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**The role of CYP2D6 and ABCB1 pharmacogenetics in drug-naïve patients
with first episode of schizophrenia treated with risperidone**

Authors:

Nikolina Jovanović[1], Nada Božina[2], Mila Lovrić[2], Vesna Medved[1], Miro Jakovljević[1], Alma Mihaljević Peleš[1]

[1] *Department of Psychiatry, School of Medicine and University Hospital Center Zagreb Kispaticeva 12, Croatia*

[2] *Clinical Institute of Laboratory Diagnosis, University Hospital Center Zagreb, Kispaticeva 12, Croatia*

Correspondence:

Nikolina Jovanovic, M.D.

University of Zagreb School of Medicine

University Hospital Center

Department of Psychiatry

Kispaticeva 12, 10000 Zagreb, Croatia

Fax: +385 1 24 21 843

E-mail: nikolina.jovanovic@gmail.com

Abstract

Purpose: To evaluate the role of cytochrome 450 2D6 (CYP2D6) and ABCB1 variants on plasma risperidone concentrations and treatment response in 83 drug naive patients with first episode of psychosis.

Methods: All patients were treated with risperidone for 8 weeks. The CYP2D6 genotyping was performed by allele-specific PCR-RFLP (for *3,*4,*6) and long PCR (for duplications and *5), while real-time PCR method was used for ABCB1 G2677T/A and C3435T. Plasma concentrations of risperidone and 9-OH risperidone were measured by high-preformance liquid chromatography.

Results: The number of patients with the CYP2D6 wild type (wt/wt), wt/mutation (mut) and mut/mut genotype was 43, 32 and 8, respectively. The number of patients with the ABCB1 2677G/G , G/T, T/T variants was 29, 42 and 12, respectively; those with the 3435C/C, C/T and T/T was 25, 37 and 21, respectively. The CYP2D6 genotype has strong effect on steady-state dose-corrected plasma levels of risperidone, its 9-OH metabolite and active moiety, while ABCB1 2677T/T and 3435T/T genotypes hold similarly strong effects on active moiety C/D. The CYP2D6 poor metabolizers had significantly higher levels of risperidone C/D and active moiety C/D, and lower levels of 9-OH risperidone C/D. The ABCB1 3435T allele and the ABCB1 2667T-3435T haplotype carriers were more frequent among subjects without extrapyramidal syndrome. Patients showed significant improvements in positive and general symptoms, but not in negative symptoms. These changes were not related to variations in genetic and drug concentration data.

Conclusion: Our findings suggest that CYP2D6 and ABCB1 G2677T and C*3435*T might be useful determinants of risperidone plasma concentrations, but the clinical implications of these associations in relation to treatment response and side-effects remains unclear.

Key words: risperidone, 9-hydroxirisperidone, CYP2D6, ABCB1, schizophrenia

Introduction

Everyday clinical experience suggests that patients with schizophrenia significantly vary in their response to antipsychotic treatment. The patients with first episode of psychosis fall into the same category. However, without confounding effects of illness chronicity and previous antipsychotic treatments, their variability in treatment response could be significantly related to drug metabolic polymorphisms [1].

Risperidone is a widely used antipsychotic drug with potent antagonistic properties for dopamine D2 and serotonin 5-HT2 receptors. Its active metabolite 9-hydroxyrisperidone (9-OH-risperidone) is approximately equipotent with the parent drug in dopamine receptor affinity. Therefore, the entire active moiety (risperidone + 9-OH-risperidone) is regarded to contribute to the full antipsychotic effect [2]. According to *in vivo* and *in vitro* studies, cytochrome CYP2D6 is responsible for the metabolism of risperidone [3, 4]. Several mutated alleles of CYP2D6 gene might cause its absent (e.g. CYP2D6*3, CYP2D6*4, CYP2D6*5), or even increased activity (CYP2D6*2xN) [5]. Risperidone has strong affinity for P-glycoprotein (P-gp), a transporter that regulates drug bioavailability by controlling intestinal drug absorption, renal excretion and transport across the blood-brain barrier [6]. P-gp is a member of the adenosine triphosphate-binding cassette (ABC) superfamily and the ABCB1 gene encodes its expression [7]. Both single-nucleotide polymorphisms, exon 21 (G2677T/A), missense polymorphism within exon 21(rs2032582) and silent mutation C3435T in exon 26 (rs1045642) are associated with a lower level of intestinal ABCB1 expression [8].

Since certain cytochrome enzyme CYP2D6 and ABCB1 variants have different functional capacity, genotyping may be a clinically useful tool for antipsychotic therapy individualization, mainly dose optimization and prevention of the drug's adverse effects. It has been shown that CYP2D6 genotypes have significant effect on steady-state plasma levels of risperidone and 9-OH risperidone [9], but ABCB1 variants' effect vary from moderate [10] to completely absent [11]. However, this is the first study to search for these effects in drug naïve patients with first episode of psychosis. Our aim was to evaluate the role of CYP2D6 and ABCB1 variants in therapeutic efficacy and safety after eight weeks of treatment with orally administered risperidone.

