

**»TREATMENT RESISTANCE« ENIGMA RESOLVED BY PHARMACOGENOMICS  
– A CASE STUDY OF CLOZAPINE THERAPY IN SCHIZOPHRENIA****ENIGMA »TERAPO-REZISTENCE« RAZREŠENA UZ POMOĆ FARMAKOGENOMIKE  
– PRIKAZ SLUČAJA TERAPIJE KLOZAPINOM U SHIZOFRENIJI**

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**Summary**

The introduction of antipsychotic medication in the 1950s forever changed the outlook on the treatment of schizophrenia, although there is still a large proportion of patients who do not reach functional recovery. At least 30% of patients do not respond to clozapine, the tricyclic dibenzodiazepine with complex pharmacological actions, which was proven to be more effective than any other antipsychotic in the treatment of schizophrenia. According to most of the therapeutic guidelines for schizophrenia, clozapine is the third line therapy for patients who did not respond to other antipsychotics. Large inter-individual variability exists for clozapine bioavailability and plasma steady-state concentrations and clearance. Clozapine is metabolized by the cytochrome P450 oxidase enzyme family (CYP450). Cytochrome P450 1A2 (CYP1A2), which is polymorphically expressed in humans, is the main enzyme of clozapine metabolism. This case report addresses the influence of CYP1A2\*1F genetic polymorphism on clozapine metabolism, explains the primary non-response of a young patient with schizophrenia due to increased gene expression in homozygous genotype \*1F/\*1F (increased metabolism of clozapine) and underlies the importance of personalizing schizophrenia treatment by means of genetic and other molecular tools, at least in the cases of »treatment resistance«.

**Keywords:** clozapine, CYP1A2, personalized medicine, pharmacogenomics, schizophrenia

**Kratak sadržaj**

Početak primene antipsihotika pedesetih godina prošlog veka zauvek je promenio perspektivu lečenja shizofrenije, mada značajan broj pacijenata sa ovom bolešću još uvek ne postiže funkcionalni oporavak. Klozapin, triciklični dibenzodijazepin sa kompleksnim farmakološkim profilom, koji se u tretmanu shizofrenije pokazao efikasnijim od ostalih antipsihotika, uglavnom je treća linija terapijskog izbora. Međutim, u najmanje 30% slučajeva obolelih od shizofrenije stanje se ne poboljšava ni na terapiji klozapinom, pa se zbog toga pristupa polifarmaciji i odstupa od racionalne primene psihofarmaka. Postoji velika interindividualna varijabilnost u bioraspoloživosti klozapina, stabilnoj koncentraciji u plazmi i njegovom klirensu. Klozapin se metaboliše preko sistema citohrom P450 oksidaza (CYP450). Citohrom P450 1A2 (CYP1A2), koji se u humanoju populaciji odlikuje genskim polimorfizmima, glavni je enzim u metabolizmu klozapina. Aktuelni prikaz slučaja ilustruje uticaj polimorfizma CYP1A2\*1F na metabolizam klozapina, gde je primarni izostanak odgovora na terapiju kod mlade pacijentkinje sa shizofrenijom objašnjen povećanjem ekspresije gena kod homozigotnog genotipa \*1F/\*1F (povišen metabolizam klozapina), i naglašava značaj personalizacije tretmana shizofrenije uz pomoć genetike i drugih molekularnih parametara, posebno u slučajevima »terapo-rezistence«.

**Ključne reči:** CYP1A2, farmakogenomika, klozapin, personalizovana medicina, shizofrenija

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## Introduction

Along with diabetes, cancer and hypertension, schizophrenia is a genetically complex disease characterized by polygenic transmission, locus heterogeneity and environmental contribution to causation. The median lifetime morbid risk for schizophrenia is 7.2/1000 persons (1). The introduction of antipsychotic medication in the 1950s forever changed the outlook on the treatment of schizophrenia, although there is still a large proportion of patients who do not reach functional recovery.

