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SEROTONINERGIC SYSTEM CONTRIBUTION TO THE DEVELOPMENT OF EARLY ONSET CHILDHOOD SCHIZOPHRENIA

Relevance. In recent years, active research on the relation rs6313 polymorphism with re-sponse and side effects of taking antipsychotics and antidepressants - established ability of these receptors to bind some antipsychotic drugs, in particular, slowing down the development of nega-tive symptoms in patients with schizophrenia. Thus, when studying the antipsychotic effect of clozapine, its relationship to 5NTR2A receptors was found, therefore, the functional variability of the gene encoding these receptors may affect the clinical effect of clozapine. It was shown that the frequency of rs6313 5NT2A is higher in the group of patients who are resistant to treatment with atypical antipsychotic drugs. The article does not cover the history of studying the genetic polymorphism 5NT2A, hypotheses “for” and “against” its participation in the pathogenesis of mental diseases. Analysis of the current available scientific literature has shown that our study on the search for associations of the rs6313 genetic polymorphism of the 5NTR2A gene with the development of schizophrenia is the first conducted not in an adult, but in a child population. Clinical and psychopathological features were studied and the genetic contribution of the 102t/S polymorphism of the 5NTR2A gene to the development of child and adolescent schizophrenia was determined.

Key words: serotonin receptor gene 5HTR2A, rs6313 of 5HT2A gene, association with schizo-phrenia, A2A2 genotype frequency, early onset childhood schizophrenia, schizophrenic dysontogenesis, pseudo-oligophrenic deficit.

Introduction

Study of schizophrenia has been historically emphasizing the role of heredity in the development of schizophrenia and searching for potential precursors and genetic markers of the disease. The development of molecular genetics gave ample opportunities to search for genetic input of various polymorphisms of so-called *candidate genes* to the risk of schizophrenia. Although the important role of the genetic component in the etiology of schizop-hrenia is now well established, the mechanism of hereditary transmission remains unclear, and the pathological genes predisposing to the disease have not yet been identified. It is known that each gene can be represented in many forms, they are called polymorphic variants of the gene, and the pheno-menon itself is termed molecular-genetic polymer-phism. Serotonin receptors type 2a (5HTR2A) are believed to be one of the candidate genes in the origin of schizophrenia and play a lead role in the emergence and development of endogenous mental diseases [1,2,3].

The studies in neurochemical effects of modern atypical antipsychotics have supported the hypothe-sis of their positive impact on the schizophrenia symptomatology, probably through combined effect on serotoninergic and dopaminergic receptors [3,4,5,6,7,8]. Still, some scientists believe that an increase in dopaminergic activity is not a direct cause of schizophrenia, and its symptoms appear as a result of a decrease in modulating effect on the dopaminergic system of other neurotransmission systems, in particular serotoninergic and glutamater-gic ones [9].

Serotonin synthesis starts with tryptophan Which successively, via the hydroxylation and decarboxylation reactions, turns into 5-hydroxyt-ryptophan and then into the final product of 5-hydroxytryptophan or serotonin (5HT). The 5-HT2A (5-HT2, HTR2A) gene encodes the 5-hydroxy-tryptamine (serotonin) receptor coupled with G-protein (GPCR) and is responsible for post-synaptic serotonin signal transduction. Serotonin-sensitive neurons mature in the deep structures of the middle lobe of the brain and then migrate to the cortex, frontal lobes and other areas of the cortex,

participate in the regulation of emotions, behavior and circadian rhythms, influence sleep, and coregulate somatic functions of the body and the activity of the autonomic nervous system [10,11].

The association of polymorphism of the HTR2A (102C> T) rs6313 gene with the development of mental disorders (depression, suicides, bipolar disorders, etc.) has been found in many populations, but the results of these studies are not unequivocal and are contradictory at times. Currently available data suggest that 5HTR2A dysfunction is also observed in affective disorders. R. Joober [12] noted an earlier age of manifestation in patients with the rs6313 of 5HTR2A gene schizophrenia and alcoholism. In her studies, N. G. Mityushina et al. showed much higher frequency of A2A2 genotype in the group of schizophrenic patients (Russian population) with pronounced negative symptomatology (decreased personality level) and hereditary burden as opposed to the group of patients with minor personality disorders [13,14,15,16].

