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GENETICS

CYP1A2 and *CYP2D6* Gene Polymorphisms in Schizophrenic Patients with Neuroleptic Drug-Induced Side Effects

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Polymorphic variants of *CYP1A2* and *CYP2D6* genes of the cytochrome P450 system were studied in patients with schizophrenia with drug-induced motor disorders and hyperprolactinemia against the background of long-term neuroleptic therapy. We revealed an association of polymorphic variant C-163A CYP1A2*1F of *CYP1A2* gene with tardive dyskinesia and association of polymorphic variant 1846G>A CY2D6*4 and genotype A/A of *CYP2D6* gene (responsible for debrisoquin-4-hydroxylase synthesis) with limbotruncal tardive dyskinesia in patients with schizophrenia receiving neuroleptics for a long time.

Key Words: *schizophrenia; tardive dyskinesia; hyperprolactinemia; gene polymorphism; cytochromes*

Long-term neuroleptic therapy is the main method for the treatment of schizophrenia improving the long-term prognosis and promoting disease transition to remission [15]. In Russia, the side effects of neuroleptic are particularly important problem from the social viewpoint, because ~70-80% schizophrenic patients receive traditional neuroleptic therapy often leading to motor disorders [3]. Motor side effects aggravate the course of the underlying disease, augment the severity of negative, cognitive, and affective disorders, and lead to additional social stigmatization of the patients [11]. New generation antipsychotics, atypical neuroleptics, are highly effective, but also produce side effects. The side effects of atypical antipsychotics used for a long time is primarily realized by the neuroendocrine mechanism; the most incident side effect of these drugs is hyperprolactinemia (HPRL) [1,7].

The pathogenesis of neuroleptic disorders remains unclear. However, the important role of genetic factors in predisposition to motor disorders and HPRL has been demonstrated [5-7,10,13]. Pharmacogenetic studies showed clinical significance of polymorphisms of the genes regulating synthesis and activity of drug biotransformation enzymes [4]. Expression of various allele variants of the genes encoding cytochrome P450 system isoenzymes leads to the synthesis of forms with modified activity, which, in turn, can be responsible for inhibition and acceleration of drug metabolism [4,8,12]. The development of methods for individual psychopharmacotherapy is one of the most important tasks of modern clinical pharmacology and biological psychiatry [2,8]. Identification of the allele variant leading to changes in drug pharmacokinetics and pharmacodynamics in a patient allows prediction of

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the pharmacological response to the drug and correction of the treatment protocol, which helps to improve significantly its efficiency and safety [2].

We studied associations of *CYP1A2* and *CYP2D6* polymorphic variants (CYP1A2*1F and CYP2D6*3, CYP2D6*4) and drug-induced tardive dyskinesia (TD) and HPRL in schizophrenic patients receiving traditional and atypical antipsychotic therapies.

MATERIALS AND METHODS

Comprehensive clinical studies were carried out in 475 patients with schizophrenia hospitalized at the Department of Endogenous Disorders of the Research Institute of Mental Health, Clinic for Biological Therapy of Mental Patients of V. M. Bekhterev Psychoneurological Research Institute, and at Departments of Regional Clinical Psychiatric Hospitals of Kemerovo and Chita. The mean age of the patients was 40 (17-80) years. The study was carried out in accordance with the Helsinki Declaration (2000). All patients signed informed consent to examination according to the protocol approved by the local ethic committee. The main criteria for including the patients in the study group were clinically verified diagnosis of schizophrenia (IDC-10: F20), Caucasian race, and the absence of organic or neurological disorders.

The severity of TD was evaluated by the Abnormal Involuntary Movement Scale (AIMS) [9]. The sample of 353 patients was divided into 2 groups: with and without incidental motor disorders against the background of neuroleptic treatment. The main group included 140 (39.7%) patients with schizophrenia treated with neuroleptics and exhibiting signs of TD. The reference group included 213 (60.3%) patients with schizophrenia treated with neuroleptics without TD. TD was diagnosed using Schooler and Kane International Criterion implying that the patients developed slight motor disorders (2 points) according to two scales or moderate disorders (3 points) according to AIMS. TD was presented by pathological involuntary movements of the face and neck (orofaciolingual TD; AIMS score 1-4), of the trunk and limbs (limbotruncal TD; AIMS score 5-7). The neuroleptic therapy was administered as monotherapy or combinations of the following drugs: traditional neuroleptics haloperidol (10-30 mg/day), chloroprotixene (100-200 mg/day), chloropromazin (100-200 mg/day), trifluoperazin (20-40 mg/day), and clopiksol (40-80 mg/day); atypical neuroleptic risperidone (4-6 mg/day); this drug, though belongs to atypical neuroleptics, modulates D2 receptors, which could promote the development of late TD in the patients.

