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Original Article

Hyperprolactinemia and *CYP2D6*, *DRD2* and *HTR2C* genes polymorphism in patients with schizophrenia

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Abstract

Introduction: Hyperprolactinemia is a common serious side effect of antipsychotic medications that are currently used in the treatment of patients with schizophrenia. Pharmacogenetic approaches offer the possibility of identifying patient-specific biomarkers for predicting the risk of this side effect. We investigated a possible relationship between variants (SNPs) in genes for cytochrome 2D6 (*CYP2D6)*, dopamine-2 receptor (*DRD2*) and serotonin-2C receptor (*HTR2C*) and antipsychotic drug-induced hyperprolactinemia in patients with schizophrenia.

Methods: Overall, 128 Russian patients with paranoid schizophrenia (61F/67M, aged 18-65 y) were included. Serum prolactin concentration was measured with ELISA. DNA analysis and genotyping of *CYP2D6* (rs3892097), *DRD2* (rs6275) and *HTR2C* (rs6318) genes was done with StepOnePlus Real-Time PCR System using TaqMan® SNP Genotyping Assays (Applied Biosystems, USA).

Results: Our study showed an association of the *CYP2D6* (rs3892097) and *HTR2C* (rs6318) gene polymorphism with hyperprolactinemia in patients with schizophrenia on the background of therapy. No associations were identified between the *DRD2* (rs6275) gene polymorphism and the risk of antipsychotic-induced hyperprolactinemia in patients with schizophrenia.

Conclusion: Our study confirms a contribution of genetic factors to the antipsychotic-induced hyperprolactinemia in patients with schizophrenia. Further studies are required to unravel the genetic predictors of antipsychotic-induced side effects and to develop the personalized treatment strategies for patients with schizophrenia.

Keywords:
Schizophrenia;
Hyperprolactinemia;
Antipsychotics;
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Introduction

The regular therapy of schizophrenia includes a maintenance antipsychotic treatment, which improves the long-term prognosis of the disease and contributes to its transition into remission (Tandon,

2011; Bruijnzeel et al., 2014). Patients with schizophrenia need to take this therapy for a long time, often throughout their entire lives (Miyamoto et al., 2005). Unfortunately, antipsychotics also have a wide spectrum of side effects, including metabolic, endocrine, cardiovascular and movement disorders (Staller, 2006; Lally and MacCabe, 2015).

One of the common side effects of these drugs is hyperprolactinemia (Ajmal et al., 2014; Peuskens et al., 2014). Prolactin secretion is known to be under the permanent inhibitory control of dopamine. Therefore, the administration of antipsychotic drugs (which are potent dopamine receptor blockers) is a foreseeable, though certainly unwanted complication.

Low efficacy of therapy along with intolerable side effects are the main causes of non-compliance and discontinuation of treatment in over 70% of patients with schizophrenia, often resulting in the relapse of psychosis (Miyamoto et al., 2005).

Unravelling the genetic predictors of therapeutic response to antipsychotics or complications in the treatment of schizophrenia is expected to elucidate the biochemical pathways leading to antipsychotic-induced side effects, and to offer possibilities for personalized treatment strategies of schizophrenic patients maximizing efficacy and minimizing the use of ineffective drugs.

The genes of some members of the drug metabolizing cytochrome system (*CYP2C19, CYP2D6 and CYP2C9*), of proteins involved in serotonin (*HTR2C, HTR2A, SLC6A4*, etc.) and dopamine (*DRD1, DRD3*, etc.) neurotransmission are considered to be suitable candidates to predict unwanted features of the clinical effects of antipsychotics (Loonen and Ivanova, 2013; Müller et al., 2013).

The present study aims to elucidate the role of three polymorphic variants of *CYP2D6*, *DRD2* and *HTR2C* genes in the pathogenesis of antipsychotic-induced hyperprolactinemia in patients with schizophrenia.

Materials and methods

The study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki 1975, revised Fortaleza, Brazil, 2013) for experiments involving humans. A total of 128 ethnic Russian patients were studied, including 61 women and 67 men (mean age 34.9 ± 1.2 years; from 18 to 65 years old) with a diagnosis of paranoid schizophrenia according to the diagnostic criteria of International Classification of Diseases-10. Patient inclusion in the study was carried out after obtaining a written consent of the patient to participate in the study. The information about drugs and study procedures was presented in a readily accessible form to each patient. None of the participants had a compromised capacity/ability to consent; hence, consent obtained from the next of kin was not necessary and not recommended by the Local Ethics Committee, which approved the study protocol.

