Translational Psychiatry

https://doi.org/10.1038/s41398-026-03853-6

Article in Press

Untargeted ^{1}H NMR-based metabolomics unveils distinct circulating biochemical signatures between treatment-resistant and non-treatment-resistant schizophrenia patients: a pilot study

Received: 29 July 2025

Revised: 15 December 2025

Accepted: 20 January 2026

Cite this article as: Marino, C., Zhang, S., De Simone, G. *et al.* Untargeted ^{1}H NMR-based metabolomics unveils distinct circulating biochemical signatures between treatment-resistant and non-treatment-resistant schizophrenia patients: a pilot study. *Transl Psychiatry* (2026). https://doi.org/10.1038/s41398-026-03853-6

Carmen Marino, Siwei Zhang, Giuseppe De Simone, Manuela Grimaldi, Anna Di Maio, Felice Iasevoli, Francesco Errico, Anna Maria D'Ursi, Andrea de Bartolomeis & Alessandro Usiello

Brief Communications

Untargeted ^{1}H NMR-based metabolomics unveils distinct circulating biochemical signatures between treatment-resistant and non-treatment-resistant schizophrenia patients: a pilot study

Carmen Marino[1,*], Siwei Zhang[2,*], Giuseppe De Simone[3,*], Manuela Grimaldi[1], Anna Di Maio[2,4], Felice Iasevoli[3], Francesco Errico[2,5], Anna Maria D'Ursi[1], Andrea de Bartolomeis[3,@], Alessandro Usiello[2,4@]

[1] Department of Pharmacy, University of Salerno, Fisciano, Salerno, Italy;

[2] CEINGE Biotecnologie Avanzate "Franco Salvatore", Naples, Italy

[3] Section of Psychiatry, Laboratory of Translational and Molecular Psychiatry and Unit of Treatment-Resistant Psychosis, Department of Neuroscience, Reproductive Sciences and Odontostomatology, University Medical School of Naples "Federico II", Naples, Italy

[4]Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, Università degli Studi della Campania "Luigi Vanvitelli", Caserta, Italy

[5] Department of Agricultural Sciences, University of Naples "Federico II", Portici, Italy.

***** share co-first authorship

[@] Prof. Alessandro Usiello, PhD
Department of Environmental, Biological and Pharmaceutical Sciences and Technologies (DISTABIF), Università della Campania, L. Vanvitelli, Caserta, Italy.
email: alessandro.usiello@unicampania.it
[@] Prof. Andrea de Bartolomeis MD, PhD

Section of Psychiatry, Laboratory of Translational and Molecular Psychiatry and Unit of Treatment-Resistant Psychosis, Department of Neuroscience, Reproductive Sciences and Odontostomatology, University Medical School of Naples "Federico II", Via Pansini 5, Naples, Italy.
email: adebarto@unina.it

Abstract

Schizophrenia is a severe psychiatric disorder that affects approximately 1% of the population. Despite the availability of antipsychotic therapies, about 30% of patients develop treatment-resistant schizophrenia (TRS), defined by a lack of response to at least two different antipsychotic trials. Although several genetic and environmental factors have been proposed to explain treatment resistance, metabolomic studies investigating circulating metabolites in TRS remain limited. In this pilot cross-sectional study, we conducted untargeted ^{1}H NMR-based metabolomics to profile serum metabolites in TRS versus non-TRS patients. Notably, multivariate analysis revealed distinct serum metabolome profiles between the two groups. Additionally, Variable Importance in Projection (VIP) analysis and robust volcano plots showed significant differences between TRS versus non-TRS patients in metabolites involved in lipid and amino acid metabolism. Specifically, serine and glycine emerged as key discriminating molecules, prompting a complementary targeted HPLC analysis in the same serum samples. Although no significant group differences were observed in L-serine, D-serine, the D-serine/total serine ratio, or glycine levels, we found a positive correlation between D-serine levels and cognitive performance, particularly in the area of executive function, across the entire patient cohort. Additionally, a significant correlation between glycine and disorganization symptoms was found selectively in TRS patients. In conclusion, our study offers new insights into potential biomarkers for TRS, highlighting serine-glycine pathway as a possible crossroad between systemic dysmetabolism, NMDA receptor dysfunction, and cognitive impairment in TRS.

Introduction

Schizophrenia (SCZ) is a severe mental disorder that impacts about 1% of the global population [1]. The primary pharmacological treatment of SCZ remains antipsychotic drugs [2]. Nevertheless, almost one-third of individuals with SCZ exhibit minimal or no response to first-line antipsychotic medications. This condition is referred to as treatment-resistant SCZ (TRS), for which clozapine remains the most efficacious medication for reducing psychotic symptoms [3, 4]. Patients with TRS experience a lower quality of life, cognitive function, and social functioning compared to non-TRS patients.

Several follow-up studies show TRS patients have unique demographic and neurobiological traits, such as different brain network connectivity and cerebral glucose metabolism, compared to non-TRS patients [5-10]. Polygenic risk score analyses with 85,490 participants show TRS patients have specific hereditary traits linked to cognitive and intelligence-related phenotypes[10]. These neurobiological findings and poor response to dopamine-related antipsychotics support that TRS may involve distinct mechanisms, including non-canonical presynaptic dopamine release and altered brain glutamate levels [3, 11, 12]. Glutamatergic abnormalities are notably relevant, indeed various magnetic resonance spectroscopy studies showed elevated glutamate in the anterior cingulate cortex of TRS patients, a pattern absent in non-TRS individuals [13-15].

Additionally, studies on neuroinflammation have identified potential immune markers in TRS patients, further distinguishing them from patients who respond to antipsychotics [16, 17]. In line with these findings, growing evidence emphasises the role of immune reactions and inflammation in TRS. Baseline levels of inflammatory markers have been strongly associated with treatment outcomes in patients with SCZ [18]. Patients with TRS exhibited increased levels of pro-inflammatory cytokines, such as IL-6, IL-8, and IL-10, as well as soluble tumour necrosis factor receptors. TRS development is also linked to Th17 activation, indicated by higher IL-12/IL-23p40, IL-17A, and beta-2 macroglobulin (B2M) [19]. Notably, these inflammatory markers affect metabolic pathways. Studies show that an imbalanced immune response in TRS may impair tryptophan metabolism and activate the kynurenine pathway, which is linked to glutamatergic dysfunction and oxidative stress [17, 20-22]. However, whether such inflammation-metabolism crosstalk contributes to treatment resistance remains underexplored.

The data described so far are the result of targeted studies carried out with the intent of identifying, in TRS individuals, signatures of resistance within distinct biological compartments. In contrast, it would be beneficial to collect data for a comprehensive understanding of the metabolic features associated with TRS patients.

Recently, metabolomics has been instrumental in dissecting disease mechanisms and biochemical pathways, allowing molecular profiling that may contribute to diagnosis and treatment strategies [23].

Nuclear magnetic resonance (NMR) and mass spectrometry (MS) spectroscopies are effective techniques for metabolomic studies, allowing simultaneous qualitative and quantitative identification of low-molecular-mass compounds in biological sample fluids [24-26]. These approaches, which provide a snapshot of the systemic condition associated with SCZ, have been explored to examine the metabolomic profile of patients versus healthy controls, gaining insight

into the molecular mechanisms of the pathology [27, 28]. Nevertheless, there is a lack of compelling metabolomic studies investigating the biochemical signatures of TRS compared to non-TRS cases. In this work, we conducted an untargeted NMR-based metabolomic analysis of serum samples from SCZ patients, specifically comparing those with treatment resistance to those without. Furthermore, targeted High Performance Liquid Chromatography (HPLC) was used to provide a reliable quantitative estimation of D- vs L-enantiomers of amino acids previously identified by NMR analysis as discriminant metabolites and investigate their associations with clinical measures, including Positive and Negative Syndrome Scale and cognitive performances assessed by the Brief Assessment of Cognition in Schizophrenia.

