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REMISSION IS NOT ASSOCIATED WITH DRD2 RS1800497 AND DAT1 RS28363170 GENETIC VARIANTS IN MALE SCHIZOPHRENIC PATIENTS AFTER 6-MONTHS MONOTHERAPY WITH OLANZAPINE

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SUMMARY

***Background**: Symptomatic remission is an achievable goal in the treatment of schizophrenia. The type of antipsychotic medication and particular genetic variants of the dopaminergic system might be associated with remission. Potential pharmacogenetic markers of the treatment response to antipsychotic medication are missing. This study assessed the possible association between dopamine receptor type 2 (DRD2 rs1800497) and dopamine transporter (DAT1 rs28363170) gene variants with symptomatic remission in schizophrenia.*

***Subjects and methods**: Olanzapine (5-20 mg/d) monotherapy was administered for 6 months to 150 male Caucasian subjects with schizophrenia. Remission was evaluated according to "Remission in Schizophrenia Working Group" criteria. Genotyping was performed by PCR-RFLP.*

***Results**: Symptomatic remission was found in 31% of patients. DRD2 rs1800497 and DAT1 rs28363170 gene variants were not significantly associated with symptomatic remission. The limitations are a relatively small sample size of patients with schizophrenia (N=150), especially of group with symptomatic remission (N=45). However, the study had moderate but adequate sample sizes for most of the comparisons. Only two dopaminergic polymorphisms were analyzed, and plasma concentration of olanzapine was not determined.*

***Conclusion**: These results revealed a lack of association between DRD2 rs1800497 and DAT1 rs28363170 genetic variants and symptomatic remission in male patients treated with olanzapine, suggesting that these genetic variants could not be used to predict symptomatic remission to olanzapine monotherapy. Negative results should be further confirmed or rejected in the larger samples, including haplotype analyses, to detect clinically useful and easy obtainable pharmacogenetic markers that might predict therapeutic response or remission in schizophrenia.*

***Key words:** schizophrenia - olanzapine - symptomatic remission - genetic variants - DRD2 - DAT*

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INTRODUCTION

Antipsychotic drugs play a key role in the treatment of schizophrenia by blocking the dopaminergic D2 receptor type 2 (DRD2). This is a property shared by all antipsychotics including olanzapine, which is one of the most prescribed second-generation antipsychotic drug (Lally & MacCabe 2015). Besides blockade of DRD2 and serotonin 2A receptors (5-HT2AR), olanzapine shows high affinity for serotonin 2C receptors (5-HT2CR), dopamine D1, D3, D4 receptors, muscarinic receptors M1-5, adrenergic alfa-1 receptor and histamine receptor H1 (Ishigooka 2004). The advantages of olanzapine include its clinical efficacy, its ability to reduce negative symptoms and its low propensity to produce movement disorders (Lieberman et al. 2005). Poor response to treatment is frequent, although novel antipsychotic medications are in use (Lally et al. 2016). Besides the important contribution of the non-genetic factors to the therapeutic response to antipsychotics, genetic variants of the genes coding for various receptors, transporters and enzymes of the dopaminergic system might play an important role in the treatment response, resistance ad remission in patients with schizophrenia (see Lally et al. 2016). However, the literature findings are inconsistent (Yoshida & Müller 2018), since the studies included different medications and different criteria for response or remission. Therefore, this study focused on the two most frequently studied genetic polymorphisms (in the dopaminergic receptors type 2 (DRD2) rs1800497 and dopaminergic transporter (DAT) rs28363170) and remission to olanzapine monotherapy, with aim to elucidate their possible use as easy obtainable pharmacogenetic markers of the remission to olanzapine.

The single nucleotide polymorphism DRD2 Taq1A (rs1800497; 2139C/T) results in a cytosine to thymine change at nucleotide position 2139, leading to the existence of two alleles, A1 and A2. Literature data on the association of this functional polymorphism (Tunbridge et al. 2019) DRD2 rs1800497 with therapeutic response to olanzapine are inconsistent (Mi et al. 2011). Meta-analysis (Zhang et al. 2011) that examined the relationship between DRD2 polymorphisms, including rs1800497, and different antipsychotic response, reported similar response rate between A1 carriers versus A2/A2 genotype, or between A2 allele carriers versus A1/A1 genotype carriers. However, this meta-analysis did not include olanzapine monotherapy (Zhang et al. 2011).

