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ORIGINAL ARTICLE

Impact of the CYP2D6 phenotype on hyperprolactinemia development as an adverse event of treatment with atypical antipsychotic agents in pediatric patients

Raluca Grădinaru[1] · Nicoleta Andreescu[1] · Laura Nussbaum[2] · Liana Suciu[3] · Maria Puiu[1]

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Abstract

Background Treatment with atypical antipsychotics is today the election therapy for different types of psychosis, but there is a high incidence of endocrine and metabolic disturbances. One of the major enzymes involved in the metabolism of antipsychotics is CYP2D6. Depending on the existing CYP2D6 alleles, the metabolic capacity of the enzyme may vary from very low to very high, so that patients can be grouped into four phenotypic groups: slow, intermediate, extensive (normal), and fast metabolizers.

Aim of the study The aim of the study is to find a relationship between the individual intervariability of CYP2D6 and the incidence of hyperprolactinemia as side effect of atypical antipsychotics.

Results A total of 81 patients with schizophrenia or bipolar disorders, median age 15.74 ± 4 years, were enrolled in the study and prescribed the following atypical antipsychotics: risperidone, aripiprazole, and olanzapine. They were evaluated at 6, 12, and 18 months after the initiation of treatment. Using the TaqMan Genotyping Assay, it has been identified the presence of the CYP2D6*4 allele in 28 patients, representing 34.56% of the total of 81 patients in the study, and CYP2D6*3 allele was identified in 15 patients and the presence of CYP2D6*41 to 11 patients. The allele CYP2D6*5 has not been present to the study patients. The study group has 44 patients which are extensive metabolizers (54%), 34 intermediate metabolizers (42%), and 3 poor (slow) metabolizers (4%).

Conclusions For the slow and intermediate metabolizers, atypical antipsychotics determined a significant increase of prolactinemia with high risk of adverse events.

Keywords Atypical antipsychotic agents · CYP2D6 phenotype · Hyperprolactinemia

Introduction

Treatment with atypical antipsychotic agents is indicated for first delirious psychotic episodes and schizophrenia. Clinical trials have shown their superiority to classical antipsychotic agents [1].

The favorable pharmacotoxicologic profile attributed to atypical antipsychotic agents originally has been reduced due to an increased risk of endocrine and metabolic disturbances. Nevertheless, not all patients have an identical predisposition for suffering adverse events from taking atypical antipsychotic agents. An antipsychotic drug often elicits a different response in terms of efficacy and adverse events even after administration of identical doses. This interindividual variability of the therapeutic response is dependent upon several physiologic factors

Raluca Grădinaru and Nicoleta Andreescu contributed equally and thus share first authorship.

✉ Liana Suciu
suciu.liana@umft.ro

[1] Discipline of Medical Genetics, Department of Microscopic Morphology, Victor Babeș University of Medicine and Pharmacy Timișoara, Timișoara, Romania

[2] Discipline of Pedopsychiatry, Department of Neurosciences, Victor Babeș University of Medicine and Pharmacy Timișoara, Timișoara, Romania

[3] Discipline of Pharmacology-Clinical Pharmacy, Faculty of Pharmacy, Victor Babeș University of Medicine and Pharmacy Timișoara, Timișoara, Romania

Table 1 Reference values for prolactinemia (according to Synevo laboratories, Timisoara, Romania)

Age	Values (μUI/ml) Males	Values (μUI/ml) Females
1–30 days	< 1720	< 2010
1–12 months	< 610	< 640
1–3 years	< 280	< 360
4–6 years	< 360	< 280
7–9 years	< 250	< 280
10–12 years	< 280	< 210
13–15 years	< 360	< 300
16–18 years	< 320	< 380
> 18 years	98–456	127–637

(age, sex, body weight, pregnancy), pathology (disease severity, comorbidity), or factors related to dietary hygiene. Interindividual variability can be due to the ability of patients to metabolize and eliminate the antipsychotic drug, resulting in variations in the plasma concentration of the drug and its metabolites [2].

One of the major enzymes involved in the metabolism of antipsychotic agents is cytochrome P_{450}2D6 (CYP2D6), which is part of the cytochrome P_{450}-dependent oxidative microsomial system. The metabolic capacity of CYP2D6 differs according to its genetic polymorphisms. Depending on CYP2D6 alleles, the metabolic capacity of the enzyme may vary from very low to very high. Hence, patients can be grouped into four phenotypic groups based on their metabolism: "slow," "intermediate," "extensive," (normal) and "fast" [3–6].