Methods

Demographic and clinical characteristics of patients with schizophrenia

Blood serum samples were obtained from SCZ patients (n = 26), divided into two groups according to treatment resistance: non-treatment-resistant SCZ (non-TRS; n=13) and treatment-resistant SCZ (TRS; n=13). Patients with SCZ were recruited at the A.O.U. "Federico II" hospital of Naples over 6 months and diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5) [29]. Inclusion criteria for patients were: age 18-60 years; no evidence of worsening psychotic symptoms in the previous 6 months; absence of other major systemic, psychiatric (e.g., addictive disorders, frequent substance use in the 6 months prior to enrollment, etc.), or neurological disorders. The treatment resistance condition was defined as a failure of at least two different antipsychotic regimens, each administered for > 6 weeks at a total daily dose equivalent

to 600 mg of chlorpromazine (CPZeq), according to the modified Treatment Response and Resistance in Psychosis Working Group (TRRIP) Consensus criteria [29]. All TRS patients were under treatment with clozapine while non-TRS patients were treated with different conventional antipsychotics, such as olanzapine, risperidone, haloperidol, amisulpride, promazine, paliperidone and aripiprazole. Clinical data were collected within 1 month from the blood sample and included the severity of psychotic symptoms measured by the Positive and Negative Syndrome Scale (PANSS) [30] and cognitive performances assessed by the Brief Assessment of Cognition in Schizophrenia (BACS) [31]. Demographic characteristics are reported in Table 1**.** Phlebotomy was conducted by a psychiatric nurse; collection was performed in fasting status, in the morning before breakfast. Serum was separated by centrifugation and stored at −20 °C until analysis. Written informed consent was obtained from all subjects, according to the Declaration of Helsinki. The study was approved by the Ethics Committee of the University "Federico II" of Naples (protocol number: 195/19).

Sample preparation and acquisition

Serum samples were prepared in accordance with the NMR metabolomics guidelines for preserving sample quality as previously reported [32]. To avoid any manipulation that might alter the metabolomic profile, the serum was diluted only in the acquisition buffer, thereby filtering out macromolecule signals using NMR acquisition spectra with a Carr-Purcell-Meiboom-Gill (CPMG) pulse, as described in the guidelines [33]. A volume of 250 µL of serum was mixed with 250 µL of phosphate buffer containing 0.075 M Na_2HPO_4· 7H2O, 4% NaN_3, and H_2O[34]. Trimethylsilylpropionic acid 2,2,3,3-d4 sodium salt (TSP at 0.1% in D2O) served as the internal reference for aligning and quantifying NMR signals, as documented previously. The biofluid

diluted in phosphate buffer was then transferred into 5 mm tubes for acquisition on the Bruker 600 MHz spectrometer[23].

NMR experiments were acquired on a Bruker Avance 600 MHz spectrometer equipped with a 5 mm triple-resonance z-gradient probe (Bruker Biospin, Rheinstetten, Germany) at 298 K. Topspin version 3.2 was used for spectrometer control and data processing. 1D experiments, with a T2 filter using the CPMG sequence, were performed with a spectral width of 12 ppm, 32 k data points, 128 scans, a total spin-echo delay of 40 msec, a relaxation delay of 5 s, and an acquisition time of 2.27 sec. A weighted Fourier transform was applied to the time-domain data, with a line width of 0.5 Hz, followed by manual step and baseline correction in preparation for targeted profiling analysis[34].

HPLC analysis

Serum sample preparation, amino acid enantiomer resolution and separation were performed according to a previously published protocol [35, 36]. Identification and quantification of L-serine, D-serine, and glycine were based on retention times and peak areas and then compared with those associated with external standards. Amino acid concentrations in the serum were expressed as µM. Amino acid ratios were expressed as a percentage (%). Quantification of enantiomers was based on peak areas using calibration curves for each enantiomer. In both NMR and HPLC detections, the experimenter was blinded to the group assignments, which were handled by a separate individual who also conducted the data analysis.

Biostatistical analysis

The metabolites' quantification matrix was analysed through a univariate method as previously described [37]. To perform the robust volcano plot, we set a fold change threshold of 1 and a p-value

threshold of less than 0.05 [38]. The matrix was normalised using sum and Pareto scaling prior to analysis. The metabolomics data were analyzed employing a multivariate analysis (MVA) methodology. Initially, unsupervised Principal Component Analysis (PCA) was conducted and validated through a permutation test (Suppl. Figure 1). Subsequently, the metabolomic profile was further examined utilizing supervised partial least squares discriminant analysis (PLS-DA) within the MetaboAnalyst 6.0 platform (http://www.metaboanalyst.ca/) [39]. The PLS-DA model's performance was evaluated using Q2 from 10-fold internal cross-validation and R^2, showing variance prediction and explanation. Discriminatory metabolites were classified by variable influence on projection (VIP) scores, which, from PLS-DA weights, indicate the importance of each variable and are significant when they exceed a specific score 1 [39]. To evaluate the potential effect of age on the metabolomic profile clustering model, a meta-analysis was conducted using Metaboanalyst 6.0, and the Pearson correlation coefficient between the drug treatment resistance condition and age was assessed (Suppl. Figure 2) [39].

We conducted pathway analysis with the Enrichment tool, utilizing the Small Molecules Pathways Database (SMPDB) for Homo sapiens. Only pathways with a false discovery rate (FDR) and adjusted p-values below 0.05, along with more than one metabolite linked to them (hits), were included. Biomarker analysis was performed by examining the univariate ROC curve to determine AUC and its 95% confidence intervals, which were calculated using 500 bootstrap samples [40].

Amino acids that were quantitatively assessed using targeted HPLC, such as D-serine, L-serine, D-serine/total serine ratio, glycine, and glycine/L-serine, were compared between groups through ANCOVA analyses adjusted for age. Then, we performed partial correlation analyses to assess potential associations between these targeted amino acids and clinical measures, which included cognitive performance and symptom severity scores, measured by BACS and PANSS, respectively. Partial Spearman correlation coefficients were computed using the *ppcor* R package [41], allowing

adjustment for age as a confounding factor. Based on the real-world nature of the study, no sample size evaluations were performed. Analyses were conducted both on the whole patient sample and within subgroups (TRS and non-TRS). Results were deemed significant whenever p-values were lower than 0.05.

Results

Patients with SCZ (n = 26) were recruited from A.O.U. "Federico II" Hospital (Table 1) and divided into two groups: non-TRS and TRS (n = 13 in each group), based on whether their symptoms persisted after at least two different antipsychotic treatments. No statistically significant differences were found in sex [$X^2 = 0$ (df=1, p = 1) while significant age-dependent variations were observed [median (min; max) of years: non-TRS = 47 (22; 60), TRS = 34 (25; 57), $t = 2.59$ (df=24), p = 0.017, Table 1] between groups.

^{1}H NMR spectroscopy identifies distinct serum metabolomic features between treatment-resistant and non-treatment-resistant schizophrenia patients.

1D ^{1}H CPMG (Carr-Purcell-Meiboom-Gill) NMR spectra (Figure 1a) were identified from the blood sera of SCZ patients. Qualitative and quantitative analyses of spectral signals were conducted using CHENOMIX and Bayesil software [42, 43]. Accordingly, data matrices containing 44 metabolite concentrations for each sample were compiled and analyzed using statistical tools available on the MetaboAnalyst 6.0 online platform (http://www.metaboanalyst.ca/) [44]. The PLSDA score scatter plot of serum metabolomic profiles of TRS and non-TRS patients showed a clear separation between TRS and non-TRS patients, confirmed by cross-validation with positive accuracy and Q2 values (0.61, 0.89) (Figure 1b).

