Molecular study of weight gain related to atypical antipsychotics: clinical implications of the CYP2D6 genotype

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Abstract
Atypical antipsychotics, especially some of them, influence cellular lipogenesis, being associated with metabolic side effects including weight gain. Due to the increasing use of atypical antipsychotics in children and adolescents, their metabolic and endocrine adverse effects are of particular concern especially within this pediatric population that appears to be at greater risk. Genetic factors with a possible influence on atypical antipsychotics adverse effects include CYP2D6 polymorphisms. Our study, performed in 2009–2014, with a two-year enrolment period during which we recruited children and adolescents with a diagnosis of schizophrenia or bipolar disorder on treatment with the antipsychotics (Risperidone, Aripiprazole or Olanzapine), included 81 patients, aged between 9 and 20 years, median age being 15.74 years. The gender percentage was 54% girls/46% boys. The CYP2D6 genotyping was performed after enrolment of the last patient. Based on the CYP2D6 genotype, three activity groups were identified and compared and we found that the patients with w*4 genotype, intermediary metabolizer (carrier of one functional and one non-functional allele) have significantly higher weight gain values than the patients who did not exhibit allele *4. The CYP2D6 genotype in children and adolescents with schizophrenia and bipolar disorder, proved to be a good predictor for the response to atypical antipsychotics and the side effects registered. The significant correlations between the CYP2D6 polymorphisms and the weight gain/BMI (body mass index) increase, as major side effects induced by antipsychotics proved the fact that the pharmacogenetic screening is needed in the future clinical practice, allowing for individualized, tailored treatment, especially for at-risk individuals.

Keywords: atypical antipsychotics, CYP2D6 genotype, child schizophrenia, pharmacogenetics, weight gain.

Introduction
Atypical antipsychotics are medication of first choice in schizophrenia, being used in the treatment of bipolar disorders, as well. Although, the atypical antipsychotics offered, since their discovery, new perspectives and many advantages over conventional neuroleptics (greater impact on negative and neuro-cognitive symptoms and better safety profile) this does not mean that they do not display considerable side effects, which can result in discontinuation of treatment [1].

Atypical antipsychotic drugs, in particular some of them, influence cellular lipogenesis and are associated with metabolic side effects including weight gain, body mass index (BMI)/blood Insulin level increase [2–5]. Antipsychotic-induced weight gain has important effects on treatment compliance and long-term health. The substantial weight gain being one of the most undesirable side effects of the atypical antipsychotics, poses the patients to other significant risks, diabetes, metabolic syndrome-lipid abnormalities and cardiovascular events [6–10].

Due to the increasing use of atypical antipsychotics in children and adolescents, their metabolic and endocrine adverse effects (weight gain, obesity, metabolic syndrome, increased levels of circulating Insulin) are of particular concern especially within this pediatric population that appears to be at greater risk [11–13].

In this frame, the pharmacogenetics of antipsychotic-induced weight gain represents a promising perspective in tailoring the individualized treatment to the needs of the patient and avoiding the significant side effects [14–19]. However, there is considerable patient-to-patient variability in therapeutic dose requirements of atypical antipsychotics and the propensity for side effects. It is needed to be noted that not all patients develop those side effects in the same extent. The inter-individual variability in the propensity of side effects can be caused by several factors such as gender, age, renal and hepatic function and genetics [20]. It is well known today, with the development of pharmacogenetics, that genetic variability can affect both pharmacokinetic and pharmacodynamic drug properties. Because the drug safety issues are becoming more important, studying the underlying genetic mechanisms of adverse drug reactions can bring important benefits to patients by helping in the process
of choosing the best drug with the least expected side effects. The pharmacogenomics enables the investigation of factors distal to drug exposure in the plasma compartment, thereby providing a more complete view of the sources of variability in psychotropic drug response. Pharmacogenetic studies have shown a significant correlation between genotype and adverse effects associated with antipsychotics, justifying once again the importance of assessing Single Nucleotide Polymorphisms (SNPs) in patients treated with antipsychotics [21–24].

