O.V. Kochetova, D.S. Avzaletdinova, T.V. Viktorova, G.F. Korytina

ANALYSIS OF POLYMORPHIC VARIANTS OF SEROTONIN AND GAMMA-AMINOBUTYRIC ACID RECEPTOR GENES IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

DOI 10.25789/YMJ.2023.84.02

УДК 612.6

An analysis of polymorphic variants of the serotonin receptor genes *HTRD rs674386, HTR1F rs56398417, HTR2A rs6313, HTR3A rs1062613, HTR2C rs6318* and the *GABRA2 rs279845* gene in T2D patients living in the Republic of Belarus was carried out. As a result of the study of 6 loci of neurotransmitter genes, protective markers *CT* and *CC* genotypes of the rs1062613 locus of the *HTR3A* gene (OR= 0.73, Pcor_FDR=0.0007) and *GC* and *CC* risk genotypes of the rs6318 locus of the *HTR2C* gene (OR=2.21, Pcor_FDR=0.0045) among women, the *CC* genotype is also risky for men (R=4.05, Pcor_FDR=0.0045). Analysis of combinations of genotypes and alleles revealed combinations of increased and decreased risk of T2D. The analysis of ROC curves showed that the studied loci and such variables as sex, age of the examined and BMI can be used to assess the prognostic significance of T2D AUC=83.4% (95% CI 83.5-87.4).

Keywords: type 2 diabetes mellitus, neurotransmitter system, serotonin receptors, gamma-aminobutyric acid receptor.

Introduction. Type 2 diabetes mellitus (T2D) is a metabolic disease characterized by elevated blood glucose levels; its development is due to the development of insulin resistance [2]. The prevalence of T2D is increasing worldwide, leading to a decrease in the quality of life and premature death [2]. Based on the concept of the psychobiosocial model of T2D pathogenesis and considering lifestyle as a trigger factor for the formation of T2D, it seems relevant and appropriate to study neurotransmitters in the development of T2D. One of the neurotransmitters of the central nervous system is serotonin (5-HT), which is mainly involved in the regulation of complex behavior such as aggression and appetite control [17]. Serotonin occurs in the body in two different pools, one in the central nervous system and the other in peripheral tissues. Approximately 90% of the total 5-HT present in the body is produced by the cells of the gastrointestinal tract; the released neurotransmitter is involved in the control of insulin secretion [11]. It has been established that altered serotonin functions cause dysfunction of pancreatic β-cells and ultimately lead to the development of T2D [4]. Bennet H., 2015 found that increased expression of the *HTR1D* and *HTR2A* genes in pancreatic β-cell tissue among T2D patients compared with healthy controls [4]. Studies of the relationship between T2D and the serotonergic system have revealed an association of polymorphic variants of the serotonin receptor genes *HTR2A, HTR2C* with the risk of developing type 2 diabetes in Caucasians [15,19], polymorphic variants of the *HTR3B* gene are associated with type 2 diabetes in Koreans [18], polymorphism of the HTR2C gene is associated with metabolic syndrome among the Greeks [3]. Polymorphic variants of the *GABRA2* gene encoding the α2 subunit of the gamma-aminobutyric acid receptor (GABA-α) have been associated with the risk of alcohol dependence [16]. Also, these receptors play an important role in the regulation of insulin secretion and glucagon release in pancreatic islet cells in both healthy and T2D patients [5]. Based on the results of GWAS studies among residents of the United Arab Emirates, markers of the risk of developing T2D were identified for GABA-α genes [6]. However, the role of neurotransmitters in the pathogenesis of T2DM remains poorly understood. In this regard, the analysis of polymorphic variants of the genes of the neurotransmitter system is an urgent problem. The aim of our study was to analyze polymorphic variants of the serotonin receptor genes *HTRD rs674386, HTR1F rs56398417, HTR2A rs6313, HTR3A rs1062613, HTR2C rs6318,* the gamma-aminobutyric acid receptor gene *GABRA2 rs279845* among patients with type 2 diabetes living in the Republic of Bashkortostan (RB).

