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L-Arginine metabolism before and after 10 weeks of antipsychotic treatment in first-episode psychotic patients

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ABSTRACT

Agmatine is an endogenous NMDA (*N*-methyl-D-aspartate) antagonist which is synthesized from L-Arginine and described as a novel neurotransmitter. Agmatine is considered to play an important role for the development of schizophrenia. The aim of the present study is to explore the role of agmatine and L-arginine metabolism in medication-naive first-episode psychosis (FEP). We conducted a case control study in medication-naive patients with FEP ($n = 40$). The healthy volunteers with no family history of schizophrenia ($n = 35$) matched for age, gender and education level were selected as a control group. The patients were recruited to the study and followed up for 10 weeks. The plasma L-arginine, L-citrulline, L-ornithine and agmatine levels were measured using modified liquid chromatography/mass spectrometry. The plasma levels of L-arginine, L-citrulline and agmatine ($p < 0.0001$), but not L-ornithine and agmatinase ($p > 0.05$), were significantly increased during baseline analysis. After 10 weeks of treatment, plasma L-arginine and L-citrulline levels were still significantly increased ($p < 0.05$) while L-ornithine and agmatinase levels remained unchanged ($p > 0.05$). Conversely, plasma agmatine levels were significantly decreased after the treatment ($p < 0.0001$). Positive and negative predictive values of agmatine used for evaluating the diagnostic accuracy were 95.1% and 97.1%, respectively ($p < 000.1$). This is the first study of arginine metabolism and agmatine in medication-naive first-episode patients and provides evidence of increased levels of an endogenous NMDA antagonist which decreases following antipsychotic treatment.

1. Introduction

Schizophrenia is one of the most debilitating psychiatric disorders characterized by positive, negative and cognitive symptoms; it affects approximately 1% of the population worldwide (Perälä et al. 2007). Despite the tremendous human, social, and economic costs of the disease, there are no known biomarkers and the pathophysiological understanding of the disease is still incomplete. Currently, two hypotheses of physiopathology dominate the schizophrenia field. The dopamine hypothesis is mainly based on the serendipitous finding that all the antipsychotics have antagonistic activity on dopamine D2 receptors (D2R) to some extent and this activity is correlated with the treatment of positive symptoms of schizophrenia (Howes and Kapur 2009; Seeman 1987; Snyder 1976). However, D2R antagonists act as antipsychotics for many types of psychosis, which may be observed in a multitude of disease states and are not sufficiently useful for the negative and cognitive symptoms of schizophrenia (Goff et al. 2011; Remington et al. 2016). Dopamine agonists, on the other hand, may cause a temporary state of nonspecific psychosis clinically—mainly consisting of positive symptoms—but are not known to produce dominant negative or cognitive symptoms (Krystal et al. 2005).

The NMDA (*N*-methyl-D-aspartate) hypothesis of schizophrenia is based on the fact that antagonists of NMDA receptors (NMDARs), such as phencyclidine (PCP) and ketamine, produce a temporary psychotic state in otherwise healthy individuals which resembles the clinical picture of schizophrenia and includes positive, negative, and cognitive symptoms (Javitt and Zukin 1991; Krystal et al. 1994; Lodge and Mercier 2015; Moghaddam and Javitt 2012; Moghaddam and Krystal 2012). Therefore, NMDAR hypofunction was proposed as a component of schizophrenia pathophysiology. However, glutamate—the endogenous agonist for NMDAR—has not been shown to decrease in schizophrenia (Moghaddam and Javitt 2012). A straightforward explanation could be the presence of an endogenous NMDAR antagonist, especially one which increases or releases at times of distress. This may trigger a first psychotic episode and possibly the subsequent exacerbations of schizophrenia. Agmatine is one such neurotransmitter that is insufficiently studied in schizophrenia.

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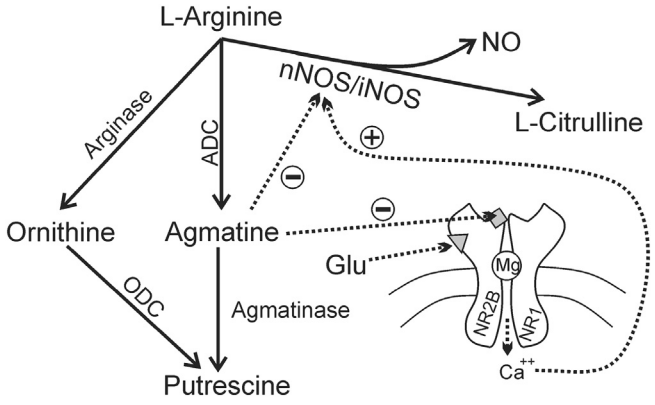

Fig. 1. L-Arginine pathway and its relationship to NMDA receptors. Agmatine can block NMDA receptor channel and inhibits its activity. Glu, glutamate; ADC, arginine decarboxylase; ODC, ornithine decarboxylase; nNOS, neuronal nitric oxide synthase; iNOS, inducible nitric oxide synthase; NO, nitric oxide; +, increased activity; −, decreased activity.

Agmatine is a product of arginine metabolism (see Fig. 1). Arginine is a conditionally essential amino acid; it becomes essential at times of rapid growth or at conditions which produce distress (Morris 2016). Agmatine is synthesized from L-arginine, stored in presynaptic vesicles and released in response to neurologic insults (Uzbay 2012). It inhibits both inducible and neuronal nitric oxide (NO) synthase to decrease the production of nitric oxide, so it potentially serves a neuroprotective function (Galea et al. 1996; Neis et al. 2017; Piletz et al. 2013). Agmatine is a selective and voltage-dependent inhibitor of NMDARs (Das et al. 1998; Krystal et al. 2005; Yang and Reis 1999). It was proposed to have antidepressant effects at certain doses, similar to ketamine (Freitas et al. 2016).

Previously, Uzbay et al. (2010a) showed that agmatine—one of the metabolites of L-arginine—disrupted prepulse inhibition (PPI) of the acoustic startle reflex in rats. PPI is a measure of the level of function of sensorimotor gating, and the disruption of PPI by using several treatments—including the administration of an NMDA antagonist such as ketamine—is a highly recognized animal model for schizophrenia. In a clinical study by Uzbay et al. (2013), they demonstrated a significantly increased plasma agmatine level in a small number of medication-free schizophrenia patients compared to matched controls. This is compatible with the post-mortem study and possibly reflects increased brain levels of agmatine, since it crosses the blood-brain barrier. Furthermore, the Uzbay et al. (2013) study suggested that agmatine could be used as a biomarker for schizophrenia. In a recent study, Liu et al. (2016) supported these findings by showing that the agmatine concentration was increased in the frontal lobe slices of the post-mortem brain tissue of chronic schizophrenia patients compared to the control group.

L-Arginine is metabolically interconvertible with the amino acids proline and glutamate, and it also serves as a precursor for the synthesis of protein, creatine, polyamines, citrulline, ornithine and urea (Morris 2007). A limited number of studies on L-arginine and its metabolites in psychosis and schizophrenia showed inconsistent results. Carl et al. (1992) compared schizophrenia patients with healthy controls and did not find any significant difference for 20 different tested amino acids, including arginine and ornithine. Rao et al. (1990) found that citrulline was increased in drug-free schizophrenia patients. Tomiya et al. (2007) did not show any difference for these three amino acid levels between the schizophrenia patients and controls but found that ornithine level was positively correlated with the length of the illness.

