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Research article

Aberrant alternative splicing of *HTR2A* exon II in peripheral blood lymphocytes of drug-naïve schizophrenic patients

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ABSTRACT

The aim of the study was to characterize the pattern of transcript isoforms of *HTR2A* exon II in lymphocytes of the blood as peripheral biomarkers of schizophrenia development and the effectiveness of antipsychotic therapy. We primarily observed an increase in mRNA levels and elevation of alternative variants in a sample of drug-naïve schizophrenic patients compared to the control group. There was no association of the expression level of *HTR2A* transcript isoforms with the effectiveness of the antipsychotic therapy. Antipsychotic-induced akathisia was associated with a significant reduction in the mRNA levels of the studied isoforms. In summary, our results suggest that levels of HTR2A exon II transcript isoforms are upregulated in patients with schizophrenia, but at the same time, elevated expression level of the studied *HTR2A* transcripts is associated with fewer side effects of the therapy.

1. Introduction

The mechanism of alternative splicing determines the diversity of eukaryotic proteome, which is a fundamental mechanism of gene evolution. The relative frequencies of the transcript isoforms generated by a particular gene are essential for the maintenance of normal cellular physiology. Changes in the expression patterns of alternative splice mRNA variants can lead to the development of pathological processes [1, 2, 3]. Schizophrenia is a progressive neurocognitive disorder that requires antipsychotic pharmacological therapy [4, 5]. Despite a century of research and evolution of antipsychotic drugs, the understanding of the etiopathogenesis of the disorder as well as increasing the effectiveness and safety of the antipsychotic therapy remains an urgent task. Presently, the involvement of the alternative splice forms of the genes, which encode the neurotransmission proteins, in the pathogenesis of schizophrenia has been demonstrated [6, 7, 8, 9]. Consequently, it has been shown that the administration of psychotropic drugs can affect the expression of the transcript isoforms [10, 11, 12].

Despite the fact that the dominant hypothesis of the pathogenesis of schizophrenia is dopaminergic, it is now definitely proved that the serotonergic and glutamatergic neurotransmitter systems also both play an important role in the pathophysiology of psychiatric disorders. The 5-hydroxytryptamine (serotonin) 2A receptor (5-HTR_{2A}), regulates the balance between excitatory and inhibitory neuronal responses, especially the modulation of dopamine activity in the brain [13, 14]. Activation of 5-HTR_{2A}, which are localized in GABA and glutamate interneurons in the prefrontal cortex and mesencephalon, leads to hyperactivity of dopaminergic neurotransmission along the mesolimbic pathway, what is associated with the development of productive symptoms of schizophrenia [15, 16, 17]. Moreover, the involvement of the 5-HTR_{2A} in psychosis has been suggested by imaging investigations: both several post mortem studies and in vivo studies (positron emission tomography (PET) of the living human brain) reported a decreased density or binding of frontal serotonin 5-HT2 receptors in schizophrenia vs control subjects [18, 19, 20]. Alternative splicing of the serotonergic system components greatly increases the complexity of the system due to the expression of

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tissue-specific transcript isoforms and diversity in phosphorylation levels [21]. Moreover, hallucinogenic drugs, such as psilocybin or lysergic acid diethylamide (LSD) trigger mental states resembling schizophrenia in healthy human through the activations of 5-HTR_{2A} [22, 23, 24]. Most of the atypical antipsychotics are classified as antagonists/inverse agonists of the 5-HTR_{2A} and have a negative effect on the basal level of constitutional activity of the receptor.

The knowledge of the different exons and mRNAs for *HTR2A* gene (which encodes the 5-HTR_{2A}) has been evolving over the past 20 years. The current gene model consists of four exons, spanning more than 65kB, and includes evidence for the alternative splicing of exon II (RefSeq accession no. NM_001165947). There are additional *HTR2A* splice variants described in the literature that are not yet annotated in RefSeq. Due to the splicing between the intron I and exon II in the mRNA of the gene, full length (E2+) and alternative (E2tr, E2-) isoforms are formed [25, 26], Figure 1. Despite of the numerous studies, the role of the genetic *HTR2A* variability in health and disease remains uncertain. As the peripheral blood lymphocytes (PBL) express the receptors of biogenic amines and are involved in the peripheral link of schizophrenia pathogenesis as well as being susceptible to systemic actions of antipsychotics, they are currently considered as an adequate tool for monitoring antipsychotic pharmacotherapy [27, 28, 29].