In spite of contradictory results of research into serotonin blood, liquor and brain structure levels, serotonin concentration in schizophrenia was found to be directly related to total brain mass. Postmortem studies of the brain revealed a decrease in the number of 5HTR2A receptors in the prefrontal region [1].

In recent years, the association of rs6313 polymorphism with the response and side effects of antipsychotic drugs and antidepressants has been actively studied. These receptors were found to bind some antipsychotic drugs, in particular, those hindering the development of negative symptoms in schizophrenic patients. For example, a study of the antipsychotic effect of clozapine revealed its affinity to 5HTR2A receptors, therefore, the functional variability of the gene encoding these receptors may influence the clinical effect of clozapine. Frequency of rs6313 5HT2A was found to be higher in the group of patients who were resistant to atypical antipsychotic drug medication [17,18,19].

This paper does not aim to cover the history of the study of 5HT2A genetic polymorphism, nor any pro and con hypotheses that it is involved in pathogenesis of mental diseases. There were quite a few publications on this topic in the early 2000s. We have reviewed the currently available scientific literature and found our research into presumable association of genetic polymorphism of rs6313 of gene 5HTR2A with development of schizophrenia to be a pioneering study of children population, not adults. Clinical and psychopathological patterns of 102T/C of 5HTR2A gene have been studied and genetic contribution of its polymorphism to development of childhood-onset and adolescent schizophrenia has been identified.

Material and Methods

In our studies, we recruited a sample of 112 probands and 104 donors (deemed mentally healthy persons) aiming to see if the allelic polymorphism of 5HT2A gene is associated with schizophrenia, and particularly, if this gene is involved in overall susceptibility to the disease, and if allelic polymorphism has an impact on the clinical diversity of the endogenous process. The accuracy of the results was assessed and confidence intervals for relative values were calculated in distribution of serotonin receptor of 5HTR2A gene types in selected clinical groups [20,21].

The age of onset of initial manifestations and age of schizophrenia debut in childhood and adolescence were taken as a basis for recruitment of proband groups into this study. Two groups were formed according to these criteria: early-onset childhood schizophrenia (code ICD-10 - F20.xx3) – 58 (51.79%). Abiding by the core principle in psychiatry, that is clinical and psychopathological analysis, we categorized the group of children with ECS into its two known clinical variants: continuous ECS (malignant and sluggish) and paroxysmal ECS (with malignant and sluggish course), 58 probands. The second group includes probands with the process onset at the age of 14 years and older, 54 (48.21%) probands. In line with the quantitative requirements for statistical sampling, we singled out paranoid schizophrenia, F-20.0, and episodic schizophrenia with stable and progressive deficit: F-20.x1; F-20.2 according to ICD-10.

Inclusion criteria for controls (104 persons) were: vocational school or university graduates; no ancestors with schizophrenia and schizoaffective psychosis, epilepsy, behavioral disorders of unidentified etiology; age 18 to 50 years [20].

To determine the statistical significance of frequencies in the control and study samples, Pearson's criterion was used, with the p value < 0.05.

Results and Discussion

First, we conducted an analysis to see if the differences in frequency distribution of allelic polymorphism of serotonin receptor 5HTR2A gene in the study vs. control groups are not random (Table 1).

It should be noted that the data we obtained are in agreement with the results of similar studies in the Russian population [1,11,13], obtained on Caucasian and ethnic white schizophrenic patients in the

United Kingdom [2], as well as studies conducted in Canada [15,16], where the frequency of the adverse homozygous A2A2 (χ_2) genotype carrier status [2] in these populations was significantly higher than in healthy individuals: χ_2 = 7,9; 6,26 и 6,54, respectively, vs. χ_2 = 11,25 (p<0,05) in our studies of the Kazakh population. The results of our studies showed that the odds ratio (OR) in homozygous adverse A2A2 genotype carriers increases the risk of schizophrenia in the Russian population by 1.9, in the UK population by 1.7, in the Kazakh population we studied - by 2.82 times.

