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Case Report

Pharmacogenetic Testing of Cytochrome P450 Metabolizing Enzymes in 28-Year-Old Man with Treatment-Resistant Schizophrenia

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Abstract: Schizophrenia is a common and socially significant mental disorder that requires long-term use of antipsychotics (APs). Long-term use of APs increases the risk of developing adverse drug reactions (ADRs) and/or therapeutic resistance in some patients. This may be due to a genetically determined impairment of APs metabolism by cytochrome P450 enzymes. Pharmacogenetic testing (PGx) is a method to identify a group of patients with a high risk of developing AP-induced ADRs. Our experience of using PGx to search for low-functional and non-functional single nucleotide variants (SNVs) / polymorphisms of the *CYP1A2*, *CYP2C9, CYP3A4, CYP3A5* and *CYP2D6*) genes encoding cytochrome P450 enzymes involved in APs metabolism demonstrates the importance of this new personalized approach to the choice of APs and its dosing in patients with pharmacogenetic profile poor metabolizer. The main purpose of the case report is to present the experience of using PGx in a 28-year-old patient with treatment-resistant schizophrenia and a medical history of AP-induced ADRs.

Keywords: *schizophrenia; therapeutic resistance; unwanted 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 yet, and the accumulated experience in targeted prevention of AP-induced ADRs suggests that most of them can be prevented or their consequences can be significantly reduced [2, 3].

This problem causes: a decrease in the quality of patients' life; reducing the adherence of patients with mental disorders to chronic AP therapy; development of pseudo-resistance of mental disorders to APs; disease progression [4]. The study of the AP-induced ADRs mechanisms is based on the change in their metabolism and transport, which depends on the following risk factors: modifiable factors (choice of AP, its dose, dosing regimen, consideration of comorbid conditions, etc.); non-modifiable factors (gender, age of patients, genetic predisposition). The study of 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, transport, cumulation, excretion of APs and their active metabolites [3-5].

Objective

The aim of the case report is to present the experience of using pharmacogenetic testing (PGx) in a 28-year-old patient with treatment-resistant schizophrenia and a medical history of AP-induced ADRs.

Materials and Methods

3.1. 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 of the *CYP1A2*, *CYP2C9, CYP3A4, CYP3A5*, *CYP2D6* genes encoding cytochrome P450 enzymes involved in APs metabolism. PGx was carried out using microchips, while the cumulative risk of developing AP-induced ADRs was assessed due to impaired 1th, 2nd and 3rd generations APs metabolism by cytochrome P450 enzymes: CYP1A2, CYP2C9, CYP3A4, CYP3A5, CYP2D6.

The features of the genotype and phenotype of patients, information of genetically determined APs metabolism was determined. Patients were divided into three phenotypes (poor metabolizers, intermediate metabolizers, extensive metabolizers, ultra-rapid metabolizers) by testing 17 low-functional and non-functional SNVs of the *CYP1A2*, *CYP2C9, CYP3A4, CYP3A5*, *CYP2D6* 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 SNVs in these genes in a patient; "use with caution" in case of heterozygous carriage of low-functional allelic variants; "use with increased caution and with more frequent monitoring" in case of homozygous carriage of low-functional allelic variants; "do not use" in case of homozygous carriage of non-functional allelic variants.

3.2. Inclusion and Exclusion Criteria

The following inclusion criteria were used in selecting patients: signed voluntary informed consent; age over 18 years; established diagnosis of schizophrenia (F20.x); taking APs for more than 6 months; the presence of AP-induced ADRs.

3.3. Patient Observation

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 candidate genes (*CYP1A2*, *CYP2C9, CYP3A4, CYP3A5*, *CYP2D6)*, and assessed the cumulative risk of AP-induced ADRs.

At visit 2, correction of AP therapy was carried out taking into account the results of PGx.

3.4. Ethical Aspects

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

Results

4.1. Medical History

Patient K., 28 y.o., has been suffering from schizophrenia since the age of 24. He 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-paranoid and negative symptoms, as well as the development of AP-induced ADRs in the treatment of various AP in monotherapy and polytherapy.

4.2. *Results of Pharmacogenetic Testing*

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

Table 1. Results of pharmacogenetic testing in a patient with treatment-resistant schizophrenia (clinically significant deviations are shown).