Atypical antipsychotic agents produce metabolic and endocrine side effects, including hyperprolactinemia. Prolactin is a peptide hormone secreted by adenohypophyseal cells and it is stimulated by increased estrogen concentrations and dopamine inhibition. Dopamine from the tuberoinfundibular neurons in the hypothalamic arcuate nucleus acts directly on lactotropic pituitary cells through dopamine D_2 receptors, thereby blocking prolactin release. Antipsychotics block dopamine receptors, thus prolactin secretion increases. This results mostly in hypogonadism and infertility in males. The

Table 2 Distribution of patients depending on the allelic variant of CYP2D6

Allele variant	Number of patients
CYP 2D6*4	28
CYP 2D6*3	15
CYP 2D6*41	11
CYP 2D6*5	Not present in any patient

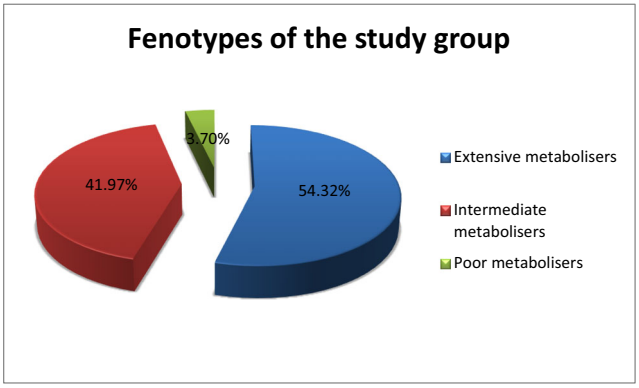

Fig. 1 Percentage of extensive, intermediate, and slow metabolizers in the study group. EM, extensive metabolizers; IM, intermediate metabolizers; PM, poor metabolizers

prevalence of gynecomastia is rather low. In children and adolescents, hyperprolactinemia can cause galactorrhea, amenorrhea, gynecomastia, growth retardation, and hypogonadism [7].

Most pharmacogenetic studies have not demonstrated a relationship between the CYP_{2D6} phenotype and the pharmacotoxicologic profile of antipsychotics.

The aims of the study were to measure the prolactin level in children and young adolescents after administration of antipsychotic agents, to determine the genetic polymorphisms of CYP2D6, and to ascertain the consequences of this polymorphism on plasma prolactin concentrations in patients treated with this kind of medication [8, 9].

Materials and methods

Ethical approval of the study protocol

The study protocol was approved by the Ethics Committee of Victor Babeş University of Medicine and Pharmacy Timişoara (Timişoara, Romania). For patients under 18 years of age, written informed consent was provided by parents or legal guardians.

Inclusion and exclusion criteria

Inclusion criteria were children and young adolescents examined and diagnosed by experienced psychiatrists using Diagnostic and Statistical Manual and Kiddie Schedule for Affective Disorders and Schizophrenia – Present and Lifetime Version; had a baseline positive and negative scale ≥ 70; had not used another type of antipsychotic medication previously and were indicated for treatment with risperidone, aripiprazole, or olanzapine; and whose cognitive capacity was unaltered.

Table 3 Variation of prolactin levels in the whole study group treated with atypical antipsychotic agents ($N = 81$)

Plasma level of prolactin (ng/mL)	Baseline	6 months	12 months	18 months	p*
(median ± SD) (min–max)	10.2 ± 6.63 (2.7–40)	14.1 ± 5.99 (5.8–39)	18.7 ± 10.73 (5.9–40)	20.3 ± 11.81 (6.3–47)	< 0.0001

p*, Freidman, non-parametric test

Exclusion criteria were children and young adolescents with mental retardation, a diagnosis or family history of diabetes mellitus, and indicated for treatment with classical antipsychotic agents.

Study design

This was a prospective, observational study. Eighty-one patients with schizophrenia or bipolar disorders aged 9–20 (median 15.74 ± 4.00) years were enrolled between 2009 and 2014 and monitored at the University Hospital for Child and Adolescent Psychiatry and Neurology (Timişoara, Romania). Among these patients, there were young adolescents, with more than 18 years old which were still studying in school at the study time. They were also included in the study. Atypical antipsychotic agents prescribed for the study group were risperidone (2–4 mg/day), aripiprazole (10–20 mg/day), and olanzapine (5–20 mg/day).

Patient evaluation

Patients underwent clinical evaluation 6, 12, and 18 months after treatment initiation. Blood samples for prolactin measurement were collected at baseline as well as 6, 12, and 18 months of treatment.

