

# Pharmacogenetic Testing of Antipsychotic Transporter Proteins: A Case Report in a 32-Year-Old Woman with Treatment-Resistant Schizophrenia

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**Citation:** Osipova, S.M.; Shnayder, N.A. Pharmacogenetic Testing of Antipsychotic Transporter Proteins: A Case Report in a 32-Year-Old Woman with Treatment-Resistant Schizophrenia. *Personalized Psychiatry and Neurology* **2022**, *2* (1): 98-106.

<https://doi.org/10.52667/2712-9179-2022-2-1-98-106>

Chief Editor: Nikolaj G. Neznanov,  
D Med Sci, Professor

Received: 28 December 2022

Accepted: 15 February 2022

Published: 15 May 2022

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**Abstract:** Schizophrenia is a common and socially significant mental disorder requiring long-term use of antipsychotics (APs). Long-term use of APs increases the risk of developing adverse drug reactions (ADRs) and / or treatment resistance in some patients. This may be due to a genetically determined impairment of APs transport across the blood-brain barrier (BBB) and the membrane of APs target neurons in the brain. Pharmacogenetic testing (PGx) is a method to identify a group of patients with a high risk of developing AP-induced ADRs. Foreign panels for PGx do not include non-functional variants of genes encoding APs transporter proteins. However, our experience of using PGx to search for low-functional and non-functional single-nucleotide variants (SNVs)/polymorphisms of three genes (*ABCB1*, *ABCG2*, *ABCC1*) encoding APs transporter proteins demonstrates the importance of this new personalized approach to the choice of APs and its dosing in patients with a slow transporter PGx profile. The main purpose of the work is to present the experience of using pharmaco-genetic testing (PGx) in a 32-year-old patient with treatment-resistant schizophrenia and a medical history of AP-induced ADRs.

**Keywords:** schizophrenia; treatment resistance; adverse reaction; antipsychotics; pharmacogenetic testing.

## Introduction

Schizophrenia is one of the most serious and socially significant mental disorders, the prevalence of which reaches millions of cases worldwide. For the treatment of mental disorders, including schizophrenia, drugs are usually used - antipsychotics (APs) [1]. Despite the generation of new APs, the problem of AP-induced adverse drug reactions (ADRs) has not been solved to date, and the accumulated experience in the targeted prevention of AP-induced ADRs suggests that most of them can be prevented or their consequences can be significantly reduced [2].

This problem is the cause of: a decrease in the quality of life of patients; reducing the adherence of patients with mental disorders to chronic APs therapy; development of pseudo-resistance of mental disorders to APs; disease progression.

The study of the mechanisms of development of AP-induced ADRs is based on changes in their metabolism and transportation, depending on the following risk factors for ADRs:

- modifiable factors (choice of APs, its dosage, dosing regimen, consideration of comorbid conditions, etc.);
- non-modifiable factors (gender, age of patients, genetic predisposition).

The study of the genetic predisposition to the development of AP-induced ADRs is based on associative genetic studies and genome-wide studies of single-nucleotide variants (SNVs) and polymorphisms of the candidate genes involved in the metabolism, transportation, cumulation, excretion of APs and their active metabolites [3].

## Objective

The main purpose of the work is to present the experience of using pharmacogenetic testing (PGx) in a 32-year-old patient with treatment-resistant schizophrenia and a medical history of AP-induced ADRs.

## Materials and Methods

### 1.1. Procedure

PGx was used based on the pharmacogenetic profile of patients with mental disorders (homo- and heterozygous carriers of risk alleles of low-functional and non-functional SNVs genes *ABCB1*, *ABCG2*, *ABCC1*) encoding APs transporter proteins of the first and new generations.

PGx was carried out using microchips, while assessing the cumulative risk of developing AP-induced ADRs due to impaired APs efflux of 1, 2 and 3 generations through the blood-brain barrier (BBB) and the membrane of neurons through APs transporter proteins: P-glycoprotein (P-gp); Breast Cancer Resistance Protein (BCRP); Multidrug Resistance-Associated Protein 1 (MRP1). The features of the genotype and phenotype of patients were determined, information on the genetically determined transportation of APs through the BBB.