Neuroendocrine effects were evaluated by the UKU side effect scale (Udvald for Klinische Undersøgelser Scale). Serum prolactin was measured by ELISA with monoclonal antibodies (normal range 96-456 μU/ml for men, 127-637 μU/ml for women). The sample of 122 patients was divided into 2 groups by serum prolactin levels: with HPRL (prolactin level >2000 μU/ml) and without HPRL (prolactin level <2000 μU/ml). The group of patients with HPRL consisted of 66 subjects (40 women and 26 men), the groups of patients with normal prolactin concentrations included 56 subjects (25 women and 31 men).

Genetic studies were carried out at Laboratory of Molecular Genetics and Biochemistry, Research Institute of Mental Health, and Laboratory of Pharmacogenomics, Institute of Chemical Biology and Basic Medicine. Venous blood for the study was collected after overnight fasting from the ulnar vein into Vacutainer tubes with EDTA. Genomic DNA was isolated from blood nuclear cells by the standard phenol-chloroform micromethod. Genotypes of CYP1A2*1F and CYP2D6*3, CYP2D6*4 polymorphic variants of the cytochrome P450 system genes were identified by real time PCR with fluorescent probes using CFX384 Touch Real-Time PCR Detection System (Bio-Rad Laboratories). Hardy–Weinberg equilibrium for genotype distribution by the studied polymorphic loci to was tested by χ^2 test. The frequency of genotypes and alleles in the groups was compared using χ^2 test. The differences were considered significant at $p<0.05$. Association of certain genotypes (or their combinations) with diseases was evaluated by the odds ratio.

RESULTS

The distribution of alleles and genotypes of the studied polymorphic variants CYP1A2*1F and CYP2D6*3, CYP2D6*4 in the total group of patients corresponded to the expected distribution at Hardy–Weinberg equilibrium.

In order to evaluate the relationship between CYP1A2*1F polymorphic locus (C163A, rs762551) and TD, we compared the frequency of various genotypes and alleles of this polymorphism in the groups of patients with and without TD (Table 1). A similar comparison was also carried out for the orofaciolingual and limbotruncal TD subtypes (Table 2). Analysis showed significant differences in the frequency of various alleles and genotypes of CYP1A2*1F polymorphic locus (C-163, rs762551) in the groups of patients with and without TD. The frequency of allele C in schizophrenics with TD was 1.3-fold higher than in those without TD ($\chi^2=4.65$, $p<0.05$). The frequency of genotype CC was 4-fold higher in the group of schizophrenic patients with TD in comparison with those without TD: 13.6% in patients with TD and 3.4% in those without TD ($\chi^2=7.30$, $p<0.05$).

TABLE 1. Frequency (%) of Genotypes and Alleles of Genes *CYP1A2* and *CYP2D6* Polymorphic Variants in Patients with Schizophrenia with and Without TD

Gene polymorphic variant	Genotype, alleles	Schizophrenic patients with TD	Schizophrenic patients without TD	χ^2
CYP1A2*1F	A/A	42.0	49.4	7.30 (*p*=**0.03**)
	C/A	44.3	47.2	
	C/C	13.6	3.4	
	A	64.2	73.0	4.65 (*p*=**0.03**)
	C	35.8	27.0	
CYP2D6*3	A/A	98.1	98.9	0.23 (*p*=0.89)
	A/del	1.9	1.1	
	del/del	0	0	
	A	99.1	99.4	0.22 (*p*=0.64)
	del	0.9	0.6	
CYP2D6*4	A/A	1.5	2.2	0.88 (*p*=0.64)
	G/A	15.5	19.1	
	G/G	83.0	78.7	
	A	9.3	11.8	0.94 (*p*=0.33)
	G	90.7	88.2	

TABLE 2. Frequency (%) of Genotypes and Alleles of Gene *CYP1A2* and *CYP2D6* Polymorphic Variants in Patients with Schizophrenia with and without Limbotruncal TD