Methods

Subjects

The study included 83 subsequently hospitalized patients who met the following criteria: (a) an ICD-10 diagnosis of first episode of schizophrenia spectrum disorder [12]; (b) drug naïve status in terms of using antipsychotics; (c) initiation of risperidone treatment based on the standard clinical practice. All were treated with orally administered risperidone and followed-up for eight weeks. No other psychotropic medication was prescribed, except anticholinergics (biperiden) for the alleviation of extrapyramidal symptoms (in 44 patients, mean dose 2.74 mg ± 1.6) and benzodiazepines (diazepam) as hypnotics (in 37 patients, mean dose 7.21mg ± 3.09). The sample was predominantly female (N=66, 79.5%) and average age was 30.3± 8.1. Majority were smokers (N=45, 54.2%), single (N=45, 54.2%) and with high school education (N=63, 75.9%). Approximately

one third had a case of psychosis in their family (N=25, 30.1%). Mean body weight was 74.26 kg ± 11.49. The clinical and demographic data for these patients are given in **Table 1**.

All participants were of Croatian origin. Out of 101 patients initially invited to participate in the study, six refused to be included and three were excluded from the final analyses due to incomplete follow-up data. In seven cases patients' condition got significantly worse several days before the study end-point and clinicians were forced to increase the drug's dose (n=4) or switch to another antipsychotic (olanzapine, n=2 and fluphenazine, n=1), thus disabling the measurement of the drug's concentration at the end-point. In order to stick to the original study design as much as possible, it was our decision to exclude these patients from the further analysis. Two additional subjects were excluded because their risperidone and 9-OH risperidone's concentrations were lower than limit of detection. Even though all participants were inpatients and drugs were given under the supervision of hospital nurses, it might be possible that they were not using the drug at all. The study was performed at the University Hospital Centre Zagreb, Department of Psychiatry, from 2006. - 2009. as part of the project "Effects of pharmacogentic variants on pathogenesis of schizophrenia and treatment response". The study protocol was approved by the institutional ethical committee and all participants gave written informed consents.

Insert Table 1 about here

Clinical assessment

Baseline clinical assessment consisted of structured psychiatric interview, collection of sociodemographic data, severity of psychopathology (using the Positive and Negative Syndrome Scale, PANSS) [13], tobacco use, physical examination and body weight measurements. PANSS was rated by two trained raters (V.M. and N.J.) and inter-rater reliability was 0.76. Both raters were blind to the genotype- and plasma concentration data. The Simpson-Angus scale was used for the evaluation of extrapyramidal symptoms and patients with score ≥ 3 were classified as the EPS+ group [14]. Patients were also assessed for other side-effects such as amenorrhea, galactorrhea and gynecomastia.

Genotyping

Patients were genotyped for CYP2D6 by means of allele-specific polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) for CYP2D6*3,*4,*6 alleles, and by long PCR for CYP2D6* duplication and CYP2D6 *5 alleles in Thermocycler 9700, Applied Biosystems [15]. For ABCB1 2677G>T/A, and 3435C>T Real-time PCR method was applied in LightCycler (Roche), with Fast Start DNA Master plus HybProbe master mix [16-18].

Plasma concentrations

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Samples were collected after eight weeks of treatment and after a minimum of 15 days of stable dose of the drug. The dosage of risperidone was completely left up to a clinician's decision, based on the rules of good clinical practice. Blood samples were obtained in the morning (07.00 h), 12 hours after the bedtime dose and before the morning dose. Plasma concentrations of risperidone and 9-hydroxyrisperidone (TRC, Canada) were measured by high performance liquid chromatography (HPLC) method with diode array detector (Shimadzu, Japan) [19]. Compounds are separated using linear gradient on Symmetry C18 (Waters) column. Solvent A was acetonitril and solvent B consisted of 0.05 M $NaH_2PO_4xH_2O$ pH 3.8. The gradient ratio of solvent B started at 25%, rising up to 60% during the 25 minute period. Flow rate was 1,5 ml/min and oven was set at 45°C. The eluted substance was detected at 200 nm (fluoxetine as internal standard) and 277 nm (risperidone and 9-OH risperidone). All compounds were confirmed with standard UV spectra 190-370 nm. One milliliter of plasma was mixed with 50 μL of the aqueous internal standard solution and 100 μL of 0.5 M NaOH. The mixture was vortexed for 3 seconds, and 5 ml of extraction solvent was added (hexan-ethyl acetate 1:1) then shaken and centrifugated. Four milliliters of organic layer evaporated to dryness at 37°C. The residue was dissolved in 150 μl of mobile phase. The method was validated for concentration range 12 – 244 nmol/L for risperidone and 12-234 nmol/L for 9-OH risperidone. Limit of detection was 6.1 and 5.8 nmol/L for analytes respectively. Accuracy at three concentrations ranged from 94.3 to 103.6 %. Inter and intra-day relative standard deviations were lower than 12 %. This analytes are included in external quality control scheme (UK NEQAS).