First-episode psychosis (FEP) presents a unique opportunity to study arginine metabolism and agmatine before the chronic disease and/or both direct and compensatory antipsychotic medication-induced effects set in. Therefore, we have designed this study to explore the role of agmatine and L-arginine metabolism in medication-naive FEP. We also followed the patients for 10 weeks after antipsychotic treatment was initiated to examine the effects of antipsychotic medications and/or recovery from psychosis on arginine metabolism. Agmatinase—the enzyme which catabolizes agmatine—was also measured in these samples.

2. Material and methods

2.1. Participants

This is a case control study conducted in medication naive patients with FEP ($n = 40$). A total of 81 patients were recruited for this study. During the follow-up period and at the beginning of the study, 40 of 81 patients were excluded from the study due to ineligibility, history of a previous psychotic episode and/or history of previous psychotic drug use. Forty-one patients were followed up for 10 weeks and the study was completed. One of these 41 patients was dropped from the study due to poor treatment compliance. Patients were diagnosed by using a semi-structured clinical interview (SCID) form for Axis 1 disorders from the Diagnostic and Statistical Manual of Mental Disorders (DSM) based on DSM-IV-TR criteria (Koroglu 2007). Psychotic symptoms and clinical conditions were evaluated by using the Scale for the Assessment of Positive Symptoms (SAPS) (Erkoc et al. 1991b), Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham 1988; Turkish version was prepared by Gulgun Yanbasti), Scale for the Assessment of Negative Symptoms (SANS) (Erkoc et al. 1991a), the Calgary Depression Scale for Schizophrenia (CDSS) (Oksay et al., 2000) and the Clinical Global Impressions (CGI) scale. The healthy volunteers with no family history of schizophrenia ($n = 35$) matched for age, gender and education level were selected as a control group and all the healthy subjects were evaluated and recruited to the study by the same clinician (BG). We have defined the first psychotic episode as the presence of a moderate level of positive symptoms that disrupt functioning for a minimum of one week but not more than one month in the absence of predominant mood symptoms. Patients who were exposed to any antipsychotic medications, any substance or drugs or previously diagnosed with mental retardation, organic brain disorders (e.g. confounding factors such as infection, inflammation, cancer, trauma, bleeding, etc.), major medical illness, pre-episode psychotic experiences or current predominant mood disorders were excluded.

Blood tests for agmatine, arginine, citrulline, ornithine and agmatinase were obtained at baseline and after 10 weeks of antipsychotic medication administration. The treatment of the FEP patients was independently selected by the psychiatrists at Gulhane Military Medical Academy. The local institutional ethical board at Kecioren Research and Training Hospital approved this study (December 12th 2012, #177 & October 15th 2014, #659). Written informed consent was obtained from all participants.

2.2. Measurement of agmatine, L-arginine, L-citrulline and L-ornithine levels

The plasma L-arginine, L-citrulline, L-ornithine and agmatine levels were measured by using a modified liquid chromatography/mass spectrometric (LC-MS/MS) method (Zhou et al. 2012). The separation was performed using an LC20AC HPLC system (Shimadzu) equipped with an autosampler, two solvent pumps and a column oven. The chromatography column was a Zorbax HILIC column (3.5 μm, 3 × 15 mm, Agilent). The column temperature was set at 30 °C. The mobile phase A consisted of ammonium formate (40 mM) and formic acid (0.2%); the mobile phase B consisted of acetonitrile. The mobile phase B concentration was gradually decreased from 75% to 35% between 2 and 4.5 min. The samples (3 μL) were delivered at a flow rate of 0.7 mL/min. The ionized analytes by electrospray ionization (ESI) in positive ion mode were analyzed by using a triple quadrupole MS (Shimadzu 8030) with a source temperature of 400 °C. The transitions (m/z) for the analytes were: L-arginine, 175.2/43.0, 175.2/70.2; L-

Table 1
Clinical assessment scores of the patients before and after antipsychotic treatment for 10 weeks.

	Before treatment	After treatment	Change (%)
SANS score	34.05 ± 2.03	46.10 ± 3.49*	36.40
SAPS score	65.23 ± 3.29	25.62 ± 2.69**	−60.50
BPRS score	53.50 ± 1.72	28.85 ± 2.15**	−46.07
CGI score	5.50 ± 0.10	3.37 ± 0.13#	−38.61
CDSS score	3.69 ± 0.70	2.56 ± 0.48	−32.29

Values for the clinical assessment scores were represented as mean ± S.E.M. For the details of the clinical assessment tests please see text (*$p < 0.001$ and **$p < 0.0001$, Student's *t*-test for paired samples; #$p < 0.0001$, Wilcoxon signed-rank test).

citrulline, 176.2/159.3, 176.2/113.0; L-ornithine, 133.2/116.1, 133.2/70.3; and agmatine, 130.1/72.0, 130.1/55.2.

2.3. Measurement of plasma agmatinase levels

The amount of agmatinase in the plasma was determined by using a commercial competitive inhibition enzyme immunoassay (ELISA) kit according to the manufacturer's instructions (Catalog No: CK-E90998, Hangzhou Eastbiopharm Co., Ltd., PRC). A volume of 100 μL of the samples was used for the assay. The absorbance was measured at 450 nm wavelength. The dynamic range of the assay was between 16 and 700 pg/mL. The assay was performed by an experienced staff blinded to the study groups.

2.4. Statistical analyses

Statistical analyses were performed using the SPSS v.17 (IBM Corp., Armonk, NY) and GraphPad Prism v.5.01 (GraphPad Software Inc., San Diego, CA) software. The data were represented as the mean ± standard error of mean (SEM). The scores obtained from clinical scales such as SAPS, BPRS, and SANS were compared before and after treatment using Student's *t*-test for paired samples; the CGI and CDSS scores were compared using Wilcoxon signed-rank test. The plasma L-arginine, L-citrulline, L-ornithine, agmatine and agmatinase levels of the healthy controls and of the patients with schizophrenia were compared using Student's *t*-test. Their levels before and after antipsychotic treatment were compared using Student's *t*-test for paired samples. A decrease in the BPRS score >40% was used to indicate a treatment response. The biochemical variables were compared between treatment responders and non-responders using Student's *t*-test.

Kolmogorov-Smirnov tests were performed, and the histograms were visually inspected to analyze the normal distribution of the variables. Correlations among biochemical variables and clinical scales were analyzed by using the Pearson test. Several multiple linear regression models with backward selection were used to identify independent biochemical predictors of the clinical symptom scales during baseline and during follow-up. The capacity of the measured biochemical variables to predict the presence of FEP was analyzed using a Receiver Operating Characteristic (ROC) curve analysis. A significant cut-off value was determined where the sensitivity and the specificity reached the highest values in ROC analysis. Then the sensitivity, specificity, and positive and negative predictive values were presented. The statistical significance was set at $p < 0.05$.

3. Results

The results of the clinical assessment scores are presented in Table 1. The patients were followed up at an inpatient unit for 3 weeks, then they were discharged. The patients were treated with mainly two antipsychotic drugs, namely risperidone (35% of the patients) and olanzapine (33% of the patients). All of the patients were treated with a single antipsychotic drug, and the treatment regime was not changed during the 10 weeks of follow-up. According to age, sex and education levels, there were no significant differences between patients and healthy control groups. The average age in patients and control groups was 21.97 ± 0.49 and 22.37 ± 0.32 years, respectively. Clinical assessment scores and percent change of these scores before and after antipsychotic treatment for 10 weeks were evaluated in patients diagnosed with FEP. After the treatment, positive psychotic symptoms (SAPS) and general psychiatric symptoms (BPRS, CGI) had shown significant improvement; the negative symptoms scale (SANS) worsened significantly after 10 weeks of treatment (Table 1). The depressive symptoms (CDSS score) showed a trend toward decreasing but did not reach a statistically significant level (Table 1).