In- and out-patients were selected from departments of the clinic of biological therapy of mental patients of St. Petersburg's V.M. Bekhterev Psychoneurological Research Institute.

Methods used in this study include the assessment of clinical-therapeutic, clinical-endocrinologic (including anthropometric and laboratory variables) and statistical analysis. Patients were treated orally with atypical and/or classical antipsychotics in a flexible dosing regimen: risperidone, 33 persons (25.78% of examined patients); olanzapine, 28 (21.87%); quetiapine, 31 (24.21%) and classical antipsychotics, 36 (28.14%). Antipsychotics were prescribed in average therapeutic doses as recommended by their package insert sheets.

For objectification of the clinical condition the following scales were used: PANSS (Positive and Negative Syndrome Scale); CGI-S (Clinical Global Impressions of Severity); CGI-I (Clinical Global Impressions of Improvement) and UKU (Udvalg for Kliniske Undersøgelser) Side-Effect Rating Scale (Lingjaerde et al., 1987).

The combined use of these scales covered the expected changes of the clinical condition during therapy. To identify and assess a possible neuroendocrine dysfunction the laboratory data were combined with the examination of the patients' anthropometric characteristics. Quantitative evaluation of the presence or absence of obesity, weight gain during treatment as well as the nature of the adipose tissue distribution ('central' or 'hip' obesities) was performed using the study of body mass index, body weight and the waist circumference.

Antipsychotic side effects were assessed with taking their main pathogenetic mechanisms into account,

including chance of development and degree. To carry out a differentiated assessment of the adverse events, the characteristic side effects of the studied antipsychotics were considered. These include neurological, psychiatric and somatic vegetative phenomena. In addition to these clinical events, the neuroendocrine dysfunction associated with pharmacotherapy was considered as well.

The prolactin serum concentration was measured using Enzyme Linked Immunosorbent Assay kits (Roche Diagnostics). Assessing of the level of prolactin in the blood serum was carried out according to the normal ranges of this test: for men 96-456 μIU/ml and for women 127-637 μIU/ml. Control of the blood glucose and lipid profile was carried out to identify the mechanisms of neuroendocrine disorders development.

The control group for genetic studies included 93

Table 1: The distribution of patients by the type of the course of schizophrenia

Type of the course of schizophrenia	Number of patients (n=128)	
	Absolute (n)	Relative (%)
Episodic with progressive defect	105	82.03
Episodic with stable defect	14	10.93
Episodic remitting	9	7.04

Table 2: The severity of side effects on the UKU scale in the examined patients with schizophrenia (n=128)

Parameter	Scores
Neurological side effects (the average score)	2.16±0.23
Psychiatric side effects (the average score)	3.1±0.63
Other side effects	
Rash	0.0
Itch	0.0
Photosensitivity	0.0
Increased pigmentation	0.0
Weight gain	1.58±0.39
Weight loss	0.0
Menorrhagia	0.0
Amenorrhea	2.93±0.23
Galactorrhea	2.23±0.19
Gynecomastia	1.02±0.34
Increased libido	0.0
The weakening of libido	2.32±0.68
Erectile dysfunction	2.09±0.23
Ejaculation dysfunction	1.98±0.09
Orgasmic dysfunction	1.96±0.33
Vaginal dryness	0.0
Headache	1.07±0.45
Physical addiction	0.0
Psychic addiction	0.0
Average score	2.16±1.6

mentally and physically healthy ethnically Russian individuals (63 women and 30 men, aged 17-65 years old).

DNA extraction was conducted according to standard protocols. Genotyping of CYP2D6 (rs3892097), DRD2 (rs6275) and HTR2C (rs6318) genes was done with StepOnePlus Real-Time PCR System using TaqMan® SNP Genotyping Assays (Applied Biosystems, USA) in the Laboratory of Molecular Genetics and Biochemistry at the Mental Health Research Institute (Tomsk).

Statistical analysis was performed using the SPSS 20.0 program. The Hardy–Weinberg equilibrium (HWE) and differences in genotype frequencies were tested using a chi-square test. As the *HTR2C* gene is X-bound, HWE was not calculated. Differences were considered significant at $P<0.05$. The associations of different genotypes with the development of the disease were evaluated by the value of the odds ratio (OR).