Patients were divided into three phenotypes (poor metabolizers, intermediate metabolizers, extensive metabolizers) by testing 26 low-functioning and non-functional SNVs of the *ABCB1*, *ABCG1*, *ABCC1* genes.

As a result of testing, a list of APs was obtained, divided into four categories: "use as directed" in case of homozygous carriage of fully functional allelic variants of genes in a patient; "use with caution" in case of heterozygous carriage of low-functional allelic variants of genes; "use with increased caution and with more frequent monitoring" in homozygous carriers of low-functioning allelic gene variants; "do not use" in case of homozygous carriage of non-functional allelic variants of genes in a patient.

### 1.2. Inclusion and Exclusion Criteria

The following inclusion criteria were used in selecting patients:

- voluntarily signed informed consent;
- age over 18 years;
- established diagnosis of schizophrenia (F20.x);
- taking AP for more than 6 months;
- the presence of AP-induced ADRs.

### 1.3. Procedure for Clinical Testing of the Technique

At visit 1, biological material (venous blood) was collected from the patient. Then the genetic analysis of the sample was carried out by PGx. PGx revealed SNVs of the *ABCB1*, *ABCG1*, *ABCC1* genes and assessed the cumulative risk of AP-induced ADRs.

#### 1.4. Ethical aspects

The study was performed in accordance with the standards of good clinical practice and the principles of the Declaration of Helsinki. Clinical testing was carried out within the framework of the state task. The participant signed a voluntary informed consent. The patient did not receive any compensation for participating in the clinical trial. Researchers did not receive any compensation for conducting clinical trials.

### Results

#### 1.5. Anamnesis of the Disease

Patient M., 32 years old, has been suffering from schizophrenia since the age of 28. She was admitted to the clinic due to the lack of a therapeutic response to APs for several years after the onset of the disease. There was an increase in hallucinatory-delusional symptoms, as well as the development of AP-induced ADRs during the treatment of various APs in monotherapy and polytherapy.

#### 1.6. Results of Pharmacogenetic Testing

The results of the performed PGx are presented in (Table 1).

**Table 1.** Results of PGx in a patient with treatment-resistant schizophrenia (clinically significant genotypes are shown).

Gene	Protein Encoded by This Gen	Rs	Normal	Result
ABCB1	P-glycoprotein	rs1045642	CC	TT
		rs1128503	CC	TT
ABCG2	Breast Cancer Resistance Protein	rs2231142	CC	AA
		rs192169063	TT	TC
		rs72552713	CC	CT
		rs1061018	TT	TC
		rs750568956	TT	TG
		rs372192400	GG	GA
ABCC1	Multidrug Resistance-Associated Protein 1	rs212090	TT	TT

Thus, the functional activity of the P-gp (responsible for the transporting of the following APs: clozapine, olanzapine, quetiapine, risperidone, paliperidone, aripiprazole, amisulpride, chlorpromazine, sulpiride, trifluoperazine) was found to be significantly reduced in the patient. Homozygous carriage of the low-functional allele T of the variant rs1045642 (C3435T) and homozygous carriage of the low-functional allele T of the variant

rs1128503 (C1236T) of the *ABCB1* (*MDR1*) gene encoding P-gp activity were found.

The functional activity of the BCRP (responsible for the transporting of the following APs: aripiprazole, clozapine, olanzapine, quetiapine, risperidone, paliperidone, chlorpromazine, haloperidol, sulpiride) is significantly reduced. Found: homozygous carriage of the low-functional allele A of the variant rs2231142 (C421A), heterozygous carriage of the low-functional allele C of the variant rs192169063 (T1465C), heterozygous carriage of the low-functional allele T of the variant rs72552713 (C376T), heterozygous carriage of the low-functional allele C of the variant rs1061018 (C376T), heterozygous carriage of the low-functional allele C of the rs1061018 variant G variant rs750568956 (T1574G), heterozygous carriage of the low-functional allele A variant rs372192400 (G88131153A) of the *ABCG2* gene encoding the activity of the BCRP. The functional activity of the MRP1 (responsible for the transporting of the following APs: clozapine) was preserved.