The plasma levels of L-arginine, L-citrulline and agmatine ($p < 0.0001$), but not L-ornithine and agmatinase ($p > 0.05$), significantly increased during the baseline analysis (Fig. 2). After 10 weeks of treatment, plasma L-arginine and L-citrulline levels were significantly increased ($p < 0.05$ and $p < 0.0001$, respectively) while L-ornithine and agmatinase levels remained unchanged ($p > 0.05$) (Fig. 2). On the other hand, the plasma agmatine level was significantly decreased after treatment ($p < 0.0001$) (Fig. 2).

According to the 40% decrease in the BPRS score, 25 of the 40 patients (62.5%) were accepted into the treatment responder group (Sanger et al. 1999). The plasma L-arginine level after 10 weeks of treatment and agmatinase levels during baseline and after treatment were significantly decreased in the treatment non-responder group compared with the treatment responder group (*p* values < 0.05) (Fig. 3).

Pearson's correlation analyses indicated that initial L-arginine levels were positively correlated with the initial SANS score ($r = 0.48$, $p < 0.01$) (Fig. 4A) and negatively correlated with the initial SAPS score ($r = -0.40$, $p < 0.01$) (Fig. 4B). The L-arginine level after treatment was negatively correlated with the follow-up BPRS score ($r = -0.36$, $p < 0.05$) (Fig. 4C). The initial L-citrulline levels were negatively correlated with the initial SAPS score ($r = -0.41$, $p < 0.01$) (Fig. 5A) and were also negatively correlated with the follow-up SANS, SAPS and BPRS scores ($r = -0.37$, -0.35, and -0.32, respectively, *p* values < 0.05) (Fig. 5B–D). The follow-up L-citrulline level was negatively correlated

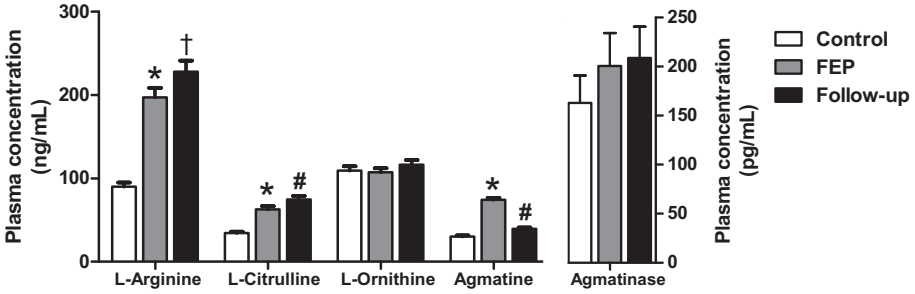

Fig. 2. Mean plasma levels of the biochemical variables in healthy controls, first episode psychosis (FEP) patients and during the follow-up after 10 weeks of antipsychotic treatment (*$p < 0.0001$, Student's *t*-test; #$p < 0.0001$ and †$p < 0.05$, Student's *t*-test for paired samples).

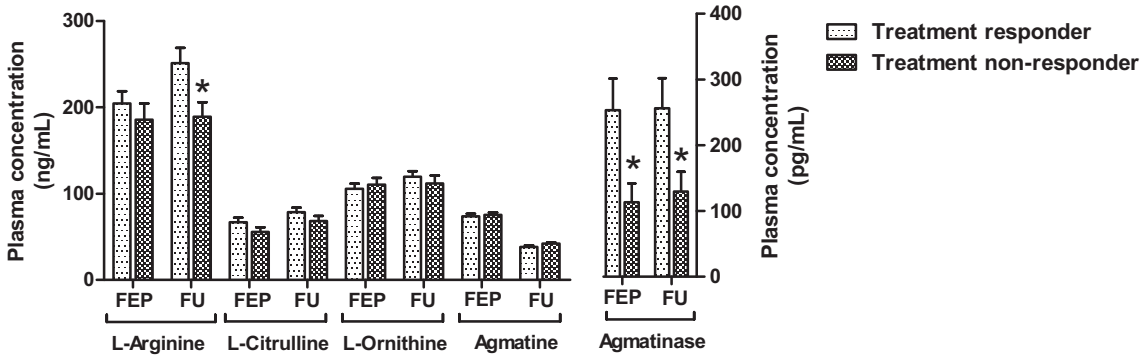

Fig. 3. Mean plasma levels of the biochemical variables in the treatment responder and non-responder patients. A decrease in the BPRS score of >40% during the follow-up (FU) after 10 weeks from the first episode psychosis (FEP) was used to indicate treatment response (*$p < 0.05$, Student's *t*-test).

with the SANS and SAPS scores during follow-up ($r = -0.41$ and -0.42, respectively, p values < 0.01) (Fig. 5E, F). The initial plasma agmatine level was negatively correlated with the SANS and SAPS scores evaluated at the end of 10 weeks ($r = -0.43$ and $r = -0.36$, respectively, $p < 0.05$) (Fig. 6A, B).

The variables were normally distributed except for agmatinase levels ($p > 0.05$, Kolmogorov-Smirnov test), and these variables were included in the multiple linear regression analysis. The main biochemical predictors for the SAPS, BPRS and SANS scores during baseline and follow-up, as well as predictors of the follow-up clinical scores during the baseline analysis, were presented in Table 2.

The ROC curve analysis was used to assess the diagnostic accuracy of the plasma L-arginine, L-citrulline and agmatine levels. When a comparison was made between FEP patients and healthy controls, the area under the curve values—with 95% confidence intervals (CIs)—proposed cut-off values at optimal sensitivity and specificity levels and positive and negative predictive values (see Table 3). The ROC curves and individual distributions of the plasma L-arginine, L-citrulline and agmatine levels are shown in Fig. 7A–C.

4. Discussion

Our results showed that plasma L-arginine and its metabolites, L-citrulline and agmatine, increased in FEP patients compared to healthy controls. After 10 weeks of antipsychotic treatment, L-arginine and L-citrulline were still elevated while agmatine plasma levels decreased compared to healthy control groups. The plasma levels of L-ornithine and agmatinase did not show any differences either at the beginning or at the end of the treatment. The results regarding increased plasma agmatine level in FEP is consistent with a previous finding in drug-free chronic schizophrenia patients (Uzbay et al. 2013). Furthermore, we showed that agmatine, but not L-arginine and L-citrulline levels, decreased after 10 weeks of antipsychotic treatment.

Plasma agmatine in psychiatric disorders is not well studied. Halaris et al. (1999) showed that plasma agmatine levels were increased in untreated depression patients and decreased after 8 weeks of bupropion treatment. The same research group reported that venlafaxine returned the increased agmatine level to the baseline level after four weeks of treatment (Halaris and Plietz, 2007). The changes seen in plasma agmatine levels in FEP or depressive disorders may be in response to stressful situations. Increased agmatine levels may be related to protective factors or a natural response to stress. Agmatine is released in response to stressful events such as immobilization (Zhu et al. 2008) and hypoxia (Kim et al. 2004). Agmatine has neuroprotective and stress-relieving effects under these conditions, and it is suggested to be an endogenous antidepressant (Halaris and Plietz 2007; Piletz et al. 2013).

