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A multi-omic dissection of molecular hallmarks of accelerated aging in schizophrenia

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Schizophrenia, a pervasive mental health condition, poses a global challenge to individual well-being. The intricate interplay of schizophrenia with the aging process is characterized by a shortened lifespan, underscoring the urgent need for an in-depth exploration of the underlying biological mechanisms that contribute to the unique aging trajectory within this specific patient population. Currently, this crucial aspect remains largely unexplored. To bridge this knowledge gap, in the present study, serum samples from of 29 subjects with schizophrenia were analysed via liquid chromatography-mass spectrometry (LC-MS) and compared with those of 30 nonpsychiatric controls. This exploratory analysis of circulating blood serum, albeit based on a limited sample size, provides valuable insights into the significantly altered molecular pathways linked to schizophrenia and clarifies the unique aspects of aging in the context of this particular disease. The data presented in this study constitute a compilation of molecular alterations associated with schizophrenia across the human lifespan. By distinguishing between schizophrenia patients and nonpsychiatric controls, we identified a panel of 342 differentially abundant metabolites and 544 differentially expressed proteins. Our pathway enrichment analysis was focused primarily on histone acetylation, the Wnt/β-catenin signaling pathway, the dopamine receptor signaling pathway, and fatty acid beta-oxidation, highlighting their pivotal roles in schizophrenia. Furthermore, we conducted a co-occurrence analysis of these metabolites and proteins, aiming to elucidate their connection with accelerated aging processes. This analysis revealed the involvement of metabolic pathways crucial for lipid metabolism, such as the peroxisome and peroxisome proliferator-activated receptor (PPAR) signaling pathways, as well as neurotransmitter-related metabolic pathways influencing tryptophan metabolism and the dopaminergic synapse pathway. The findings significantly increase our understanding of the intricate molecular mechanisms underlying schizophrenia and its co-occurring comorbidities across the spectrum of aging, providing insights into the dynamics of the progression of this complex disorder. However, this study has certain limitations, particularly the lack of its findings and the relatively small sample size, especially in the proteomic analysis.

Keywords Schizophrenia, Metabolomics, Proteomics, Multi-omics, Aging

Schizophrenia, a persistent condition with an uncertain origin, affects approximately 1% of the global population. This psychiatric disorder has significant implications for families and society and results in substantial economic burdens[1,2]. Approximately 5% of individuals diagnosed with schizophrenia display suicidal ideation, with a potential reduction in life expectancy of 8–16 years[3]. Notably, the mortality rate among individuals with schizophrenia surpasses that of the general population, with premature deaths often attributed to cardiovascular diseases[4]. The intricate interplay of diverse patient characteristics and the multifaceted aetiology of schizophrenia, encompassing genetic, environmental, and neurobiological factors, has impeded the elucidation of the molecular mechanisms driving the pathogenesis of schizophrenia[5,6]. Although approximately 20% of patients achieve significant recovery, 50% continue to experience symptoms throughout their lives, and the increasing lifetime prevalence highlights ongoing challenges in managing this complex disorder[7]. Currently, there is limited research on the molecular underpinnings and comprehensive extent of disease risk related to aging and metabolic disorders in individuals diagnosed with schizophrenia[8]. Elevated levels of chronic inflammation have been reported to be closely associated with aging, suggesting the potential role of inflammation as a driving factor in multiple age-related diseases[9]. Another significant class of inflammatory indicators that may be linked to accelerated aging are mediators of oxidative stress[10,11]. Researchers have also delved into salivary proteomic

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expression profiles to elucidate the relationship between aging and schizophrenia. Their findings revealed differentially expressed proteins that are involved in processes such as the immune response and oxidative stress[12]. Thus, individuals with schizophrenia display alterations in peripheral biomarkers potentially related to aging and that this aging process is possibly associated with increased somatic morbidity and mortality. The findings of these studies have opened a new chapter in the exploration of the relationship between aging and schizophrenia.

In the field of systems biology research, metabolomic and proteomic analyses reveal changes at the metabolic and proteomic levels within organisms, respectively[13,14]. By integrating data from these two analyses, a more comprehensive understanding of the physiological and pathological processes in organisms can be achieved, thereby providing essential insights into the complex mechanisms underlying biological systems[8].