Thus, the use of PGx to assess the functional activity of transporter proteins made it possible to explain the development of AP-induced ADRs and pseudo-resistance to previously used APs in this patient.

The cumulative pharmacogenetic risk of reducing the transport of psychotropic drugs, including APs, with the participation of P-gp and BCRP in the patient is regarded as high.

Given that the functional activity of two of the three studied transporter proteins, which ensure the transporting of psychotropic drugs across the BBB and the membrane of target cells of a wide range of APs, is significantly reduced, the phenotype of this patient was assessed as "poor metabolizers" (or "poor transporter").

### 1.7. Recommendations for the Patient

We recommended that the patient use the following APs with increased caution and more frequent therapeutic drug monitoring: aripiprazole, clozapine, olanzapine, quetiapine, risperidone, paliperidone, chlorpromazine, trifluoperazine, haloperidol, sulpiride, amisulpride. The starting and target dose of these APs should be reduced by an average of 50% compared to the average therapeutic dose (according to the instructions for these drugs);

The AP of choice for this patient was cariprazine, whose transporting through the BBB does not involve the above transporter proteins.

There was a positive clinical effect after the correction of psychopharmacotherapy in the form of a reduction in hallucinatory-delusional symptoms and a reduction in the patient's previously AP-induced ADRs.

## Discussion

Membrane transport protein (transporter protein) is a membrane protein that is involved in the movement of ions, small molecules and macromolecules, such as other proteins, across a biological membrane. Transport proteins are integral transmembrane proteins. They constantly exist inside the cell and cover the membrane through which they carry substances. Transporter proteins can promote the movement of substances using facilitated diffusion or active transport [4].

Currently, 15 transporter proteins involved in the transport of drugs across cell membranes and tissue barriers are the most studied (**Table 2**).

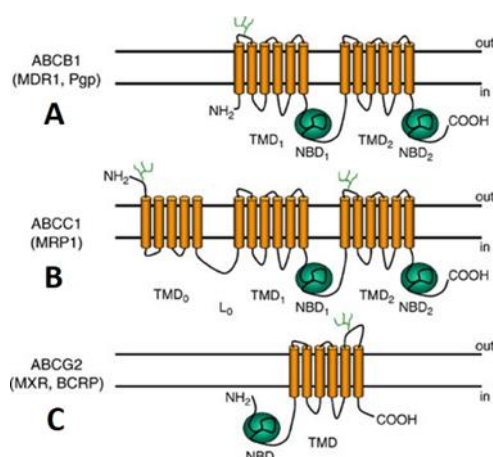
**Table 2.** Transporter proteins and their encoding genes.

Gene	Location	Protein	Aminoacid content
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ABCB1	chr7:87,503,017-87,713,323(GRCh38/hg38)	MDR1 Multidrug Resistance Protein 1 P-Gp P-Glycoprotein 1	1280
ABCB2	chr6:32,845,209-32,853,816(GRCh38/hg38)	TAP1 Transporter 1	808
ABCB3	chr6:32,821,831-32,838,770(GRCh38/hg38)	TAP2 Transporter 2	653
ABCB4	chr7:87,398,988-87,480,435(GRCh38/hg38)	MDR2 Multidrug Resistance Protein 3 P-Glycoprotein 3	1279
ABCB11	chr2:168,915,468-169,031,396(GRCh38/hg38)	BSEP Bile Salt Export Pump	1321
ABCC1	chr16:15,949,138-16,143,257(GRCh38/hg38)	MRP1 Multidrug Resistance-Associated Protein 1	1531
ABCC2	chr10:99,782,602-99,853,741(GRCh38/hg38)	MRP2 Multidrug Resistance-Associated Protein 2	1545
ABCC3	chr17:50,634,777-50,692,253(GRCh38/hg38)	MRP3 Multidrug Resistance-Associated Protein 3	1527
ABCC4	chr13:95,019,835-95,301,475(GRCh38/hg38)	MRP4 Multidrug Resistance-Associated Protein 4	1325
ABCC5	chr3:183,919,934-184,018,010(GRCh38/hg38)	MRP5 Multidrug Resistance-Associated Protein 5	1437
ABCC6	chr16:16,149,565-16,223,617(GRCh38/hg38)	MRP6 Multidrug Resistance-Associated Protein 6	1503
ABCC10	chr6:43,427,366-43,451,994(GRCh38/hg38)	MRP7 Multidrug Resistance-Associated Protein 7	1464
ABCC11	chr16:48,164,842-48,249,973(GRCh38/hg38)	MRP8 Multidrug Resistance-Associated Protein 8	1382
ABCC12	chr16:48,080,882-48,156,018(GRCh38/hg38)	MRP9 Multidrug Resistance-Associated Protein 9	1359
ABCG2	chr4:88,090,150-88,231,628(GRCh38/hg38)	BCRP Breast Cancer Resistance Protein	655

