [24]. *GRIN2B* rs1805476 and rs1805502 (functionally significant substitution in regulatory region of the *GRIN2B* gene) were reported to be in linkage disequilibrium, and influenced the *GRIN2B* gene expression and glutamate releasing in the brain [27]. Magnetic resonance spectroscopy detected that the carriers of rs1805476 C variant have decreased concentration of glutamatergic substance in anterior cingulated cortex, therefore this polymorphism may contribute to the regulation of *GRIN2B* expression and affect glutamate release in the brain [28].

Our data are consistent with the findings of Narita et al. who did not find significant association between four *GRIN2B* polymorphisms (including rs7301328) and personality traits, but this may be due to low statistical power [29].

GRIN1 rs6293 polymorphic variant was associated with impaired eating behavior, namely external eating. According to the externality theory, people with external eating experience higher reactivity when exposed to food cues, which in turn increases the probability of overeating.

Our results in some extent are consistent with literature data, and the association with depression and disruptive behavior have been reported for polymorphic variants in *GRIN1* (rs4880213) gene [26]. *GRIN1* rs6293 polymorphism has been implicated in encephalitis, age of manifestation in patients with Huntington's disease, determining cortical plasticity and excitability [30–32]. However, Mori et al. have demonstrated the lack of influence of *GRIN1* rs6293 polymorphism on excitability of brain cortex [32]. Hazer and Tunal have identified the association of *GRIN1* rs6293

GG genotype with risk of Huntington's disease in Turkish population [31].

Analyzing T2D complications, we identified the association of polymorphic variants rs534131 in *GRIK3* and rs2195450 in *GRIA1* with diabetic retinopathy. Our study has shown the increased proportion of GG homozygotes for the rs534131 in *GRIK3* gene among T2D patients with diabetic retinopathy. Diabetic retinopathy, the main cause of visual loss and blindness among working population, is a complication of T2D, which has been described as a major public health challenge, so it is important to identify biomarkers to predict and to stratify patient´s possibility for developing retinopathy.

The main reasons for this decline in visual acuity are blood-retinal barrier disruption with consecutive diabetic macular edema and retinal angiogenesis, but the exact mechanism that leads to vascular disruption is not defined [33]. Both, microvascular pathologies and changes in the neural retina add to the final pathway for vision loss. This is why diabetic retinopathy is no longer solely considered a microvascular disease, but also recognized as a neurodegenerative disorder of the retina [34].

It has long been proposed that in diabetic retinopathy, glutamate-mediated activation of NMDARs and subsequent NMDAR signalling contribute to neuroretinal dysfunction and death. Increased concentrations of glutamate were detected in the retina of streptozotocine-induced diabetic rats and in vitreous fluid samples of patients [35]. Excess glutamate is neurotoxic, and the degree of its toxicity is dependent on the concentration of glutamate and duration of the exposure. T2D changes the expression of genes related to glutamate neurotransmission and transport in retina of eye [36].

It can be hypothesized that allelic variants of NMDAR genes *GRIN1* and *GRIN2B* are associated with diabetic retinopathy. However, the results of our study did not confirm the association of polymorphisms in NMDAR genes with diabetic retinopathy but revealed the impact of genes coding kainate and propionate glutamate receptors.

GRIK3 belongs to the family of glutamate kainate receptors consisting of four subunits and functioning as ligand-activated ionic channels. The polymorphic variants in this gene are associated with schizophrenia, alcoholism, major depressive disorders and suicides [37, 38].

These findings suggested that *GRIK3* rs534131 is associated with elevated expression and could serve as an independent prognostic marker and a novel potential treatment target for patients with diabetic retinopathy.

Intronic polymorphism rs2195450 in *GRIA1* gene was associated with the migraine risk, especially in Asian populations. According to the data of several authors, this polymorphism is not functionally significant, and its association with the disease is likely due to the tight linkage with other functionally significant polymorphism, or this polymorphism results in alternative splicing and changes the degree of receptor desensitization [39].

Our study is the first to perform the analysis of polymorphic loci of glutamatergic genes in T2D. Meanwhile, more and more evidence appears that insulin secretion by pancreatic beta-cells is regulated by glutamate [40]. There is direct correlation between insulin production and glutamate. In addition to NMDAR and other glutamate receptors, pancreatic beta-cells express vesicular transporters of glutamate VGLUT1 и VGLUT3, actively regulating intra- and extracellular glutamate signaling. Beta-cells can also release glutamate during insulin secretion.

The results of our study indicate the existence of association of *GRIN1* gene with eating behavior disorder that can also cause T2D and association of *GRIK3* and *GRIA1* genes with diabetic retinopathy. Thus, our findings support the hypothesis that glutamatergic system is the novel mechanism involved in the pathogenesis of T2D and obesity and a potential target for developing new medicines for treatment of T2D and its complications. Further investigations of polymorphisms in the gene encoding glutamate receptors in T2D patients with different genetic setting are needed to clarify their role in the pathogenesis of T2D.

Compliance with ethical standards

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