Statistical Analysis

All statistical analyses central to the aim of this study were performed with the following genotype groups: CYP2D6 wt/wt, wt/mut and mut/mut, ABCB1 *2677G/G, G/T, T/T* and ABCB1 *3435C/C, C/T, T/T*. Furthermore, ABCB1*2677* alleles G and T, and ABCB1 *3435* alleles C and T were analysed separately in a logistic regression model described in details below. Haplotype analysis was performed on the *C3435T* and *G2677T* variants on the basis of linkage disequilibrium observed between both positions. Steady-state plasma concentrations of risperidone and 9-hydroxyrisperidone were adjusted by drug dosage (dose-corrected values) and presented as geometric means and 95% confidence interval. Active moiety concentration stands for the sum of risperidone and 9-OH risperidone concentrations. Differences in plasma drug concentrations (C/Ds) between different genotypes were analysed using Kruskal-Wallis test with Mann Whitney test for post hoc comparisons. Due to multiple comparisons, level of statistical significance for the Mann Whitney test is decreased to $p<0.017$. Interval measures were presented as means with standard deviations. Chi-square tests were used to compare the groups for different categorical variables. Differences in psychopathology (measured by PANSS) were examined using one-way ANOVA (all results statistically non significant) and paired sample t-test. A binary logistic regression model was created to identify which genetic variant predicted best the patients' clinical response to treatment. The latter was dichotomized depending on weather the individual value was below or above 50% improvement in total PANSS score and used as a dependent variable in the model. Independent variables were: age, gender, CYP2D6, ABCB1 *G2677T* and ABCB1 *C3435T* genotypes (each as wt/wt, wt/mut and mut/mut), ABCB1*2677* alleles (G and T), ABCB1 *3435* alleles (C and T). The Statistical Package for the Social Sciences (SPSS, Chicago, IL) version 12 was used for all the abovementioned analyses.

Results

The CYP2D6 and ABCB1 genotype distribution

The distribution of the analyzed CYP2D6 genotypes wt/wt, wt/mut and mut/mut was 43, 32 and 8, respectively. Only one patient carried more than two copies of a functional CYP2D6 allele (ultrarapid metabolizer) and was excluded from the final analysis. Their risperidone concentration was 0 nmol/L and 9-OH risperidone was 56 nmol/L which is in agreement with their ultraextensive metabolic phenotype. Concerning the ABCB1 2677G/G, 2677G/T, 2677T/T genotypes, the distribution was 29, 42 and 12, respectively. None of the participants carried a 2677A allele. Furthermore, a total of 25 patients were the ABCB1 3435C/C carriers, while 37 carried the 3435C/T and 21 the 3435T/T. Data presented in **Table 2**. The two single nucleotide polymorphisms were in linkage disequlibrium (D'=0.78) and the most frequent ABCB1 haplotype was 2677G - 3435C (0.318), followed by the 2677T - 3435T (0.265), the 2677G - 3435T (0.238) and the 2677T - 3435C (0.179). There were no differences between these genotype subgroups in regard to any of the studied sociodemographic or clinical variables, including PANSS scores at baseline and end-point.

Insert Table 2 about here

The steady-state plasma concentrations

The drug was prescribed at the mean dose of 3.96 mg ± 1.5 (range 1-8 mg). The schemes were as follows: "8 mg/day" (n=3), "7 mg/day" (n=1), "6 mg/day" (n=7), "5 mg/day" (n=4), "4 mg/day" (n=30), "3 mg/day" (n=22), "2 mg/day" (n=15) and "1 mg/day" (n=1). Dose-corrected plasma levels (C/Ds) of risperidone, 9-OH risperidone and active moiety C/D (risperidone + 9-OH risperidone) are given in **Table 2**. In general, the CYP2D6 genotype holds a statistically significant effect on risperidone C/D (Kruskal-Wallis Test: 16.161, df=2 $p<0.001$), 9-OH risperidone C/D (9.699, df=2, p=0.008) and active moiety C/D (9.142, df=2, p=0.01). The ABCB1 G2677TA genotypes have significant effect only on the active moiety C/D (Kruskal-Wallis Test: 13.478, df=2, p=0.001). The ABCB1 C3435T genotypes have statistically significant effect on risperidone C/D (6.351, df=2, p=0.042) and active moiety C/D (14.744, df=2, p=0.001).

The role of the CYP2D6 poor metabolizers (PMs)

A total of 8 patients were genotyped as the CYP2D6 poor metabolizers (genotype CYP2D6 mut/mut). They had significantly higher levels of risperidone C/D than homozygous EMs (genotype CYP2D6 wt/wt; Mann Whitney U=1.00, Z = -3.83, p<0.001) and heterozygous EMs (genotype CYP2D6 wt/mut; U=8.00, Z = -3.38, p<0.001). The same pattern was observed in regard to active moiety C/D – PMs had significantly higher levels of active moiety C/D than homozygous EMs (U=30.00, Z = -2.73, p=0.004) and heterozygous EMs (U=34.00, Z = -2.12, p=0.033). However, PMs have significantly lower levels of 9-OH risperidone C/D than homozygous EMs

(U=49.000, Z = -2.03, p=0.045) or heterozygous EMs (U=21.000, Z = -2.8, p=0.004). The Risperidone/9-OH risperidone ratio was >1 in PMs, thus indicating lack of CYP2D6 activity. Furthermore, the same ratio was higher in PMs than in homozygous EMs (U=0.00, Z= - 3.864, p<0.001) or heterozygous EMs (U=6.00, Z= - 3.457, p<0.001). Please see **Figure 1** and **Table 2**.