On the other hand, increased plasma agmatine level could be a trigger factor for developing psychotic symptoms caused by an antagonism

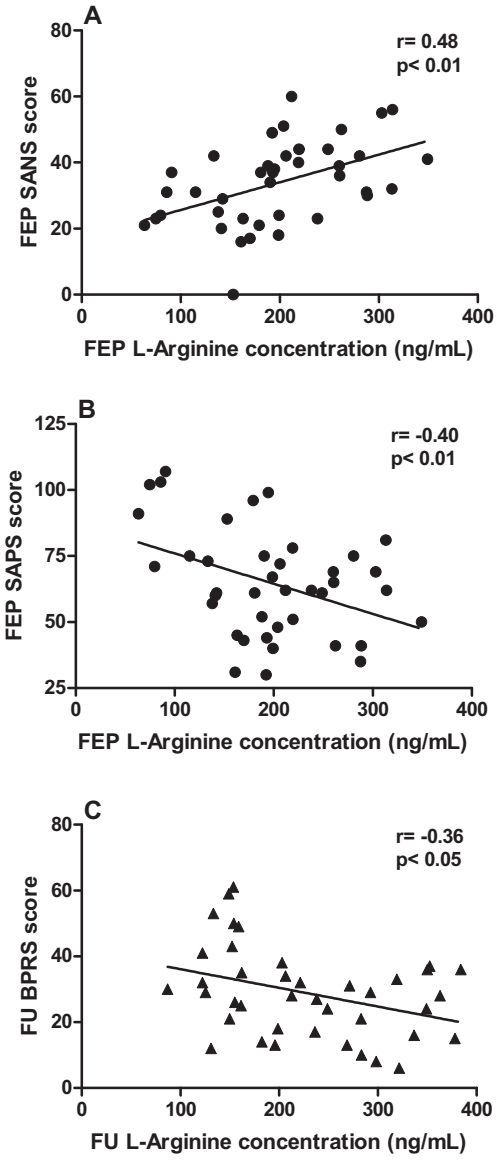

Fig. 4. Plasma L-arginine levels correlated with SANS (A) and SAPS (B) scores during first-episode psychosis (FEP); there was a correlation between plasma L-arginine level and the BPRS score (C) during follow-up (FU). Please see text for the explanation of symptom scales (r, Pearson's correlation coefficient).

of NMDARs (Yang and Reis 1999). The glutamatergic theory of schizophrenia is based on the findings that NMDAR antagonists such as PCP and ketamine induce schizophrenia-like symptoms. Studies of glutamatergic models have shown that NMDA dysfunction on pre- and postsynaptic glutamate receptors may be the trigger inducing schizophrenia-like symptoms—including negative and cognitive symptoms—by increasing synaptic activity of glutamate primarily in the prefrontal cortex (Javitt 2010). On the other hand, endogenous NMDA antagonists such as agmatine may play a role in the pathophysiology of schizophrenia and the onset of psychotic disorders alone or in interactions with other neurotransmitters found in the CNS. The disruptive effects of agmatine at high doses (e.g. 160 mg/kg) on PPI of the startle reflex in rats may support the hypothesis of agmatine having an NMDA antagonism function (Uzbay et al. 2010a).

As indicated by the ROC analysis (Table 3 and Fig. 7), measuring plasma agmatine levels during an acute episode is a strong tool for discriminating patients from healthy controls. In addition, the levels of agmatine in FEP negatively correlated with the follow-up SAPS and SANS scores (Fig. 6). This indicates that patients with higher agmatine levels at the beginning of treatment had less severe positive and negative symptoms after 10 weeks of treatment. Regression analysis also showed that agmatine level for FEP is the only predictor for follow-up SANS scores and one of the predictors for follow-up SAPS scores (Table 3). Thus, agmatine may have a protective effect, and the high agmatine level at baseline may be associated with improved clinical findings at the end of 10 weeks of treatment.

The agmatine-catabolizing enzyme agmatinase is believed to be the rate-limiting enzyme responsible for regulating the agmatine level in the brain (Sastre et al. 1996). In the present study, plasma agmatinase levels were similar in the control and patient groups; however, treatment responder patients—according to a decrease in BPRS score of >40%—had significantly more agmatinase than non-responders and this difference did not change with the treatment. Agmatine levels were similar in treatment responder and non-responder patients before