Material and Methods Diagnosis of T2D was based on a set of ICD-10 codes, data on quantitative parameters that determine levels of T2D risk such as age, BMI, waist circumference, hip circumference, low-density lipoprotein, high-density lipoprotein levels, triglycerides. The characteristics of the groups are presented in Table 1. Statistics The correspondence between the frequency of genotypes and alleles and the Hardy-Weinberg equilibrium was assessed using the χ2 test. Analysis of associations with the development of T2D was performed using the PLINK v.1.9 program [14]. The Pcor_FDR comparison multiplicity correction was estimated using the online calculator https://www.sdmproject.com/utilities/?show=FDR. The association was considered significant when the Pcor_FDR level was less than 0.05, and the 95% confidence interval did not cross one. Analysis of associations calculated for the main group is presented in models: codominate and additive, as well as

KOCHETOVA Olga Vladimirovna – PhD in Biology, Senior Researcher, Institute of Biochemistry and Genetics of Ufa Federal Research Centre of the Russian Academy of Science, Ufa, https://ORCID.org/0000-0003-2071-0969, SPIN code: 3461-3952, Web of Science Researcher ID A-2035-2014, Olga_mk78@mail.ru; **AVZALETDINOVA Diana Shamilevna** – PhD, Associate Professor of Bashkir State Medical University, Ufa, ORCID: 0000-0002-1590-6433; eLibrary SPIN: 5540-6951, ecolab_203@mail.ru; **VIKTOROVA Tatyana Viktorovna** – MD, Professor, Head of the Department of Bashkir State Medical University, https://ORCID.org/0000-0001-8900-2480, SPIN code: 2705-0468, Author ID: 90113, ecolab_203@mail.ru; **KORYTINA Gulnaz Faritovna** – Doctor of Biological Sciences, Associate Professor, chief researcher, Institute of Biochemistry and Genetics of Ufa Federal Research Centre of the Russian Academy of Science, SPIN code: 1200-2906, Web of Science ResearcherID A-mail: Olga_mk78@mail.ru

Table 1

Characteristics of the samples included in the study

Parameters	Healthy n=1096	T2D n=691	*P* value
Age, mean±Std.Dv	51.82±9.70	58.08±12.28	0.05
Men, n (%)	263 (23.9)	158 (22.2)	0.483
Women, n (%)	833 (76.1)	555 (77.8)	
Body mass index (BMI) (kg/m2), mean±SD	26.72±2.78	31.12±5.82	<0.0001
Obesity, n (%)	–	612 (85.8)	–
Duration of DM2, median [Q1;Q3]	–	7.23 [2; 15]	–
Arterial hypertension, n (%)	–	602 (84.5)	–
Cardiovascular disease, n (%)	–	256 (36.0)	–
HbA1C (%), median [Q1;Q3]	4.9 [3.8; 5.90]	9.20 [7.10; 14.00]	<0.0001
Fasting glucose (mmol/l), median [Q1;Q3]	4.80 [3.20; 5.90]	8.36 [8.31; 15.00]	<0.0001
Total cholesterol (mmol/l), median [Q1;Q3]	4.50 [3.30; 6.12]	5.30 [3.20; 10.30]	0.0008
LDL (mmol/l), median [Q1;Q3]	2.70 [0.79; 3.99]	3.20 [1.60; 7.09]	<0.005
HDL (mmol/l), median [Q1;Q3]	1.10 [0.87; 1.40]	1.10 [0.85; 1.30]	0.08
Triglycerides (mmol/l), median [Q1;Q3]	1.32 [1.10; 2.07]	1.67 [1.15; 2.17]	0.029

Note. LDL – low density lipoproteins, HDL – high density lipoproteins, Std. dv. – standard deviation.

in the form of an allelic test. Multiple logistic regression analysis and ROQ analysis were performed using SPSS v.22. Analysis of gene-gene associations with T2DM between allele and or/genotype was calculated using the APSampler 3.6.0 program (http://apsampler.sourceforge.net).

Results and discussion. To carry out the analysis of associations, we initially calculated whether the distribution of genotype frequencies of the studied polymorphic loci corresponded to the Hardy–Weinberg equilibrium, taking into account the rare allele frequency (MAF) among sick and healthy subjects (Table 2). The locus of the *HTR2C* gene located on the X chromosome was analyzed separately in women and men. The analysis was carried out in the codominant and additive models, and the allele test was also evaluated, the data are presented in Table 3. Statistically significant differences were obtained for the rs1062613 locus of the *HTR3A* gene in the codominant model for the *CT* and *CC* genotypes (OR=0.71 and OR=0.59, Pcor_FDR=0.004). In the additive model, the OR was 0.73, Pcor_FDR=0.0007. For the rs6318 polymorphic locus of the *HTR2C* gene, statistically significant differences were obtained in the group of women. In the codominant model, the association with the risk of developing T2D was determined for the *GC* and *CC* genotypes (OR=2.37 (95% CI 1.74-3.24), Pcor_FDR=0.0045, OR=2.77 (95% CI 0.93-8.25), Pcor_FDR=0.0045). Given that the confidence interval crossed 1 in the second case, the most statistically significant model is the additive OR=2.21 (95% CI 1.66-2.94), Pcor_FDR=0.0045). Among men, the CC genotype was also associated with the risk of developing T2D (OR=4.05, Pcor_FDR=0.0045). Analysis of the *rs279845* locus of the GABRA2 gene revealed a trend towards an increase in the frequency of the TT genotype among patients up to 37.2% compared with 31.0% in the control (OR=0.75 and OR=0.79, Pcor_FDR=0.046), respectively, for projective CT and CC genotypes.