Results

Patient characteristics

The mean PANSS total score in examined patients was 86.32 ± 5.03 points (ranged from 70 to 120 points). The average duration of disease was 2.82 ± 0.93 years. The average duration of actual exacerbation was 8.39 ± 4.03 weeks. The average number of previous exacerbations was 2.48 ± 1.72.

The distribution of patients by leading clinical syndrome was as follows: 97 patients (75.78%) had the leading hallucinatory-paranoid syndrome and 31 (21.22%) patients - clinical paranoid syndrome. The distribution of patients by type of the disease is presented in Table 1. Patients with episodic course of the disease with a gradual increase in the severity of the defect predominated.

According to the study design, the evaluation of the incidence and severity of side effects when used antipsychotics was performed using anthropometric surveys and collecting the peripheral blood of patients to determine series of biochemical and hormonal parameters. To assess the severity of side effects occurring in patients the data of UKU Side-Effect Rating Scale were analyzed (Table 2).

Prolactin-related side effects

Evaluation of side effects of neuroendocrine spectrum was carried out with the help of the relevant paragraphs of the scale UKU, united under the heading "other side effects", which also contains indicators of toxic-allergic genesis. As shown in Table 2, the structure of the clinical syndrome of hyperprolactinemia in women on acute treatment with all medications was characterized by menstrual irregularities, galactorrhea, decreased libido, engorgement and tenderness of breast. In men, the clinical syndrome structure included decreased libido, disturbances of erectile, ejaculatory function,

Table 3: The metabolic profile of the examined patients with schizophrenia

Index	Level
Body mass index (kg / m^2)	29.02±2.14
Waist circumference (cm)	89.03±5.19
Hip circumference (cm)	113.92±10.64
Glucose (mmol/ L)	5.46±0.97
Cholesterol (mmol/L)	7.19±1.29
Triglycerides (mmol/ L)	2.17±0.46
High-density lipoproteins	1.09±0.32
Low-density lipoproteins	3.82±1.02
Blood pressure (systolic, diastolic) (mm Hg)	121±10; 82±8
Prolactin (μIU/ml) Men women	 1983±129 2189±312

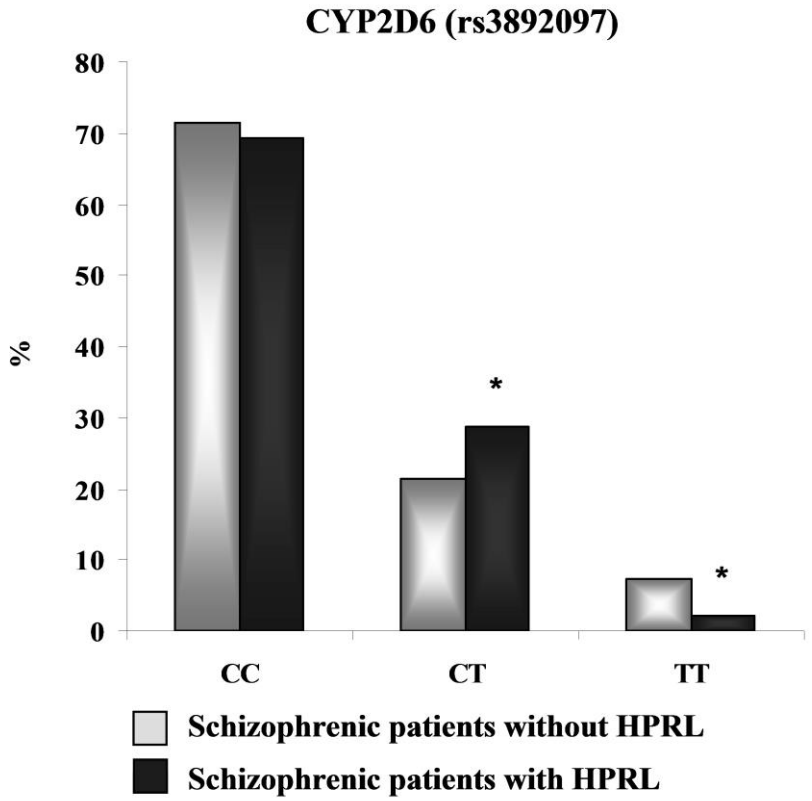

Fig.1. Comparison of genotype frequencies of *CYP2D6* (rs3892097) gene polymorphism in schizophrenic patients with and without hyperprolactinemia (HPRL). * *P*<0.05 compared to the schizophrenic patients without hyperprolactinemia.