In the clinical practice of a psychiatrist, knowledge of the role of transporter proteins and changes in their functional activity and expression at the level of cell membranes of neurons and the BBB can help assess the risk of developing ADRs and treatment resistance to a wide range of drugs, including APs, mood stabilizers, antidepressants, anticonvulsants, etc.

The analysis of fundamental molecular and pharmacogenetic studies showed that 3 transporter proteins play the most important role in APs transporting: P-gp or multidrug resistance protein 1 (MDR1); BCRP - "breast cancer resistance protein"; MRP1, a protein associated with multidrug resistance 1 (**Figure 1**) [5].



**Figure 1.** Sheme of transporter proteins that play a leading role in the transporting of antipsychotiks through biological membranes (A - P-gp or MDR1; B - MRP1; C - BCRP) [6].

P-gp is a membrane protein that is an ATP-dependent efflux pump for drugs and other xenobiotics with broad substrate specificity. It performs the function of a carrier of drugs, including APs, through the BBB and the membrane of target neurons for the effect of these drugs. This is important to consider if long-term pharmacotherapy of mental disorders is needed. It may be associated with the development of multidrug resistance. The involvement of P-gp has been described in the transporting of APs of the 1st generation (chlorpromazine, amisulpride, trifluoperazine), 2nd generation (clozapine, olanzapine, quetiapine, risperidone, paliperidone) and 3rd generation (aripiprazole). At the same time, P-gp is involved in the efflux of other drugs that are often prescribed together with APs, including antidepressants and mood stabilizers [6], [7].

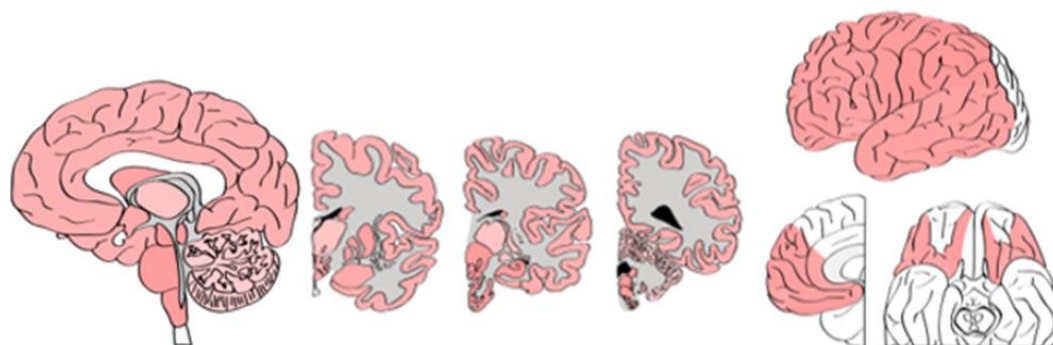
P-gp expression in the brain is highest at the level of the frontal, medial and medio-basal cortex, in the hippocampus, tail of caudate nucleus, and other organs: adrenal glands, liver, gallbladder, small and large intestines, kidneys, ovaries, and fallopian tubes (in women) (Figure 2).