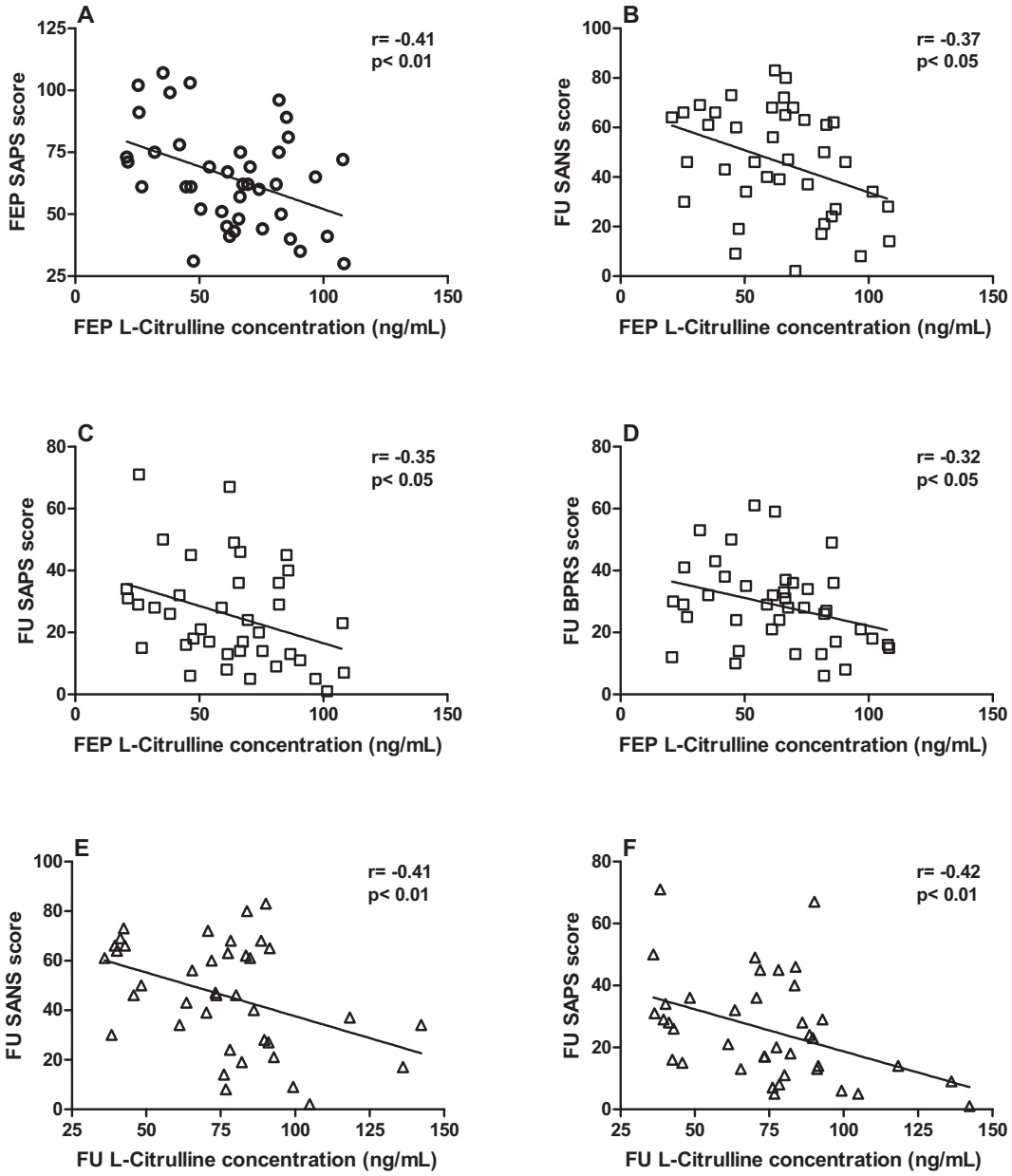

Fig. 5. Plasma L-citrulline levels correlated with SAPS score (A) during first-episode psychosis (FEP). Plasma L-citrulline levels measured during FEP correlated with SANS (B), SAPS (C) and BPRS (D) scores assessed during follow-up (FU). Plasma L-citrulline levels measured during FU correlated with SANS (E) and SAPS (F) scores assessed consecutively. Please see text for the explanation of symptom scales (r, Pearson's correlation coefficient).

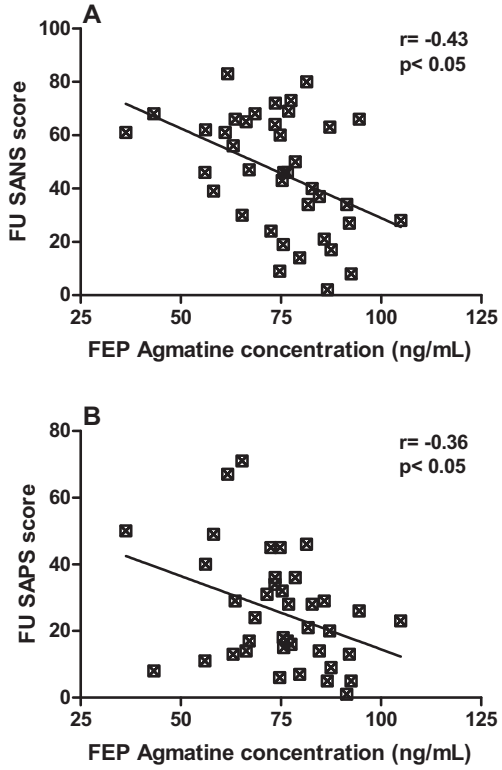

Fig. 6. Plasma agmatine levels measured during first-episode psychosis (FEP) correlated with SANS (A) and SAPS (B) scores assessed during follow-up (FU). Please see text for the explanation of symptom scales (r, Pearson's correlation coefficient).

and after treatment. The observed difference in agmatinase may be related to the downward regulation of the polyamine system instead of agmatine metabolism. However, we did not measure the subsequent metabolites of agmatine, and so we cannot test this possibility in our current data set. Moreover, the other enzymes catabolizing L-arginine, such as arginase, arginine decarboxylase (ADC) and nitric oxide synthase (NOS) may play important roles in regulating the L-arginine and the amino acid levels derived from L-arginine in humans. Under certain conditions, these enzymes may compete to direct the metabolite production toward a specific pathway (Morris 2007). To our knowledge there is no report on how these enzymes are regulated in FEP and schizophrenia patients; the present study showed significant changes in the plasma levels of L-arginine and its derived amino acids, but the enzyme regulation of these pathways remains to be investigated.

Table 2
The statistically significant models from multiple regression analyses in relation to clinical assessment scores during the first episode psychosis (FEP) and follow-up after 10 weeks of antipsychotic treatment.

Clinical Scores	Predictors	B	SE	β	t	*p*-Value	R^2
FEP	*FEP*						
SANS	L-Arginine	0.121	0.031	0.688	3.941	<0.0001	0.30
	L-Citrulline	−0.181	0.091	−0.346	−1.982	=0.05	
SAPS	L-Citrulline	−0.344	0.125	−0.406	−2.741	=0.009	0.17
BPRS	–	–	–	–	–	–	–
Follow-up	*Follow-up*						
SANS	L-Citrulline	−0.353	0.130	−0.408	−2.717	=0.010	0.17
SAPS	L-Citrulline	−0.272	0.097	−0.420	−2.819	=0.008	0.18
BPRS	L-Arginine	−0.057	0.024	−0.355	−2.344	=0.024	0.13
Follow-up	*FEP*						
SANS	Agmatine	−0.672	0.232	−0.430	−2.899	=0.006	0.19
SAPS	Agmatine	−0.314	0.180	−0.258	−1.740	0.091	0.32
	L-Citrulline	−0.237	0.105	−0.341	−2.251	0.031	
	L-Ornithine	0.211	0.080	0.377	2.644	0.012	
BPRS	L-Citrulline	−0.181	0.087	−0.320	−2.085	=0.044	0.10

Please see text for the explanation of symptom scales; B, unstandardized beta; SE, standard error for the B; β, standardized beta; t, *t*-test statistics; R^2, regression coefficient.

Previous reports investigating the levels of amino acids in the plasma or brain in schizophrenia mainly focused on measuring the levels of L-glutamate, homocysteine, proline, L-tryptophan, L-serine (De Luca et al. 2008) and D-serine (Kantrowitz et al. 2015). Rao et al. (1990) showed that L-arginine increased in drug-naive and treated schizophrenia patients, while L-citrulline increased only in patients under treatment with typical antipsychotics. Our results with L-arginine and L-citrulline levels extend these findings to medication-naive first-episode patients. Also, as indicated by the ROC analysis (Table 3 and Fig. 7), measuring L-arginine and L-citrulline levels during acute episodes is a useful method for discriminating patients from healthy controls.

In schizophrenia literature, L-arginine and L-citrulline have been mentioned often because of their role in NO metabolism. In the present study, because L-ornithine level remained unchanged, the increase in L-arginine may be responsible for the increase in the metabolites agmatine and L-citrulline. We showed that L-arginine and L-citrulline levels were elevated both pre- and post-treatment, and NO is produced as a byproduct during L-arginine and L-citrulline conversion. Moreover, after the decrease in agmatine with the treatment, increased levels of L-citrulline seen during follow-up would be primarily connected to L-arginine. Thus, a concurrent increase in the production of NO is highly expected. We did not directly measure NO or nitrite and nitrate levels in the present study, but our results indirectly support the previous studies indicating the role of NO in FEP and schizophrenia.

The reasons for increased metabolism of L-arginine remains unclear, and at least two distinct possibilities can be discussed. First, this increase may cause the psychosis and subsequent pathologies. Recent publications emphasize the role of inflammation and oxidative stress in the pathogenesis of schizophrenia, emphasizing upregulated inflammatory status reflected by elevated levels of IL-1beta, IL-6, and TNF-alpha (Bergink et al. 2014). A decrease in total antioxidant capacity was also proposed as an important factor in early-onset FEP (Micó et al. 2011) and symptom severity in schizophrenia patients (Pazvantoglu et al. 2009). NO is one of the primary signaling molecules mediating the inflammatory response. It causes an anti-inflammatory effect under normal physiological conditions, but it is also considered to be a pro-inflammatory mediator when it is produced excessively (Sharma et al. 2007). Thus, disturbances in its modulation might cause pathologies at cellular and behavioral levels.