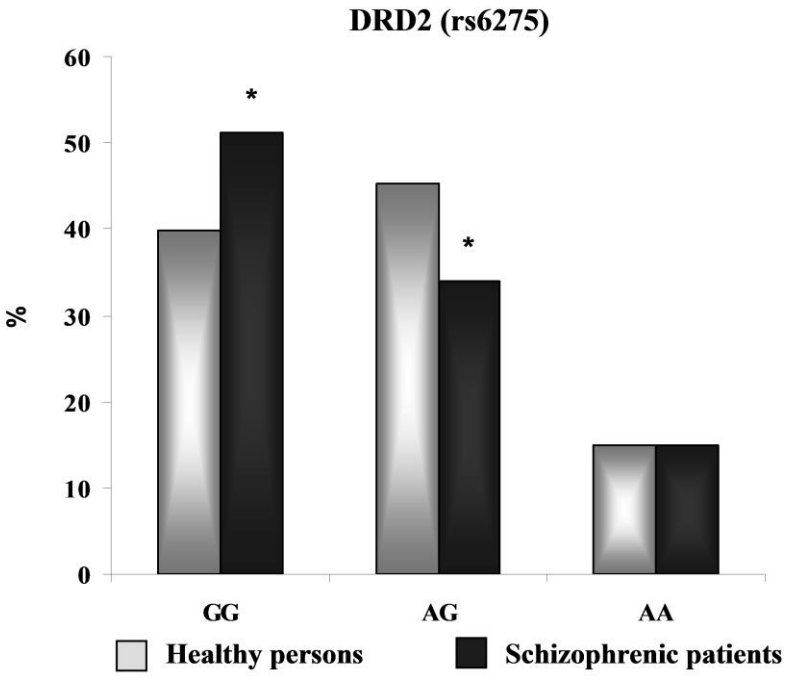

Fig.2. Comparison of genotype frequencies of *DRD2* (rs6275) gene polymorphism in patients with schizophrenia and healthy persons. * *P*<0.05 compared to the healthy persons.

gynecomastia and galactorrhea. The metabolic profile of patients is presented in Table 3.

Based on the data obtained, all patients with schizophrenia were divided into two groups: those with hyperprolactinemia (serum prolactin is more than 2000 μIU/ml) and without hyperprolactinemia (serum prolactin is less than 2000 μIU/ml). Genotyping of DNA samples was carried out in these groups of