The aim of this study was to define the pattern of the alternatively spliced transcript isoforms of exon II of *HTR2A* gene in PBL as a peripheral biomarker of schizophrenia development and the effectiveness of the antipsychotic therapy.

2. Materials and methods

2.1. Participants

This study was conducted according to the principles expressed in the Declaration of Helsinki (revised in 1983). Written informed consent was obtained from all patients participated in the study, and all procedures were approved (protocol N9, 18.09.2014) by a local ethics committee of the V.M. Bekhterev National Medical Research Center Psychiatry and Neurology. This research included 60 neuroleptic-naïve first-episode schizophrenic male patients according to the F20.X ICD-10 criteria (WHO, 2011). We randomly assigned patients in a 1:1 ratio to either olanzapine monotherapy or haloperidol monotherapy utilizing stratified permuted-block method. Stratification was based upon Positive and Negative Syndrome Scale (PANSS) total score and age. Randomization was conducted and monitored by "random number generator" software (randomizeR, version 1.3). Demographic and clinical characteristics of schizophrenic patients are presented in Table 1. All assessments of schizophrenic patients were conducted at two points: before treatment and at 28th day of medication.

The control group consisted of 40 healthy males who were recruited from a group of blood donors, hospital staff members of the Bekhterev Medical Center, and students, on a voluntary basis. They reported on questioning to be mentally and somatically healthy individuals without autoimmune and infectious pathologies.

2.2. Evaluation of treatment response and development of adverse drug reactions

The effectiveness of therapy was assessed using Positive and Negative Syndrome Scale (PANSS). The primary measure of efficacy, ΔPANSS-Total, was the difference between baseline and last observation (28th day of medication). Patients with reduction of PANSS more than 20% attributed to the group of effective therapy. Adverse side effects were evaluated such as antipsychotic-induced weight gain (AIWG) and antipsychotic-induced extrapyramidal symptoms (EPS). Resolution of EPS with Barnes Akathisia Rating Scale (BARS, for akathisia) and Simpson Angus Scale (SAS, for parkinsonism) was evaluated. Akathisia was diagnosed if a patient scored 2 or more on the global clinical assessment of BARS. If a patient had a total SAS score of 3 or more, parkinsonism was diagnosed. AIWG defined as $\geq$ 7% increase in initial (baseline) body weight.

2.3. Measurement of the HTR2A exon II spliced transcript isoform mRNA levels

PBLs were isolated from the blood samples by means of the Ficoll-Paque gradient method (d = 1.077, GE Healthcare Biosciences). Extraction of total RNA was performed on PBLs using the "RNeasy Mini Kit" (Qiagen, GmbH) according to the manufacturer's instructions. Total RNA was reverse-transcribed into cDNA with the Fermentas cDNA synthesis kit (RevertAidTM, Fermentas, USA).

Quantitative real-time polymerase chain reaction (qRT) PCR for alternative splicing of the exon II of *HTR2A* was performed using Bio-Rad CFX96 Real-Time PCR Thermocycler (Bio-Rad, USA) and EVA GREEN method. Differences in total cDNA per reaction were normalized using 2 reference (housekeeping) genes *GNB2L1* and *GAPDH*. The sequences of primes are shown in Table 2. qRT-PCR was carried out with 1 μL of cDNA templates using 1.5 μL of pairs of primers, (100 nM, target isoform and *GNB2L1* or *GAPDH*), 10x Reaction Buffer with Eva GREEN (Syntol JSC, Russia), $MgCl_2$, 10 mM dNTP Mix and Taq polymerase for a final reaction volume of 50 μL. The reaction conditions for all genes were 95 °C for 15 min for pre-denaturation, 94 °C for 15 s for denaturation, 60 °C for 30 s for annealing, 72 °C for 30 s for extension and cycling for 40 rounds.