DNA analyses

Genomic DNA was extracted from blood samples using a QIAamp® DNA Mini Kit (Qiagen, Stanford, VA, USA). DNA samples were stored at − 80 °C.

CYP2D6 genotyping involved identification of CYP2D6*3, *4, *41 (rs 35742686, rs 3892097, rs 28371725) alleles encoding the synthesis of non-functional proteins or with low enzyme activity using real-time polymerase chain reaction (PCR) and TaqMan™ (Thermo Fisher Scientific, Waltham, MA, USA) detection. Specific kits for each single-nucleotide polymorphism in part of the TaqMan Drug Metabolism Genotyping Assay series were provided by Thermo Fisher Scientific (C__32407232_50, C__27102431_D0, and C__34816116_20, respectively). The real-time PCR of the target DNA fragments was done on a 7900HT Fast Real-Time PCR System according to manufacturer (Thermo Fisher Scientific) protocols.

The CYP2D6*5 polymorphism is associated with complete deletion of the gene, so these patients cannot synthesize the CYP2D6 protein. For detection of the CYP2D6*5 deletion, the PCR was done using specific primers to identify the deletion. Primers from Life Technologies (Carlsbad, CA, USA) were selected based on the Hersberger method [10]. The resulting products were analyzed directly using electrophoretic migration on 0.8% agarose gel, and DNA was visualized with ethidium bromide.

Fasting blood sample was taken from each patient. Serum was separated and preserved at – 20 °C and serum level of prolactin was measured by using chemiluminescent microparticle immunoassay (CMIA). Reference values are age and sex related, according to the next table (Table 1); conversion for the measurement units is as follows: $mUI/mL = mUI/L$; $mUI/L \times 0.0472 = ng/mL$; $ng/mL \times 21.2 = mUI/L$, according to the analysis laboratory Synevo, Timisoara, Romania.

Statistical analyses

Statistical analyses were undertaken using Excel™ 2003 (Microsoft, Redmond, WA, USA) and SPSS v17 (IBM, Armonk, NY, USA). Data are expressed as a mean ± standard deviation, the value of $p < 0.05$ was considered significant. For comparing the values of prolactin level at different time points, the Friedman non-parametric test (for paired values) was used, and for comparing the prolactin levels between groups of different metabolizers, the Mann-Whitney non-parametric test was used.

Table 4 Prolactin values at different times for slow metabolizers ($N = 3$)

Plasma level of prolactin (ng/mL)	Baseline	6 months	12 months	18 months	p*
(median ± SD) (min–max)	8.4 ± 6.2 (4–16)	14.2 ± 4.20 (11–19)	32.20 ± 13.25 (17–40)	34.8 ± 12.45 (20–42)	0.0263

p*, Freidman non-parametric test

Table 5 Prolactin values at different times for intermediate metabolizers ($N = 34$)

Plasma level of prolactin (ng/mL)	Baseline	6 months	12 months	18 months	p*
(median ± SD) (min–max)	7.7 ± 6.76 (3–39)	16.0 ± 5.72 (6–39)	24.9 ± 11.18 (6–40)	28 ± 11.71 (6–47)	< 0.0001

p*, Friedman non-parametric test

Results

Of 81 patients, 37 (46%) were female and 44 (54%) were male. For the study period, 44 patients used risperidone, two patients used aripiprazole, and one patient used olanzapine. A total of 34 patients (42%) had to change two or even three antipsychotic drugs due to adverse events or unsatisfactory control of disease depending on the view of the treating physician. Risperidone was replaced by olanzapine in six patients, olanzapine was replaced by aripiprazole in six patients, and risperidone was replaced by aripiprazole in 14 patients. For the remaining cases, various other combinations were used and, indeed, successive administration of these three antipsychotic agents was employed in some patients.

The TaqMan Genotyping Assay identified the CYP2D6*4 allele in 28 patients (35%). This type of CYP2D6 gene is a null allele which encodes a non-functional protein without metabolic enzymatic activity. The CYP2D6*3 allele was identified in 15 patients and CYP2D6*41 in 11 patients. The allele CYP2D6*5 was not present in any patient.

Upon receipt of these results, patients were classified into three groups based on phenotype (Table 2). Extensive metabolizers had only the normal wt allele. Intermediate metabolizers had a normal allele wt and allele *4 (heterozygote genotype wt/*4), a normal allele wt and allele *3 (genotype wt/*3), a normal allele wt and allele *5 (genotype wt/*5)—no patient had this genotype—and a normal allele wt and allele *41 (genotype wt/*41). In slow metabolizers, only one allele was identified: *4 (genotype *4/*4). Thus, 44 were extensive metabolizers (54%), 34 were intermediate metabolizers (42%), and 3 were slow metabolizers (4%) (Fig. 1).