The cytochrome P450 (CYP450) genotype testing is recommended in order to pinpoint therapeutic dose based on genotype in patients for whom antipsychotic therapy is necessary [25–27]. Cytochrome P450 2D6 (CYP2D6) genotypes allow the classification of the patients based on their metabolizer phenotype. Four categories were described: poor metabolizer (PM), intermediate (IM), extensive (EM) and ultra-rapid metabolizer (UM). Pharmacokinetic studies have hypothesized that poor CYP450 activity could be linked to increased serum levels of antipsychotics that may lead to increased weight gain [15, 28, 29]. Genetic factors with a possible influence on antipsychotics adverse effects include CYP2D6 polymorphisms, CYP2D6 being an isoenzyme with important role in hepatic metabolism of different antipsychotic drugs [30, 31].

In our study, an original theme of clinical interest is approached through the genetic information linked to the clinical issues related to the patient’s side effects – atypical antipsychotics-induced weight gain. We aimed to underline the role of individual genetic variation, in correlation with the CYP2D6 genotype, for weight gain in patients treated with atypical antipsychotics. We also targeted the evaluation of the effectiveness of genetic markers-CYP2D6 polymorphisms for the clinical practice.

Patients and Methods

Our study was performed in 2009–2014 and the first scheduled step consisted in a two-year enrolment period during which we recruited children and adolescents with a diagnosis of schizophrenia or bipolar disorder, being patients of the University Hospital for Child and Adolescent Psychiatry and Neurology, Timișoara, Romania.

The inclusion criteria in this study were: inpatients or outpatients with a diagnosis of schizophrenia or bipolar disorder, according to DSM IV and confirmed by a child and adolescent psychiatrist through K-SADS-PL; the age <18 years or less than 21 years if the patients were still studying in school; patients being on treatment with the following atypical antipsychotics: Risperidone, Aripiprazole or Olanzapine; children from mono- or bi-parental families, being raised in the family.

For the selection of the cases, we took also as inclusion criteria, the accessibility of children and their families in order to apply our instruments and also the procedures. The patients under treatment with other antipsychotics and the parents/patients who refused the participation in this study and to sign the informed consent, were excluded.

We obtained for each patient less than 18 years, the informed consent from the parents/legal guardians and the assent from the child and for the patients over 18 years we obtained the informed consent signed by them. Our study is in accordance with the Ethical Committee regulations of the “Victor Babeș” University of Medicine and Pharmacy, Timișoara and with the GCP (Good Clinical Practice) regulations.

Our study group included 81 patients, aged between 9 and 20 years, median age being 15.74 years. The gender percentage in the sample was 54% girls/46% boys.

Clinical evaluation of the patients

The patients in the study group had a diagnosis of schizophrenia or bipolar disorder, reconfirmed through the K-SADS-PL at baseline. Also, the PANSS (Positive and Negative Syndrome Scale) was applied by an authorized rater, in order to offer an objective measure for the psychiatric symptoms, the positive, negative and general symptoms.

Because the children were still developing at the time of exposure to the antipsychotics, in the context of physiological changes in hormonal and endocrine level and body composition, all the values were adjusted for gender, age and growth charts.

All the patients were receiving one of the chosen atypical antipsychotics: Risperidone, Aripiprazole or Olanzapine. It must be mentioned that in their psychiatric history, some of the patients have changed their treatment through switching between those antipsychotics, sometimes because of lack of efficacy or because of adverse events. Also, the majority of the patients had a significant genetic loading, being offspring of psychotic parents (with schizophrenia or bipolar disorder).

The compulsory evaluations included: the phase evaluation of the clinical, neurobiological markers and reevaluations applying the standardized procedures. We measured for each patient their body weight, their height and calculated their BMI for different timepoints – at baseline, three months, six months, one year and 18 months.

Blood was also withdrawn, in order to dose their Insulin levels at different time points. We evaluated the BMI and the blood Insulin variations for these patients in different time points during the treatment with atypical antipsychotics.