In this study, we analyzed normalized expression ΔΔ Cq relative to control sample using 2 reference genes *GNB2L1* and *GAPDH* in order to compare all samples in proper manner. First, we selected one control sample (control) and calculated relative quantity (RQ) of every sample with this formula:

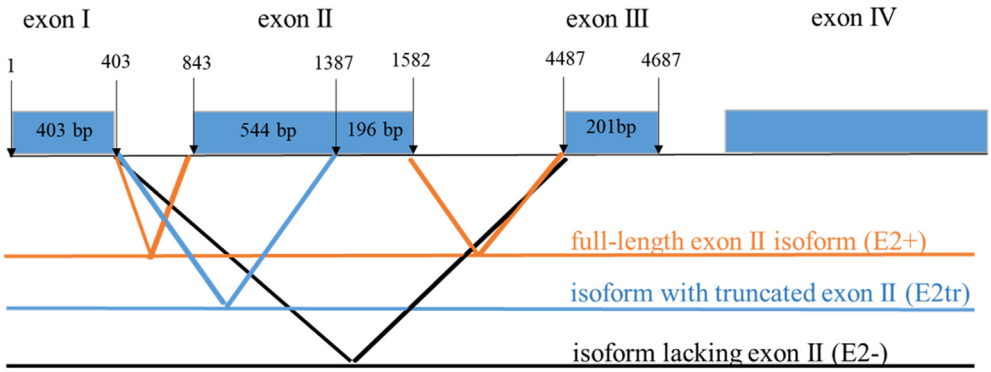

Figure 1. Gene map of the *HTR2A* region and transcripts identified in the current study.

Table 1. The biological and clinical characteristics of patients with schizophrenia and healthy individuals.

Characteristics[†]	First-episode patients with schizophrenia		Healthy persons (n = 40)
	treated with haloperidol (n = 30)	treated with olanzapine (n = 30)	
Age (years) [Range]	29.4 ± 8.07 [19–53]	26.6 ± 6.24 [18–43]	32.1 ± 11.55 [18–58]
Optimal therapeutic dosage of antipsychotic (mg/day) [range]	20.0 ± 5.61 [5–30]	18.5 ± 3.88 [5–20]	N/A
Familial history (yes/no)	5/25	5/25	N/A
Education (lower secondary/secondary/higher/incomplete higher)	3/16/8/3	5/10/8/7	0/0/36/4
Marriage (yes/no)	5/25	1/29	
Diagnostic types (paranoid SCZ/APPD with symptoms SCZ/different subtypes SCZ)	21/9/0	14/11/6	N/A
BMI (kg/m^2) [range]			
Pretreatment	23.4 ± 3.76 [17.0–31.7]	22.7 ± 4.08 [16.2–35.3]	25.2 ± 3.55 [16.4–32.9]
Posttreatment	23.1 ± 3.53 [17.4–31.1]	23.4 ± 3.91 [17.6–36.4]	N/A
Weight (kg) [range]			
Pretreatment	74.33 ± 14.18 [47.10–101.30]	72.48 ± 12.83 [53.00–114.50]	80.47 ± 13.48 [55.00–110.00]
Posttreatment	73.32 ± 13.09 [46.50–93.65]	74.56 ± 12.56 [54.00–117.80]	N/A
PANSS total			
Pretreatment	91.4 ± 13.63	87.3 ± 18.62	N/A
Posttreatment	72.2 ± 17.51**	64.38 ± 18.75**	N/A
Positive scale total			
Pretreatment	23.7 ± 5.49	20.5 ± 4.54	N/A
Posttreatment	14.2 ± 3.44**	13.0 ± 3.97**	N/A
Negative scale total			
Pretreatment	25.0 ± 6.06	24.4 ± 7.54	N/A
Posttreatment	22.2 ± 7.14*	20.0 ± 6.49*	N/A
General psychopathology scale total			
Pretreatment	43.0 ± 8.15	42.3 ± 10.95	N/A
Posttreatment	35.5 ± 9.21*	31.4 ± 10.35**	N/A
BARS (total scores) [range]			
Pretreatment	0.0 ± 0.0	0.0 ± 0.0	N/A
Posttreatment	0.32 ± 0.69 [0–2]	0.32 ± 0.82 [0–4]	N/A
SAS (total scores) [range]			
Pretreatment	0.17 ± 0.54 [0–2]	0.07 ± 0.25 [0–1]	N/A
Posttreatment	6.44 ± 4.24 [0–14]	0.86 ± 1.46 [0–6]	N/A