The 40-bp variable-number tandem repeat (VNTR) in the 3'-untranslated region of the dopamine transporter gene (DAT1 or SLC6A3) is a frequently studied polymorphism (DAT1 VNTR, rs28363170) with 2 most common alleles, the 9-repeat (9R) and the 10-repeat (10R). Its functionality has been recently questioned (Tunbridge et al. 2019). A limited number of studies elucidated an association between DAT1 rs28363170 and antipsychotic response, including olanzapine, generally with negative findings (Szekeres et al. 2004; Zhang et al. 2007; Xu et al. 2010; Tybura et al. 2012).

Since the results on the possible relationship between DRD2 and DAT1 genetic variants with treatment response are still inconsistent (Yoshida & Müller 2018), and patients included in previous studies were not separated by gender (Sagud et al. 2018), and were treated with different antipsychotic drugs, this pharmacogenetic study, with a longitudinal design, aimed to evaluate the association between genetic variants of the DRD2 rs1800497 and DAT1 rs28363170 with symptomatic remission in Croatian male patients with schizophrenia. Remission was determined according to the "Remission in Schizophrenia Working Group" /RSWG/ criteria (Andreasen et al. 2005) to 6-months monotherapy with olanzapine. The hypothesis of the study was that particular genetic variants of the DRD2 rs1800497 and DAT1 rs28363170 will be associated with olanzapine-induced remission in schizophrenia.

SUBJECTS AND METHODS

Sample

The present study included 150 male patients with schizophrenia and was carried out at the Neuropsychiatric Hospital Dr. Ivan Barbot, Popovaca, Croatia, and at the University Hospital Centre Zagreb, Zagreb, Croatia. Inclusion criteria were: male subjects, age range 19 to 60 years (median 33, IQR 28-42), body mass index (BMI) < 30 (median 25.7, IQR 23.1-28.5), DSM-IV diagnosis of schizophrenia, monotherapy with olanzapine, acute exacerbation of schizophrenia, and Caucasian ethnicity living in Croatia. Exclusion criteria were: serious somatic illnesses, neurologic disorders, previous therapy with clozapine and electroconvulsive therapy, and a history of drug use during the previous 6 months. The study was approved by the Ethics Committee of the Neuropsychiatric Hospital Dr. Ivan Barbot (Popovaca, Croatia) and the Zagreb School of Medicine (Zagreb, Croatia). All subjects provided written informed consent with the committee's guidelines. Therefore, all human studies have been approved by the corresponding Ethics Committees, confirming the provisions of the Declaration of Helsinki in 1995 (as revised in Edinburgh 2000).

Genotyping of the DRD2 rs1800497 and DAT rs28363170

Genomic DNA was extracted from peripheral blood (5-10 ml) using standard salting-out method (Miller et al. 1988). Genotyping for DRD2 Taq1ARFLP (rs1800497) was performed by polymerase chain reaction (PCR) - restriction fragment length polymorphism (RFLP) methods. The 6 alleles of the VNTR polymorphism in DAT1 (rs28363170), consisting of 6, 7, 8, 9, 10, or 11 copies of the 40-base-pair repeat sequence were determined.

Psychiatric evaluation

The study was conducted between April 2008 and May 2011. Participants were evaluated two times with the Positive and Negative Syndrome Scale /PANSS/ (Kay et al. 1987) during the first day of admission, and after 6 months, by psychiatrists with extensive clinical experience. Raters were blind to DRD2 rs1800497 and DAT1 rs28363170 genotypes of patients. Most of the patients were taking atypical antipsychotics (except clozapine) before the inclusion in the protocol (89.3%), whereas a smaller number of patients was drug-naïve (10.7%). However, prior to study (4-6 months), 118 patients (78.7%) did not take any medication, while 32 patients (21.3%) were on different medications. Since all patients were in the exacerbation, no wash out was performed. All patients received olanzapine monotherapy (5-20 mg/d). Only one brand of olanzapine was used throughout the trial to ensure bio-availability. After titration of the dose of olanzapine during the first few days of admission (7±2 days), participants remained at a fixed dose of olanzapine to the end of the study. During the study no concomitant medication was allowed except benzodiazepines for insomnia and anxiety. Patients were excluded from the study in case of correction of the dose of olanzapine or switching to another antipsychotic. Treatment response was defined according to the remission criteria by the RSWG, consisting of a reduction to mild levels on the key 8 symptoms on the PANSS scale (items P1, P2, P3, N1, N4, N6, G5, G9) for at least 6 months (Andreasen et al. 2005). Patients were divided into two groups, remitted and non-remitted, depending whether they meet the remission (RSWG) criteria.