Plasma levels of prolactin from the study are shown in Table 3. From baseline to 18 months, there was a significant increase in prolactin levels. In slow metabolizers, the use of atypical antipsychotic agents resulted in a significant increase in prolactin levels from baseline to 18 months ($p = 0.015$) (Table 4). In intermediate metabolizers, the use of atypical antipsychotic agents resulted in a significant increase in prolactin levels (Table 5). An increase in prolactin level values was also observed in extensive metabolizers (Table 6). A slight increase in prolactin levels was observed in extensive metabolizers ($p = 0.834$) (Fig. 2). The mean level of prolactin was higher for intermediate metabolizers than that for extensive metabolizers at each time point except baseline (Table 7). The mean concentration of prolactin for CYP2D6 metabolizers was lower than that for extensive metabolizers but, after 6 months of treatment with antipsychotic agents, intermediate metabolizers (carriers of just one functional allele) showed a significant increase in prolactin level over extensive metabolizers (carriers of two functional alleles).

Discussion

The present study demonstrated a diminishing of CYP2D6 metabolic capacity to be associated with a higher risk of hyperprolactinemia as an adverse effect of treatment with atypical antipsychotic agents.

Troost et al. generated results inconsistent with those presented in our study. They claimed that being an ultra-fast metabolizer was a risk factor for hyperprolactinemia. They analyzed the correlation between hyperprolactinemia in 25 children treated with risperidone and the CYP2D6 phenotype by identification of deletion of the non-functional alleles *3, *4, *6, *7, and 2D6*5 and *CYP2D6* amplification. After 8 weeks of treatment with risperidone, a positive correlation between prolactin levels, the number of CYP2D6 functional genes, and plasma concentration of 9-HO-risperidone was found [11]. In health people, the risperidone, 9-HO-risperidone ratio, is 1:3. However, in rapid metabolizers, due to the increased biotransformation of risperidone to the active metabolite 9-HO-risperidone, the plasma concentration of metabolites increases and the ratio of active substance metabolite decreases. The hyperprolactinemia observed in ultra-fast metabolizers is because 9-HO-risperidone, being less lipophilic, exerts more potent actions on the pituitary glands and causes increased release of prolactin.

Table 6 Prolactin levels at different times for extensive metabolizers ($N = 44$)

Plasma level of prolactin (ng/mL)	Baseline	6 months	12 months	18 months	p*
(median ± SD) (min–max)	12.2 ± 5.97 (6–35)	12.6 ± 5.96 (6–35)	13.0 ± 5.88 (7–36)	13.3 ± 5.90 (7–36)	0.834

p*, Friedman non-parametric test

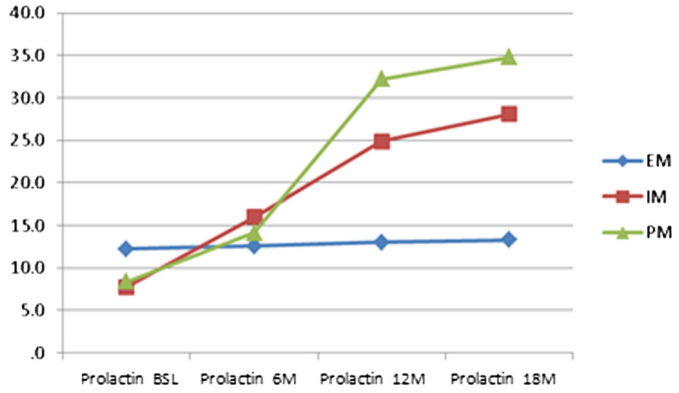

Fig. 2 Development of hyperprolactinemia for the three phenotypes identified in study patients at baseline, 6 months, 12 months, and 18 months

EM – extensive metabolizers; IM – intermediate metabolizers; PM – poor metabolizers

However, other studies and the company that markets 9-HO-risperidone (as paliperidone) claim that there are no differences between the two molecules in the degree of hyperprolactinemia induction [11]. In the study of Troost et al., only two patients were fast metabolizers, with prolactin levels of 78 ng/mL and 39 ng/mL. Another limitation of the study by Troost et al. was that four patients used methylphenidate (a dopaminergic drug that could theoretically lower prolactin levels) concomitantly. Hence, the prolactin level could have been underestimated; there was no indication of the CYP2D6 genotype presenting in these four patients [11].