Note. BMI = Body mass index was calculated as weight in kilograms divided by height squared in metres; SCZ = schizophrenia; APPD - acute polymorphic psychotic disorder; N/A – not applicable.

*p = 0.001.

**p < 0.001.

[†] All values are reported as the mean ± standard deviation.

Table 2. List of primer sequences used for quantitative real-time PCR.

	Localization	Sequence (5` - 3′)
HTR2A E1 FOR	Exon1	CTGTGAGAGATGCAGCGAGTC
HTR2A E2+Rev	Exon2- Exon1	TCGGGAAGATAAATGTCATTTGTC
HTR2A E2-Rev	Exon3- Exon1	GCCACCGGTACCATTTGTC
HTR2A E2trRev	Exon2tr- Exon1	CAGACCAGTTTTTTTCATTTGTCTTC
GAPDH For	Exon4	GAAATCCCATCACCATCTTCCAGG
GAPDH Rev	Exon5	GAGCCCCAGCCTTCTCCATG
GNB2L1 For	Exon3	GAATACCCTGGGTGTGTGCAA
GNB2L1 Rev	Exon4	GGACACAAGACACCCACTCTGA

Exon1 is marked by underlining.

$$RQ\ \ sample = E^{Cq\ control—Cq\ sample} = 2^{Cq\ control—Cq\ sample}$$

Where:

E = Efficiency of primer and probe set. The efficiency was decided to be = 2, in our experiment.

Cq = quantification cycle (the cycle of (qRT) PCR in the moment, when it is on its exponential growth phase

Average Cq = $\sqrt[3]{Cq1*Cq2*Cq3}$ of every sample, which we measured in 3 different test tubes

$Cq_{control}$ = Average Cq for the control sample

Cq sample = Average Cq for any samples with gene of interest

Normalized Expression (ΔΔ Cq) is relative quantity of target gene (*HTR2A*) to the quantities of reference targets (*GNB2L1*, *GAPDH*). We calculated ΔΔ Cq of every sample with this formula:

$$\Delta\Delta Cq\ \ HTR2A = \ RQ\ \ HTR2A \Big/ \sqrt{RQ\ \ GNB2L1*RQ\ \ GAPDH}$$

Where:

RQ *HTR2A* = Relative Quantity of *HTR2A*
RQ *GNB2L1* = Relative Quantity of *GNB2L1*
RQ *GAPDH* = Relative Quantity of *GAPDH*

According to these formulas, we estimated normalized expression ΔΔ Cq of *HTR2A* and of every *HTR2A* isoform: *E2*+, E2-, E2tr. ΔΔ Cq represents relative gene expression, but not absolute values of expression levels of the studied gene and its mRNA isoforms. All gene expression calculations were made with Bio-Rad CFX96 Manager 3.1 software (Bio-Rad, USA).

2.4. Statistical analysis

The statistical analysis was performed using software SPSS version 21 (IBM, USA). Because the data for mRNA levels were not normally distributed, non-parametric tests were used. The Mann-Whitney (U) test was used for pair-wise comparison, Kruskal-Wallis test was performed when comparing three indicators. The comparison between repeated measures at the longitudinal trajectory was performed using Friedman's test. Data are presented as median with interquartile range (25th to 75th percentilies). $P < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1. Differential expression of splicing HTR2A exon II mRNA isoforms according health states E2tr