CYP2D6 genotyping

Genomic DNA was extracted from EDTA blood using QIAamp DNA Mini Kit (Qiagen, Germany). DNA samples were stored at -80°C. The CYP2D6 genotyping was performed after enrollment of the last patient, and the laboratory staff was blinded to the patients’ data.

CYP2D6*4 allele identification was performed by using TaqMan Drug Metabolism Genotyping Assay for Allelic Discrimination CYP2D6*4 and TaqMan® PCR Master Mix (Applied Biosystems) according to the protocol provided by the producer.

Allelic discrimination was carried out on Applied Biosystems 7900HT Fast Real-Time PCR System in a reaction volume of 25 μL, containing TaqMan® Drug Metabolism Genotyping Assay for Allelic Discrimination CYP2D6*4 and TaqMan® PCR Master Mix and DNA probe.

CYP2D6 genotyping was done in the presence of
two controls, AL-1 (wild type) and AL-2 (CYP2D6*4). The AL1 probe corresponding to the wild type was VIC dye-labeled while the AL2 probe corresponding to the mutant type (*4) was FAM dye-labeled. Genotypes were determined by measuring allele-specific fluorescence using the software for allelic discrimination (Applied Biosystems).

Based on the CYP2D6 genotype, three activity groups were identified and compared: group 1 included subjects that were not carrying the functional allele (Figure 1), group 2 included subjects carrying one functional (wt) and one non-functional allele (*4) (Figure 2) and group 3 subjects carrying the wild type allele and had missing the non-functional allele (*4) (Figure 3).

Figure 1 – Amplification curve indicating the presence of allele 2 (*4).

Figure 2 – Amplification curve indicating the presence of both alleles (wt/*4).
Figure 3 – Amplification curve indicating the presence of wild type allele (wt).

Statistical analysis

In order to correlate the CYP2D6 genotype with the weight gain and Insulin level at different time points we used the Friedman non-parametric test for pair values. For comparing the weight gain and Insulin variation/increase between groups, we applied the non-parametric test Mann–Whitney. For comparing the median of BMI and the Insulin level at two different timepoints, the non-parametric test Wilcoxon signed-rank was used. All analyses were carried out using SPSS software (version 17.0, Chicago, IL, USA) and Microsoft Excel.

Results

The descriptive statistics is summarized in the Table 1. In order to compare the BMI values for the five time points, for the sample of patients with schizophrenia and bipolar disorder, we applied the Friedman non-parametric test for pair values and we obtained statistically significant values ($p<0.001$). Comparing the timepoints each two with two, using the Wilcoxon signed-rank non-parametric test, we obtained in each of the 10 comparisons statistically significant differences ($p<0.001$). So that, the BMI increase from the start moment until 18 months is significant, with a significance threshold $\alpha=0.00$.

We also took into account another very important value, the blood Insulin, which is in direct correlation with the metabolic, hormonal state and the weight/BMI variations of psychotic patients under treatment with atypical antipsychotics.

Insulin values for four different timepoints in the whole sample of patients were compared. We obtained for Insulin statistically significant differences between the timepoints ($p<0.001$), the values of Insulin being globally much more increased during the treatment with the atypical antipsychotics. So that the Insulin values increased from baseline to 18 months, with a significance threshold $\alpha=0.001$.

Table 1 – Comparisons between the different timepoints for the sample of patients with schizophrenia and bipolar disorder: descriptive statistics of study group

<table>
<thead>
<tr>
<th>Timepoint</th>
<th>No. of cases</th>
<th>BMI [kg/m$^2$]</th>
<th>Insulin values [μU/mL]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. dev.</td>
<td>Min.</td>
</tr>
<tr>
<td>BASELINE</td>
<td>81</td>
<td>20.82</td>
<td>2.57</td>
</tr>
<tr>
<td>3 months</td>
<td>81</td>
<td>21.47</td>
<td>2.60</td>
</tr>
<tr>
<td>6 months</td>
<td>81</td>
<td>23.33</td>
<td>2.69</td>
</tr>
<tr>
<td>1 year</td>
<td>81</td>
<td>24.53</td>
<td>3.33</td>
</tr>
<tr>
<td>18 months</td>
<td>81</td>
<td>25.18</td>
<td>4.11</td>
</tr>
</tbody>
</table>

BMI – Body Mass Index; Std. dev. – Standard deviation; Min. – Minimum; Max. – Maximum.