Statistical analysis

All confidence intervals were given at the 95% level. Normality of distribution of continuous variables, like age, BMI, or PANSS score, was tested by Kolmogorov-Smirnov test for samples greater than 30, or by Shapiro-Wilk test for samples smaller than 30. Median and interquartile range were used as measures of central tendency and variability when distribution did not significantly deviate from the normal one. Homogeneities of variances of continuous variables were tested by Levene test. Differences in mean values for the continuous, numeric variables between more than two categories of a nominal variable were analyzed by ANOVA, if distribution did not significantly deviate from the normal one, and if variances were homogenous. Standard measure of effect size given with ANOVA was η^2. To analyze which of two genotype/allele groups differ significantly, Bonferroni post hoc test was used. Univariate and multivariate prediction of symptomatic remission were carried by means of logistic regression and odds ratios with 95% confidence intervals given for each variable. Genotype or allelic frequencies between remitted and non-remitted patients and Hardy-Weinberg equilibrium (HWE) were evaluated using Pearson's χ^2 test and Yates correction for continuity. To test the possibility of gene-gene interaction, a series of hierarchical multivariate prediction of remission was made. In each analysis, all the polymorphisms were entered in analysis at first step, then the product of two polymorphisms was added, and we tested statistical significance of its contribution to predict remission. Procedure was repeated for all possible 2- and 3-way interactions. All the analyses were carried out Sigma Stat 3.5 (Jandel Scientific Corp. San Rafael, CA, USA).

Due to multiple comparisons (testing the association of 2 polymorphisms), a correction was performed and p value was set to p=0.05/2=0.025. G*Power 3 Software (Faul et al. 2007) was used to determine the required sample size and statistical power. For multiple regression, with P=0.025; medium effect size=0.15; and power (1-β) =0.800; number of predictors= 2; the required sample size was 66. For the Mann-Whitney test, with P=0.025; medium effect size=0.5; and power (1−β) =0.800; the required sample size was 128. For χ^2 test, with P=0.025; medium effect size=0.3; and power (1−β) =0.800; the required sample size was 128. As the study included 150 subjects at the beginning, and 145 subjects after 6 months, it had a needed sample size. Only for ANOVA, the study did not include enough subjects since with P=0.025; medium effect size =0.25; and power (1−β) =0.800; the required sample size was 189. Therefore, the study, with corrected P value (0.025) and needed power of 0.800, had adequate sample size for the most of the comparisons, except ANOVA, to detect significant differences among the groups if they existed.

RESULTS

From 150 male patients with schizophrenia that were assessed initially, five dropped out during the study, and therefore the remaining 145 subjects were included in further analysis. Distribution of age and BMI values significantly deviated from normal distribution (age: Kolmogorov-Smirnov z=0.098; p=0.001, BMI: Kolmogorov-Smirnov z=0.087; p=0.007), and therefore median and interquartile range were used as measures of central tendency and variability (age: median 33; IQR 28-42, BMI: median 25.7; IQR 23.1-28.5).

Symptomatic remission was achieved in 45 patients (31%), according to proposed criteria in all 8 key items in the PANSS scale after six months (Table 1). It was not significantly related to smoking status since smokers and no-smokers had had similar odds (OR=1.6; 95% CI=0.74-3.36; univariate logistic regression) to achieve remission.