The results showed significant differences in *HTR2A* exon II spliced transcript isoform mRNA levels in PBL across all patient statuses (schizophrenic patients vs healthy donors) (Figure 2). Schizophrenia disorder was associated with the increase in expression levels of E2tr, E2+, E2-mRNA isoforms of *HTR2A* (1.27 (0.74÷1.86) vs 0.69 (0.38÷1.16), $p = 0.007$; 1.19 (0.85÷1.99) vs 0.69 (0.31÷1.34), $p = 0.018$; 1.46 (0.9÷2.5) vs 0.73 (0.35÷1.16), $p < 0.0001$, respectively). Moreover, Kruskal-Wallis test showed no significant differences ($p = 0.670$) in expression levels E2tr, E2-and E2+ in healthy donors, when in patients with schizophrenia the expression pattern was significantly different ($p = 0.034$): expression of the alternative E2-isoform was 1.3 times higher than the constitutive E2+ isoform. Spearman rank correlation analysis also supported that the isoform ratio in schizophrenic patients is disturbed, while in the control group E2+ isoform mRNA level is associated with both the E2-isoform and the E2tr isoform mRNA levels (Table 3). At the same time, 28 days of therapy with both the olanzapine and the haloperidol had no effect on the mRNA levels of the studied splicing variants of the HTR2A gene (data is presented in Figure 2 without dividing the patients into groups according to pharmacotherapy). The expression levels did not decrease during the improvement in mental states of patients.

3.2. Association of exon II HTR2A spliced transcript isoforms mRNA levels with treatment response

Consequently, the splice isoform mRNA expression was investigated in association with the effectiveness of the therapy and the development of adverse side effects (Table 4).

Significant associations were determined between E2tr, E2-and E2+ isoform expressions and the akathisia status ($p < 0.05$), with significantly decreased transcript isoform mRNA level both before the treatment and 28 days after the administration of the antipsychotic drug in patients with antipsychotic-induced akathisia. Furthermore, when comparing the expression levels of the mRNA of the *HTR2A* exon II isoforms according to a 7% body weight increase as a result of the therapy, by day 28 of antipsychotic administration there was a significant increase in *HTR2A* E2-splice variant mRNA level in patients with AIWG ($p = 0.040$). However, both the effectiveness of the therapy and the antipsychotic-induced parkinsonism have no significant effect on the expression of the transcript isoforms under study.

4. Discussion

In this work, we have demonstrated the differential expression of the splicing exon II of *HTR2A* mRNA isoforms in PBL among the schizophrenia patients and the control group. The majority of studies examining the role of the alternative splicing of exons in the pathogenesis of psychiatric pathologies have been carried out on brain tissue (post-mortem) [8]. However, dysregulation of the peripheral monoamine system components including the decrease in mRNA level of *HTR2A* in PBL independent from brain biomarkers is associated with schizophrenia [29, 30]. Moreover, Oldmeadow et al demonstrated contribution of a splicing mechanism to the variation in psychiatric disorder risk and the differential expression of the various transcript isoforms between the blood and the brain tissue [31]. This data, as well as the results of our work, show that isoform-level dysregulation in PBL of the schizophrenic patients can constitute an independent element of disorder pathogenesis.

A recent study by [11] also demonstrated an increase in the expression of the three transcript isoforms of the *NRG1* gene in the blood cells of patients with schizophrenia receiving clozapine, compared with the control group. The authors conclude not only about the association of