Table 1 – Distribution of allele and genotypes frequencies of the serotonin 5HTR2A receptor gene in the studied groups, %

Groups	N	Genotype Frequency						Allele frequency	
		A1A1		A1A2		A2A2		pA1	pA2
		N	%	N	%	N	%	%	%
Cases	112	8	7.14 ±	59	52.68 ±	45	40.18* ±	33.48 ±	66.52* ±
Healthy	104	10	9.62 ±	74	71.15 ±	20	19.23 ±	45.19 ±	54.81 ±
Note: *- the differences are statistically significant in relation to the compared group (p < 0.05)									

The association of the adverse A2A2 genotype carriership of the 5HTR2A gene with schizophrenia determined by us gave rise to a number of studies related to clinical and psychopathological peculiarities of different clinical forms of ECS and genetic polymorphism of the gene under study. Thus, the risk of empirical development of schizophrenia for the adverse A2A2 genotype carriers in the ECS group as a whole, and for female patients in the ECS proband group could not be detected, while for male patients it equaled 2.38 (no association with the disease is assumed at OR=1).

However, it should be noted that similar studies of the Chinese, Irish, Swedish and Italian populations [22,23,24] have found no association with the rs6313 polymorphism of the 5HTR2A gene with the risk of schizophrenia. It is assumed that frequency variability of alleles and genotypes in different ethnic populations, as well as extreme clinical and genetic heterogeneity of mental diseases could be responsible for such contradictory results [1].

As presented in Table 1, the frequency of carrying a homozygous A2 minor allele in the main group was 40.18 ± 4.6%, significantly higher than the similar frequency in the healthy individuals group, 19.23 ± 3.9% (p<0.05), suggesting its possible association with the development of schizophrenia in the Kazakh population.

Apparent gender differences in schizophrenia found by researchers made them seek rationale for this, more based on clinical observations. Some researchers believe the clinical differences between schizophrenic patients of different sexes reflect a different balance of etiological factors or different frequency of subtypes (i.e. strong genetic influence in women and greater environmental dependence in men). V.M. Bashina [25], an ECS researcher, has found male predominance among patients with continuous sluggish schizophrenia and Kanner's non-progredient autism syndrome (2.9:1), and higher proportion of females among paroxysmal schizophrenia patients with low level of progredience (2.1:1) vs. 1.6:1 and 2:1, respectively, in our studies (Table 2).

The rs6313 carriers of 5HTR2A gene of malignant form of continuous schizophrenia display the disease odds ratio (OR) 2.5 times higher than controls.

The distribution of allelic polymorphism genotypes of the serotonin 5HTR2A receptor gene in the group of studied probands with continuous malignant ECS was as follows: A1A1 genotype could not be detected; A1A2 genotype identified in 21 (75%) and A2A2 genotype in 7 (25%) probands.

Table 2 – Distribution of allele and genotypes frequencies of the serotonin 5HTR2A receptor gene by gender and ESC course type, %

Genotype	Continuous ECS								Total	
	malignant				sluggish					
	m		f		m		F			
	n	%	n	%	n	%	n	%	n	%
A1A1	-		-		1	1.72±	-		1	1.72±
A1A2	15	25.86±	6	10.34±	4	6.89 0.6	1	1.72±	26	44.82±
A2A2	6	**10.34***±	1	1.72±	3	5.17 0.65	4	6.89±	14	**24.13***±
N	21	36.21±	7	12.06±	8	13.79±	5	8.62±	41	70.68±
	Paroxysmal ECS									
	malignant				sluggish				Total	
A1A1	-		2	3.44±	-		2	3.44±	4	6.89±
A1A2	1	1.72±	8	66.67±	-		-	-	9	52.94±
A2A2	2	3.44±	2	3.44±	-		-	-	4	6.89*±
N	3	5.17±	12	20.68±			2	3.44±	17	29.31±
Total	24	41.37±	19	32.756.16	8	13.79±	7	12.06±	58	51.784.72