Group 1

For the comparison of the values between different timepoints for each group, we applied the Friedman non-parametric test for pair values. For the patients from group 1, the BMI values increased significant since the baseline, even if the sample with this genotype was not so numerous ($p=0.017$, $\alpha=0.05$). For Insulin values, the differences between the timepoints were also statistically
18 months is statistically significant. We obtained the expressed through weight gain and BMI increase.

Group 2

Friedman non-parametric test for pair values used to compare the BMI values between the five timepoints for the patients from group 2 indicated statistically significant differences ($p<0.001$, $\alpha=0.001$) (Table 3).

For the patients from group 2, the increase of the Insulin values from baseline until 18 months is statistically significant ($\alpha=0.001$), meaning that the patients with this genotype were most prone and exposed to adverse effects of the atypical antipsychotics – increased Insulin values or even hyperinsulinism, with high morbidity consequences (Table 3).

The increase of the BMI values from baseline until 18 months is statistically significant, with a threshold of significance $\alpha=0.001$. The BMI increase was much higher than for the group 3, meaning that the patients from group 2 were much prone and exposed to adverse effects, expressed through weight gain and BMI increase.

Group 3

Concerning the patients from group 3, the comparisons between the different timepoints are summarized in Table 4.

The increase of the BMI values from baseline until 18 months is statistically significant. We obtained the threshold of significance $\alpha=0.001$ for the comparisons baseline, three months, six months, one year, of $\alpha=0.01$ for the comparison six months–one year and $\alpha=0.05$ for the comparison six months–18 months. The BMI increase was much lower than for patients from group 2, meaning that the patients from group 3 were not so prone and exposed to adverse effects, expressed through weight gain. The patients in the group 3 registered for the period one year and 18 months, no significant BMI increase.

For the patients from group 3, the increase of the Insulin values from baseline until 18 months is not statistically significant, proving the fact that the patients with this genotype are not so exposed to the side effects of the atypical antipsychotics (Table 4).

We compared the differences between group 2 and group 3 as regard the evolution of the BMI and Insulin level, the results being summarized in Table 5.

It is important to note that at moment of treatment initiation, there were no statistically significant differences between the BMI of patients from groups 2 and 3. We found that the differences of BMI are statistically significant after six months of administration of atypical antipsychotics ($p<0.001$). It was observed that patients from group 2 present higher BMI as compared with patients from group 3. For Insulin values, statistically significant differences were found for each timepoint.

Table 2 – Comparisons between the different timepoints for the group 1

<table>
<thead>
<tr>
<th>Timepoint</th>
<th>No. of cases</th>
<th>BMI [kg/m²]</th>
<th>Insulin values [μU/mL]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. dev.</td>
<td>Min.</td>
</tr>
<tr>
<td>BASELINE</td>
<td>3</td>
<td>19.47</td>
<td>1.50</td>
</tr>
<tr>
<td>3 months</td>
<td>3</td>
<td>19.73</td>
<td>1.53</td>
</tr>
<tr>
<td>6 months</td>
<td>3</td>
<td>23.83</td>
<td>0.15</td>
</tr>
<tr>
<td>1 year</td>
<td>3</td>
<td>28.50</td>
<td>1.04</td>
</tr>
<tr>
<td>18 months</td>
<td>3</td>
<td>29.33</td>
<td>1.27</td>
</tr>
</tbody>
</table>

BMI – Body Mass Index; Std. dev. – Standard deviation; Min. – Minimum; Max. – Maximum.