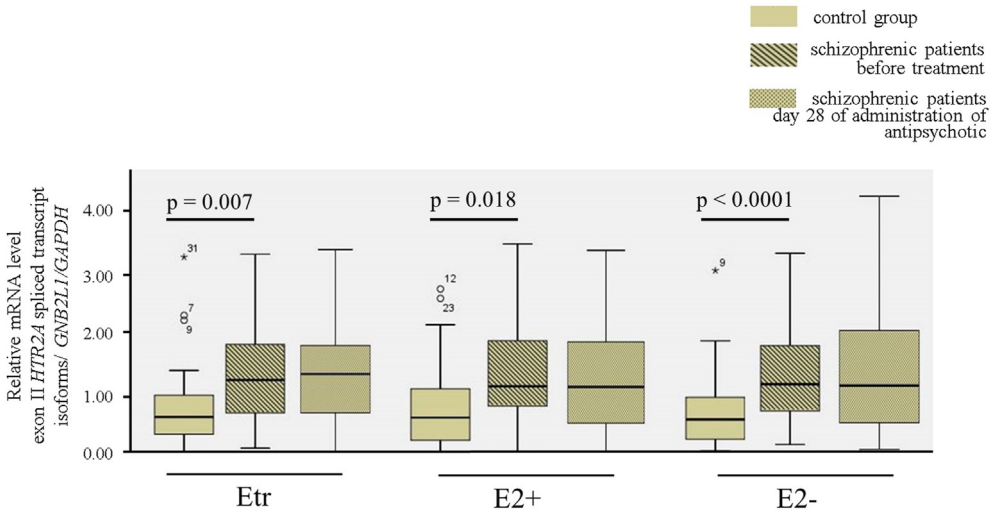

Figure 2. Expression of the exon II HTR2A spliced transcript isoforms mRNA in peripheral blood lymphocytes from schizophrenic patients and control group.

Table 3. Spearman rank correlation analysis between exon II *HTR2A* spliced transcript isoforms mRNA levels in patients with schizophrenia and control group.

	patients with schizophrenia (n = 60)			control group (n = 40)		
	E2tr	E2+	E2-	E2tr	E2+	E2-
E2tr R	1.000	0.530**	0.198*	1.000	0.645**	0.612**
E2+ R	0.530**	1.000	0.531**	0.645**	1.000	0.784**
E2-R	0.198*	0.531**	1.000	0.612**	0.784**	1.000

r – Spearman's correlation coefficient.
*p = 0.139, **p < 0.0001.

Table 4. Distribution of exon II *HTR2A* spliced transcript isoforms mRNA levels according to evaluation of treatment response and development of adverse drug reactions.

Administration of antipsychotic	Spliced transcript isoforms of *HTR2A*	First-episode patients with schizophrenia (n = 60)		p, value
		Total PANSS score ≥20%, effective therapy (n = 35)	Total PANSS score <20%, lowed effective (n = 25)	
before treatment	E2tr	1.28 (0.67÷1.81)	1.25 (0.75÷1.80)	0.843
	E2+	1.34 (0.72÷2.08)	1.17 (0.92÷1.82)	0.973
	E2-	1.69 (0.96÷3.02)	1.39 (0.83÷1.80)	0.309
day 28	E2tr	1.30 (0.65÷1.97)	1.36 (0.84÷1.76)	0.896
	E2+	1.07 (0.37÷2.46)	1.27 (0.72÷1.97)	0.623
	E2-	0.98 (0.51÷2.53)	1.33 (0.88÷2.17)	0.400
		weight change <7% (n = 47†)	weight increase by ≥ 7%, AIWG (n = 11)	
before treatment	E2tr	1.28 (0.71÷1.74)	1.02 (0.69÷2.56)	0.908
	E2+	1.14 (0.76÷1.91)	1.68 (0.84÷2.30)	0.430
	E2-	1.44 (0.88÷2.32)	1.46 (0.85÷5.09)	0.255
day 28	E2tr	1.16 (0.71÷1.83)	1.60 (0.70÷1.87)	0.553
	E2+	1.19 (0.60÷1.66)	1.97 (0.55÷2.60)	0.855
	E2-	0.98 (0.45÷1.85)	2.29 (1.18÷4.05)	**0.040**
		BARS score <2 (n = 53)	BARS score ≥2, akathisia (n = 7)	
before treatment	E2tr	1.28 (0.78÷1.92)	0.51 (0.36÷0.78)	**0.011**
	E2+	1.28 (0.96÷2.01)	0.68 (0.21÷0.90)	**0.011**
	E2-	1.51 (0.94÷2.94)	0.59 (0.36÷1.07)	**0.017**
day 28	E2tr	1.48 (0.84÷1.89)	0.50 (0.29÷1.21)	**0.030**
	E2+	1.24 (0.71÷2.34)	0.39 (0.05÷0.39)	**0.044**
	E2-	1.33 (0.75÷2.52)	0.48 (0.38÷0.48)	0.052
		SAS score <3 (n = 35)	SAS score ≥3, parkinsonism (n = 25)	
before treatment	E2tr	1.27 (0.75÷2.01)	1.28 (0.64÷1.74)	0.778
	E2+	1.24 (0.93÷2.08)	1.25 (0.82÷1.87)	0.773
	E2-	1.36 (0.88÷3.32)	1.46 (0.86÷2.26)	0.942
day 28	E2tr	1.52 (0.82÷2.15)	1.35 (0.72÷1.64)	0.219
	E2+	1.19 (0.61÷2.40)	1.07 (0.61÷1.92)	0.551
	E2-	1.17 (0.62÷2.42)	1.32 (0.90÷2.55)	0.585