Considering the age differences in our samples and their potential impact on metabolomic profiles, we conducted a meta-analysis with age included as an additional variable to distinguish between groups. The findings indicated no relationship between serum metabolomic profiles of TRS and non-TRS patients and their age, implying that age is unlikely to be a confounding factor (Suppl. Figure 2).

Variable importance project (VIP) analysis was used to identify the metabolites responsible for the serum metabolome discrimination between groups. Remarkably, data analysis highlighted several amino acid metabolites driving the separation. Notably, serine (VIP: 2.17), proline (VIP: 2.13), glycine (VIP: 1.86), glutamine (VIP: 1.53), lysine (VIP: 1.30), and histidine (VIP: 1.10) exhibited significant discriminative power (Figure 1c). Quantitative robust volcano plot further indicated significant downregulation of proline and serine serum levels, together with increased glutamine concentrations in TRS, compared to non-TRS patients. Moreover, betaine (VIP: 1.35) emerged as another distinguishing metabolite, showing markedly higher serum levels in TRS patients in the volcano plot compared to non-TRS individuals (Figure 1d).

MVA also identified bioenergetic metabolites that differentiated the metabolome profiles of TRS from non-TRS patients. Accordingly, VIP analysis identified formate (VIP: 2.17), malonate (VIP: 1.33), glycerol (VIP: 1.31), and lactic acid (VIP: 1.29) as significant contributors (Figure 1c). Consistent with this evidence, the volcano plot showed upregulation of formate in the serum of TRS patients (Figure 1d).

Heatmap analysis of mean metabolomic serum profiles further revealed higher concentrations of glycine, formate, glutamine, betaine, and lactic acid, as well as reduced levels of serine and proline in TRS compared to non-TRS patients (Figure 1e).

We subsequently conducted enrichment analyses to identify the possible biochemical pathways affected by drug resistance in SCZ (Figure 1f). Data analysis suggested that dysregulation involved

membrane phospholipid, particularly in sphingolipid metabolism and phosphatidylethanolamine biosynthesis pathways, mainly driven by serine reductions in TRS group (Figure 1f; Suppl. Table 1). Metabolic perturbations were also detected in amino acid-related pathways, including selenoamino acid metabolism, methionine metabolism, and ammonia recycling (Figure 1f; Suppl. Table 1). Finally, we identified dysfunctional metabolism of arginine and proline in TRS compared to non-TRS patients (Figure 1f; Suppl. Table 1).

Importantly, ROC curve analysis identified serine as a potential biomarker for TRS, with an area under the curve (AUC) value of 0.81, supporting the role of this amino acid in differentiating the serum profiles of TRS and non-TRS patients (Figure 2).

Collectively, these findings suggest that antipsychotic treatment resistance is associated with a distinct serum metabolomic signature in SCZ patients. The alterations primarily involve amino acids influencing NMDA receptor activity, lipids related to membrane structure, and energy metabolism. Our current NMR-based metabolomic findings provide evidence for candidate serum metabolites that could serve as biomarkers for drug resistance in SCZ.

HPLC analysis and clinical data reveal significant correlations between NMDA receptor-related amino acids and cognitive functions in schizophrenia patients

Based on untargeted NMR findings, which highlighted serine as one of the most affected metabolites and a potential biomarker of treatment resistance, we performed a targeted HPLC analysis to quantitatively assess serum levels of serine and its metabolic derivative glycine. This approach also enabled the enantiomeric separation of D-serine and L-serine, given their distinct biological relevance. First, we compared L-serine, D-serine, and glycine levels, as well as D-serine/total serine and glycine/L-serine ratios, between TRS and non-TRS patients using ANCOVA with age as a covariate. This analysis revealed no significant differences across groups

(Suppl. Table 2). Next, we explored the association between these amino acid levels and clinical parameters (Figure 3 and Suppl. Tables 3-8). In the whole patient cohort, D-serine levels were positively correlated with cognitive performance in the executive function domain (r = 0.44, p = 0.04) (Figure 3a; Suppl. Tables 3,4). Subgroup analyses in TRS patients revealed a significant positive correlation between the D-serine/total serine ratio and cognitive performance in the executive function domain (r = 0.77, p = 0.006) (Suppl. Tables 7,8), as well as a negative correlation between glycine and disorganization symptoms (r = -0.62, p = 0.04) (Figure 3b; Suppl. Tables 7,8). No significant associations emerged in the non-TRS subgroup (Suppl. Tables 5,6).

Discussion

In this pilot study, we conducted an untargeted NMR-based metabolomic analysis to identify the serum metabolic signatures associated with TRS, compared to non-TRS condition. Notably, MVA showed different serum metabolomic profiles between TRS and non-TRS patients, with amino acid changes ranking among the most distinctive biomolecules.

Among the metabolites identified through NMR data analysis, serine demonstrated the most significant discriminating power, as shown by the VIP score. Specifically, the robust volcano plot revealed lower serum levels of serine in TRS compared to non-TRS patients. Consistent with this evidence, ROC curve analysis further validated a role for serine as a suitable biomarker of TRS. Moreover, to define the relevance of serine metabolism in TRS, we also employed an HPLC approach that allowed the enantiomeric discrimination of L- and D-serine, along with the quantification of the L-serine derivative, glycine.

Physiologically, L-serine plays a key role in intermediary metabolism and serves as the biosynthetic precursor for the endogenous NMDA receptor ligands, glycine and D-serine [45-48]

whose dysfunctions are well-established in SCZ pathophysiology [35, 36, 49-51]. Overall, we did not observe significant differences in D-serine, L-serine, or glycine levels between TRS and non-TRS groups following age correction. However, we identified a positive correlation between D-serine, and cognitive performance in the executive function domain in the entire cohort of SCZ patients. In contrast, only in TRS patients, we detected a significant positive correlation between the D-serine/total serine ratio and cognitive performance in the executive function domain, as well as a negative correlation between glycine and disorganization symptoms. These results support the association between the glycine-serine metabolism and clinical symptoms in SCZ, including both cognitive and psychopathological domains. Although the limited sample size may represent a limitation, the results underscore a significant link between NMDA receptor-related amino acids and TRS. In keeping with this, our data are in line with previous findings demonstrating a critical role of D-serine and glycine in modulating cognitive functions in SCZ [51]. Through its co-agonist action at the glycine binding-site of NMDA receptors, D-serine regulates synaptic plasticity [52-54], which is critical for higher-order cognitive operations. Accordingly, preclinical studies have shown that reduced D-serine availability impairs NMDA receptor-mediated signaling in the prefrontal cortex, resulting in deficits in working memory, cognitive flexibility, and decision-making [55, 56]. In the same way, clinical evidence indicates that peripheral and central D-serine levels correlate with cognitive performance in SCZ patients [57, 58], and that adjunctive treatment with D-serine can ameliorate specific cognitive symptoms [59, 60]. Further, a recent large-cohort study found that TRS patients exhibit impaired performance in executive-related areas, such as working memory, processing speed, learning, and attention measures, along with biochemical evidence of dysregulated D-serine metabolism [61]. Hence, integrating previous findings with our current evidence of a selective association between D-serine/total serine ratio and executive performance in TRS patients supports a pathophysiological mechanism in which impaired NMDA receptor-

mediated D-serine metabolism contributes to treatment resistance by sustaining cognitive dysfunction.