The role of ABCB1 genotypes on differences in drug concentrations

The ABCB1 2677T/T genotype is associated with higher levels of dose-corrected active moiety than ABCB1 2677G/G genotype (Mann Whitney U=25.00, Z = -3.151, p=0.001) and than ABCB1 2677G/T genotype (Mann Whitney U=30.00, Z = -3.356, p<0.001). The ABCB1 3435T/T genotype is associated with higher levels of risperdone C/D than the 3435C/C genotype (Mann Whitney U=73.00, Z = -2.529, p=0.011). Similarly, the carriers of the 3435T/T genotype have higher levels of active moiety C/D than those who carry the 3435C/C (Mann Whitney U=43.00, Z = -3.508, p<0.001) or the 3435C/T genotype (Mann Whitney U=88.00, Z = -2.830, p=0.004).

The clinical response

No statistically significant difference was found when comparing genotype groups for baseline and end-point (after 8 weeks of treatment) PANSS scores (**Table 3**). Likewise, no statistically significant correlations were found between the PANSS scores and plasma concentrations (p>0.05). However, majority of our patients did show decrease in symptom severity. In comparison to baseline, greatest benefits were seen in reduced scores on the positive symptoms subscale (t-test: t=14.356, df=82, p<0.001), general psychopathology subscale (t=10.558, p<0.001) and total PANSS score (t=10.181, p<0.001), but the difference was not significant for the negative symptoms score (t=1.6, p=0.114). Our next step was to search for the predictive value of these genotypes (*CYP2D6 wt/wt, wt/mut, mut/mut*, ABCB1 *C3435T* and *G2677T/A)* and ABCB1 alleles (ABCB1 *3435* G and T, ABCB1 *2677* C and T) on the clinical response (defined as 50% improvement from baseline in total PANSS). Following this criteria, a total of 21 participants (25.3%) were responders. After adjusting for confounding factors, our regression model showed no statistically significant predictors (p>0.05; data not shown). The only significant difference was that fewer people with EPS were among the responders (6/21, chi square 5.697, df=1, p=0.017). No other differences in genotype, plasma concentrations or general data were present.

Insert Table 3 about here

The extra-pyramidal and other side-effects

We found that 44 patients (53.01%) had EPS (Simpson-Angus scale > 3). Interestingly, when analyzing the EPS-group (N=39, 46.9%), there were significantly more ABCB1 2667T-3435T haplotype carriers (N=17, chi square

4.140, df=1, p=0.042) and ABCB1 *3435*T allele carriers (N=29, chi square 5.8, P=0.016). No other difference in respect to demographic, clinical or genetic data was found. No statistically significant correlations were found between the score on Simpson-Angus scale and plasma concentrations (p>0.314). Considering other serious side-effects, eleven women reported amenorrhea (13.3 %), three suffered from galactorrhea (3.6 %) and one had gynecomastia (1.2 %). Two patients had EPS, amenorrhea and galactorrhea at the same time.

Discussion

Our results suggest that CYP2D6 genotype has significant influence on the steady-state dose-corrected levels of risperidone, 9-OH risperidone and active moiety which is in accordance with some previously conducted studies on Italian [10], Japanese [11] and Korean [20] subjects with schizophrenia. This is the first time to replicate these results on a Croatian (Slavic) population.

Out of all CYP2D6 phenotypes, poor metabolizers are of highest significance for clinicians because this group is at risk for adverse reactions to medications and may reach toxic levels of medications at relatively low clinical doses. As expected, the poor metabolizers in the present study were characterized with significantly higher levels of steady state dose-corrected risperidone and lower levels of dose-corrected 9-OH risperidone. Furthermore, the CYP2D6 poor metabolizer phenotype was associated with the risperidone/9-OH risperidone ratio >1 which indicates the lack of CYP2D6 activity. What we also found was that the frequency of PMs in our sample was 9.6% which is three times higher than for the general population in Croatia (3%), while the frequencies of other two CYP2D6 genotypes did not differ much from the general population data [21]. This was a surprising and difficult to explain finding especially since these are patients with the first episode of psychosis and not treatment resistant or high level of adverse effects group. We did not show that they have significantly more side-effects or different treatment response than others, but our results should be interpreted with caution due to small number of subjects. A study on much larger sample of 325 persons stabilized on risperidone therapy found that the CYP2D6 poor metabolizer phenotype triples the risk for moderate-to-marked adverse drug reactions and drug discontinuation. [22].