Currently, psychotropic drugs are administered by trial and error. Personalized medicine is one of the most promising aspects of contemporary medicine, which may be achieved by the adaptation of treatments to individual patients by means of genetic and other molecular tools (2). To reduce suffering and minimize costs, it is desirable to know in advance whether a drug is likely to be effective and tolerable. Unfortunately, clinical and anamnestic variants have not been found to be sufficiently helpful in this respect (3), while the genetically determined investigation of pharmacological responses could be more promising (4).

Pharmacogenomics (PGx) is the study of how an individual's genetics affects their response to drugs, combining traditional pharmaceutical sciences, such as biochemistry, with annotated knowledge of genes, proteins and single-nucleotide polymorphisms (SNPs). It has been found that the variability of pharmacotherapeutic reply may be the result of different genetic polymorphisms, which can further influence the pharmacokinetics (absorption, distribution, metabolism and the drug excretion) and pharmacodynamics (determined by the targeted spot of the drug treatment – the receptors). The main objective of PGx is individualization and treatment optimization. Thus, PGx should be used as a tool for getting the right drug, to reach an effective dose earlier and to ensure patient safety.

Clozapine is a second generation (SGA) tricyclic dibenzodiazepine with complex pharmacological actions including an affinity for a wide variety of receptors (e.g. dopaminergic, alpha adrenergic, muscarinic and serotonergic). Large inter-individual variability exists for clozapine bioavailability and plasma steady-state concentrations and clearance. Clozapine is metabolized by the cytochrome P450 oxidase enzyme family (CYP450). The estimated contributions of different isoenzymes involved in clozapine metabolism are: 30%, 24%, 22%, 12%, and 6% for CYP1A2, CYP2C19, CYP3A4, CYP2C9, and CYP2D6, respectively (5). Therefore, CYP1A2 is the main enzyme involved in clozapine metabolism, whereas the majority of other antipsychotics are primarily metabolized by CYP2D6 (6). Studies have revealed that clozapine treatment non-responders have the CYP1A2\*1F/\*1F genotype, suggesting that

this variation is associated with resistance to treatment (7).

In the current treatment guidelines, clozapine is recommended as the third-line treatment option for schizophrenia, mostly due to its potential to induce agranulocytosis (8). Although this side effect is not common and is now managed by systematic blood testing, clozapine is still reserved for patients who did not respond to at least two other antipsychotics from two different classes (9) – e.g. treatment-resistant patients. »Treatment resistant schizophrenia« is defined as schizophrenia in patients who have failed to show an acceptable response to treatment with two different standard antipsychotics given separately in adequate doses for an adequate time (6–8 weeks) (10).

It has been found that up to 60% of neuroleptic-resistant patients respond to clozapine, half of them within the first six weeks of treatment, while the other half respond within six months of treatment (11). However, at least 30% of patients do not respond to clozapine (12). Those patients remain undertreated and are at great risk for serious consequences of their illness, including suicide. In most of these cases the add-on approaches are used, but this practice increases treatment expenses and often leads to the off-label polypharmacy (or polypragmasia) without adequate evidence-based support. With this in mind, alongside the fact that clozapine is the final monotherapy option for patients with treatment-resistant schizophrenia, efforts should be made to make schizophrenia treatment personalized at least in the case of clozapine.

In the personalization of clozapine treatment two main interventions should be considered respectively: monitoring clozapine serum levels (13) and conducting pharmacogenomic analyses to test variations in clozapine metabolism (14). To illustrate the need for personalized clozapine therapy, we will present a case of a young patient with »treatment-resistant« schizophrenia who did not respond to the common clozapine dosage during a sufficiently long treatment period. We will explain the therapeutic dilemmas and suggest a pathway towards elucidation of the pharmacogenomic nature of non-response to clozapine.