An increasing number of investigations have been focused on the use of emerging omic techniques to uncover previously unknown disease mechanisms and define biomarkers associated with clinical variables[15]. For schizophrenia, such studies not only have the power to reveal a wealth of information about the biological basis of the disorder but also herald new strategies for the management of metabolic and inflammatory dysfunction associated with schizophrenia and its treatment. In this study, nontargeted metabolomic analyses based on mass spectrometry (MS) data-independent acquisition (DIA) techniques were applied to capture metabolite and protein alternations from individuals with schizophrenia patients and nonpsychiatric controls to obtain a more comprehensive view of the metabolic profile. The resulting data indicate a compendium of molecular factors associated with increased disease risk in individuals with schizophrenia, including lipid metabolism disorders and increased broad-scale inflammatory factors.

Materials and methods

Serum sample collection

Five milliliters of fasting blood were collected from 29 schizophrenia and 30 nonpsychiatric comparison subjects, with strict adherence to the guidelines set forth by the Hulunbuir Mental Health Centre. The research protocol for this study received approval from the ethics committee of Hulunbuir Mental Health Centre (No.2021NO.02), and informed consent was obtained from all the subjects. All methods were carried out in accordance with relevant guidelines and regulations. Following centrifugation, the serum samples were stored at − 80 °C until analysis. Specific inclusion criteria were rigorously followed for the recruitment of individuals with schizophrenia for this study. These criteria entailed a formal diagnosis of schizophrenia, as defined in the Diagnostic and Statistical Manual of Mental Disorders Fifth Edition (DSM-5), made by a qualified clinician[16]. The exclusion criterion was the presence of severe medical conditions, other neuropsychiatric disorders, brain structural abnormalities, brain trauma, a history of continuous illicit drug use, or movement disorders. Also in accordance with these criteria, asymptomatic age-matched nonpsychiatric controls with no family history of mental illness or blood transfusion within the preceding three months were selected from among participants who underwent annual hospital staff examinations. A set of standard laboratory assays were conducted for triglyceride, apolipoprotein, total bilirubin, albumin, cholesterol, low-density lipoprotein, and high-density lipoprotein levels, all assays were performed at Hulunbuir Third People's Hospital by technicians adhering to established and stringent standard procedures.

Protein isolation

The samples were thawed on ice, and segregated into 100 µL aliquots for further analysis. These aliquots were suspended in lysis buffer composed of 1% sodium deoxycholate and 8 M urea and further supplemented with an adequate amount of 1× protease inhibitor cocktail to effectively hinder activity. The mixture was then vigorously vortexed to ensure thorough mixing and subsequently processed three times through a high-throughput tissue homogenizer. The mixture was subsequently allowed to settle undisturbed at 4 °C for 30 min, during which it was gently vortexed every 10 min to maintain homogeneity. Subsequently, the mixture was centrifuged at 14 000 g for 20 min at 4 °C, which enabled the separation of the protein-rich supernatant.

The protein concentration in the supernatant was determined via the bicinchoninic acid (BCA) method with a BCA protein assay kit, which ensures accurate and reliable quantification according to the standard protocol of the manufacturer. Sample preparation encompasses critical steps such as protein denaturation, reduction, and alkylation, along with tryptic digestion and peptide cleanup. We adhered to the specified protocols while utilizing the commercially available iST Sample Preparation kit (PreOmics, Germany). Initially, 50 µL of lyse buffer was added to the sample, followed by heating at 95 °C for 10 min while agitating at 1000 rpm. Once the sample cooled to room temperature, trypsin digestion buffer was added, and the sample was incubated at 37 °C for 2 h with shaking at 500 rpm. The digestion process was subsequently halted with stop buffer. For sample cleanup and desalting, an iST cartridge was employed, utilizing the recommended wash buffers. The peptides were then eluted with elution buffer (2 × 100 µL) and subsequently lyophilized with a SpeedVac.

Extraction of compounds

The serum samples were processed on ice, where 100 µL aliquots were combined with 400 µL of cold methanol/acetonitrile (ACN) (1:1, v: v) to remove proteins. The mixture was subsequently centrifuged for 15 min at 14,000×*g* and 4 °C. The resulting supernatant was then dried in a vacuum centrifuge. Prior to LC-MS analysis, the samples were reconstituted in 100 µL of a mixture of ACN/water (1:1, v/v). To assess the stability of the experimental process, quality control (QC) samples were prepared by incorporating equal volumes of all the analyzed samples, Subsequently, nine control samples were injected in a randomized sequence.