Second, increases in L-arginine metabolism may originate from a compensatory mechanism. Glutamatergic hypofunction is a prominent theory in schizophrenia pathogenesis (Coyle 2012). NO is a second messenger for NMDARs and the NO concentration reflects the activity level of glutamatergic neurotransmitters (Nasyrova et al. 2015). L-Arginine metabolism may be stimulated to compensate for abnormal glutamatergic function. Hallak et al. (2013) showed in a randomized, placebo-controlled study that intravenous infusion of sodium nitroprusside, a NO donor in low doses, to schizophrenia patients significantly improved the symptoms (Hallak et al. 2013). This effect appeared after 2 h and persisted for four weeks.

Our results mostly support the second explanation, based on the correlation between the symptom scales and the L-arginine and L-citrulline levels. Our analyses revealed that all the significant correlations were in the negative direction except for the positive correlation between L-arginine and the SANS score at FEP (Figs. 4 and 5). This means that patients with higher levels of L-arginine and L-citrulline during baseline and follow-up had less severe psychotic symptoms. Additionally, regression analysis revealed that L-arginine and L-citrulline levels could predict the concurrent SAPS, BPRS, and SANS scores, and initial L-citrulline level was the predictor for the follow-up BPRS score and one of the predictors for the follow-up SAPS score (Table 3).

Table 3
The capacity of the variables in predicting first episode psychosis.

	AUC ± S.E.M. (95% CI)	*p*-Value	Proposed cut-off value	Sensitivity% (95% CI)	Specificity% (95% CI)	Positive predictive value (%)	Negative predictive value (%)
L-Arginine	0.90 ± 0.037 (0.832–0.976)	<0.0001	127.6 ng/mL	85.00 (70.16–94.29)	91.43 (76.94–98.20)	91.9	84.2
L-Citrulline	0.84 ± 0.048 (0.746–0.935)	<0.0001	44.6 ng/mL	77.50 (61.55–89.16)	85.71 (69.74–95.19)	86.1	76.9
L-Ornithine	0.52 ± 0.067 (0.384–0.648)	>0.05	–	–	–	–	–
Agmatine	0.99 ± 0.006 (0.982–1.005)	<0.0001	41.3 ng/mL	97.50 (86.84–99.94)	94.29 (80.84–99.30)	95.1	97.1
Agmatinase	0.56 ± 0.067 (0.433–0.695)	>0.05	–	–	–	–	–

AUC, area under the curve; CI, confidence interval.

The present study has several limitations. Our results were only obtained from male subjects. This situation limits the generalization of the findings to the whole population. The sexual hormones can modulate many of the metabolic pathways, including those of the amino acids (Comitato et al. 2015). Generally, men and women have discrepancies in their protein metabolism processes (Markofski and Volpi 2011). L-

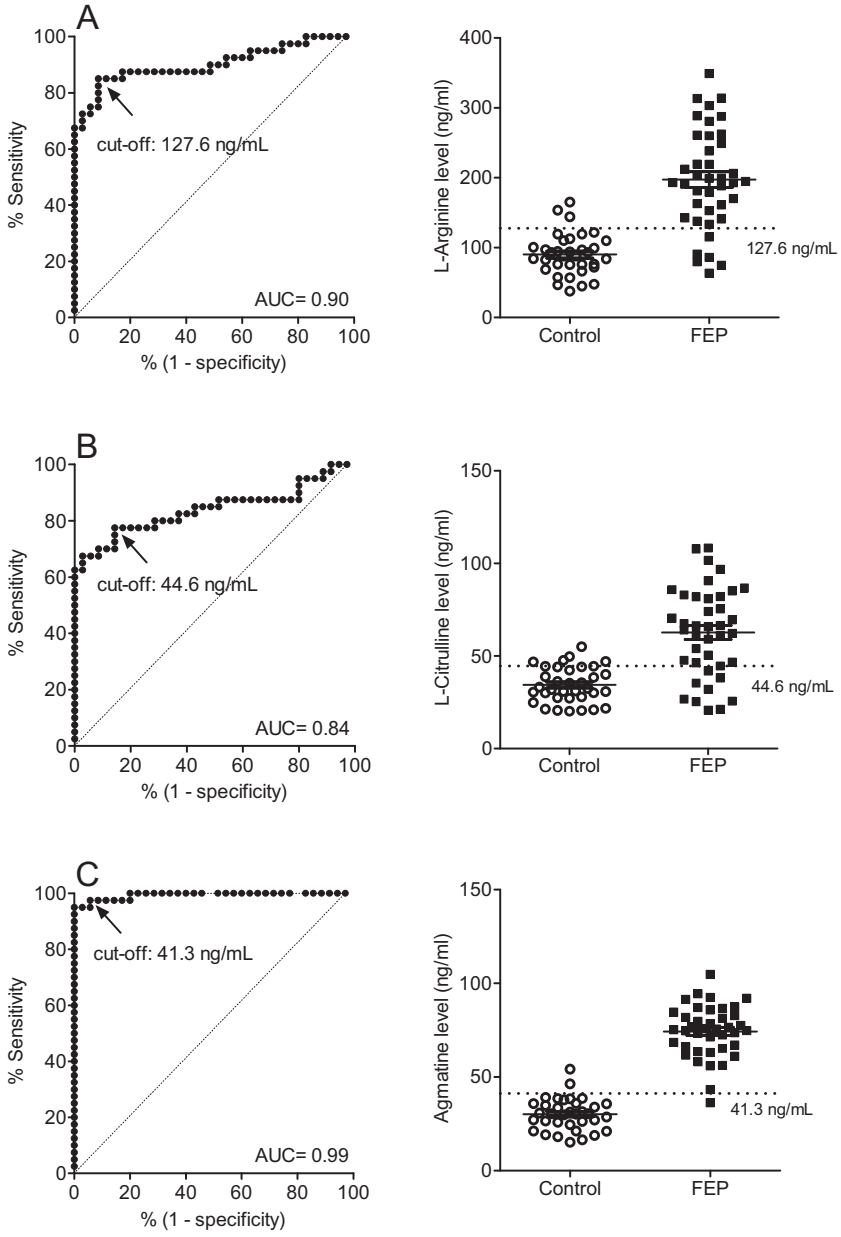

Fig. 7. The receiver-operator characteristic (ROC) curve (left panel) and the individual distribution of plasma levels (right panel) of L-arginine (A), L-citrulline (B) and agmatine (C) in healthy controls and in first-episode psychosis (FEP) patients (AUC, area under the curve).

Arginine metabolism in particular was investigated for gender differences because of its role in cardiovascular system, and women were found to have some advantages in utilizing L-arginine and producing more NO in their vascular systems (Forte et al. 1998). Also, animal studies pointed out several different effects of agmatine in males and females (Uzbay et al. 2010b). On the other hand, using male subjects in the present study may positively contribute to our results by decreasing variability.

In the present study, amino acid levels were measured in the peripheral blood; thus, how or at what grade they reflect the metabolism of the central nervous system could be assessed. However, collecting peripheral blood samples is the less invasive and more practical way to determine metabolic changes. Alternatively, cerebrospinal fluid (CSF) from the lumbar area could have been sampled and screened, but a similar problem would still exist; CSF represents the brain metabolism when it is collected from brain ventricles or posterior fossa, not from the lumbar area (Segal et al. 1990).