Gene	Protein	Rs	Normal	Result
CYP1A2	CYP1A2 enzyme	rs2069514	GG	AA
		rs56276455	GG	AG
CYP2C9	CYP2C9 enzyme	rs9332131	AA	delA/delA
CYP2D6	CYP2D6 enzyme	rs35742686	AA	delA/delA
		rs5030655	TT	delT/delT

Thus, the patient showed that the functional activity of the CYP1A2 enzyme (responsible for the metabolism of the following APs: asenapine, clozapine, olanzapine, thiothixene, trifluoperazine, chlorpromazine, haloperidol, perphenazine, quetiapine, thioridazine) was significantly reduced. Homozygous carriage of the low-functional allele A of the rs2069514 variant (3860G>A) and heterozygous carriage of the low-functional allele A of the rs56276455 variant (2385G>A) of the *CYP1A2* gene encoding the activity of the CYP1A2 enzyme.

The functional activity of the CYP2C9 enzyme (responsible for the metabolism of the following APs: haloperidol, perphenazine, promazine, clozapine, olanzapine) is shut off. Found: homozygous deletion of the functional allele A of the rs9332131 (818delA) variant of the *CYP2C9* gene encoding the activity of the CYP2C9 enzyme.

The functional activity of the CYP3A4 enzyme (responsible for the metabolism of the following APs: aripiprazole, cariprazine, lurasidone, pimavanserin, quetiapine, ziprasidone, haloperidol, perphenazine, alimemazine, zuclopenthixol, promazine, clozapine, sertindol, risperidone, paliperidone) was preserved.

The functional activity of the CYP3A5 enzyme (responsible for the metabolism of the following APs: quetiapine, clozapine, olanzapine, paliperidone, pimozide, risperidone, aripiprazole, haloperidol) was preserved.

Functional activity of the CYP2D6 enzyme (responsible for the metabolism of the following APs: aripiprazole, risperidone, chlorpromazine, fluphenazine, haloperidol, perphenazine, pimozide, thioridazine, alimemazine, promazine, clozapine, zuclopenthixol, trifluperazine, levomepromazine, flupentixol, pipotiazine, quetiapine, paliperidone, sertindol, cariprazine) is shut off. Found: homozygous deletion of the functional allele A

of the rs35742686 variant (2549delA) and homozygous deletion of the functional allele T of the rs5030655 variant (1707delT) of the *CYP2D6* gene encoding the activity of the CYP2D6 enzyme.

Thus, the use of PGx to assess the functional activity of cytochrome P450 enzymes made it possible to explain the development of AP-induced ADRs and pseudoresistance to previously used APs in this patient.

The cumulative pharmacogenetic risk of reducing the metabolism of psychotropic drugs, including APs, with the participation of cytochrome P450 enzymes CYP1A2, CYP2C9, CYP2D6 in the patient is regarded as high.

Given that the functional activity of three out of the five studied cytochrome P450 enzymes, which provide the metabolism of psychotropic drugs of a wide range of APs, was significantly reduced, the phenotype of this patient was assessed as a "poor metabolizer".

4.3. Recommendations for the Patient

We recommended the patient use the following APs with increased caution and more frequent therapeutic drug monitoring: asenapine, thiothixene. 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).

We advised the patient to do not use the following APs: haloperidol, perphenazine, promazine, clozapine, olanzapine, aripiprazole, risperidone, chlorpromazine, fluphenazine, perphenazine, pimozide, thioridazine, alimemazine, zuclopenthixol, trifluperazine, levomepromazine, flupentixol, pipotiazine, quetiapine, paliperidone, sertindol, cariprazine.

The APs of choice for this patient was lurasidone or ziprasidone, whose metabolism does not involve the mentioned low-functional and non-functional enzymes of the CYP 450.

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

Discussion

Of great clinical interest is the study of the role of hepatic metabolism of APs, since most of the typical and atypical APs used in real clinical practice have a hepatic or predominantly hepatic metabolism. Hepatic metabolism of APs can be carried out in various ways, including oxidation, glucuronization, N-deamination, etc. Oxidation is the leading mechanism of hepatic metabolism of most APs used in clinical practice and is carried out with the participation of liver cytochrome P450 isoenzymes.

Cytochrome P450 (cytochrome P450-dependent monooxygenase) is the common name for enzymes of the P450 family, which are part of the class of hemoproteins and belong to type b cytochromes.

In different people, the set of the cytochrome P450 isoenzymes in the endoplasmic reticulum (ER) and on the inner mitochondrial membrane differs due to genetic characteristics. In this regard, the study of genetically determined changes in the expression of the functional activity of CYP family isoenzymes is of great clinical importance in psychiatry and clinical pharmacology [5-7].

In humans, 57 genes and more than 59 pseudogenes encoding CYP isoenzymes have been identified. These isozymes are divided into 18 families and 43 subfamilies. The following enzymes are mainly involved in the metabolism of 1th, 2nd and 3rd generations APs through the oxidation process: CYP1A2, CYP2C9, CYP2D6, CYP3A4, CYP3A5 [5].