Table 3 – Comparisons between the different timepoints for the patients from group 2

<table>
<thead>
<tr>
<th>Timepoint</th>
<th>No. of cases</th>
<th>BMI [kg/m²]</th>
<th>Insulin values [μU/mL]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. dev.</td>
<td>Min.</td>
</tr>
<tr>
<td>BASELINE</td>
<td>25</td>
<td>21.18</td>
<td>2.92</td>
</tr>
<tr>
<td>3 months</td>
<td>25</td>
<td>22.21</td>
<td>2.78</td>
</tr>
<tr>
<td>6 months</td>
<td>25</td>
<td>25.05</td>
<td>1.99</td>
</tr>
<tr>
<td>1 year</td>
<td>25</td>
<td>27.53</td>
<td>1.93</td>
</tr>
<tr>
<td>18 months</td>
<td>25</td>
<td>29.30</td>
<td>2.14</td>
</tr>
</tbody>
</table>

BMI – Body Mass Index; Std. dev. – Standard deviation; Min. – Minimum; Max. – Maximum.

Table 4 – Comparisons between the different timepoints for patients from group 3

<table>
<thead>
<tr>
<th>Timepoint</th>
<th>No. of cases</th>
<th>BMI [kg/m²]</th>
<th>Insulin values [μU/mL]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. dev.</td>
<td>Min.</td>
</tr>
<tr>
<td>BASELINE</td>
<td>53</td>
<td>20.73</td>
<td>2.44</td>
</tr>
<tr>
<td>3 months</td>
<td>53</td>
<td>21.23</td>
<td>2.51</td>
</tr>
<tr>
<td>6 months</td>
<td>53</td>
<td>22.49</td>
<td>2.67</td>
</tr>
<tr>
<td>1 year</td>
<td>53</td>
<td>22.89</td>
<td>2.71</td>
</tr>
<tr>
<td>18 months</td>
<td>53</td>
<td>23.00</td>
<td>3.13</td>
</tr>
</tbody>
</table>

BMI – Body Mass Index; Std. dev. – Standard deviation; Min. – Minimum; Max. – Maximum.
In the actual general context, the approach of pharmacogenomic variability in pertinent drug targets—children and adolescents exposed to atypical antipsychotics, proved to be a fruitful pathway especially because of the lack of consistent research for these age groups. The pharmacokinetic of atypical antipsychotics and their impact on the adverse effects, in correlation with the CYP2D6 genotype, have been approached in some studies in adults, but there is a lack of research involving children and adolescents, although this medication is extensively used in this population [18, 23, 26].

The pharmacokinetics of atypical antipsychotics (Risperidone, Aripiprazole, Olanzapine) is not affected only by age, but its half-life varies depending on the activity of the CYP2D6 enzyme [32, 33]. There is lack of data regarding the impact of CYP2D6 isoenzyme polymorphisms on the short and long-term adverse effects, including the weight gain in the case of the child and adolescent patients using atypical antipsychotic drugs [1, 34, 35].

Nowadays, in accordance with the pediatric regulations of EMA (European Medicines Agency) and the development of the PIP (Pediatric Investigational Plan), the drug safety must be investigated in the case of psychotic patients being children and adolescents. Our study is especially valuable in the light of personalized pharmacotherapy, tailored to the genetic variability, correlated with the CYP2D6 genotype of the patient [36, 37].

The implementation of the CYP2D6 genotype testing to this category of patients before choosing the suitable antipsychotics, in the clinical practice could avoid the severe side effects, like morbid weight gain or the non-response to medication, being a valuable perspective for the future [15, 26, 27, 38, 39]. This could be a way of improving the quality of life of children and adolescents with a diagnosis of schizophrenia or bipolar disorder.

It was reported that the serum concentration of Risperidone, Aripiprazole and Olanzapine varies considerably between patients with different CYP2D6 genotypes. There is a substantial phenotypical difference between patients carrying one functional allele (wt) and one nonfunctional allele (*3, *4, *5) and the patients exhibiting two CYP2D6 variant alleles (two reduced-function or one reduced-function in combination with a nonfunctional variant allele).