Another limitation of the present study was the short follow-up duration for the patients. After the 10 weeks of follow-up, we observed significant improvements in several symptom scales—such as SAPS and BPRS—but we also observed that negative symptoms (SANS) worsened. Moreover, we only observed a significant decrease in agmatine during the follow-up, but it did not significantly correlate with any of the symptom scales. One of the possible explanations for this finding is that a 10-week duration was insufficient for observing the changes in clinical manifestations derived from the changes in agmatine level. Longer follow-up durations with multiple measurement points are needed to better show the clinical progress of the FEP patients.

Endogenous NMDA antagonists may play an important role in the pathophysiology of schizophrenia. The exact mechanism is not well understood, but agmatine is thought to be a new neuromodulator and neurotransmitter in the CNS and is described as an endogenous NMDA antagonist. Since NMDA antagonism produces schizophrenia-like symptoms in preclinical and clinical studies (Krystal et al. 1994; Moghaddam and Krystal 2012), agmatine may be related to NMDA dysfunction and the production of psychotic symptoms. Agmatine may also be used as a diagnostic tool to differentiate patients from healthy individuals. Future studies are needed to investigate brain levels of agmatine and its relation to NMDARs as well as symptoms.

Conflict of interest

The authors have no potential conflicts of interest to declare.

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CRediT authorship contribution statement

Beyazit Garip: Conceptualization, Data curation, Funding acquisition, Methodology, Writing - original draft, Writing - review & editing. **Hakan Kayir:** Conceptualization, Formal analysis, Methodology, Project administration, Writing - original draft, Writing - review & editing. **Ozcan Uzun:** Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Writing - original draft.

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References

Bergink, V., Gibney, S.M., Drexhage, H.A., 2014. Autoimmunity, inflammation, and psychosis: a search for peripheral markers. Biol. Psychiatry 75 (4), 324–331.
Carl, G.F., Brogan, M.P., Young, B.K., 1992. Is plasma serine a marker for psychosis? Biol. Psychiatry 31 (11), 1130–1135.
Comitato, R., Saba, A., Turrini, A., Arganini, C., Virgili, F., 2015. Sex hormones and macronutrient metabolism. Crit. Rev. Food Sci. Nutr. 55 (2), 227–241.
Coyle, J.T., 2012. NMDA receptor and schizophrenia: a brief history. Schizophr. Bull. 38 (5), 920–926.
Das, I., Ramchand, C.N., Gliddon, A., Hirsch, S.R., 1998. Nitric oxide, free radicals and polyamines may have a role in the membrane pathology of schizophrenia. Neuropsychobiology 37 (2), 65–67.
De Luca, V., Viggiano, E., Messina, G., Viggiano, A., Borlido, C., Viggiano, A., Monda, M., 2008. Peripheral amino acid levels in schizophrenia and antipsychotic treatment. Psychiatry Investig. 5 (4), 203–208.
Erkoc, S., Arkonca, O., Ataklı, C., Ozmen, E., 1991a. Negatif semptomlari degerlendirme olceginin güvenirliği ve gecerliligi. Dusunan Adam 4 (2), 14–15.
Erkoc, S., Arkonca, O., Ataklı, C., Ozmen, E., 1991b. Pozitif semptomlari degerlendirme olceginin güvenirliği ve gecerliligi. Dusunan Adam 4 (2), 20–24.
Forte, P., Kneale, B.J., Milne, E., Chowienczyk, P.J., Johnston, A., Benjamin, N., Ritter, J.M., 1998. Evidence for a difference in nitric oxide biosynthesis between healthy women and men. Hypertension 32 (4), 730–734.
Freitas, A.E., Neis, V.B., Rodrigues, A.L.S., 2016. Agmatine, a potential novel therapeutic strategy for depression. Eur. Neuropsychopharmacol. 26 (12), 1885–1899.
Galea, E., Regunathan, S., Eliopoulos, V., Feinstein, D.L., Reis, D.J., 1996. Inhibition of mammalian nitric oxide synthases by agmatine, an endogenous polyamine formed by decarboxylation of arginine. Biochem. J. 316 (Pt 1), 247–249.
Goff, D.C., Hill, M., Barch, D., 2011. The treatment of cognitive impairment in schizophrenia. Pharmacol. Biochem. Behav. 99 (2), 245–253.
Halaris, A., Plietz, J., 2007. Agmatine: metabolic pathway and spectrum of activity in brain. CNS Drugs 21 (11), 885–900.
Halaris, A., Zhu, H., Feng, Y., Piletz, J.E., 1999. Plasma agmatine and platelet imidazoline receptors in depression. Annals of the New York Academy of Sciences. 881, pp. 445–451.
Hallak, J.E.C., Maia-de-Oliveira, J.P., Abrao, J., Evora, P.R., Zuardi, A.W., Crippa, J.A.S., Belmonte-de-Abreu, P., Baker, G.B., Dursun, S.M., 2013. Rapid improvement of acute schizophrenia symptoms after intravenous sodium nitroprusside: a randomized, double-blind, placebo-controlled trial. JAMA Psychiatry 70 (7), 668–676.
Howes, O.D., Kapur, S., 2009. The dopamine hypothesis of schizophrenia: version III-the final common pathway. Schizophr. Bull. 35 (3), 549–562.
Javitt, D.C., 2010. Glutamatergic theories of schizophrenia. Isr. J. Psychiatry Relat. Sci. 47 (1), 4–16.
Javitt, D.C., Zukin, S.R., 1991. Recent advances in the phencyclidine model of schizophrenia. Am. J. Psychiatry 148 (10), 1301–1308.
Kantrowitz, J.T., Woods, S.W., Petkova, E., Cornblatt, B., Corcoran, C.M., Chen, H., Silipo, G., Javitt, D.C., 2015. D-Serine for the treatment of negative symptoms in individuals at clinical high risk of schizophrenia: a pilot, double-blind, placebo-controlled, randomised parallel group mechanistic proof-of-concept trial. Lancet Psychiatry 2 (5), 403–412.
Kim, J.H., Yenari, M.A., Giffard, R.G., Cho, S.W., Park, K.A., Lee, J.E., 2004. Agmatine reduces infarct area in a mouse model of transient focal cerebral ischemia and protects cultured neurons from ischemia-like injury. Exp. Neurol. 189 (1), 122–130.
Koroglu, E., 2007. American Psychiatric Association. (2000). Diagnostic and Statistical Manual of Mental Disorders – Turkish. 4th ed. (text rev.).
Krystal, J.H., Karper, L.P., Seibyl, J.P., Freeman, G.K., Delaney, R., Bremner, J.D., Heninger, G.R., Bowers, M.B., Charney, D.S., 1994. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans: psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch. Gen. Psychiatry 51 (3), 199–214.
Krystal, J.H., Perry, E.B., Gueorguieva, R., Belger, A., Madonick, S.H., Abi-Dargham, A., Cooper, T.B., MacDougall, L., Abi-Saab, W., D'Souza, D.C., 2005. Comparative and interactive human psychopharmacologic effects of ketamine and amphetamine: implications for glutamatergic and dopaminergic model psychoses and cognitive function. Arch. Gen. Psychiatry 62 (9), 985–994.
Liu, P., Jing, Y., Collie, N.D., Dean, B., Bilkey, D.K., Zhang, H., 2016. Altered brain arginine metabolism in schizophrenia. Transl. Psychiatry 6 (64), e871–e879.
Lodge, D., Mercier, M.S., 2015. Ketamine and phencyclidine: the good, the bad and the unexpected. Br. J. Pharmacol. 172 (17), 4254–4276.
Markofski, M.M., Volpi, E., 2011. Protein metabolism in women and men: similarities and disparities. Curr. Opin. Clin. Nutr. Metab. Care 14 (1), 93–97.
Micó, J.A., Rojas-Corrales, M.O., Gibert-Rahola, J., Parellada, M., Moreno, D., Fraguas, D., Graell, M., Gil, J., Irazusta, J., Castro-Fornieles, J., Soutullo, C., Arango, C., Otero, S., Navarro, A., Baeza, I., Martínez-Cengotitabengoa, M., González-Pinto, A., 2011. Reduced antioxidant defense in early onset first-episode psychosis: a case-control study. BMC Psychiatry 11 (1), 26.
Moghaddam, B., Javitt, D., 2012. From revolution to evolution: the glutamate hypothesis of schizophrenia and its implication for treatment. Neuropsychopharmacology 37 (1), 4–15.
Moghaddam, B., Krystal, J.H., 2012. Capturing the angel in "angel dust": twenty years of translational neuroscience studies of NMDA receptor antagonists in animals and humans. Schizophr. Bull. 38 (5), 942–949.
Morris, S.M., 2007. Arginine metabolism: boundaries of our knowledge. J. Nutr. 137 (6 Suppl 2), 1602S–1609S.
Morris, S.M., 2016. Arginine metabolism revisited. J. Nutr. 146 (12), 2579S–2586S.
Nasyrova, R.F., Ivashchenko, D.V., Ivanov, M.V., Neznanov, N.G., 2015. Role of nitric oxide and related molecules in schizophrenia pathogenesis: biochemical, genetic and clinical aspects. Front. Physiol. 6, 139.
Neis, V.B., Rosa, P.B., Olescowicz, G., Rodrigues, A.L.S., 2017. Therapeutic potential of agmatine for CNS disorders. Neurochem. Int. 108, 318–331.
Oksay, S.E., Aksaray, G., Ve Kaptanoglu, C., 2000. Calgary Depresyon Olceginin sizofreni hastalarında gecerlik ve guvenirlilik çalışması. Türk Psikiyatri Dergisi 11 (4), 278–284.
Overall, J.E., Gorham, D.R., 1988. The brief psychiatric rating scale (BPRS): recent developments in ascertainment and scaling. Psychopharmacol. Bull. 24, 97–99.