Gene polymorphic variant	Genotype, alleles	Schizophrenic patients and limbtruncal TD	Schizophrenic patients without limbtruncal TD	χ^2
CYP1A2*1F	A/A	43.9	46.5	0.49 (*p*=0.78)
	C/A	45.0	42.3	
	C/C	11.0	11.2	
	A	66.4	67.7	0.22 (*p*=0.64)
	C	33.6	32.3	
CYP2D6*3	A/A	97.8	98.1	0.51 (*p*=0.78)
	A/del	2.2	1.5	
	del/del	0	0.4	
	A	98.9	98.8	0.01 (*p*=0.93)
	del	1.1	1.2	
CYP2D6*4	A/A	4.3	0.8	5.25 (*p*=**0.047**)
	G/A	17.2	16.1	
	G/G	78.5	83.1	
	A	12.9	8.8	2.52 (*p*=0.11)
	G	87.1	91.2	

The CYP1A subfamily in humans and other mammals consists of two enzymes denoted as CYP1A1 and CYP1A2 according to the standard nomenclature of P450. These enzymes are involved in metabolic activation of many procarcinogens and are induced by a series of compounds interesting for toxicology, including dioxin. More than 15 alleles (*1B-*16) of *CYP1A2* gene have been identified, including several promoter variants. The best studied are polymorphic variants -3860G>A (CYP1A2*1C), -2467delT (*1D), -739T>G (*1E), and -163C>, located in intron 1 (*1F), which lead to changes in the CYP1A2 enzymatic activity. Polymorphic variant CYP1A2*1F (-163C>A) in intron 1 is associated with high inducibility of the enzyme and more intense drug metabolism. High inducibility of this enzyme in tobacco smoking is associated with low blood level of clozapine in schizophrenic patients receiving standard therapy. Therapy with such drugs as leflunomide and olanzepin is fraught with a high risk of toxic effect.

Our results indicate that *CYP1A2* gene polymorphic variant CYP1A2*1F with homozygous allele C can be associated with drug-induced TD in patients with schizophrenia.

About 25% of all drugs serve as substrates for CYP2D6, coded for by the gene on chromosome 22. These drugs include beta-blockers, tricyclic antidepressants, neuroleptics, morphine derivatives, and other drugs, for which FDA in the USA recommends genetic testing for correcting the drug dose. At least 40 genetic variants of *CYP2D6* are known associated with low metabolic activity of the enzyme [14]. However, the main alleles are *1-6, *9, *10, *17. Enzyme activity is not found in carriers of alleles CYP2D6*3 and CYP2D6*4. For amitryptiline therapy, the drug dose is recommended to be reduced by 25%, while for carriers of CYP2D6*4 allele, other analgesics (other than codeine) are recommended.

No differences in the frequency of alleles and genotypes of CYP2D6*3 and CYP2D6*4 polymorphic variants were detected in schizophrenic patients with and without TD (Table 1). However, we showed the association of CYP2D6*4 polymorphic variant with

TABLE 3. Frequency (%) of Genotypes and Alleles of Gene *CYP1A2* and *CYP2D6* Polymorphic Variants in Patients with HPRL and with Normal Prolactin Level

Gene polymorphic variant	Genotype, alleles	Patients with HPRL	Patients without side effects	χ^2
CYP1A2*1F	A/A	51.5	39.3	3.61 (p=0.16)
	A/C	34.8	51.8	
	C/C	13.6	8.9	
	A	68.9	65.2	
	C	31.1	34.8	
CYP2D6*3	A/A	98.5	91.1	3.69 (p=0.16)
	A/del	1.5	7.1	
	del/del	0.0	1.8	
	A	99.2	94.6	
	del	0.8	5.4	
CYP2D6*4	A/A	0.0	5.4	3.62 (p=0.16)
	A/G	21.2	19.6	
	G/G	78.8	75.0	
	A	10.6	13.6	
	G	89.4	86.4	

limbotruncal TD (Table 2). The frequency of allele A (*4) in patients with TD was 1.5 times higher than in the group without TD. The frequency of allele AA was 4.3% – 5-fold higher than in patients without TD (χ^2=5.25, p<0.05).

Comparison of patients with HPRL and with normal prolactin concentrations showed no appreciable differences in the genotype and allele distribution for the studied genes (Table 3).

Hence, the study has detected association of CYP1A2*1F polymorphic variant with the development of motor disorders and of CYP2D6*4 polymorphic variant with limbotruncal TD in patients with schizophrenia receiving neuroleptics for a long time. These alleles are associated with reduction of the activities of the relevant enzymes, which can result in inhibition of the neuroleptic biotransformation, their long circulation in the blood, and risk of motor disorders.

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