When analyzing differences in baseline PANSS scores between subjects who achieved symptomatic remission and those who did not achieve remission, a significant difference was found in the PANSS negative (Mann-Whitney U=1285.5; Z=-4.139, P<0.001; AUC=0.29), PANSS general psychopathology (Mann-Whitney U=1407.5; Z=-3.604, P<0.001; AUC=0.31) and PANSS total (Mann-Whitney U=1427; Z=-3.519, P<0.001; AUC=0.33) scores. Namely, all patients that achieved symptomatic remission had lower values in PANSS total scores, and PANSS negative and general psychopathology subscales at baseline than patients who did not achieve remission (Table 2).

Table 1. Results of symptomatic remission according to proposed criteria (8 items of PANSS< 3 during 6 months follow up, n=150) in male patients with schizophrenia treated for 6 months with olanzapine monotherapy

Remission criteria	N=150	%
Remission after 6 months treating with olanzapine		
remission	45	31.0
no remission	100	69.0
total	145	100
Remission criteria		
remission (at all 8 items result < 3)	45	30.0
no remission (items >3)	80	53.3
positive response, but less than 6 months	18	12.0
positive response, but deteriorated after 6 weeks; added another antipsychotic	2	1.3
lost to follow-up	5	3.3

N = number of subjects; %= percent of subjects; PANSS = Positive and Negative Syndrome Scale

DRD2 rs1800497 and DAT1 rs28363170 genotypes did not deviate from the HWE (Pearson's χ^2 test). Symptomatic remission was evaluated in patients with schizophrenia subdivided according to the DRD2 and DAT genotypes (Table 3) and alleles (Table 4). DRD2 rs1800497 and DAT1 rs28363170 genotypes or alleles were not significantly associated (P>0.025) with symptomatic remission (univariate logistic regression analysis).

To further asses this lack of association, the frequency of the DRD2 rs1800497 and DAT1 rs28363170 genotypes and alleles was evaluated in subjects with or without symptomatic remission. No significant differences in the distribution of the DRD2 rs1800497 (χ^2=2.702; df=2; P=0.259) or DAT1 rs28363170 (χ^2=0.758; df=2; P=0.684) genotypes, or DRD2 rs1800497 (χ^2=1.361; df=1; P=0.243) or DAT1 rs28363170 (χ^2=0.575; df=1; P=0.448) alleles were detected between patients with or without symptomatic remission, respectively.

In addition, no significant enhancement to the prediction of symptomatic remission was shown when the interaction of DRD2 rs1800497 and DAT1 rs28363170 (χ^2=2.76; P=0.598) was tested.

The PANSS total, positive, negative and general psychopathology scores, corrected by smoking status (ANOVA), did not differ significantly (P>0.025) after 6 months of olanzapine treatment in 45 patients with symptomatic remission, subdivided into carriers of the DRD2 and DAT1 genotypes (Table 5). Accordingly, no significant (P>0.025) changes (Mann-Whitney U test) were found in the total, positive, negative and general psychopathology PANSS scores after 6 months of olanzapine treatment in 45 patients with symptomatic remission, after they were subdivided into carriers of the DRD2 and DAT1 alleles (Table 6).

Table 2. Symptomatic remission in male patients with schizophrenia in relation to PANSS scores at baseline assessment

PANSS subscale scores	Remission achieved		Remission not achieved		P	Effect
	Median	IQR	Median	IQR		
Positive symptoms	37	32-40	36	32-39	0.646	
Negative symptoms	34	29-35	36	34-39	<0.001	0.29
General psychopathology	59	54-63	63	59-69	<0.001	0.31
PANSS total score	127	119-135	136.5	126.5-142.8	0.001	0.32

IQR = interquartile range, P = Mann-Whitney U test for two independent groups; effect = standardized measure of size effect for statistically significant results; PANSS = Positive and Negative Syndrome Scale

Table 3. Symptomatic remission in patients with schizophrenia subdivided according to the DRD2 rs1800497 and DAT1 rs28363170 genotypes

Genotypes	Remission achieved		Remission not achieved		Total		P	OR_{uv}	95% CI
	N	%	N	%	N	%			
DRD2									
A1A2	11	22.4	38	77.6	49	100.0		1	
A2A2	31	36.0	55	64.0	86	100.0	0.104	2.0	0.87-4.34
A1A1	3	30.0	7	70.0	10	100.0	0.610	1.5	0.33-6.70
DAT									
9/10	17	27.0	46	73.0	63	100.0		1	
10/10	24	33.8	47	66.2	71	100.0	0.393	1.4	0.66-2.90
9/9	3	33.3	6	66.7	9	100.0	0.692	1.4	0.30-6.02