Nano-HPLC-MS/MS analysis

The UltiMate 3000 (Thermo Fisher Scientific, MA, USA) LC system was connected to a time TOF Pro2, an ion mobility spectrometry quadrupole time-of-flight mass spectrometer (Bruker Daltonics). Prior to analysis, the samples were reconstituted in a solution containing 0.1% formic acid (FA) and 200 ng of peptide was subjected to separation on an analytical column (15 cm length, 75 µm inner diameter (i.d), 1.7 µm particle size, and 120 Å pore size; IonOpticks) with an optimized 60-min gradient. This gradient commenced with the addition of 4% buffer B (80% ACN with 0.1% FA), which was gradually increased to 28% over 25 min, followed by a steeper rise to 44% in 10 min, which culminated in 90% in 10 min and remained there for 7 min. Finally, the column was equilibrated back to 4% for 8 min. The column flow rate was maintained at 400 nL/min, with a column temperature of 50 °C to ensure optimal separation conditions.

DIA data were acquired via the DIA PASEF mode. We defined 22 × 40 Th precursor isolation windows spanning the m/z range from 349 to 1229. To optimize the MS1 cycle time, we incorporated variable repetition steps (ranging from 2 to 5) into the 13-scan DIA PASEF scheme during our experiment. During the PASEF MS/MS scanning, the collision energy was systematically ramped in a linear fashion, adapting to the mobility from 59 eV at 1/K0 = 1.6 Vs/cm2 to 20 eV at 1/ K0 = 0.6 Vs/cm2.

LC-MS/MS analysis

The analysis was performed via a UHPLC system (1290 Infinity LC, Agilent Technologies) paired with a quadrupole time-of-flight mass spectrometer (AB Sciex TripleTOF 6600) at Shanghai Applied Protein Technology Co., Ltd. For reversed-phase liquid chromatography (RPLC) separation, an ACQUITY UPLC HSS T3 column (1.8 µm, 2.1 mm × 100 mm, Waters, Ireland) was employed. To obtain more information about the metabolites, both positive and negative ionization modes were used. In electrospray ionization (ESI) positive mode, the mobile phase consisted of A (water with 0.1% FA) and B (Acetonitrile with 0.1% FA); and in ESI negative mode, the mobile phase consisted of A (0.5 mM ammonium fluoride in water) and B (ACN). The corresponding liquid elution procedure and MS acquisition procedure were provided in Supplementary Table 1. The gradient was set at a flow rate of 0.3 mL/min, with the column temperature maintained at 25 °C. A 2 µL aliquot of each sample was injected.

Data analysis

The raw data were processed and analysed via Spectronaut (Biognosys AG, Switzerland) with default settings, and the retention time prediction type was set to dynamic iRT. Data extraction was performed via Spectronaut 17 via extensive mass calibration, dynamically adjusting the ideal extraction window according to iRT calibration and gradient stability. A Q value (false discovery rate (FDR)) cut-off of 1% was applied at both the precursor and protein levels. The decoy generation was set to mutated, simulating scrambled sequences but introducing random amino acid swaps at a minimum of 2 and a maximum of half the peptide length. For normalization, a local normalization strategy was chosen to ensure accurate quantification. Peptides that surpassed the 1% Q value threshold were subsequently used to calculate protein abundances via the MaxLFQ method. To gain insights into protein functions and classification, searches were conducted against comprehensive databases, including Gene Ontology (GO), EuKaryotic Orthologous Gr Ok.oups (KOG)/Cluster of Orthologous Groups of Proteins (COG), and the Kyoto Encyclopedia of Genes and Genomes (KEGG) databases[17]. Differentially expressed proteins were identified through statistical analysis, employing a p-value threshold of 0.05 for student′s t-test and a fold change (FC) criterion of ≤ 0.5 or ≥ 2. The use of these thresholds ensured the identification of proteins with significant expression differences, thus providing a reliable dataset for further analysis and interpretation.

The raw MS data were converted to MzXML files via Proteo Wizard MSConvert software and then imported into XCMS software for further analysis. During peak picking, the following parameters were adopted: centWave with m/z tolerance set at 10 ppm, a peak width ranging from 10 to 60 scans, and a prefilter spanning 10 to 100 scans. For peak grouping, the bandwidth (bw) was set to 5, the mz width (mzwid) was set to 0.025, and the minimum fraction (minfrac) required for grouping was 0.5. Collection of Algorithms of Metabolite Profile Annotation (CAMERA) was employed to annotate isotopes and adducts within the extracted ion features. Among these features, only variables that exhibited more than 50% nonzero measurement values in at least one group were retained for further analysis. For the identification of metabolites, we relied on comparing the accuracy of m/z values (within 10 ppm) and MS/MS spectra against an internal database comprising authenticated standards. Missing data were addressed via the K-nearest neighbor (KNN) method, and extreme values were identified and appropriately handled. Finally, to ensure parallelism between samples and metabolites, the total peak area of the data was normalized.