As a result of the multilocus analysis of associations, five combinations of genotypes and alleles were identified that showed statistical significance with T2DM. The C allele of the rs1062613 locus of the HTR3A gene was included in two models of increased T2D risk, and the T allele was included in three reduced risk

Table 2

Description of the studied genes and the Hardy-Weinberg equilibrium

Polymorphism	Gene	Localization	P_{X-W} control	P_{X-B} patients	MAF (European)	MAF
rs279845	*GABRA2*	chr 4:46327706	0.1	0.045	44.8	44.0
rs1062613	*HTR3A*	chr11:113975284	0.061	0.42	22.6	25.05
rs6318	*HTR2C*	chrX:114731326	0.52	0.26	16.2	8.0
rs6313	*HTR2A*	chr 13:46895805	0.11	0.39	42.0	48.0
rs674386	*HTRD*	chr1:23192984	0.28	0.79	22.1	27.0
rs56398417	*HTR1F*	chr3:87975836	0.42	0.014	28.8	17.0

Note: PX-W level of significance in determining the Hardy-Weinberg equilibrium, MAF (European) frequency of the minor allele in the population of Caucasians (Project 1000 genomes), MAF – frequency of the minor allele in the control group.

Table 3

Association of studied polymorphic loci of serotonin and *GABBRA2* receptor genes

Gene, polymorphism	Model	Allele, genotype	T2D n=691 Individuals (%)	Control n=1096 Individuals (%)	OR (95 CI)	P	P_{FDR}
HTR1D rs674386	Co-dominant	*GG/AG/AA*	330 (47.8)/ 296 (42.9)/ 63 (9.1)	591 (53.9)/ 418 (38.1)/ 87 (7.9)	00 1.27 (1.04-1.56) 1.30 (0.91-1.84)	0.045	0.062
HTR1D rs674386	Allelic test	*G/A*	957 (69.0)/ 423 (31.0)	1600 (73.0)/ 592 (27.0)	1.19 (1.02-1.39)	0.01965	0.046
HTR1D rs674386	Additive	---	---		1.19 (1.03-1.38)	0.02067	0.046
HTR1F rs56398417	Co-dominant	*CC/CT/TT*	499 (72.3)/ 165 (23.9)/ 26 (3.8)	780 (71.2)/ 294 (26.8)/ 22 (2)	1.00 0.88 (0.70-1.09)/ 1.85 (1.04-3.30)	0.041	0.062
HTR1F rs56398417	Allelic test	*C/T*	1163 (84.0)/ 217 (16.0)	1854 (85.0)/ 338 (17.0)	1.02 (0.85-1.23)	0.843	0.932
HTR1F rs56398417	Additive		---	---	1.02 (0.85-1.23)	0.812	0.932
GABRA2 rs279845	Co-dominant	*TT/AT/AA*	257 (37.2)/ 307 (44.4)/ 127 (18.4)	340 (31)/ 542 (49.5)/ 214 (19.5)	1.00/ 0.75 (0.60-0.93) /0.79 (0.60-1.03)	0.023	0.046
GABRA2 rs279845	Allelic test	*T/A*	821 (59.0)/ 561 (41.0)	1222 (56.0)/ 970 (44.0)	0.86 (0.75-0.98)	0.034	0.055
GABRA2 rs279845	Additive		---	---	0.86 (0.76-0.99)	0.034	0.055
HTR3A rs1062613	Co-dominant	*CC/CT/TT*	440 (63.7)/ 227 (32.9)/ 24 (3.5)	604 (55.1)/ 435 (39.7)/ 57 (5.2)	1.00 0.71 (0.58-0.87) 0.59 (0.36-0.96)	0.0012	**0.004**
HTR3A rs1062613	Allelic test	*C/T*	1107 (80.0)/ 275 (20.0)	1643 (74.95)/ 549 (25.05)	0.74 (0.63-0.88)	0.0001	**0.00045**
HTR3A rs1062613	Additive		---	---	0.73 (0.64-0.88)	0.0002	**0.0007**
HTR2A rs6313	Co-dominant	*CC/CA/AA*	177 (25.6)/ 356 (51.6)/ 157 (22.8)	290 (26.5)/ 559 (51)/ 247 (22.5)	1.00 / 1.04 (0.83-1.31)/ 1.04 (0.79-1.37)	0.931	0.931
HTR2A rs6313	Allelic test	*C/A*	710 (51.0)/ 670 (49.0)	1139 (52.0)/ 1053 (48.0)	1.02 (0.89-1.17)	0.931	0.931
HTR2A rs6313	Additive		---	---	1.02 (0.89-1.17)	0.761	0.931
HTR2C rs6318	Co-dominant, female	*GG/GC/CC*	554 (68.7)/ 235 (29.1) / 18 (2.2)	341 (84)/ 61 (15)/ 4 (1)	1.00 2.37 (1.74-3.24)/ 2.77 (0.93-8.25)	0.0001	**0.00045**
HTR2C rs6318	Allelic test, female	*G/C*	1343 (83.0)/ 271 (17.0)	743 (92.0)/ 69 (8.0)	2.17 (1.64-2.87)	0.0001	**0.00045**
HTR2C rs6318	Additive, female		---	---	2.21 (1.66-2.94)	0.0001	**0.00045**
HTR2C rs6318	Male	*G/C*	114 (75.5) / 37 (24.5)	237 (92.6)/ 19 (7.4)	1.00/ 4.05 (2.23-7.35)	0.0001	**0.00045**