OR = odds ratio; 95% CI = 95% confidence interval for odds ratio; uv = univariate logistic regression;
DRD2 = dopaminergic receptor type 2; DAT: dopaminergic transporter

Table 4. Symptomatic remission in patients with schizophrenia subdivided according to the DRD2 rs1800497 and DAT1 rs28363170 alleles

Alleles	Remission achieved		Remission not achieved		P	OR_{uv}	95% CI
	n	%	n	%			
DRD2							
A2A2	73	81.1	148	74.0			
A1A1	17	18.9	52	26.0	0.191	1.50	0.92-2.79
DAT							
10/10	65	73.9	140	68.6			
9/9	23	26.1	64	31.4	0.370	1.29	0.74-2.26

OR = odds ratio; 95% CI = 95% confidence interval for odds ratio; uv = univariate logistic regression;
DRD2 = dopaminergic receptor type 2; DAT: dopaminergic transporter

Table 5. Differences in total, positive, negative and general psychopathology PANSS scores after 6 months of olanzapine treatment in 45 patients with symptomatic remission subdivided according to the DRD2 rs1800497 and DAT1 rs28363170 genotypes

Genotypes	N	PANSS total			PANSS positive			PANSS negative			PANSS general		
		Mean	SD	P	Mean	SD	P	Mean	SD	P	Mean	SD	P
DRD2				0.976			0.934			0.967			0.970
A2A2	31	51.61	12.48		11.03	3.47		15.58	4.13		25.00	6.26	
A2A1	11	52.00	10.19		11.27	2.37		15.55	3.86		25.18	5.55	
A1A1	3	52.33	17.21		12.00	5.00		15.00	5.29		25.33	7.23	
DAT				0.158			0.311			0.140			0.410
10/10	24	51.04	12.04		10.71	3.08		15.38	4.30		24.96	5.92	
9/10	17	55.18	11.48		12.18	3.54		16.59	3.46		26.41	6.10	
9/9	3	44.67	7.37		10.33	2.08		13.33	2.08		21.00	4.58	

P = ANOVA for more than two independent groups; PANSS = Positive and Negative Syndrome Scale;
DRD2 = dopaminergic receptor type 2; DAT = dopaminergic transporter

Table 6. Differences in total, positive, negative and general psychopathology PANSS scores after 6 months of olanzapine treatment in 45 patients with symptomatic remission according to DRD2 rs1800497 and DAT1 rs28363170 alleles

Alleles	N	PANSS total			PANSS positive			PANSS negative			PANSS general		
		Median	IQR	P	Median	IQR	P	Median	IQR	P	Median	IQR	P
DRD2				0.853			0.582			0.844			0.885
A2	73	49	43-62		11	8-14		15	13-19		23	21-30	
A1	17	54	43-61		11	8-14		15	14-19		25	20-30	
DAT				0.712			0.835			0.447			0.458
10	65	49	44-62		11	8-14		16	13-19		24	22-30	
9	25	49	42-59		11	8-14		15	13-19		24	19-20	

P = Mann-Whitney U test for two groups; PANSS = Positive and Negative Syndrome Scale;
DRD2 = dopaminergic receptor type 2; DAT = dopaminergic transporter

DISCUSSION

Present study is the first to simultaneously evaluate the association between DRD2 rs1800497 and DAT1 rs28363170 gene variants and symptomatic remission after 6-months olanzapine monotherapy in male Caucasian patients with schizophrenia. Our results revealed that DRD2 rs1800497 and DAT1 rs28363170 genetic variants are not associated with symptomatic remission, either alone, or in interaction with each other. These genetic variants were also not related to symptoms evaluated by the PANSS. Therefore, DRD2 rs1800497 and DAT1 rs28363170 variants might not be used to predict the response to 6 months of monotherapy with olanzapine in male schizophrenic patients.