### Case report

A 26-year-old Caucasian woman, diagnosed with »Undifferentiated Schizophrenia« using the ICD-10 criteria (International Statistical Classification of Diseases and Related Health Problems, 10<sup>th</sup> Revision, F 20.3) was referred to the Clinic for Psychiatry, Clinical Center of Serbia, with an observed »treatment resistant« illness. Medical records revealed that she had been previously treated with fluphenazine, both in oral and depot formulations (5 mg/day at the

beginning, then 25 mg every four weeks, respectively) for a period of 16 months. However, the aforementioned treatment resulted in only a minor improvement in the positive and negative symptoms of the illness, while causing several serious adverse events: hyperprolactinaemia, amenorrhoea and considerable extra-pyramidal symptoms. As second-line treatment, the SGA risperidone was considered, but it was not introduced due to its potential to produce similar side effects, particularly hyperprolactinaemia and amenorrhoea. Another SGA, olanzapine, was considered instead, but administration of the drug was not commenced due to socio-economic reasons (it was not covered by the patient's health insurance). Therefore, clozapine was added in a dosage of 100 mg as an adjuvant to the fluphenazine depot.

At this point, the patient presented to our outpatient service with delusions of reference, disorganized behavior, social withdrawal, apathy and global functional impairment. Upon admission, the score on the Positive and Negative Symptoms Scale (PANSS) (15) was 99, reflecting a severe illness. During the later outpatient treatment at our clinic, the dosage of clozapine was gradually increased to 300 mg/day, and fluphenazine was terminated. The patient was regularly monitored, and three months later no significant improvement was observed. As the first step, measurement of clozapine serum levels was performed showing that the plasma drug levels were below the therapeutic range (concentration 0.12 mg/l; reference range: 0.35–0.5 mg/L) (16).

To control for potential non-compliance, we admitted the patient to the ward and monitored medication adherence. After two weeks of strict monitoring, the clozapine serum levels were still below the therapeutic threshold: 0.03 mg/L. At this point, we suspected that the patient might be a rapid clozapine metabolizer, so we analyzed genetic polymorphisms for enzymes *CYP1A2* and *CYP2D6*, members of the cytochrome P450 oxidase family (*CYP450*) responsible for the metabolism of most antipsychotic drugs (17). Genomic DNA was isolated from 5 ml peripheral blood and collected in Na-EDTA vacutainers using a QIAamp DNA mini Kit (QiagenGmbH, Hilden, Germany). *CYP1A2\*1F*, *CYP2D6\*3* and *CYP2D6\*4* were genotyped using the previously published PCR-restriction fragment length polymorphisms method (18). Briefly, 200 ng of genomic DNA was subjected to PCR amplification using appropriate primers and 2xPCR MasterMix (Fermentas). Amplified PCR products were subjected to restriction digestion with appropriate enzymes, *MspI*, *MvaI* and *Bsp120I* respectively for *CYP2D6\*3*, *CYP2D6\*4* and *CYP1A2\*1F*. The digested products were separated by gel electrophoresis in a 3% agarose gel. Then, they were identified by the unique patterns, characteristic to their specific genotypes. The patient was a carrier of alleles in the homozygous genotype *CYP1A2\*1F/\*1F*, which is an indicator of increased gene

expression and increased metabolism of clozapine, and was heterozygous for *CYP2D6\*4/\*1*, a phenotype of intermediary metabolism.

In the next step, we increased the dosage of clozapine to 450 mg/day. After eight weeks, the serum clozapine levels rose to 0.49 mg/l, thus reaching the therapeutic range for the first time since clozapine was prescribed. At the same time, the patient showed symptomatic improvement – the PANSS score decreased by 29 points (PANSS total score = 70, overall symptom intensity moderate). The improvement was sustained at the follow-up visits three and six months later.