Cytochrome P450 enzyme 1A2 is an enzyme encoded in humans by the *CYP1A2* gene. The CYP1A2 enzyme is expressed in the liver (**Figure 1**) [8]. The CYP1A2 enzyme is

localized in the ER and its expression is induced by several polycyclic aromatic hydrocarbons (PAHs), some of which are found in cigarette smoke. The endogenous substrate of the enzyme is unknown. However, it is able to metabolize some PAHs to carcinogenic intermediates. Other xenobiotic substrates for this enzyme include caffeine, aflatoxin B1, and paracetamol. The CYP1A2 enzyme is associated with 72 reactions in 10 different subsystems: cytosol, extracellular, mitochondria, nucleus, peroxisome. The CYP1A2 enzyme is involved in the metabolism of a wide range of drugs, including APs [9].

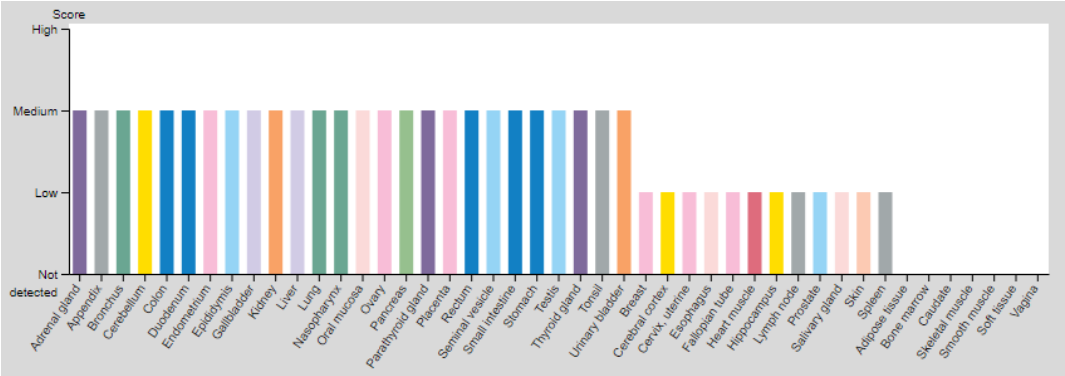

Figure 1. Expression level of CYP1A2 in human organs and systems.

Cytochrome P450 enzyme 2C9 (CYP2C9) is an enzyme that in humans is encoded by the *CYP2C9* gene. The CYP2C9 enzyme is expressed in the liver, to a lesser extent in the duodenum, small intestine, stomach, large intestine, and gallbladder (**Figure 2**) [8]. CYP2C9 is an enzyme of cytochrome P450, which plays an important role in the oxidation of both xenobiotics and endogenous compounds. CYP2C9 accounts for about 18% of cytochrome P450 enzymes in liver microsomes. About 100 drugs are metabolized by CYP2C9, including drugs with a narrow therapeutic index, such as warfarin and phenytoin. The CYP2C9 enzyme is involved in metabolism of a wide range of drugs, including Aps. The *CYP2C9* gene is highly polymorphic. For CYP2C9 substrates such as warfarin and phenytoin, a decrease in the activity of this enzyme due to genetic polymorphism or drug interaction can lead to toxicity at normal therapeutic doses of drugs, including many APs [10].

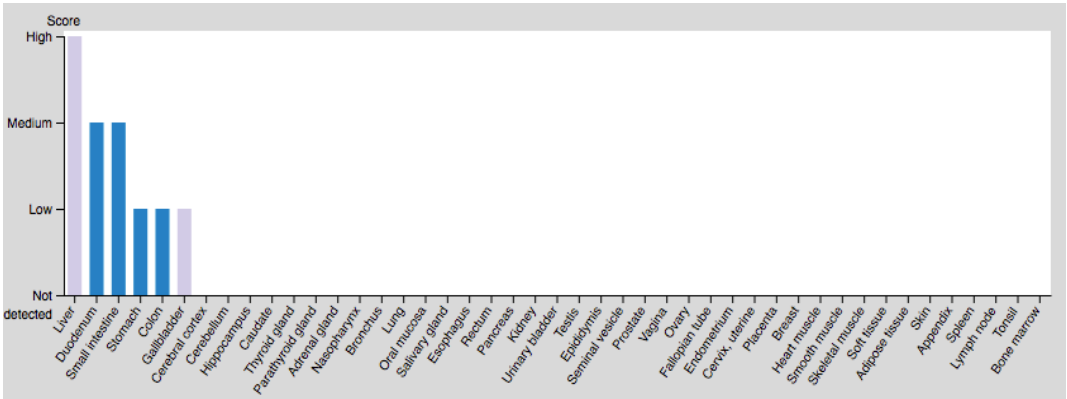

Figure 2. Expression level of CYP2C9 in human organs and systems.