The unsupervised multivariate approach of principal component analysis (PCA) and principal coordinates analysis (PCoA) were applied to LC-MS data as dimensionality reduction methods, based on the underlying data, to examine clustering patterns among nonpsychiatric controls and schizophrenia patients. PCA was used not only to validate the data quality but also to facilitate visualization of differences between sample groups during unsupervised analysis. To further distinguish between the schizophrenia patients and the nonpsychiatric controls, we employed supervised multivariate analysis along with orthogonal least squares data analysis (OPLS) to identify potential metabolites. The selection of potential differentially abundant metabolites was based on variable importance in projection (VIP) values, wherein variables with a VIP score exceeding 1 significantly contributed to sample separation in the OPLS-DA analysis. Additionally, a student's t-test was conducted to determine the statistical significance of these differentially abundant metabolites between groups, setting the threshold at $p < 0.05$. The fold change (FC) threshold further served as an indicator for differentiating the groups.

Integrated analysis of metabolites and proteins

MetaboAnalyst v6.0 (https://www.metaboanalyst.ca/) was used to support the comprehensive pathway analysis of both the proteomic and metabolomic data through enrichment analysis or co-occurrence. These analyses were carried out on the basis of a user-defined list of metabolites and proteins.

Results

Multi-omic analysis for assessing extensive alterations associated with schizophrenia

In this study, we conducted a comprehensive analysis of serum samples from 29 individuals diagnosed with schizophrenia and 30 age-matched nonpsychiatric controls (Fig. 1a). Using MS techniques, we performed metabolomic analysis of all participants and proteomic analysis of a total of 6 individuals with schizophrenia and 4 nonpsychiatric controls. Our cohort was balanced in terms of age and sex across both groups (Supplementary