Concerning the ABCB1 genotypes, the strongest effect was seen between ABCB1 2677T/T and ABCB1 3435T/T genotypes and increased level of risperidone active moiety C/D. The presence of 3435T/T genotype might reduce expression and activity of P-gp, thus limiting intestinal drug absorption and central nervous system penetration [8, 23]. We have also found that half of our subjects had extrapyramidal symptoms. However, the other half (EPS- group) was characterized with significantly more ABCB1 3435T allele and ABCB1 2667T-3435T haplotype carriers. There was no difference in plasma levels in regard to these features. This finding suggests that ABCB1 3435T allele and/or ABCB1 2667T-3435T haplotype variants may not be such strong mediators of drug's penetration into central nervous systems after all. Even though two other studies reported that higher plasma concentrations of active moiety tends to be associated with a higher rate of extrapyramidal symptoms [24, 25], it might be hard to establish a straightforward and firm association between drug's levels peripherally and centrally. Since 9-OH risperidone has been marketed as a separate antipsychotic (paliperidone), we would like to discuss our findings about 9-OH risperidone in more details. In an attempt to combine our

results and effects of CYP2D6 and ABCB12677T/T and 3435T/T genotypes, we can say that the level of dose-corrected 9-OH risperidone is significantly lower in CYP2D6 PMs, while ABCB1 genotype holds no effect on this metabolite. Therefore, there are no additive effects between these genetic variants and it might be suggested that determination of these ABCB1 genotypes has very little clinical importance considering individualization of paliperidone treatment. However, these findings need to be replicated since two other studies on Caucasian men reported that the ABCB1 2677T/T carriers had significantly lower 9-OH risperidone levels [10, 26].

We found no clear association between the genotype and plasma concentration differences and treatment response to eight weeks of risperidone, thus leaving this extremely important question still opened. Results from a recent study on 59 Slovenian patients with the first episode of schizophrenia, also treated with risperidone, indicate that heterozygous carriers of G2677T/A and C3435T polymorphism scored somewhat higher on scales measuring psychopathology and extrapyramidal side effects, but the difference was not significant and there was no tendency of gene-dose effect [27]. Interestingly, in our previous study we did find positive associations between ABCB1 2677T allele and 2677T/T genotype and better treatment response in female patients with schizophrenia who were treated with another atypical antipsychotic – olanzapine [28]. What we found here was that after 8 weeks of risperidone treatment a quarter of our patients had 50% lower level of symptom severity (responders). There were significantly fewer people with EPS were among them. Significant improvements were observed in positive and general symptoms, but not in negative symptoms. This finding is probably contributable to the fact that risperidone may not be very efficient in reducing negative symptoms, rather than to not so high level of negative symptoms early in the course of schizophrenia in our patients. Secondly, this finding holds an important message to clinicians working with this group of patients especially because it has been shown that early non-response (as early as 1 or 2 weeks) can reliably predict subsequent non-response to treatment of schizophrenia [29, 30].

Main study limitation is relatively small sample size. It might be necessary to analyse much larger samples to show clear effects of drug metabolic polymorphisms on clinical outcome. However, strengths are related to the sample type (drug naive patients) and naturalistic design (hopefully this might help clinicians in translating these results into reality of their clinical practice).

In conclusion, the present study suggests that CYP2D6 genotype has strong effect on steady-state dose-corrected plasma levels of risperidone, its 9-OH metabolite and active moiety, while ABCB1 2677T/T and 3435T/T genotypes hold similarly strong effects on active moiety. Additionally, the ABCB1 3435T/T genotype has somewhat weaker influence on risperidone C/D. Clinically significant group of CYP2D6 poor metabolizers were characterized with significantly higher levels of dose-corrected risperidone and active moiety, and lower levels of dose-corrected 9-OH risperidone. Those with extrapyramidal syndrome did not differ in drug plasma concentrations. However, the ABCB1 3435T allele and the ABCB1 2667T-3435T haplotype carriers were more frequently present among subjects without extrapyramidal syndrome. Even though patients did show significant improvements in positive and general symptoms, but not in negative symptoms, these changes were not related to variations in genetic and drug concentration data. Therefore, our findings suggest that CYP2D6 and ABCB1

G2677T and C*3435*T might be useful determinants of risperidone plasma concentrations, but clinical implications of these findings in relation to treatment response and side-effects remains unclear.

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Table 1 – General, clinical and side-effects data for all subjects included in the study

	All subjects N=83
Gender, M/F, N (%)	17, 20.5 / 66, 79.5
Age, years (mean ± SD)	30.3 ± 8.1
Marital status, N (%)	
- Single	45 (54.2)
- Married	31 (37.3)
- Divorced	7 (8.4)
Education, N (%)	
- primary school	7 (8.4)
- high school	63 (75.9)
- college	13 (15.7)
Smokers, N (%)	45 (54.2)
Body weight, kg (mean ± SD)	74.26 ± 11.5
Family history of psychosis, N (%)	25 (30.1)
Baseline total PANSS score (mean ± SD)	95.3 ± 17.7
End-point total PANSS score (mean ± SD)	62.97 ± 23.4
Percentage of change in PANSS total score (mean ± SD)	32.09 ± 29.01
Responders to 8 weeks of risperidone treatment, N (%)	23 (27.7)
Dosage of risperidone at the study end-point (mean ± SD)	3.96 ± 1.5
Concomitant therapy, mg (mean ± SD)	
- anticholinergic	2.74 ± 1.6
- diazepam	7.21 ± 3.1
Side-effects to treatment, N (%)	
- EPS+ subjects	44 (53.01)
- amenorrhea	11 (13.3)
- galactorrhea	3 (3.6)
- gynecomastia	1 (1.2)

Note. PANSS stands for Positive and Negative Syndrome Scale. Response to treatment is defined as 50% improvement from baseline total PANSS score. EPS+ subjects are those experiencing extrapyramidal symptoms (score ≥ 3 on the Simpson-Angus scale for extrapyramidal symptoms)

Table 2 – The difference in dose-corrected plasma concentrations (C/D) of risperidone, 9-hydroxyrisperidone, active moiety and risperidone/9-OH risperidone ratio between the CYP2D6, ABCB1 *C3435T* and *G2677T/A* genotypes.