Cytochrome P450 enzyme 2D6 (CYP2D6) is an enzyme encoded in humans by the *CYP2D6* gene. The CYP2D6 enzyme is primarily expressed in the liver, to a lesser extent - in the small intestine, duodenum, colon, and testes (**Figure 3**). It is also strongly expressed in neurons of the central nervous system, including nigrostriatal neurons, which are target

neurons of some APs [8]. The CYP2D6 enzyme, a member of the cytochrome P450 mixed oxidase system, is one of the most important enzymes involved in the metabolism of xenobiotics in the body and in a wide range of APs. This enzyme is responsible for the metabolism and excretion of approximately 25% of drugs used in clinical practice. The *CYP2D6* gene shows the greatest variability among other genes of the CYP family, mainly due to genetic polymorphism [11].

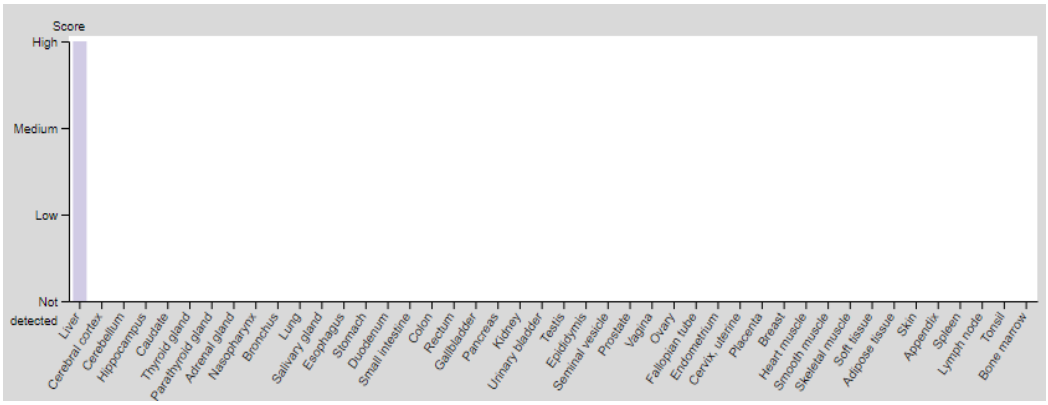

Figure 3. Expression level of CYP2D6 in human organs and systems.

Cytochrome P450 enzyme 3A4 (CYP3A4) is an enzyme that in humans is encoded by the *CYP3A4* gene. The CYP3A4 enzyme is expressed predominantly in the liver, duodenum, and small intestine (**Figure 4**) [8]. This is one of the most important enzymes involved in the metabolism of xenobiotics and drugs in the human body. Its purpose is to oxidize small foreign organic molecules such as toxins or drugs so that they can be removed from the body. CYP3A4 is one of a large group of the cytochrome P450 enzymes. This protein is localized in the ER of hepatocytes, and its expression is induced by glucocorticoids and some other drugs. The CYP3A4 enzyme is involved in the metabolism of 1th, 2nd and 3rd generations APs [12].

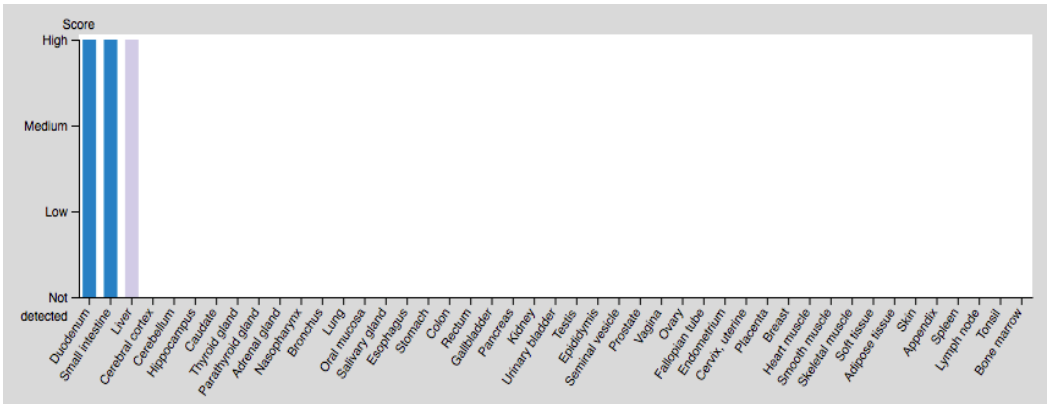

Figure 4. Expression level of CYP3A4 in human organs and systems.