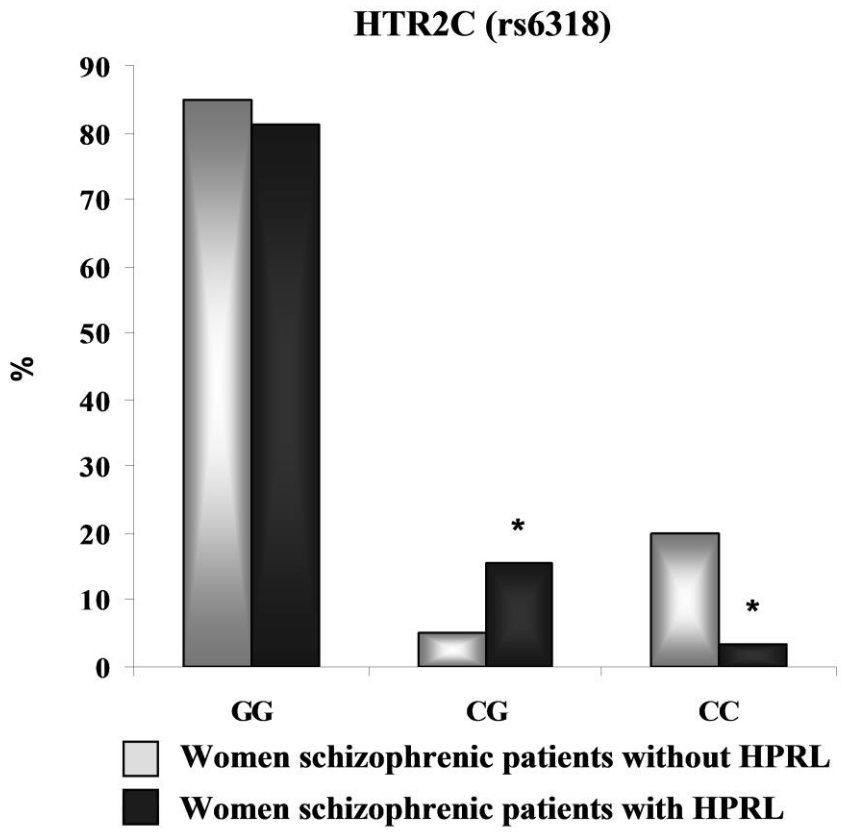

Fig.3. Comparison of genotype frequencies of *HTR2C* (rs6318) gene polymorphism in women schizophrenic patients with and without hyperprolactinemia (HPRL). **P*<0.05 compared to the women schizophrenic patients without hyperprolactinemia.

patients and in the group of healthy individuals.

Hyperprolactinemia and genotypes

The analysis of the prevalence of *CYP2D6* (rs3892097) and *DRD2* (rs6275) genotypes in patients and control sample did not reveal deviance from Hardy–Weinberg equilibrium.

Genotyping of the control group for the *CYP2D6* (rs3892097) marker showed a high frequency of homozygous GG genotype (75.2%), and then in descending order go genotypes AG (23.6%) and AA (1.2%). The major G and a minor A alleles were represented in the control group, with frequencies of 92.6% and 7.4%, respectively.

The prevalence of genotypes of the *HTR2C* (rs6318) gene polymorphic variant in the control group was as the following: GG, 84.9%; GC, 10.8%; CC, 4.3%. Alleles were presented by the following frequencies: G, 92% and C, 8%. In the control group for the *DRD2* (rs6275) gene the following prevalence of the genotypes was identified: CC, 39.8%; CT, 45.2%; TT, 15%. The major C and a minor T were presented in the control group with frequencies of 65.9% and 34.1%, respectively.

A comparison of the genotype frequencies between patients and healthy individuals revealed no differences for the *CYP2D6* (rs3892097) polymorphism, leading to the conclusion about the absence of the association of this gene with the development of schizophrenia ($\chi 2 = 2.1$, $P>0.05$). However, a comparison of the genotype frequencies between patients with hyperprolactinemia and the group with normal level of prolactin revealed differences for the *CYP2D6* (rs3892097) gene polymorphism, thus providing the evidence for the association of this gene variant with the development of hyperprolactinemia in patients with schizophrenia on the background of antipsychotic therapy ($\chi 2 = 7.2$, $P<0.005$; Fig. 1). Also, it can be speculated that the TT genotype has a protective effect regarding antipsychotic-induced hyperprolactinaemia in schizophrenic patients (OR = 0.75; 95% CI: 0.634-0.887; $P = 0.0008$).

Comparison of the groups of patients and healthy individuals showed an association of *DRD2 (rs6275)* gene polymorphism with schizophrenia ($\chi 2 = 7.85$,

P<0.005). In the group of patients with schizophrenia, the GG genotype of the *DRD2* gene is the most common followed by AG and AA genotypes (Fig. 2). In the control group, in contrast, AG is the most common genotype.

The prevalence of genotypes in patients with and without hyperprolactinemia for allelic variants of *DRD2* (rs6275) gene does not differ. Thus, according to our study, rs6275 is not associated with the development of hyperprolactinemia in schizophrenic patients on the background of antipsychotic therapy ($\chi 2 = 0.467$, $P = 0.49$).

Due to the fact that serotonin receptor gene *HTR2C* is localized on X-chromosome, the prevalence of genotypes and alleles of polymorphic variant rs6318 was analyzed separately in women and men. We did not find any statistically significant differences for rs6318 of *HTR2C* gene between patients with schizophrenia and healthy individuals separated by gender. Thus, according to our data, the polymorphic variant rs6318 is not associated with the development of schizophrenia ($\chi 2 = 7.2$, $P>0.05$).

When comparing men and women with schizophrenia, with and without hyperprolactinemia we revealed an association of *HTR2C* (rs6318) gene polymorphism with the development of hyperprolactinemia in women schizophrenic patients, on the background of antipsychotic therapy ($\chi 2 = 8.3$; $P<0.005$) (Fig. 3).