In a study by Knegtering et al., the prolactin level in plasma was correlated with the plasma concentration of 9-HO-risperidone, but not with risperidone. This finding suggested that slow metabolizers with a lower concentration of the active metabolite carried a lower risk of hyperprolactinemia induction compared with extensive or ultra-fast metabolizers [12].

In 2016, Sukasem and colleagues failed to establish an association between CYP2D6 and the prolactin concentration in children and adolescents given risperidone for the treatment of autism spectrum disorder. One hundred forty-seven patients were genotyped for CYP2D6*4, *10, and *41 and gene deletion of *5. No significant correlation between prolactin levels and genotype, or significant differences in the response to prolactin, was found in intermediate or extensive metabolizers of CYP2D6 [13].

Most studies have observed that CYP2D6 polymorphisms do not influence the plasma level of risperidone or 9-HO-risperidone. A possible explanation for the increase in hyperprolactinemia risk in slow and intermediate metabolizers could be the different affinity to dopamine D2 receptors and dissociation rate of risperidone and 9-HO-risperidone.

Also, a correlation between prolactin levels and CYP2D6 polymorphism, or the concentration of aripiprazole or dehydroaripiprazole, or the sum of the two, was established after aripiprazole administration in a study by Hendset and colleagues. In patients using aripiprazole, correlations were found between prolactin levels and intermediate metabolizers of CYP2D6. CYP2D6-defective alleles caused increased plasma concentrations of aripiprazole and dehydroaripiprazole and thus, more potent adverse effects [14].

Olanzapine is metabolized by CYP2D6 to a metabolite without a pharmacodynamic action: 2-hydroxymethylolanzapine [15]. Hence, it can be assumed that a slow or intermediate metabolizer of CYP2D6 would have an increased plasma concentration of olanzapine. However, the effect would be minimal because olanzapine uses the CYP2D6 system as a secondary metabolic pathway. Another aspect that could have influenced our results is CYP1A2 polymorphism, which is a major metabolic pathway for olanzapine or CYP3A4.

The present study had an observational approach based primarily on ethical considerations. The choice of medication

Table 7 Comparison of prolactin levels for intermediate ($N = 34$) and extensive ($N = 44$) metabolizers

Time	Genotype	Prolactin level (ng/mL)	p*	Alfa significance level
Baseline	EM	12.2 (5.97 ± 0.90)	< 0.001	0.001
	IM	7.7 (6.76 ± 1.16)		
6 months	EM	12.6 (5.96 ± 0.90)	< 0.001	0.001
	IM	16.0 (5.72 ± 0.98)		
12 months	EM	13.0 (5.88 ± 0.89)	< 0.001	0.001
	IM	24.9 (11.18 ± 1.92)		
18 months	EM	13.3 (5.90 ± 0.89)	< 0.001	0.001
	IM	28.0 (11.71 ± 2.01)		

EM, extensive metabolizer; *IM*, intermediate metabolizer; p*, Mann-Whitney non-parametric test

was strictly at the discretion of the treating physician, so that each patient had the most appropriate treatment. Hence, in some patients, it was necessary to change the atypical antipsychotic agent to obtain better control of symptoms or because of adverse reactions. Hence, a limitation of our study was that we did not focus on a single atypical antipsychotic agent because of the medical needs of the patient.

It was the first study of this type in a population from Romania. Hence, the frequency of polymorphisms identified, the results obtained, and the conclusions formulated cannot be compared with other studies undertaken in Romania. The limited number of studies in the literature and their contradictory results necessitate further research to help identify the benefit that CYP2D6 phenotyping brings to "personalizing" antipsychotic treatment in order to achieve maximum efficacy with minimal adverse events. Such an approach could increase the quality of life of children and adolescents with schizophrenia and bipolar disorder.

Conclusions

We found that CYP2D6 polymorphisms could predict predisposition to hyperprolactinemia following treatment with atypical antipsychotic agents. Thus, a patient found to be a slow or intermediate metabolizer of CYP2D6 could benefit from taking an antipsychotic agent that does not use this enzymatic pathway for its metabolism.

The interindividual variability of the therapeutic response to antipsychotic medications in the general population and pediatric patients is evident. Hence, investigation of the cause of this variability is an important unmet need.

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Compliance with ethical standards The study protocol was approved by the Ethics Committee of Victor Babeș University of Medicine and Pharmacy Timișoara (Timișoara, Romania). For patients under 18 years of age, written informed consent was provided by parents or legal guardians.

Conflict of interest The authors declare that they have no conflict of interest.

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