Cytochrome P450 enzyme 3A5 (CYP3A5) is an enzyme encoded in humans by the *CYP3A5* gene. The CYP3A5 enzyme is expressed in the liver, small intestine, stomach, duodenum, colon, and to a lesser extent in the pancreas, bladder, kidneys, esophagus, rectum, prostate, skin, tonsils, vagina, lungs, tongue, appendix, bridge and medulla oblongata, adrenal glands, cervix, breast, adipose tissue (**Figure 5**) [8]. This enzyme is localized in ER cells and its expression is induced by glucocorticoids and some APs. The enzyme metabolizes drugs such as nifedipine and cyclosporine, as well as steroid hormones

(testosterone, progesterone and androstenedione). Enzymes CYP3A4, 3A5 are a group of gem-thiolate monooxygenases. In liver microsomes, they are involved in the nicotinamide adenine dinucleotide phosphate (NADP)-H-dependent electron transport pathway. CYP3A5 oxidizes a variety of structurally unrelated compounds, including steroids, fatty acids, and xenobiotics. The human CYP3A subfamily (CYP3A4, CYP3A5, CYP3A7 and CYP3A43) is one of the most versatile biotransformation systems involved in the metabolism of a wide range of drugs (37% of the 200 most commonly prescribed drugs in the US). CYP3A4 and CYP3A5 together make up approximately 30% of hepatic cytochrome P450, and approximately half of the lipoproteins that are oxidatively metabolized by P450 are CYP3A substrates. The CYP3A5 enzyme is involved in the metabolism of many APs [13].

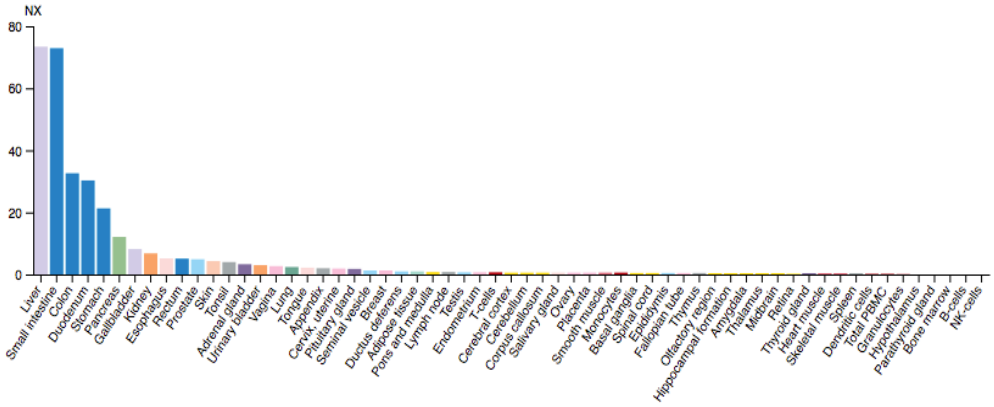

Figure 5. Expression level of CYP3A5 in human organs and systems.

Thus, the use of PGx to assess genetically determined changes in the functional activity and expression of these cytochrome P450 enzymes 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 therapeutic resistance in some patients. This may be due to a genetically determined disorder of APs metabolism in the liver and other organs and systems. PGx is a method that allows to identify a group of patients with a high risk of developing AP-induced ADRs. Foreign panels GeneSight Psychotropic test (GeneSight) [14] and Genecept Assay (Genecept) [15] for PGx do not include many significant non-functional variants of the genes encoding enzymes of the CYP family. When using the GeneSight test, analysis is performed on 59 allelic variants of 8 genes - *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP3A4*, *CYP2B6*, *CYP2D6*, *HTR2A* and *SLC6A4*. 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 was developed in the USA. The study is carried out on allelic variants in 20 genes, including *5HT2C, MC4R, DRD2, COMT* and 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 five genes (*CYP1A2*, *CYP2C9*, *CYP2D6*, *CYP3A4*, *CYP3A5*) encoding enzymes that are centrally involved in APs metabolism demonstrates the importance of this new personalized approach to the choice of APs and its dosing in patients with a pharmacogenetic profile, a poor metabolizer.

Conclusion

Our experience of the PGx use based on the pharmacogenetic profile of cytochrome P450 enzymes metabolizing APs 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 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.

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