Regarding the other major NMDA receptor co-agonist, glycine, we found a selective negative correlation between its levels and disorganization symptoms in the TRS group. This finding is relevant, as several modulators of the glycine B site at NMDA receptor, including sarcosine, a potent and selective glycine transporter 1 (GlyT-1) inhibitor, have been proposed as augmentation strategies for SCZ [62]. Furthermore, clozapine, the only approved and effective drug for TRS [63], may exert at least part of its therapeutic action through GlyT-1 inhibition, thus increasing glycine levels in the synaptic cleft [64]. On the other hand, the disorganization domain has been considered as a predictive factor for the identification of TRS [65] , highlighting the importance of this correlation within the TRS group. Although the correlations identified between D-serine or glycine and clinical parameters are of notable interest, their interpretation remains preliminary due to the small sample size, underscoring the need for confirmation in larger and independent populations.

Beyond its role in the biosynthesis of NMDA receptor co-agonists, serine also contributes to multiple metabolic pathways. In particular, it is involved in sphingolipid metabolism and phosphatidylethanolamine biosynthesis, serving as a precursor for complex lipids and as a methyl donor in phospholipid remodeling [45-48]. Consistent with a prominent downregulation of serine reported by NMR results, pathway enrichment analysis indicates significant disruption of sphingolipid metabolism and phosphatidylethanolamine biosynthesis in TRS patients.

In this regard, it has been reported that the high lipophilicity of clozapine could influence membrane lipid composition and related metabolic processes [64], potentially modulating synaptic membrane integrity and neuroinflammatory responses, and thereby contributing to TRS-specific neuroprogression [66, 67]. Importantly, prior research has identified a selective reduction in L-serine

synthesis among patients who did not respond to clozapine treatment, suggesting a distinct biochemical and pathophysiological feature of treatment resistance in SCZ [68]. Taken together, our observations strengthen the hypothesis that amino acid dysregulation, particularly involving serine, represents a central mechanism underlying TRS.

Besides serine, NMR analysis revealed notable alterations in other amino acids, including reduced proline and increased glycine and glutamine in TRS compared to non-TRS patients. In keeping with this, pathway enrichment analysis highlighted a significant dysregulation of the arginine and proline metabolism pathway between TRS and non-TRS patients. Arginine has been previously implicated in SCZ pathophysiology, especially for its contribution to nitric oxide (NO) modulation[69-75]. Furthermore, its metabolism has been posited to play a role in microglial activation,[76] a process also documented in the context of SCZ pathophysiology. Following microglial activation, release of pro-inflammatory cytokines, including IL-6, TNF-α, and IFN-γ, has been consistently reported in SCZ [77]. Proline metabolism is also relevant as SCZ has been linked to hyper-prolinaemia, due to decreased activity of proline-degrading enzymes, particularly proline dehydrogenase [78, 79]. Our metabolomic findings support an association with SCZ, showing lower proline levels in TRS patients compared with non-TRS. Interestingly, if translated at the central nervous system level, the combination of reduced proline and increased glycine levels may indirectly perturb NMDA receptor-mediated glutamatergic neurotransmission [80]. Indeed, low proline could reduce competition at amino acid transporters or allosteric sites, while higher glycine levels provide greater co-agonist availability, collectively promoting an enhanced NMDA receptor activation.

Our metabolomic work also revealed increased serum betaine in TRS compared to non-TRS patients,-which may reflect an adaptive mechanism to counteract the greater oxidative stress and

inflammation, reported in these patients[81-85 86, 87]. Moreover, since the peripheral metabolism of choline to betaine and subsequently to methionine occurs primarily in the liver [88], the biochemical serum alterations observed in TRS patients may also be caused by altered multiorgan and multisystem metabolism driven by diet or antipsychotic drugs [89, 90].

Despite its novelty, our pilot real-world study has some limitations. First, the relatively small sample size substantially reduces the statistical power and may limit the generalizability of the findings, underscoring the need for replication in larger independent cohorts. Moreover, the lack of sex-stratified analyses represents another constraint, given the well-documented sex-specific differences in SCZ phenotypes, which could mask potential associations with metabolites. In addition, the cross-sectional design precludes causal inference between serum metabolomic changes and clinical phenotypes in TRS patients. Finally, the potential confounding role of diet should be acknowledged, as dietary intake can significantly influence circulating metabolite levels and thereby interfere with the interpretation of metabolomic alterations. Future studies in larger, sex-stratified cohorts with controlled dietary assessments are required to validate these findings and clarify whether the observed metabolic alterations represent causal mechanisms or adaptive responses.

In summary, our untargeted NMR-based metabolomics study demonstrates for the first time that TRS patients can be distinguished from non-TRS based on their systemic metabolome signatures. Intriguingly, serine emerges as a central metabolite, linking amino acid dysregulation, NMDA receptor hypofunction, and lipid dynamics, and shows promising diagnostic potential as suggested by ROC curve analysis. Furthermore, the correlation of glycine with disorganization symptoms in TRS highlights a potential mechanistic link between altered NMDA receptor signaling and cognitive impairment in these patients. In conclusion, our work provides a foundation for future

metabolomic investigations in larger cohorts, which could ultimately help to enhance antipsychotic treatment response.

Ethics approval and consent to participate

The studies involving humans were approved by Ethics Committee of the University "Federico II" of Naples (protocol number: 195/19). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Acknowledgments

The study was supported by the Italian Ministry of Universities and Research (Ministero dell'Università e della Ricerca, MUR) through PRIN 2017 - Project nr 2017M42834 (to F.E., A.d.B. and A.U.), PRIN 2020 - Project nr 2020K53E57 (to A.U.) and PRIN PNRR 2022 financed by the European Union – Next Generation EU - Project nr P2022ZEMZF (to F.E. and A.U.). The work of A.U., A.d.B., F.I. and G.D.S. was supported by #NEXTGENERATIONEU (NGEU) and funded by MUR, National Recovery and Resilience Plan (NRRP), project MNESYS (PE0000006) – A Multiscale integrated approach to the study of the nervous system in health and disease (DN. 1553 11.10.2022).

Conflict of Interest

A.d.B. has received research support from Janssen, Lundbeck, and Otsuka and lecture fees for unrestricted educational meeting from Chiesi, Lundbeck, Roche, Sunovion, Viatris, HIKMA,

Tabuk, Recordati, Angelini, Gedeon RIchter and Takeda; he has served on advisory boards for Eli Lilly, Jansen, Lundbeck, Otsuka, Roche, Takeda, Chiesi, Recordati, Angelini, Viatris, Newron, Gedeon Richter. None of the above competing interests is related directly or indirectly to the work presented here. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be considered as a potential conflict of interest.

Author contributions

C.M.: Investigation, Writing – original draft. S.Z.: Writing – original draft. G.D.S.: Investigation, Writing – review & editing M.G.: Investigation. A.D.M.: Investigation; F.I.: Writing – review & editing, F.E.: Writing – review & editing, Funding acquisition. A.M.D.: Writing – review & editing.; A.d.B.: Writing – review & editing, Funding acquisition. A.U.: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Data availability

Metabolomics data have been deposited in the EMBL-EBI MetaboLights database (https://www.ebi.ac.uk/metabolights/) with the identifier **MTBLS12709**.

References

1. Howes OD, Murray RM. Schizophrenia: an integrated sociodevelopmental-cognitive model. The Lancet. 2014;383(9929):1677-87.