Genotype	Total sample N=83 (%)	Risperidone C/D [nmol L⁻¹ mg⁻¹]	9-OH risperidone C/D [nmol L⁻¹ mg⁻¹]	Active moiety C/D [nmol L⁻¹ mg⁻¹]	Risperidone/9-OH risperidone ratio
CYP2D6					
wt/wt	43 (51.8)	4.4 (2.8 – 5.9)	14.4 (11.6 – 17.2)	5.7 (4.1 – 7.4)	0.4 (0.2 – 0.6)
wt/mut	32 (38.6)	7.6 (3.9 – 11.3)	18.6 (14.8 – 22.4)	7.2 (5.3 – 9.0)	0.8 (0.08 – 1.6)
mut/mut	8 (9.6)	35.1 (19.5 – 50.5)	7.9 (3.9 – 11.8)	13.4 (5.7 – 21.1)	5.4 (2.4 – 8.4)
Statistics		16.161, df=2, p<0.001	9.699, df=2, p=0.008	9.142, df=2, p=0.01	15.268, df=2, p<0.001
ABCB1 *G2677T/A*					
GG	29 (34.9)	6.1 (2.8 – 9.5)	15.2 (11.1 – 19.1)	5.8 (3.7 – 7.9)	0.5 (0.2 – 0.9)
GT	42 (50.6)	9.9 (4.9 – 14.9)	14.1 (11.5 – 16.5)	6.3 (4.8 – 7.9)	1.4 (0.4 – 2.4)
TT	12 (14.5)	9.3 (3.3 – 15.3)	22.5 (13.4 – 31.2)	13.2 (8.2 – 18.1)	0.8 (0.3 – 1.9)
Statistics		3.840, df=2, p=0.147	4.760, df=2, p=0.093	13.478, df=2, p=0.001	0.246, df=2, p=0.884
ABCB1 *C3435T*					
CC	25 (30.1)	4.5 (2.3 – 6.7)	14.9 (10.6 – 19.2)	4.8 (3.2 – 6.3)	0.4 (0.1 – 0.6)
CT	37 (44.6)	9.9 (4.7 – 15.1)	14.5 (11.8 – 17.2)	6.5 (4.8 – 8.2)	1.4 (0.4 – 2.5)
TT	21 (25.3)	11.9 (4.5 – 19.5)	18.7 (12.7 – 24.6)	11.9 (7.9 – 15.9)	1.0 (0.2 – 1.9)
Statistics		6.351, df=2, p=0.042	2.240, df=2, p=0.326	14.744, df=2, p=0.001	1.470, df=2, p=0.480

Note. Data are shown as geometric means (95% confidence interval). Active moiety stands for sum of risperidone and 9-OH risperidone.
Statistics: Kruskal-Wallis Test (intragroup comparisons described in the text).

Table 3 – Influence of CYP2D6, ABCB1 *G2677T/A* and ABCB1 *C3435T* genotypes on symptom severity on admission (baseline) and after 8 weeks of risperidone treatment (study end-point).

				ANOVA*
CYP2D6	wt/wt N=43	wt/mut N=32	mut/mut N=8	
Baseline PANSS				
Positive subscale	26.08±6.6	25.9±6.3	27.6±5.7	0.782
Negative subscale	21.21±8.34	22.4±11.13	19.4±3.96	0.699
General psychopathology subscale	46.84±9.67	48.97±11.9	47.8±12.2	0.731
Total score	94.39±15.41	97.14±14.6	94.8±16.3	0.822
Study end-point PANSS				
Positive subscale	12.31±6.4	13.43±5.41	15.25±5.8	0.418
Negative subscale	18.87±11.24	19.11±10.55	4.9±1.74	0.959
General psychopathology subscale	30.63±10.13	29.89±8.79	32.6±11.8	0.786
Total score	61.82±16.3	62.81±16.5	65.3±10.9	0.931
ABCB1 *G2677T*	G/G N=29	G/T N=42	T/T N=12	
Baseline PANSS				
Positive subscale	27.56±5.21	25.64±6.9	24.33±6.13	0.274
Negative subscale	20.81±8.24	20.64±7.5	25.81±14.75	0.236
General psychopathology subscale	48.37±11.02	48.43±10.03	44.00±12.6	0.460
Total score	96.74±17.71	95.10±18.29	93.72±18.31	0.883
Study end-point PANSS				
Positive subscale	13.03±5.6	13.69±6.34	11.00±5.6	0.428
Negative subscale	18.63±12.3	16.42±7.03	21.00±13.09	0.120
General psychopathology subscale	31.74±9.23	29.63±10.57	30.72±8.43	0.702
Total score	63.4±18.93	59.75±12.81	68.72±14.98	0.515
ABCB1 *C3435T*	C/C N=25	C/T N=37	T/T N=21	
Baseline PANSS				
Positive subscale	26.54±6.1	26.52±6.6	24.63±6.04	0.521
Negative subscale	20.7±8.5	20.4±7.7	24.6±8.24	0.269
General psychopathology subscale	46.4±11.2	49.23±9.3	46.7±12.9	0.561
Total score	93.6±18.3	96.44±17.5	96.23±18.9	0.828
Study end-point PANSS				
Positive subscale	12.7±5.5	13.4±6.6	12.94±5.5	0.900
Negative subscale	19.4±8.95	15.5±6.7	24.35±8.8	0.136
General psychopathology subscale	31.2±9.3	29.7±10.9	31.4±7.96	0.790
Total score	63.2±12.8	58.6±12.6	68.7±8.8	0.340