The difference between values was considered to be significante in case p < 0.05, marked bold.
† two patients had a weight loss of more than 7%.

elevated mRNA of *NRG1* transcripts with the development of schizophrenia, but also associate the differential expression of isoforms with the current equivalent dose of chlorpromazine and clozapine levels in plasma. The researchers have confirmed their conclusions by means of in vitro experiments - by cultivation of the peripheral blood mononuclear cells isolated from healthy donors under the influence of clozapine.

Other researchers have shown the induction of the expression of certain isoforms of the *Dclk1* kinase gene by means of antipsychotic drugs, which is involved in the synaptic plasticity and the neurogenesis processes, in the mouse's nucleus accumbens and the prefrontal cortex [12]. Drug-naïve first-episode schizophrenic patients were included in our study, which excludes the effect of antipsychotics on the expression of the gene isoforms, allowing the identified increased expression of the *HTR2A* transcript isoforms to be associated exclusively with mental illnesses. In addition, the effects of both the haloperidol and the olanzapine during the 28 days of administration had no effect on the level of transcript isoforms studied.

Notably, there are limitations of studies involving the brain tissue associated with the inaccessibility of the material during its lifetime, and variety of genes with differential splicing that have been identified (more than 1000 genes according to some authors) associated with schizophrenia [9, 32, 33]. These facts complicate the selection of the individually reliable biomarkers associated with the development of mental pathologies. At the same time, a small number of alternatively transcript isoforms of genes in blood cells were associated with mental illness that suggest success in identifying them as peripheral biomarkers for disorders. Currently, work on peripheral biomarkers for the safety and effectiveness of the antipsychotic therapy associated with the differential

expression of the gene splicing variants is practically absent. In our work, for the first time, we have demonstrated reliable associations between the expression of E2tr, E2-and E2+ isoforms and the development of antipsychotic-induced akathisia. Despite statistical significance, these results require confirmation with an extended sample of patients with the drug akatisia and a data replication on an independent cohort. This study had other limitations. Primarily, the systemic inflammation (dysregulation of innate and adaptive responses, disturbance of inflammatory cytokine production) that accompanies schizophrenia has not been evaluated, which can independently affect the expression of alternative isoforms. Secondly, there was no assessment of the genetic variant effects and the epigenetic changes of gene regulation (DNA methylation and other), which can lead to disruption of gene transcription processes and alternative splicing. Finally, since there is variability in psychiatric consortia in accepted cutoffs regarding remission (at least 20% and 50% reduction of the baseline score PANSS), the cutoff we have chosen may not be sufficient to assess the effect of the studied parameters on the effectiveness of the therapy.

5. Conclusion

The present study showed that over-expression and pattern disruption of *HTR2A* exon II spliced transcript isoforms were detected in drug-naïve schizophrenic peripheral blood lymphocytes, as well as antipsychotic-induced akathisia was associated with reduced levels of E2tr, E2+, E2- *HTR2A* isoform expression. This data indicates the contribution of the alternative forms of the *HTR2A* transcripts to the pathogenesis of mental disorders, and the levels of the mRNA splicing gene variants in lymphocytes can be considered as potential biomarkers for the safety of the therapy (development of antipsychotic-induced negative effects).

Declarations

Author contribution statement

M. Grunina: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

A. Taraskina: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

R. Nasyrova, E. Krupitsky: Contributed reagents, materials, analysis tools or data.

M. Belinskaia, A. Zhuravlev: Performed the experiments; Contributed reagents, materials, analysis tools or data.

A. Zabotina: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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