Note: m – male; f – female; * – the differences are statistically significant in relation to the compared group ($p < 0.05$)

The frequency of the adverse A2A2 genotype of the 5HTR2A gene was found highest in males as evidenced by a statistically significant excess of this genotype frequency in the ECS group boys when the rs6313 frequencies were compared in pairs with those of the male control group: χ_2=10,13 (p<0,05). This may indicate the possible impact of this genotype on a number of insidiously evolving negative symptoms which in early childhood would have, instead of typical presentation, the schizophrenic dysontogenesis symptomatology (in essence, these are negative symptoms), intertwined with age-related ontogenesis.

Drawing on available scientific data on the heterogeneous distribution of genders in different forms of schizophrenia, we completed a comparative analysis in the proband group based on gender differences.

Distribution of patients by age of onset of initial manifestations of schizophrenia depending on the diagnosed carriership of the serotonin 5HTR2A receptor gene genotypes is indicative of differences related to the carriership of a particular genotype (see Table 3).

For rs6313 carriers, the mean age of overt signs of schizophrenic dysontogenesis was 3±0.26 years. Proband children under study displayed reduced activity at this stage, most noticeably, listless indifferent attitude to feeding, dysontogenesis of play and speech activity, denial of games and communication, slowly breaking family ties, unresponsiveness to caress, lack of initiative in establishing communication, privatism, loss of acquired skills of speech and game activity: all negative symptomatology. The mean period of non-overt (negative symptomatology) to full blown signs of the disease equaled 4.25±0.35 years for rs6313 vs. 4.66±0.33 years for A1A2 genotype.

At the full-blown signs stage (mean age 5.5±0.43 years), the main characteristics of proband's mental state were pronounced autistic behavior, primarily its negativistic variant, unpronounced affective, neurosis-like and catatonic disorders, with symptoms of terminal states in the form of oligophreniform deficit.

It was interesting to know whether there are age differences in different variants of the ECS for rs6313 carriers. When comparing the frequency

distribution of the serotonin 5HTR2A receptor gene genotypes in continuous malignant and paroxysmal ECS for rs6313 carriers, we identified significant differences in their distribution depending on the age of probands. The age of probands, in both initial and manifest stages of development, was younger in the continuous malignant ECS, the t-criterion was 2.89 and 2.60, respectively, with 95% CI ($p<0.05$).

Table 3 – Average age and frequency distribution of the serotonin 5HTR2A receptor gene genotypes

Genotype	Continuous ECS			
	sluggish		malignant	
	i	m	i	m
A1A1	10±4.0	15±2	-	-
A1A2	6.25±1.49	11.25±2.39	3.27±0.21	6.05±0.45
A2A2	8.29±1.41	14.57±1.04	3.00±0.26	5.5±0.43
Note: i – initial period; m – manifest period				

It should be noted that clinical presentation of malignant continuous ECS has been most thoroughly described by clinical scientists, and its description in our probands did not differ much from that in children in other populations. The most common symptoms were: early on presentation of initial catatonic disorders, more often in the form of catatonic excitation (prolonged repetitive monotonous movements: rocking, bumping against objects, continuously knocking one's head against a wall, brandishing objects and monotonous unprovoked long crying, mutism), less often as stupor (in the form of «withdrawal «, «freezing») and rapidly evolving terminal state as oligophreniform deficit.

Conclusion

So, in Kazakh population we established association of the rs6313 5HT2A genetic polymorphism with ECS. In malignant form of continuous disease, we found the rs6313 5HT2A to be associated with the following: negative symptoms manifesting at the initial stage in the course of the disease; with significant predominance of males in malignant course; with younger age of probands in both initial, and manifest stages of ECS course; and with rapidly developing oligophreniform deficit in the malignant continuous ECS.

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