2. Pham M, Caglayan A. A Comprehensive Review of Schizophrenia and Antipsychotic Metabolism as a Predictor of Treatment Response. Cureus. 2024;16(7):e65279.
3. de Bartolomeis A, Ciccarelli M, De Simone G, Mazza B, Barone A, Vellucci L. Canonical and Non-Canonical Antipsychotics' Dopamine-Related Mechanisms of Present and Next Generation Molecules: A Systematic Review on Translational Highlights for Treatment Response and Treatment-Resistant Schizophrenia. Int J Mol Sci. 2023;24(6).
4. Siskind D, McCartney L, Goldschlager R, Kisely S. Clozapine v. first- and second-generation antipsychotics in treatment-refractory schizophrenia: systematic review and meta-analysis. The British journal of psychiatry : the journal of mental science. 2016;209(5):385-92.
5. Smart SE, Kępińska AP, Murray RM, MacCabe JH. Predictors of treatment resistant schizophrenia: a systematic review of prospective observational studies. Psychological medicine. 2021;51(1):44-53.
6. Zhang A, Yao C, Zhang Q, Zhao Z, Qu J, Lui S, et al. Individualized multi-modal MRI biomarkers predict 1-year clinical outcome in first-episode drug-naïve schizophrenia patients. Front Psychiatry. 2024;15:1448145.
7. Yesilkaya UH, Gica S, Ilnem MC, Sen M, Ipekcioglu D. Evaluation of IGF-1 as a novel theranostic biomarker for schizophrenia. J Psychiatr Res. 2021;140:172-9.
8. De Simone G, Iasevoli F, Barone A, Gaudieri V, Cuocolo A, Ciccarelli M, et al. Addressing brain metabolic connectivity in treatment-resistant schizophrenia: a novel graph theory-driven application of 18F-FDG-PET with antipsychotic dose correction. Schizophrenia. 2024;10(1):116.
9. Iasevoli F, D'Ambrosio L, Ciccarelli M, Barone A, Gaudieri V, Cocozza S, et al. Altered Patterns of Brain Glucose Metabolism Involve More Extensive and Discrete Cortical Areas in Treatment-resistant Schizophrenia Patients Compared to Responder Patients and Controls: Results From a Head-to-Head 2-[18F]-FDG-PET Study. Schizophrenia bulletin. 2023;49(2):474-85.
10. Pardiñas AF, Smart SE, Willcocks IR, Holmans PA, Dennison CA, Lynham AJ, et al. Interaction Testing and Polygenic Risk Scoring to Estimate the Association of Common Genetic Variants With Treatment Resistance in Schizophrenia. JAMA psychiatry. 2022;79(3):260-9.

11. Okubo R, Okada M, Motomura E. Dysfunction of the NMDA Receptor in the Pathophysiology of Schizophrenia and/or the Pathomechanisms of Treatment-Resistant Schizophrenia. Biomolecules. 2024;14(9).
12. Vellucci L, Ciccarelli M, Buonaguro EF, Fornaro M, D'Urso G, De Simone G, et al. The Neurobiological Underpinnings of Obsessive-Compulsive Symptoms in Psychosis, Translational Issues for Treatment-Resistant Schizophrenia. Biomolecules. 2023;13(8).
13. Egerton A, Griffiths K, Casetta C, Deakin B, Drake R, Howes OD, et al. Anterior cingulate glutamate metabolites as a predictor of antipsychotic response in first episode psychosis: data from the STRATA collaboration. Neuropsychopharmacology. 2023;48(3):567-75.
14. Huang LC, Lin SH, Tseng HH, Chen KC, Abdullah M, Yang YK. Altered glutamate level and its association with working memory among patients with treatment-resistant schizophrenia (TRS): a proton magnetic resonance spectroscopy study. Psychological medicine. 2023;53(7):3220-7.
15. Matrone M, Kotzalidis GD, Romano A, Bozzao A, Cuomo I, Valente F, et al. Treatment-resistant schizophrenia: Addressing white matter integrity, intracortical glutamate levels, clinical and cognitive profiles between early- and adult-onset patients. Progress in neuro-psychopharmacology & biological psychiatry. 2022;114:110493.
16. Chen W, Gou M, Wang L, Li N, Li W, Tong J, et al. Inflammatory disequilibrium and lateral ventricular enlargement in treatment-resistant schizophrenia. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology. 2023;72:18-29.
17. Leboyer M, Godin O, Terro E, Boukouaci W, Lu CL, Andre M, et al. Immune Signatures of Treatment-Resistant Schizophrenia: A FondaMental Academic Centers of Expertise for Schizophrenia (FACE-SZ) Study. Schizophrenia bulletin open. 2021;2(1):sgab012.
18. Fenn-Moltu S, Deakin B, Drake R, Howes OD, Lawrie SM, Lewis S, et al. The association between peripheral inflammation, brain glutamate and antipsychotic response in Schizophrenia: Data from the STRATA collaboration. Brain, behavior, and immunity. 2023;111:343-51.
19. Jiao S, Cao T, Cai H. Peripheral biomarkers of treatment-resistant schizophrenia: Genetic, inflammation and stress perspectives. Frontiers in pharmacology. 2022;13:1005702.

20. Chen W, Tian Y, Gou M, Wang L, Tong J, Zhou Y, et al. Role of the immune-kynurenine pathway in treatment-resistant schizophrenia. Progress in neuro-psychopharmacology & biological psychiatry. 2024;130:110926.

21. Schwieler L, Larsson MK, Skogh E, Kegel ME, Orhan F, Abdelmoaty S, et al. Increased levels of IL-6 in the cerebrospinal fluid of patients with chronic schizophrenia--significance for activation of the kynurenine pathway. Journal of psychiatry & neuroscience : JPN. 2015;40(2):126-33.

22. De Picker L, Fransen E, Coppens V, Timmers M, de Boer P, Oberacher H, et al. Immune and Neuroendocrine Trait and State Markers in Psychotic Illness: Decreased Kynurenines Marking Psychotic Exacerbations. Front Immunol. 2019;10:2971.

23. Marino C, Grimaldi M, Sommella EM, Ciaglia T, Santoro A, Buonocore M, et al. The Metabolomic Profile in Amyotrophic Lateral Sclerosis Changes According to the Progression of the Disease: An Exploratory Study. Metabolites. 2022;12(9).

24. Ghini V, Quaglio D, Luchinat C, Turano P. NMR for sample quality assessment in metabolomics. New Biotechnology. 2019;52:25-34.

25. Ravanbakhsh S, Liu P, Bjordahl TC, Mandal R, Grant JR, Wilson M, et al. Accurate, Fully-Automated NMR Spectral Profiling for Metabolomics. PloS one. 2015;10(5):e0124219.

26. Aslam N, Pfender M, Neumann P, Reuter R, Zappe A, Fávaro de Oliveira F, et al. Nanoscale nuclear magnetic resonance with chemical resolution. Science. 2017;357(6346):67-71.

27. Cloarec O, Dumas M-E, Craig A, Barton RH, Trygg J, Hudson J, et al. Statistical total correlation spectroscopy: an exploratory approach for latent biomarker identification from metabolic 1H NMR data sets. Analytical chemistry. 2005;77(5):1282-9.

28. Pedrini M, Cao B, Nani JVS, Cerqueira RO, Mansur RB, Tasic L, et al. Advances and challenges in development of precision psychiatry through clinical metabolomics on mood and psychotic disorders. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2019;93:182-8.

29. Howes OD, McCutcheon R, Agid O, de Bartolomeis A, van Beveren NJ, Birnbaum ML, et al. Treatment-Resistant Schizophrenia: Treatment Response and Resistance in Psychosis (TRRIP) Working Group Consensus Guidelines on Diagnosis and Terminology. The American journal of psychiatry. 2017;174(3):216-29.

30. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophrenia bulletin. 1987;13(2):261-76.

31. Keefe RS, Goldberg TE, Harvey PD, Gold JM, Poe MP, Coughenour L. The Brief Assessment of Cognition in Schizophrenia: reliability, sensitivity, and comparison with a standard neurocognitive battery. Schizophr Res. 2004;68(2-3):283-97.

32. Grimaldi M, Palisi A, Marino C, Montoro P, Capasso A, Novi S, et al. NMR-based metabolomic profile of hypercholesterolemic human sera: Relationship with in vitro gene expression? PloS one. 2020;15(4):e0231506.

33. Emwas AH, Zacharias HU, Alborghetti MR, Gowda GAN, Raftery D, McKay RT, et al. Recommendations for sample selection, collection and preparation for NMR-based metabolomics studies of blood. Metabolomics. 2025;21(3):66.

34. Marino C, Imarisio A, Gasparri C, Napolitano E, Di Maio A, Avenali M, et al. 1H-NMR-based metabolomics identifies disrupted betaine metabolism as distinct serum signature of pre-frailty. npj Aging. 2025;11(1):26.

35. Garofalo M, De Simone G, Motta Z, Nuzzo T, De Grandis E, Bruno C, et al. Decreased free D-aspartate levels in the blood serum of patients with schizophrenia. Front Psychiatry. 2024;15:1408175.

36. Rampino A, Garofalo M, Nuzzo T, Favia M, Saltarelli S, Masellis R, et al. Variations of blood D-serine and D-aspartate homeostasis track psychosis stages. Schizophrenia (Heidelb). 2024;10(1):115.

37. Valsecchi V, Errico F, Bassareo V, Marino C, Nuzzo T, Brancaccio P, et al. SMN deficiency perturbs monoamine neurotransmitter metabolism in spinal muscular atrophy. Communications Biology. 2023;6(1):1155.

38. Kumar N, Hoque MA, Sugimoto M. Robust volcano plot: identification of differential metabolites in the presence of outliers. BMC Bioinformatics. 2018;19(1):128.

39. Pang Z, Lu Y, Zhou G, Hui F, Xu L, Viau C, et al. MetaboAnalyst 6.0: towards a unified platform for metabolomics data processing, analysis and interpretation. Nucleic Acids Res. 2024;52(W1):W398-w406.

40. Xia J, Sinelnikov IV, Han B, Wishart DS. MetaboAnalyst 3.0--making metabolomics more meaningful. Nucleic Acids Res. 2015;43(W1):W251-7.

41. Kim S. ppcor: An R Package for a Fast Calculation to Semi-partial Correlation Coefficients. Commun Stat Appl Methods. 2015;22(6):665-74.

42. van Zyl CDW, van Reenen M, Osthoff G, du Preez I. Evaluation of BAYESIL for automated annotation of 1H NMR data using limited sample volumes: application to African elephant serum. Metabolomics. 2023;19(4):31.

43. Zheng C, Zhang S, Ragg S, Raftery D, Vitek O. Identification and quantification of metabolites in 1H NMR spectra by Bayesian model selection. Bioinformatics. 2011;27(12):1637-44.

44. Pang Z, Lu Y, Zhou G, Hui F, Xu L, Viau C, et al. MetaboAnalyst 6.0: towards a unified platform for metabolomics data processing, analysis and interpretation. Nucleic acids research. 2024;52(W1):W398-W406.

45. Murtas G, Marcone GL, Sacchi S, Pollegioni L. L-serine synthesis via the phosphorylated pathway in humans. Cell Mol Life Sci. 2020;77(24):5131-48.

46. Holecek M. Serine Metabolism in Health and Disease and as a Conditionally Essential Amino Acid. Nutrients. 2022;14(9).

47. Maugard M, Vigneron PA, Bolanos JP, Bonvento G. l-Serine links metabolism with neurotransmission. Prog Neurobiol. 2021;197:101896.

48. Pollegioni L, Kustrimovic N, Piubelli L, Rosini E, Rabattoni V, Sacchi S. d-amino acids: new functional insights. FEBS J. 2025;292(17):4395-417.

49. Coyle JT. Passing the torch: The ascendance of the glutamatergic synapse in the pathophysiology of schizophrenia. Biochem Pharmacol. 2024;228:116376.

50. Errico F, Nuzzo T, Carella M, Bertolino A, Usiello A. The Emerging Role of Altered d-Aspartate Metabolism in Schizophrenia: New Insights From Preclinical Models and Human Studies. Front Psychiatry. 2018;9:559.

51. Coyle JT, Ruzicka WB, Balu DT. Fifty Years of Research on Schizophrenia: The Ascendance of the Glutamatergic Synapse. Am J Psychiatry. 2020;177(12):1119-28.

52. Souza INO, Roychaudhuri R, de Belleroche J, Mothet JP. d-Amino acids: new clinical pathways for brain diseases. Trends Mol Med. 2023;29(12):1014-28.

53. Fossat P, Turpin FR, Sacchi S, Dulong J, Shi T, Rivet JM, et al. Glial D-serine gates NMDA receptors at excitatory synapses in prefrontal cortex. Cereb Cortex. 2012;22(3):595-606.

54. Dallerac G, Li X, Lecouflet P, Morisot N, Sacchi S, Asselot R, et al. Dopaminergic neuromodulation of prefrontal cortex activity requires the NMDA receptor coagonist d-serine. Proc Natl Acad Sci U S A. 2021;118(23).

55. Balu DT, Coyle JT. The NMDA receptor 'glycine modulatory site' in schizophrenia: D-serine, glycine, and beyond. Curr Opin Pharmacol. 2015;20:109-15.

56. Nava-Gomez L, Calero-Vargas I, Higinio-Rodriguez F, Vazquez-Prieto B, Olivares-Moreno R, Ortiz-Retana J, et al. Aging-Associated Cognitive Decline Is Reversed by D-Serine Supplementation. eNeuro. 2022;9(3).

57. Hashimoto K, Engberg G, Shimizu E, Nordin C, Lindstrom LH, Iyo M. Reduced D-serine to total serine ratio in the cerebrospinal fluid of drug naive schizophrenic patients. Prog Neuropsychopharmacol Biol Psychiatry. 2005;29(5):767-9.

58. Kantrowitz JT, Malhotra AK, Cornblatt B, Silipo G, Balla A, Suckow RF, et al. High dose D-serine in the treatment of schizophrenia. Schizophr Res. 2010;121(1-3):125-30.

59. Kantrowitz JT, Woods SW, Petkova E, Cornblatt B, Corcoran CM, Chen H, et al. 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. 2015;2(5):403-12.

60. Zhang T, Liu C, Zhong N, Wang Y, Huang Y, Zhang X. Advances in the Treatment of Cognitive Impairment in Schizophrenia: Targeting NMDA Receptor Pathways. Int J Mol Sci. 2024;25(19).

61. Camporesi S, Xin L, Golay P, Eap CB, Cleusix M, Cuenod M, et al. Neurocognition and NMDAR co-agonists pathways in individuals with treatment resistant first-episode psychosis: a 3-year follow-up longitudinal study. Mol Psychiatry. 2024;29(11):3669-79.

62. de Bartolomeis A, Vellucci L, Austin MC, De Simone G, Barone A. Rational and Translational Implications of D-Amino Acids for Treatment-Resistant Schizophrenia: From Neurobiology to the Clinics. Biomolecules. 2022;12(7).

63. Murray RM, Egerton A, Gao Y, Grace AA, Howes O, Jauhar S, et al. Why Is Clozapine Uniquely Effective in Treatment-Resistant Schizophrenia? A Review and Proposal. Biol Psychiatry. 2025.