Allele C has a protective effect with respect to hyperprolactinemia development (OR = 0.930; CI = 0.306-2.828; $P = 0.89798$), and allele G is a predisposing allele (OR = 1.075; CI = 0.354-3.271; $P = 0.89798$), but these data do not reach the level of statistical significance.

Discussion

Our study provided the evidence for the association of the *CYP2D6* (rs3892097) gene polymorphism with the development of hyperprolactinemia in patients with schizophrenia as a respond to the antipsychotic therapy. Also, it can be concluded that the TT genotype has a protective effect regarding antipsychotic-induced hyperprolactinemia in schizophrenic patients. Homozygosity for null alleles and heterozygosity for low activity alleles is seen in most clinically important phenotype of poor metabolizer lacking the enzymatic activity of *CYP2D6*. Ultrarapid metabolizers carry three or more normal copies of the gene in their genotype (Steen et al., 1995), although ultrarapid metabolizers without duplication are described. The poor metabolizer phenotype for *CYP2D6* is associated with adverse effects of psychotropic drugs. In this study, we did not determine the metabolic phenotypes; however, the analyzed SNP rs3892097 is known to lead to an inactive form of the enzyme and therefore results in reduction of CYP2D6 function (Van der Merwe et al., 2012).

According to our study, the GG genotype of the *DRD2* (rs6275) gene polymorphism is the most common followed by AG and AA genotypes in the group of patients with schizophrenia. It is possible to make an assumption that the GG genotype predisposes to schizophrenia, since its frequency in patients is higher in comparison to the control group. Also we have shown that *DRD2* (rs6275) gene polymorphism is not associated with the development of hyperprolactinemia in schizophrenic patients on the background of antipsychotic therapy. It is worth noting the controversial data in the literature about the associations of *DRD2* gene with the hyperprolactinemia development. Perhaps a series of genetic and pharmacokinetic factors is behind the phenomenon of antipsychotic-induced hyperprolactinemia.

According to our data, the polymorphic variant *HTR2C* (rs6318) is not associated with the development of schizophrenia. At the same time, the serotonergic system is considered to be one of the most important neurotransmitter systems contributing to the pathogenesis of schizophrenia or at least determining an important part of the response to antipsychotic drugs. A number of hypotheses of the pathogenesis of schizophrenia is associated with the assumption of impairment of specific links of metabolism, in particular of the biogenic amines. For example, the indolamine hypothesis postulates involvement of serotonin and its metabolites, as well as other indole derivatives in the mechanisms of mental activity, an impairment of which may lead mental disorders, in particular to the development of schizophrenic symptoms (Nothern et al., 1995).

HTR2C (rs6318) gene polymorphism results in the Cys23Ser amino acid substitution associated with the dysfunction of the serotonin neurotransmission in various psychiatric disorders and the development of

side effects of antipsychotic and antidepressant therapy (Drago and Serretti, 2009). The Ser23 allele is associated with weight gain in women with schizophrenia receiving therapy with atypical antipsychotics (Houston et al., 2012). With respect to motor side effects of antipsychotic therapy it has been shown the protective value of the Ser23 allele in the development of tardive dyskinesia (Al Hadithy et al., 2009). The *HTR2C* is an excitatory G-protein coupled receptor which has been described to have constituitive activity in the absence of neurotransmitter (Aloyo et al., 2009). This means, that a complete blockade of the receptor results in a decrease of the excitatory activity of the receptor and therefore also a decrease of the activation of the cell carrying these receptors. The presence of less active HTR2C on prolactin producing cells of the pituitary gland in C-allele carriers could result in a decrease of prolactin secretion due to under stimulation of these cells.

Conclusion

Thus, our study identified an association of *CYP2D6* (rs3892097) and *HTR2C* (rs6318) gene polymorphisms with antipsychotic induced hyperprolactinemia in patients with schizophrenia. We demonstrated that hyperprolactinemia in our patient population is related to gynecological disturbances in women and sexual disorders in men. Further search for genetic markers associated with the development of side effects of antipsychotic treatment will allow developing effective methods for diagnosis and treatment of schizophrenia and will contribute to compliance of patients with mental health problems to the psychotropic therapy for improvement of their physical condition and quality of life.

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Conflict of interest

None of the authors has any conflict of interest to disclose

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