Note: Statistically significant differences are in bold.

models. In three models, the rs674386 locus of the HTRD gene was found, in this case, the A allele is represented in the increased risk model of the disease, the G allele was determined in the reduced risk models (Pcor_FDR=0.007 and (Pcor_FDR=0.001). In individual analysis, the association of the G allele was also more common among The most significant association was found for the combination of HTR3A rs1062613 allele C + HTR2A rs6313 allele A + HTRD rs674386 allele A + HTR1F rs55639841 CC genotype (OR=1.74, Pcor_FDR=0.0004).

When analyzing ROC curves to assess the prognostic significance of the identified risk values in the development of DM2, two models were built, for the first model only polymorphic loci were taken into account: *HTRD rs674386, HTR1F rs56398417, HTR3A rs1062613, GABRA2 rs279845*, the same loci were included in the second model, and also variables such as sex, age of the subjects and BMI. Prediction performance was measured using the area under the curve (AUC). ROC analysis showed an AUC of 56.7% (95% CI 53.9-59.4) for a model including only the studied polymorphs. For the second model, AUC was 83.4% (95% CI 83.5-87.4), indicating a high ability of the indicators included in the analysis to correctly classify individuals with T2DM and healthy individuals.

The rs10623613 polymorphic variant of the *HTR3A* gene demonstrated the largest number of associations. This polymorphism is located in the 5'UTR region of the gene; it was found that the C allele of this polymorphic locus affects the binding affinity of the transcription factor CTCF to the promoter region of

the *HTR3A* gene [12]. According to a number of authors, allele C is associated with low expression of the *HTR3A* gene [7] and a high level of methylation [13]. Low expression, in turn, causes a decrease in the level of serotonin in the central nervous system, leads to a change in eating behavior and the development of hyperphagia, and subsequently obesity, which provokes the development of T2D [2]. An association with the development of T2D was shown for the *rs6318* locus of the *HTR2C* gene. This polymorphism is due to the substitution of the amino acid Cys for Ser at position 23. It has been shown that the protein encoded by the Ser23 or C allele has a reduced affinity for serotonin [20]. A number of authors have established an association of this locus with the risk of developing depression [8], as well as obesity and DM2, which confirms our data [9, 10, 15, 19]. The *rs279845* locus of the *GABRA2* gene is associated with alcoholism; carriers of the *T* allele suffer from alcoholism to a lesser extent [16]. On the other hand, no relationship was found for the *rs279845* locus of the *GABRA2* gene when studying quantitative traits of personality, temperament, and character [1].

Conclusion. In this study, we assessed the effect of polymorphic variants of neurotransmitter genes on the risk of developing T2D by analyzing combinations of genotypes and alleles. It has been shown that a selected set of genetic variants and indicators such as gender, age, and BMI can be used to predict T2DM among residents of the Republic of Belarus.

The study was partly supported by the Russian Science Foundation (No. 22-25-00010).