Pazvantoglu, O., Selek, S., Okay, I.T., Sengul, C., Karabekiroglu, K., Dilbaz, N., Erel, Ozcan, 2009. Oxidative mechanisms in schizophrenia and their relationship with illness subtype and symptom profile. Psychiatry Clin. Neurosci. 63 (5), 693–700.
Perälä, J., Suvisaari, J., Saarni, S.I., Kuoppasalmi, K., Isometsä, E., Pirkola, S., Partonen, T., Tuulio-Henriksson, A., Hintikka, J., Kieseppä, T., Härkänen, T., Koskinen, S., Lönnqvist, J., 2007. Lifetime prevalence of psychotic and bipolar I disorders in a general population. Arch. Gen. Psychiatry 64 (1), 19.
Piletz, J.E., Aricioglu, F., Cheng, J.T., Fairbanks, C.A., Gilad, V.H., Haenisch, B., Halaris, A., Hong, S., Lee, J.E., Li, J., Liu, P., Molderings, G.J., Rodrigues, A.L.S., Satriano, J., Seong, G.J., Wilcox, G., Wu, N., Gilad, G.M., 2013. Agmatine: clinical applications after 100 years in translation. Drug Discov. Today 18 (17–18), 880–893.
Rao, M.L., Gross, G., Strebel, B., Bräunig, P., Huber, G., Klosterkötter, J., 1990. Serum amino acids, central monoamines, and hormones in drug-naive, drug-free, and neuroleptic-treated schizophrenic patients and healthy subjects. Psychiatry Res. 34 (3), 243–257.
Remington, G., Foussias, G., Fervaha, G., Agid, O., Takeuchi, H., Lee, J., Hahn, M., 2016. Treating negative symptoms in schizophrenia: an update. Curr. Treat. Options Psychiatry 3 (2), 133–150.
Sanger, T.M., Lieberman, J.A., Tohen, M., Grundy, S., Beasley, C.J., Tollefson, G.D., 1999. Olanzapine versus haloperidol treatment in first-episode psychosis. Am. J. Psychiatry 156 (1), 79–87.
Sastre, M., Regunathan, S., Galea, E., Reis, D.J., 1996. Agmatinase activity in rat brain: a metabolic pathway for the degradation of agmatine. J. Neurochem. 67 (4), 1761–1765.
Seeman, P., 1987. Dopamine receptors and the dopamine hypothesis of schizophrenia. Synapse 1 (2), 133–152.
Segal, M.B., Preston, J.E., Collis, C.S., Zlokovic, B.V., 1990. Kinetics and Na independence of amino acid uptake by blood side of perfused sheep choroid plexus. Am. J. Phys. 258 (5 pt 2), F1288–F1294.
Sharma, J.N., Al-Omran, A., Parvathy, S.S., 2007. Role of nitric oxide in inflammatory diseases. Inflammopharmacology 15 (6), 252–259.
Snyder, S.H., 1976. The dopamine hypothesis of schizophrenia: focus on the dopamine receptor. Am. J. Psychiatry 133 (2), 197–202.
Tomiya, M., Fukushima, T., Watanabe, H., Fukami, G., Fujisaki, M., Iyo, M., Hashimoto, K., Mitsuhashi, S., Toyo'oka, T., 2007. Alterations in serum amino acid concentrations in male and female schizophrenic patients. Clin. Chim. Acta 380 (1–2), 186–190.
Uzbay, T., 2012. A new target for diagnosis and treatment of CNS disorders: agmatinergic system. Curr. Med. Chem. 19 (30), 5116–5121.
Uzbay, T., Kayir, H., Goktalay, G., Yildirim, M., 2010a. Agmatine disrupts prepulse inhibition of acoustic startle reflex in rats. J. Psychopharmacol. 24 (6), 923–929.
Uzbay, T., Kose, A., Kayir, H., Ulusoy, G., Celik, T., 2010b. Sex-related effects of agmatine on caffeine-induced locomotor activity in Swiss Webster mice. Eur. J. Pharmacol. 630 (1–3), 69–73.
Uzbay, T., Goktalay, G., Kayir, H., Eker, S.S., Sarandol, A., Oral, S., Buyukuysal, L., Ulusoy, G., Kirli, S., 2013. Increased plasma agmatine levels in patients with schizophrenia. J. Psychiatr. Res. 47 (8), 1054–1060.
Yang, X.C., Reis, D.J., 1999. Agmatine selectively blocks the N-methyl-D-aspartate subclass of glutamate receptor channels in rat hippocampal neurons. J. Pharmacol. Exp. Ther. 288 (2), 544–549.
Zhou, B., Xiao, J.F., Tuli, L., Ressom, H.W., 2012. LC-MS-based metabolomics. Mol. BioSyst. 8 (2), 470–481.
Zhu, M.Y., Wang, W.P., Huang, J., Feng, Y.Z., Regunathan, S., Bissette, G., 2008. Repeated immobilization stress alters rat hippocampal and prefrontal cortical morphology in parallel with endogenous agmatine and arginine decarboxylase levels. Neurochem. Int. 53 (6–8), 346–354.