64. de Bartolomeis A, Vellucci L, Barone A, Manchia M, De Luca V, Iasevoli F, et al. Clozapine's multiple cellular mechanisms: What do we know after more than fifty years? A

systematic review and critical assessment of translational mechanisms relevant for innovative strategies in treatment-resistant schizophrenia. Pharmacol Ther. 2022;236:108236.

65. Barone A, De Prisco M, Altavilla B, Avagliano C, Balletta R, Buonaguro EF, et al. Disorganization domain as a putative predictor of Treatment Resistant Schizophrenia (TRS) diagnosis: A machine learning approach. J Psychiatr Res. 2022;155:572-8.

66. Wang S, Dong Y, Qiu Y, Sun X, Jiang C, Su Q, et al. Prediction of treatment response in drug-naïve schizophrenia patients from the perspective of targeted metabolomics. Schizophr Res. 2025;278:9-16.

67. Xu CX, Huang W, Shi XJ, Du Y, Liang JQ, Fang X, et al. Dysregulation of Serum Exosomal Lipid Metabolism in Schizophrenia: A Biomarker Perspective. Molecular neurobiology. 2025;62(3):3556-67.

68. Hons J, Vasatova M, Cermakova E, Doubek P, Libiger J. Different serine and glycine metabolism in patients with schizophrenia receiving clozapine. J Psychiatr Res. 2012;46(6):811-8.

69. Pitsikas N. The role of nitric oxide donors in schizophrenia: Basic studies and clinical applications. European journal of pharmacology. 2015;766:106-13.

70. Lauer M, Johannes S, Fritzen S, Senitz D, Riederer P, Reif A. Morphological abnormalities in nitric-oxide-synthase-positive striatal interneurons of schizophrenic patients. Neuropsychobiology. 2005;52(3):111-7.

71. Xing G, Chavko M, Zhang L-X, Yang S, Post RM. Decreased calcium-dependent constitutive nitric oxide synthase (cNOS) activity in prefrontal cortex in schizophrenia and depression. Schizophrenia Research. 2002;58(1):21-30.

72. Liu P, Jing Y, Collie ND, Dean B, Bilkey DK, Zhang H. Altered brain arginine metabolism in schizophrenia. Translational psychiatry. 2016;6(8):e871-e.

73. Mete G, Fidan Ç, Demirci A, Ozen Yalcin D, Devrim E, Elgun Ulkar S, et al. Evaluation of serum arginine metabolic pathway markers in patients with bipolar disorder and schizophrenia. International journal of psychiatry in clinical practice. 2024;28(3-4):218-23.

74. Yin B, Cai Y, Teng T, Wang X, Liu X, Li X, et al. Identifying plasma metabolic characteristics of major depressive disorder, bipolar disorder, and schizophrenia in adolescents. Translational psychiatry. 2024;14(1):163.

75. Ustundag MF, Ozcan H, Gencer AG, Yilmaz ED, Uğur K, Oral E, et al. Nitric oxide, asymmetric dimethylarginine, symmetric dimethylarginine and L-arginine levels in psychotic exacerbation of schizophrenia and bipolar disorder manic episode. Saudi medical journal. 2020;41(1):38-45.

76. Hajji N, Garcia-Revilla J, Soto MS, Perryman R, Symington J, Quarles CC, et al. Arginine deprivation alters microglial polarity and synergizes with radiation to eradicate non-arginine-auxotrophic glioblastoma tumors. The Journal of clinical investigation. 2022;132(6).

77. Hartmann S-M, Heider J, Wüst R, Fallgatter AJ, Volkmer H. Microglia-neuron interactions in schizophrenia. 2024;Volume 18 - 2024.

78. Volk DW, Gonzalez-Burgos G, Lewis DA. l-Proline, GABA Synthesis and Gamma Oscillations in Schizophrenia. Trends in Neurosciences. 2016;39(12):797-8.

79. Guo X, Tang P, Yang C, Li R. Proline dehydrogenase gene (PRODH) polymorphisms and schizophrenia susceptibility: a meta-analysis. Metabolic brain disease. 2018;33(1):89-97.

80. Cummings KA, Popescu GK. Glycine-dependent activation of NMDA receptors. The Journal of general physiology. 2015;145(6):513-27.

81. Yang YS, Smucny J, Zhang H, Maddock RJ. Meta-analytic evidence of elevated choline, reduced N-acetylaspartate, and normal creatine in schizophrenia and their moderation by measurement quality, echo time, and medication status. NeuroImage Clinical. 2023;39:103461.

82. Yoshihara S, Jiang X, Morikawa M, Ogawa T, Ichinose S, Yabe H, et al. Betaine ameliorates schizophrenic traits by functionally compensating for KIF3-based CRMP2 transport. Cell Reports. 2021;35(2):108971.

83. Zeisel SH. Choline: clinical nutrigenetic/nutrigenomic approaches for identification of functions and dietary requirements. Journal of nutrigenetics and nutrigenomics. 2010;3(4-6):209-19.

84. Carotenuto A, Andreone V, Amenta F, Traini E. Effect of Treatment of the Cholinergic Precursor Choline Alphoscerate in Mild Cognitive Dysfunction: A Randomized Controlled Trial. 2024;60(6):925.

85. Bekdash RA. Choline and the Brain: An Epigenetic Perspective. Advances in neurobiology. 2016;12:381-99.

86. Wu X, Ao H, Wu X, Cao Y. Sulfur-containing amino acids and risk of schizophrenia. Schizophr Res. 2023;262:8-17.

87. Ohnishi T, Balan S, Toyoshima M, Maekawa M, Ohba H, Watanabe A, et al. Investigation of betaine as a novel psychotherapeutic for schizophrenia. EBioMedicine. 2019;45:432-46.
88. Abu Ahmad N, Raizman M, Weizmann N, Wasek B, Arning E, Bottiglieri T, et al. Betaine attenuates pathology by stimulating lipid oxidation in liver and regulating phospholipid metabolism in brain of methionine-choline-deficient rats. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2019;33(8):9334-49.
89. Aarøe ASK, Odgaard Maeng K, Leifsdottir Jacobsen R, Eggert Jensen S, Graff C, Polcwiartek C, et al. Hepatic steatosis in patients with schizophrenia: a clinical cross-sectional study. Nordic journal of psychiatry. 2022;76(2):114-9.
90. Stanbridge AJ, Cranshaw T, Paul MM. Clozapine metabolism may be affected by Gilbert's syndrome: case report and discussion. Pharmacogenetics and genomics. 2021;31(9):221-4.

Table 1. Demographic characteristics of non-TRS and TRS patients enrolled in the study.

Demographic characteristics	***nTRS***	***TRS***	***Statistic***	***p-value***
Subjects (total number)	13	13	-	-
Age (years, median [IQR])	47 [22; 60]	34 [25; 57]	t = 2.59 (df=24)	0.017
Sex (M/F)	10/3	11/2	X^2 = 0 (df=1)	1
Antipsychotics	Aripiprazole (23.1%), Haloperidol (38.5%), Olanzapine (38.5%), Paliperidone (15.4%), Risperidone (7.7%)	Amisulpride (7.7%), Aripiprazole (7.7%), Clozapine (100%), Haloperidol (7.7%)	-	-
CPZeq (mean±SD)	379.3 ± 211.7	631.5 ± 519.2	t = 1.5 (df=21)	0.15
Disease duration (mean±SD)	20.4 ± 11.1	14.6 ± 9.2	t = -1.37 (df=21)	0.19

Abbreviations: nTRS, non-treatment-resistant schizophrenia; TRS, treatment-resistant schizophrenia; IQR, interquartile range; CPZeq, chlorpromazine equivalent.