Non-response to treatment is frequent in schizophrenia (Lally et al. 2016). The proportion of responders varies between studies (Thomas et al. 2008, Novick et al. 2007, Levine et al. 2012). Large number of non-genetic confounding factors, like diagnosis, illness course, antipsychotic drugs prescribed, treatment duration and adherence, assessment of efficacy and adverse effects, concomitant medication and co-morbidities, can affect individual response (Arranz et al. 2011). In line with previous data (Levine et al. 2012, Terzic et al. 2016), 31% of participants in our study achieved symptomatic remission. Remission was not influenced by smoking. In the recent systematic review, patients with multiple episodes had remission rates between 16% and 62%, with a weighted mean of 37% (AlAqeel & Margolese 2012), which is higher than in our patients. Therefore, this difference might be induced by chronicity and/or previous pharmacological treatment. Namely, longer treatment duration, higher number of hospitalizations and longer illness course (van Haren et al. 2007, Levine et al. 2012), different definitions and different criteria of symptomatic remission (Andreasen et al. 2005, Lieberman et al. 2003), different duration of the study period, high dropout rates, variations in sample selection (Lambert et al. 2010, AlAqeel & Margolese 2012) might influence treatment outcome. Present study applied RSWG criteria (Andreasen et al. 2005) with lower stringency (low threshold of the 8 core PANSS symptoms) and longer time (6 months) criterion, that contain positive and negative symptoms, core dimensions of schizophrenia (Cassidy et al. 2010). Our patients, who have achieved symptomatic remission, had lower values in PANSS negative, general psychopathology and PANSS total scores at baseline. This suggests that increased severity of symptoms, except on the PANSS positive subscale, is associated with lower chance to achieve symptomatic remission. Reduced baseline illness severity is one of the most relevant predictors of the symptomatic remission (AlAqeel & Margolese 2012). Another less clear predictor is male gender, since female gender was a predictor of remission (Anderson et al. 2017, Mihaljevic Peles et al. 2016). In our study middle-aged male patients with multiple episodes displayed low remission frequency (31%) during 6 months' follow-up, which was similar to 32% remission found in Slovenian patients with schizophrenia (Terzic et al. 2016).

In our study DRD2 rs1800497 was not associated with symptomatic remission after 6 months of monotherapy with olanzapine in male schizophrenic patients. Variations in the DRD2 gene have been investigated in relation to treatment response (Yoshida & Müller 2018, Kaur et al. 2017, Terzic et al. 2016, Huang et al. 2016a,b, Zhang et al. 2015, Kang et al. 2015, Blasi et al. 2015), but not remission, and there are no data related to olanzapine monotherapy. Significant association between three SNPs on *DRD2* gene (rs180498, rs2514218 and rs1079597) and antipsychotic treatment response was reported (Kaur et al. 2017, Zhang et al. 2015, Huang et al. 2016a,b, Kang et al. 2015). Other polymorphism, DRD2 rs1799732 (-141C Ins/Del), was not related to treatment response (Terzic et al. 2016, Bishop et al. 2015). In line with no relationship between the rs1800497 polymorphism and response to different antipsychotics (Zhang et al. 2011, Kang et al. 2015, Escamilla et al. 2018), the results of the present study revealed no association between DRD2 rs1800497 and symptomatic remission, or symptoms of schizophrenia, in male schizophrenic patients treated for 6 months with olanzapine monotherapy. Previous studies that included treatment with various typical and atypical antipsychotics revealed inconsistent findings (Alenius et al. 2008, Shen et al. 2008, Tybura et al. 2012, Vehof et al. 2012). The lack of association between symptomatic remission to olanzapine and DRD2 rs1800497 was confirmed in other studies showing that DRD2 rs1800497 was not related to the response to different antipsychotics (Escamilla et al. 2018), such as amisulpride (Kang et al. 2015), perazine, olanzapine or ziprasidone (Tybura et al. 2012).