**Figure 2.** Level of P-gp expression in the brain of human [13].

BCRP is a membrane protein, ATP-binding cassette transporter. This protein is involved in the transport of drugs (including APs) and other xenobiotics, and may play a role in the development of multidrug resistance to chemotherapeutic agents. The involvement of BCRP has been described in the transporting of APs of the 1st generation (haloperidol, chlorpromazine), 2nd generation (clozapine, olanzapine, quetiapine, risperidone, paliperidone), and 3rd generation (aripiprazole) [8].

BCRP expression in the brain is highest at the level of the frontal, medial and medio-basal cortex, in the hippocampus, tail of caudate nucleus, and other organs: seminal vesicles, testicles (in men), small and large intestines, placenta, lungs, thyroid gland, adrenal glands, myocardium (Figure 3).



**Figure 3.** Level of BCRP expression in the brain of human [13].

MRP1, a multidrug resistance-associated protein, is a member of the ATP-binding cassette transporter superfamily. The participation of this transporter protein in the transport of 2nd generation APs (clozapine) was studied [9].

MRP1 expression in the brain is highest at the level of the cortex, as well as other organs (Figure 4).



**Figure 4.** Level of MRP1 expression in the brain of human [13].

Thus, the use of PGx to assess genetically determined changes in the functional activity and expression of these transporter proteins in patients with mental disorders is promising and justified.

Schizophrenia is a common and socially significant mental disorder that requires long-term use of APs. Long-term use of APs increases the risk of developing ADRs and / or treatment resistance in some patients. This may be due to a genetically determined impairment of APs transporting across the BBB and the membrane of APs target neurons in the brain. PGx is a method that allows the identification of a group of patients with a high risk of developing AP-induced ADRs. Foreign panels GeneSight Psychotropic test (GeneSight) [10] and Genecept Assay (Genecept) [11] for PGx do not include non-functional variants of the genes encoding APs transporter proteins. When using the GeneSight test, analysis is performed on 59 allelic variants of 8 genes: the *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP3A4*, *CYP2B6*, *CYP2D6*, *HTR2A* and *SLC6A4* genes. The attending physician is provided with information already analyzed by the program based on the results of the patient's genotyping. The conclusion contains a list of antipsychotics and antidepressants divided into 3 categories: "use as directed", "use with caution", and "use with increased caution and more frequent monitoring". The Genecept Assay test was developed in the USA. The study is carried out on allelic variants in 20 genes, including the *5HT2C*, *MC4R*, *DRD2*, *COMT* and other genes of the cytochrome p450 system. The conclusion is provided in the form of a detailed table with recommendations for prescribing medicines for a particular patient.



Our experience of using PGx to search for low-functional and non-functional SNVs/polymorphisms of three genes (*ABCB1*, *ABCG2*, *ABCC1*) encoding APs transporter proteins demonstrates the importance of this new personalized approach to the selection of APs and its dosing in patients with a poor metabolizer pharmacogenetic profile [3],[14],[15].

## Conclusion

Our experience with the use of PGx based on the pharmacogenetic profile of transporter proteins in patients with mental disorders and the presented clinical case demonstrate the promise of its use in adult patients not only in the case of ADRs development, but also before the start of APs therapy. This is important for improving the personalized strategy for choosing APs, their dosing regimen, the rate of dose increase, and the possibility of combination with other APs if polytherapy of mental disorders is required. In addition, PGx can be useful in cases where additional prescription of drugs of other pharmacological groups is necessary with a decrease in the risk of ADRs in a particular patient.

**Author Contributions:** Conceptualization, S.M.O.; methodology, S.M.O.; software, S.M.O.; formal analysis, S.M.O.; investigation, S.M.O.; resources, S.M.O.; data curation, S.M.O.; writing original draft preparation, S.M.O.; writing—review and editing, N.A.S.; visualization, S.M.O.; supervision, N.A.S.; project administration, N.A.S.

All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Conflicts of Interest:** The authors declare no conflict of interest.

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