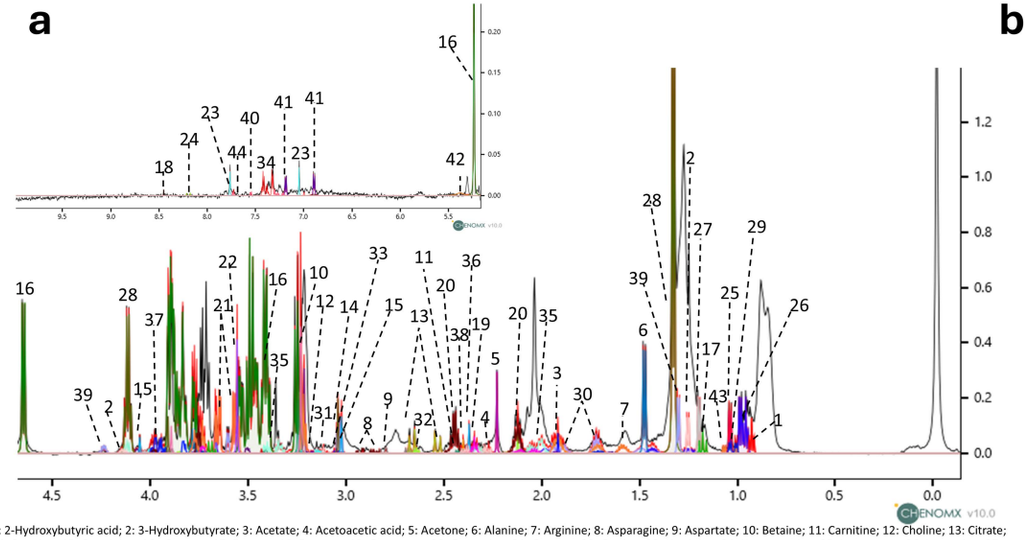
Figure legends

Figure 1. NMR-based metabolomic analysis reveals a distinct serum metabolite signature that distinguishes TRS from non-TRS patients. a) 1D ^{1}H CPMG spectrum of SCZ patients' serum. The spectrum is acquired at 600 MHz and dT = 298 K. Forty-four metabolites are identified and annotated as follows:1: 2-Hydroxybutyric acid; 2: 3-Hydroxybutyrate; 3: Acetate; 4: Acetoacetic acid; 5: Acetone; 6: Alanine; 7: Arginine; 8: Asparagine; 9: Aspartate; 10: Betaine; 11: Carnitine; 12: Choline; 13: Citrate; 14: Creatine; 15: Creatinine; 16: Glucose; 17: Ethanol; 18: Formate; 19: Glutamate; 20: Glutamine; 21: Glycerol; 22: Glycine; 23: Hystidine; 24: Hypoxanthine; 25: Isobutyrate; 26: Isoleucine; 27: Isopropyl alcohol; 28: Lactate; 29: Leucine; 30: Lysine; 31: Malonate; 32: Methionine; 33: Ornithine; 34: Phenylalanine; 35: Proline; 36: Pyruvate; 37: Serine; 38: Succinate; 39: Threonine; 40: Tryptophan; 41: Tyrosine; 42: Urea; 43: Valine; 44: 1-Methylhistidine. **b)** Score plot for the supervised analysis conducted using PLS-DA indicating the separation between non-treatment resistant (non-TRS) and treatment-resistant (TRS) schizophrenia patients' serum profile. The Cartesian space is described by the principal component 1 (PC1), equal to 9.3%, and the secondary component (PC2), equal to 7.2%. the model was validated by cross-validation and reports accuracy values for the first and second components of 0.89 and Q2 values of 0.62 and 0.64 for the first and second component, respectively. **c)** VIP graph showing the metabolites responsible for clustering (VIP >1). **d)** Robust volcano plot analysis of metabolic changes in treatment-resistant SCZ patients compared to non-treatment-resistant patients' serum. Each point on the volcano plot was based on p- and fold-change values, set at 0.05 and 1.0, respectively. Red and blue circles identify upregulated and downregulated metabolites, respectively. Variations are expressed as a function of the serum profiles of treatment-resistant patients. **e)** Hierarchical heat maps are generated by MetaboAnalyst 6.0 software using Euclidean distance and Ward's algorithm. These heatmaps represent the concentrations of treatment-resistant

and non-treatment-resistant schizophrenia (SCZ) patients. Each bar represents a metabolite, coloured according to its abundance intensities on a normalized scale that ranges from blue (low level) to red (high level). **f)** Enrichment analysis of pathways was conducted on ^{1}H NMR data regarding the serum metabolomics of treatment-resistant SCZ patients compared to those without treatment resistance. The bars represent the number of hits, indicating metabolites detected in the spectra and linked to specific pathways. Pathways were considered statistically significant if they had Hits > 1, a p-value < 0.05, with p-values adjusted via the Holm-Bonferroni test (Holm p) and the False Discovery Rate (FDR) < 1. Darker colours signify lower, and thus more significant, p-values. The significance range is displayed at the top. Pathway exploration was conducted using the Small Molecules and Pathways Database (SMPDB), with *Homo sapiens* selected as the organism.

Figure 2. Diagnostic performance of serum serine in TRS. a) Receiver operating characteristic (ROC) curves comparing TRS and non-TRS based on serum serine levels. The x-axis represents the false positive rate, and the y-axis the true positive rate. The ROC curve has two components: the empirical ROC curve, obtained by connecting the points representing sensitivity and specificity for different cutpoints, and the chance diagonal, represented by a 45-degree line drawn through the coordinates (0,0) and (1,1). The red dot indicates the cut-off value for serine and reports its sensitivity and specificity. **b)** Violin plot showing serum serine concentrations in non-TRS (red) and TRS patients (green). The yellow diamonds in the bars show the average values, while black dots represent individual concentrations. The lower section of the violin plot displays the AUC value, significance indicated by the p-value, and the fold change (FC) in relation to TRS patients.

Figure 3. Correlation of D-serine with executive functions in schizophrenia patients. a) Scatter plot showing the relationship between D-serine levels and executive function scores in the whole patient sample. Data points are color-coded by diagnostic subgroup (TRS and non-TRS). The blue line represents the overall regression line adjusted for age. Reported statistics correspond to the partial correlation coefficient (r = 0.44) and p-value (p = 0.04) for the whole sample. **b)** Partial correlation between glycine and disorganization symptoms in the TRS group (r = -0.62; p = 0.04) adjusted for age.



1: 2-Hydroxybutyric acid; 2: 3-Hydroxybutyrate; 3: Acetate; 4: Acetoacetic acid; 5: Acetone; 6: Alanine; 7: Arginine; 8: Asparagine; 9: Aspartate; 10: Betaine; 11: Carnitine; 12: Choline; 13: Citrate; 14: Creatine; 15: Creatinine; 16: Glucose; 17: Ethanol; 18: Formate; 19: Glutamate; 20: Glutamine; 21: Glycerol; 22: Glycine; 23: Hystidine; 24: Hypoxanthine; 25: Isobutyrate; 26: Isoleucine; 27: Isopropyl alcohol; 28: Lactate; 29: Leucine; 30: Lysinhe; 31: Malonate; 32: Methionine; 33: Ornithine; 34: Phenylalanine; 35: Proline; 36: Pyruvate; 37: Serine; 38: Succinate; 39: Threonine; 40: Tryptophan; 41: Tyrosine; 42: Urea; 43: Valine; 44: 1-Methylhistidine.