Reference

1. Marusin A.V. [et al.] Association of susceptibility genes to alcoholism, schizophrenia and Alzheimer's disease with psychodiagnostic features in the Russian population. Bull. Siberian Medicine. 2016; 15 (5): 83-96. DOI 10.20538/1682-0363-2016-5-83–96

2. Dedov I.I. [et al.]. The role of neurotransmitters in the regulation of energy homeostasis and the possibility of drug correction of its disorders in obesity. Obesity and metabolism. 13(1): 9-15 doi. org/10.14341/omet201619-15

3. Rizos E. [et al.] Acute necrotizing pancreatitis following olanzapine treatment and 759C/T polymorphism of *HTR2C* gene: a case report. In vivo. 2015; 29 (5): 529-531.

4. Bennet H. [et al.] Altered serotonin (5-HT) 1D and 2A receptor expression may contribute to defective insulin and glucagon secretion in human type 2 diabetes. Peptides. 2015; 71:113-120 doi.org/10.1016/j.peptides.2015.07.008

5. Taneera J. [et al.] γ-Aminobutyric acid (*GABA*) signalling in human pancreatic islets is altered in type 2 diabetes. Diabet. 2012; 55 (7): 1985-1994. doi.org/10.1007/s00125-012-2548-7

6. Al Safar H.S. [et al.] A genome-wide search for type 2 diabetes susceptibility genes in an extended Arab family. An. of human genetics. 2013; 77 (6): 488-503. doi.org/10.1111/ahg.12036

7. Niesler B. [et al.] Association between the 5′ UTR variant C178T of the serotonin receptor gene HTR3A and bipolar affective disorder. Pharm. and Genomics. 2001; 11 (6): 471-475.

8. Brummett B.H. [et al.] A putatively functional polymorphism in the *HTR2C* gene is associated with depressive symptoms in white females reporting significant life stress. PloS one. 2014; 9 (12): e114451. doi.org/10.1371/journal.pone.0114451

9. Drago A., Serretti A. Focus on *HTR2C*: A possible suggestion for genetic studies of complex disorders. Am.J.Med.Gen. Neurop. Gen. 2009; 150(5): 601-637. https://doi.org/10.1002/ajmg.b.30864

10. Bah J. [et al.]. Further exploration of the possible influence of polymorphisms in *HTR2C* and *5HTT* on body weight. Metabol. 2010; 59(8): 1156-1163. Doi.org/10.1016/j.metabol.2009.11.07

11. Gershon M.D., Tack J. The serotonin signaling system: from basic understanding to drug development for functional GI disorders. Gastro. 2007; 132(1): 397-414. DOI:10.1053/j.gastro.2006.11.002

12. Methylation of a HTR3A promoter variant alters the binding of transcription factor CTCF/ Jajodia A. [et al.]//RSC Advances. – 2015. – Т. 5. – №. 57. – С. 45710-45717. DOI:10.1039/C5RA04455C

13. Perroud N. [et al.] Methylation of serotonin receptor 3A in ADHD, borderline personality, and bipolar disorders: link with severity of the disorders and childhood maltreatment. Depression and anxiety. 2016; 33 (1): 45-55.

14. Purcell S. [et al.]. PLINK: a tool set for whole-genome association and population-based linkage analyses. The Amer. J hum. genetics. 2007; 81(3): 559-575. doi.org/10.1086/519795

15. Kring S.[et al.] Polymorphisms of serotonin receptor 2A and 2C genes and COMT in relation to obesity and type 2 diabetes. PLoS One. 2009; 4(8): e6696. doi:10.1371/journal.pone.0006696.

16. Russell M.A. [et al.]. PROSPER intervention effects on adolescents' alcohol misuse vary by *GABRA2* genotype and age. Preven. sci. 2018; 19(1): 27-37. doi: 10.1007/s11121-017-0751-y

17. Cataldo Bascuñan L.R. [et al.] Serotonergic regulation of insulin secretion. Acta Physiologica. 2019; 225(1): e13101. doi.org/10.1111/apha.13101

18. Kwon Y.J. [et al.]. Serotonin receptor 3B polymorphisms are associated with type 2 diabetes: The Korean Genome and Epidemiology Study. Diab. Res.clin. prac. 2019; 153: 76-85. doi.org/10.1016/j.diabres.2019.05.032

19. Iordanidou M. [et al.]. The-*759C*/T polymorphism of the *5-HT2C* receptor is associated with type 2 diabetes in male and female Caucasians. Pharm. and gen. 2008; 18: 153-159. doi:10.1097/FPC.0b013e3282f4ae93

20. Yildirim B. O., Derksen J.J. Systematic review, structural analysis, and new theoretical perspectives on the role of serotonin and associated genes in the etiology of psychopathy and sociopathy. Neur. Biobeh. Reviews. 2013; 37(7): 1254-1296. doi: 10.1016/j.neubiorev.2013.04.009