In our study, we have found that the patients with the genotype wt/*4, intermediate metabolizer (carrier of one functional and one non-functional allele) have significantly higher weight gain values than the patients included in the group 3 (patients without *4 allele). Our study proves to be a successful pathway towards assessing the genetic liability and its plausible clinical application for children and adolescents [40].

It is obvious that the delay in finding an adequate treatment for psychosis, especially in the case of children, has a detrimental effect on their development, on prognosis and the chances of recovery. The genetic determination of the patient’s status brings many benefits by helping in choosing a suitable antipsychotic, in adjusting the therapeutic doses and reducing the adverse effects [41, 42].

Although the CYP2D6 has a minor pathway for Olanzapine, in our research it proved to be a reliable predictor for the correlation between Olanzapine and weight gain in psychotic patients. We also noted in our study that the weight gain, independently of the atypical antipsychotic used (Risperidone, Olanzapine, Aripiprazole) was highest correlated with the wt/*4 genotype. In comparison with the results obtained in other researches in the last years, which showed that after a longer period on atypical antipsychotics (six months–one year), the patients reach a weight gain plateau, in our study the patients with the wt/*4 genotype proved to have a progressive weight gain even after 18 months [1].

This could be because our study is on children and adolescents, who are highly metabolically and hormonally sensitive during the period of development. This proves that in the case of children with schizophrenia and bipolar disorder, even more attention and care should be paid to this category, when choosing the antipsychotic [41]. In the same time, from the clinical point of view, we must pay attention to the fact that, in some cases, the results could be false negative because of the weight gain plateau.

It is important to note the fact that excessive weight gain in children and adolescents brings other deleterious effects like more stigmatization, further social withdrawal non-compliance with medication, but also the high-risk state for cardiovascular morbidity and mortality. The attention is now shifting towards a focus on individuali-
zation of pharmacotherapy and elucidation of the mechanistic basis of interindividual variability in drug response with use of pharmacokinetic and pharmacodynamic biomarkers [42].

In this context, the pharmacogenetics provides a valuable tool to fulfill the promise of personalized interventions by adopting the most indicated treatment based on the genetic markers of the patients. It is obvious that it would be much easier and cost-effective to prevent the weight gain and other major side effects through choosing the suitable atypical antipsychotic treatment from the beginning, than changing and switching the antipsychotics because of major adverse events or non-compliance. This, also, would be much more ethical [23, 43–45].

Our study suggests that the pharmacogenetic testing is a feasible, reliable prediction tool of the antipsychotic-induced weight gain. Structured procedures should be used in predicting the probability of the psychotic patient to gain weight under atypical antipsychotics in correlation with their genotype, considering the genetic polymorphisms. So that, the pharmacogenetics holds the promise of predicting treatment emergent side effects and for the future of personalized antipsychotic treatment and we are one-step closer to a routine clinical utilization of pharmacogenetic testing in children and adolescents.

Conclusions

In our study, the CYP2D6 genotype in children and adolescents with schizophrenia and bipolar disorder, proved to be a good predictor for the response to atypical antipsychotics, for the metabolic ratio and the side effects registered. The significant correlations between the CYP2D6 polymorphisms and the weight gain/BMI and or blood Insulin increase, as major side effects induced by antipsychotics proved the fact that the pharmacogenetic screening is needed in the future clinical practice, allowing for individualized, tailored treatment, especially for at-risk individuals. A priori identification, of high-risk subjects, who are prone and exposed to develop major adverse effects, could lead to alternative treatment strategies in this population. In the case of children and adolescents, being in development and very exposed, it is ethical and cost-effective to prevent the adverse effects through choosing the suitable atypical antipsychotic treatment from the beginning than permanently switching antipsychotics to manage the consequences. More research is required in order to develop a genetically informed, personalized medicine, although some promising steps towards assessing genetic liability and its fruitful clinical applications have been already made.

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