Note. PANSS stands for Positive and Negative Syndrome Scale.
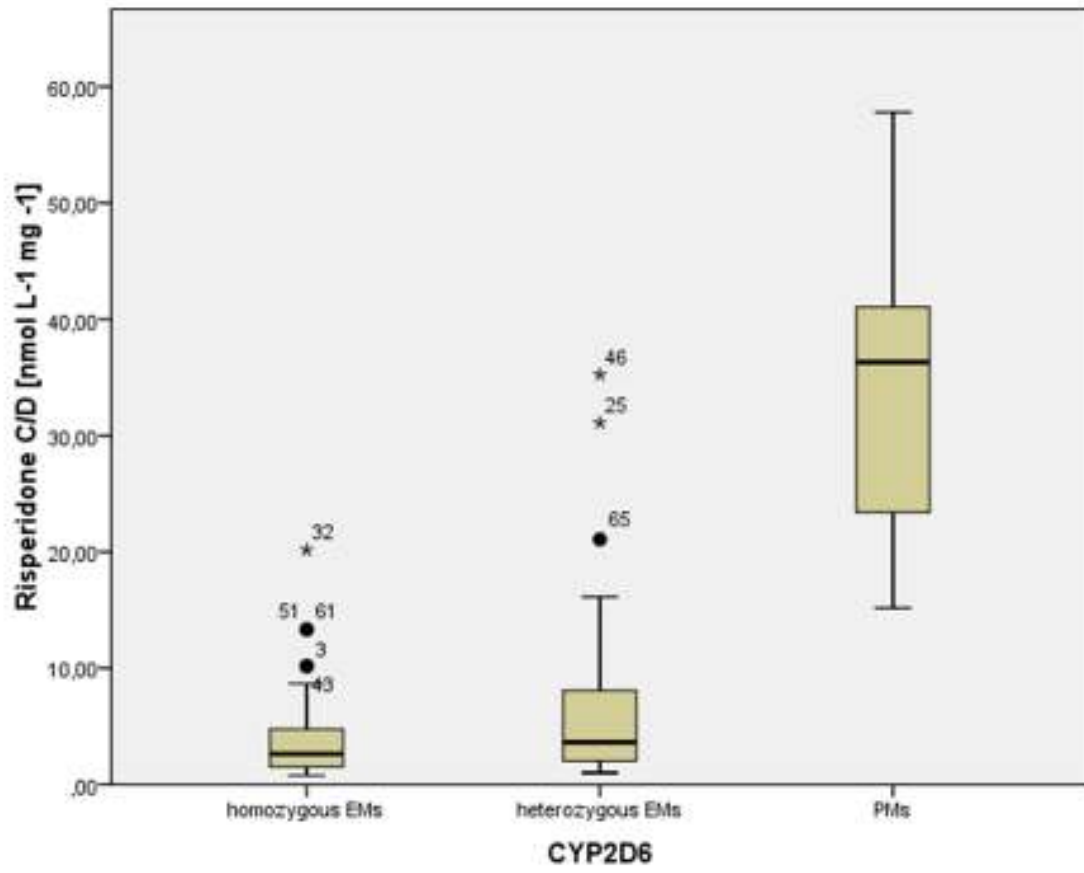*All results are statistically non-significant ($p > 0.05$).