In agreement with previously published studies (Zhang et al. 2007, Xu et al. 2010, Tybura et al. 2012) and results from different Croatian patients (Bilic et al. 2014), no significant association was detected between DAT1 rs28363170 and symptomatic remission to olanzapine, or with PANSS scores in remitted patients. Negative findings might be explained by the different DAT tissue distribution (Piccini 2003). However, the DAT density was not affected by olanzapine (Kim et al. 2006) or by other antipsychotics or illness duration (Fusar-Poli & Meyer-Lindenberg 2013). Furthermore, negative findings might be due to the interactive effects of DAT1 rs28363170 with other DAT polymorphisms or with other dopaminergic gene polymorphisms, that could influence transcription and stability of mRNA or translational efficiency (Heinz et al. 2000). The interaction with DRD2 rs1800497 was not confirmed in the present study. DAT1 rs28363170 might interact with other gene polymorphisms such as serotonin transporter polymorphism (5HTTLPR), since schizophrenic patients, carriers of the DAT 10/10 or 10/12 genotypes and S carriers of the 5HTTLPR, were more likely to be treatment resistant compared to L carriers (Bilic et al. 2014). Besides differences in the treatment response and remission, as we did not evaluate 5HTTLPR gene variants in this study, we cannot confirm these findings.

The limitations of the study should be acknowledged: a relatively small sample size of patients with schizophrenia (N=150), especially in a group with symptomatic remission (N=45). However, RSWG criteria (Andreasen et al. 2005) of 6 months' duration might explain this low rate of remission. To overcome this limitation, we corrected p value to 0.025, and calculated in advance the needed sample size and statistical power: for power of 0.800, and expected moderate effect sizes, the study had moderate but adequate sample sizes for most of the comparisons; however, the results were negative. In addition, previously published studies evaluating olanzapine response were conducted using smaller samples, including both genders (Thomas et al. 2008). Only two dopaminergic polymorphisms were analyzed, and plasma concentration of olanzapine was not determined. The concentration-dependent therapeutic failures between studies might be explained by the large inter-individual variation in the pharmacokinetics of olanzapine.

Strengths of the present study include olanzapine monotherapy, inclusion of ethnically homogenous Caucasian patients with schizophrenia, only male subjects, usage of RSWG criteria and the longitudinal study design of 6 months follow up, which is well suited and powerful to address issues related to treatment response. Divergent pharmacogenetic results could be explained by the varieties in the study designs, small sample sizes, ethnic differences in the frequency of studied genotypes, small effect sizes of most genetic variants, uncompleted coverage of the most genes, lack of control of environmental and clinical confounders, differences in definition of outcome parameters, or insufficient incorporation of gene-gene and gene-environment interaction (Brandl et al. 2014, Yoshida & Müller 2018, Tunbridge et al. 2019). This longitudinal study tried to control for most of these confounders and reported negative findings.

CONCLUSION

In conclusion, the results of the present study revealed a lack of association between DRD2 rs1800497 and DAT1 rs28363170 genetic variants and symptomatic remission and/or symptoms of schizophrenia in male patients treated for 6 months with olanzapine monotherapy. Our previous study reported that COMT rs4680 was not significantly associated with symptomatic remission in male patients with schizophrenia treated with olanzapine monotherapy (Zivkovic et al. 2019). These data collectively suggest that these genetic variants of the dopaminergic system could not be used to predict remission after olanzapine monotherapy. However, negative results should be either rejected or confirmed in the larger samples (Tunbridge et al. 2019), including haplotype analyses (Sagud et al. 2018), to detect clinically useful and easy obtainable pharmacogenetic markers that might predict therapeutic response or remission in schizophrenia.

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Part of the statistical analysis was carried out by Biometrika Healthcare Research, Croatia. All authors contributed to and have approved the final manuscript.

Conflict of interest: None to declare.

Contribution of individual authors:

Maja Zivkovic, Alma Mihaljevic-Peles & Dorotea Muck-Seler were responsible for the idea and design of the study.

Maja Zivkovic & Nela Pivac were involved in the interpretation of findings and prepared the first draft of the manuscript.

Nela Pivac wrote the final draft of the manuscript.

Maja Zivkovic, Alma Mihaljevic-Peles, Marina Sagud & Suzana Vlatkovic were responsible for collection of data, explained the research goals and described protocol in details to the patients; explained the inclusion/exclusion criteria, insured participant adherence for the participation in the study, motivated, selected, diagnosed, evaluated and sampled patients and did psychiatric diagnoses and evaluation of the remission.

Lana Ganoci & Nada Bozina did the genotyping.

Lucija Tudor did the statistical analyses.

All authors have read and approved the final version and have contributed substantially to the design, performance, analysis, and reporting of this study.

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