## Discussion

In this report, we presented the case of a young patient suffering from schizophrenia, with possible treatment resistance to the most potent antipsychotic – clozapine, where the therapeutic resolution was provided by pharmacogenomic testing (PGx). The patient was homozygous for *CYP1A2\*1F* allele (*\*1F/\*1F* genotype), and thus classified as a rapid metabolizer of clozapine. The genetic information was used to provide the right therapeutic decision and to continue with monotherapy towards illness remission. The *CYP1A2\*1F* allele is the result of a single point mutation (-163 C>A) and is associated with increased induction, particularly in smokers, in comparison to the wild-type *CYP1A2\*1A* allele. The distribution of *CYP1A2* genotypes is as follows: *\*1F/\*1F* (nucleotide sequence A/A) ~ 46%; *\*1A/\*1F* (nucleotide sequence C/A) ~ 44%; and *\*1A/\*1A* (nucleotide sequence C/C) ~ 10%, indicating that high induction is the most common phenotype (19–20). The allelic frequencies refer to the Caucasian population. However, to our best knowledge, there are no studies on the distribution of *CYP1A2* polymorphism in the Serbian population.

PGx is based on a single blood sampling and thus has considerable advantage in the case of clozapine therapy. As it was mentioned before, the drug itself is associated with potentially fatal blood disorders (i.e. agranulocytosis) which necessitate the laborious and time-consuming blood monitoring process. Blood samples for blood counts are taken every 2–4 weeks from the beginning of clozapine treatment and the majority of patients hesitate to consent to any additional serial blood monitoring regimen. In addition, routine therapeutic drug monitoring of clozapine is not feasible in many clinical settings as it is usually associated with additional administration and requirements (21).

When access to drug monitoring is limited and pharmacogenomic testing is not performed, the most likely scenario is the addition of a second antipsychotic or mood stabilizer, or a combination of several psychiatric drugs. Such off-label polypharmacy polyphrag-

masia without adequate evidence-based support is usually followed by more side effects and more non-compliance. The aforementioned strategy, very common in everyday practice in the region (22, 23), negatively impacts the overall prognosis of the illness and highly increases the burden of the disease both on the individual and on the community.

Considering all this, at least as far as the »last choice« drug such as clozapine is concerned, PGx based on a single blood sampling has considerable advantage as an important tool for earlier drug efficacy and ensured safety of the patient.

Although pharmacogenomics is gradually becoming an important part of routine clinical practice in developed countries, the developing world still does not have access either to education and/or consensus guidelines, or to the calculations of cost-effectiveness. As recently summarized by Babić (24), the costs of genomic sequencing are constantly decreasing and they are already affordable for consumer testing, so it is an imperative for the health care system to co-evolve with the technology.

If pharmacogenomics (PGx) cannot be globally applicable, it is important to make at least a meaningful choice of the criteria for personalized medicine in terms of PGx. In line with the present case report, our suggestion is that all patients prescribed antipsychotic medications should be carefully monitored by routine methods during the first and second antipsychotic trial; however, once prescribed clozapine, the patient needs additional assessment. Beyond monitoring the side effects (as mentioned earlier), achieving the maximum efficacy of this very potent drug acting on the dopaminergic, alpha adrenergic, serotonergic, and muscarinic receptors requires the psychiatrist to con-

sider the possible pharmacokinetic variations and to try to customize the therapeutic intervention and improve the outcome. In future, combining the assessment of pharmacokinetic factors with the analysis of pharmacodynamic markers – for example serotonin HTR2 and HTR3A gene polymorphisms (25, 26), might be the best approach to improve our clinical practice (25–27).

In conclusion, at least in the case of clozapine as a third-line therapy in psychiatry, PGx should be considered both by practitioners and by legislators. Resolution of the treatment resistance enigma in any case of schizophrenia will alleviate the marked burden of disease on the patients, their families and the society as a whole.

### Consent

Informed consent was obtained from the patient for the publication of this case report.

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### Conflict of interest statement

The authors stated that have no conflicts of interest regarding the publication of this article.

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