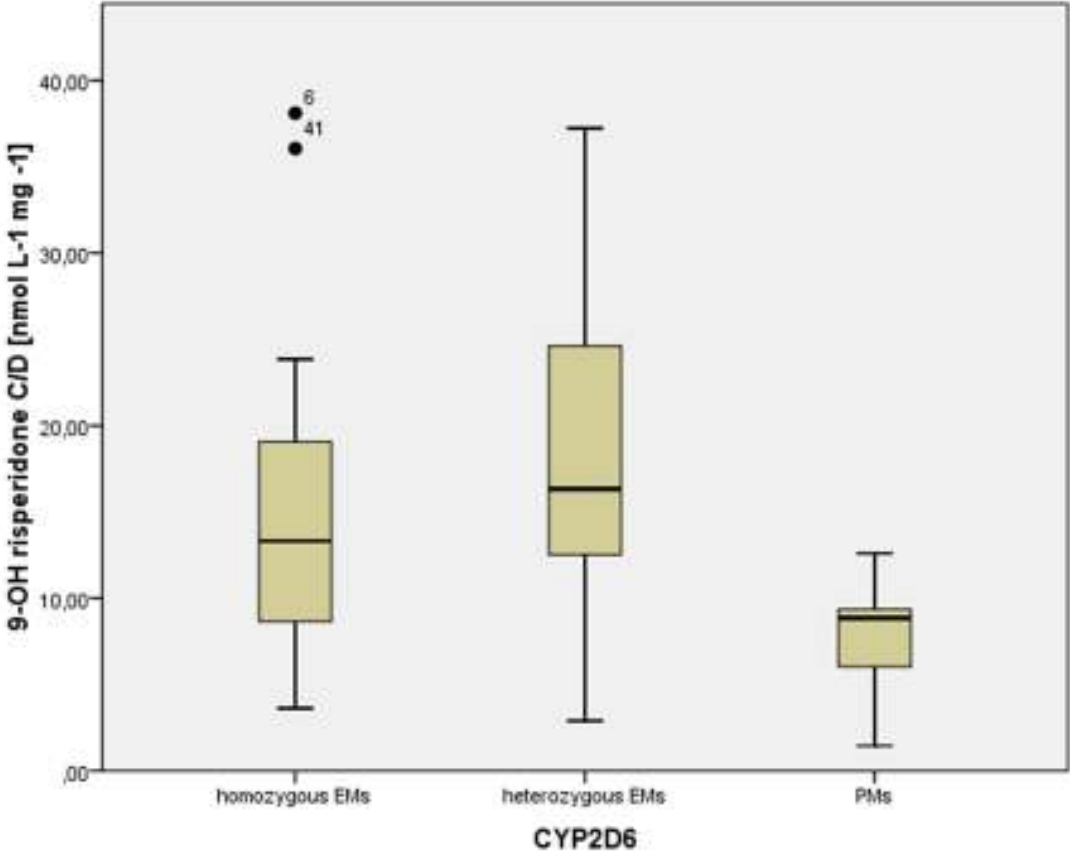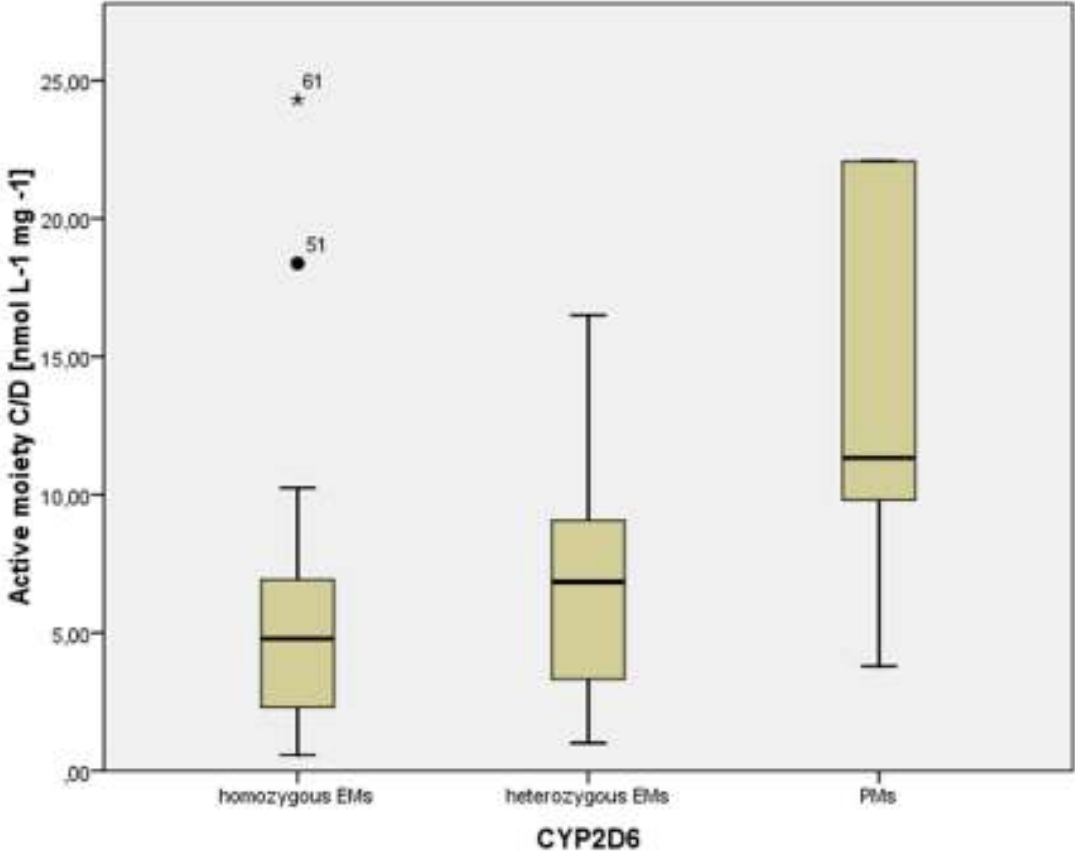
Figure 1A

Figure 1B

Figure 1C

Figure 1D