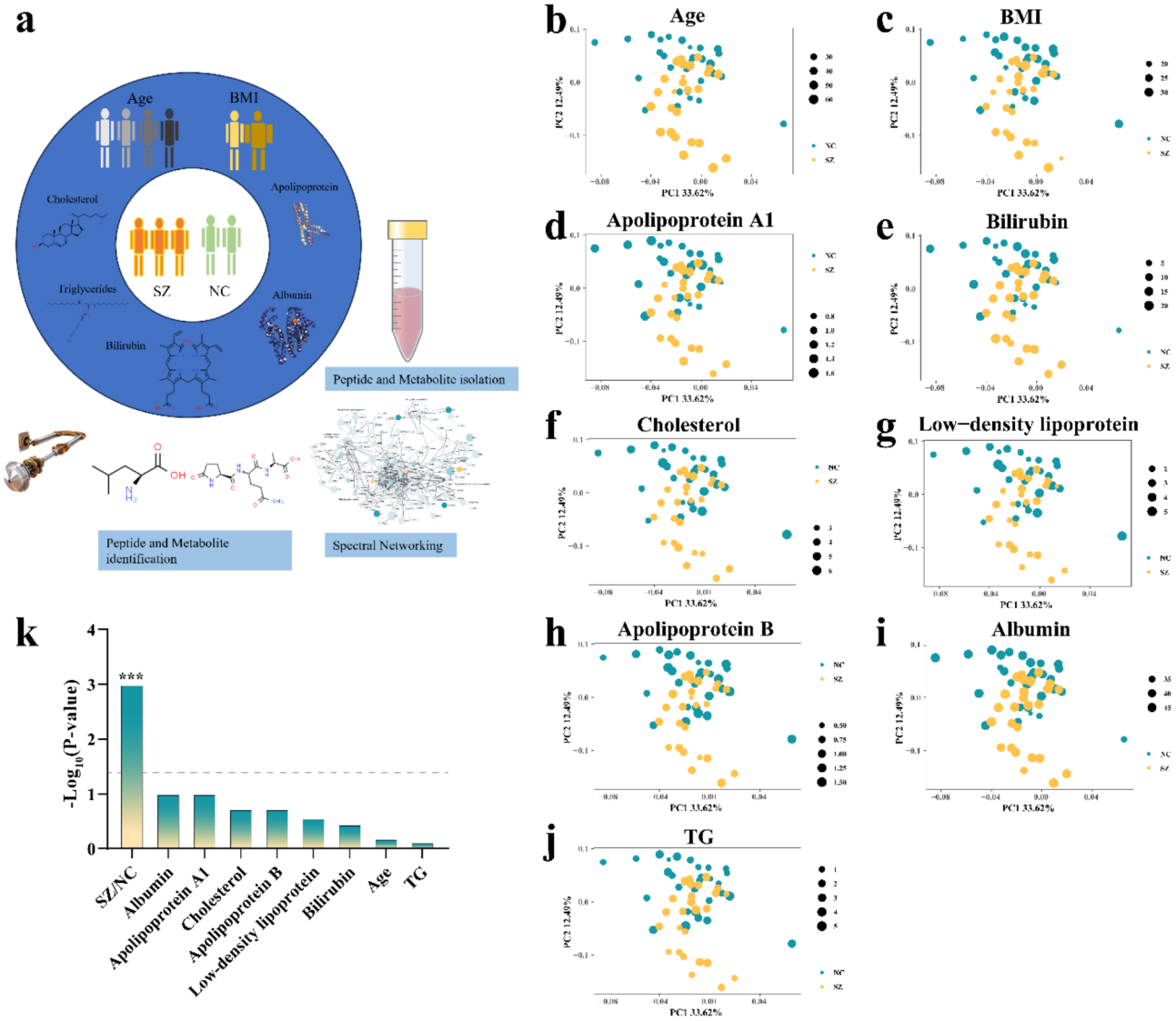


Fig. 1. Multi-omic analysis of circulating molecular variations in serum: A comparision of schizophrenia (SZ) and nonpsychiatric control (NC) subjects utilizing proteomic and metabolomic analyses. (**a**) Multi-omic mass spectrometry-based strategy for profiling molecular signatures in human schizophrenia patients. Serum samples from 29 schizophrenia patients and 30 age-matched (22–64 years) nonpsychiatric controls were subjected to proteomic and metabolomic analyses for the characterization of proteins and small molecules for identification. Data on patient clinical features such as lipid metabolism, age and body mass index (BMI) were collected. Principal coordinates analysis (PCoA) of the measurable influence of annotated metabolomic data were conducted for the (**b**) ages, (**c**) BMIs, (**d**) serum apolipoprotein A1 levels, (**e**) bilirubin levels, (**f**) cholesterol levels, (**g**) low – density lipoprotein levels, (**h**) apolipoprotein B levels, (**i**) albumin levels, (**j**) triglyceride, (TG) levels of subjects. (**k**) Significance measurements for the impact of metadata variables on the PCoA distribution of metabolomic data. The data are presented as -log10(p value) values (the dotted line threshold indicates p value<0.05). *SZ* schizophrenia patients, *NC* nonpsychiatric controls.

Fig. 1). The corresponding clinical information and clinical indicators were provided in Supplementary Table 2. The reliability of the experimental procedures, as well as the stability and reproducibility of the analysis, were assessed utilizing QC samples, as illustrated in Supplementary Fig. 2a-b. The clustering of QC samples validated the credibility of the analytical method, and the well-defined separation between the schizophrenia and nonpsychiatric control groups also serves as evidence for the effectiveness of our modeling approach. Despite the relatively small number of samples included in the proteomics-based study, the PCA plot reveals a clear separation between the schizophrenia group and the nonpsychiatric control group. Moreover, the model's explanatory power stands at 55.7% (PC1 = 49.7%, PC2 = 6%) (Supplementary Fig. 2c).

Proteomics analysis revealed a total of 2991 proteins in both the schizophrenia and nonpsychiatric control groups, providing an overview of the proteomic landscape, whereas metabolomic analysis revealed 22,913 metabolites, 2468 of which were successfully matched to known annotations.

To visualize the influence of clinical variables on the collected data, we performed a PCoA on the metabolomics dataset. The annotated metabolomic data exhibited a distinct separation based on schizophrenia status (Fig. 1b-j), whereas the proteomic analysis was hampered by the limited sample size, and a similar segregation was unable to be achieved. The metadata encompassed diverse variables, including schizophrenia diagnosis, age, body mass index (BMI), triglyceride level and cholesterol level. To assess the extent to which these metadata variables influenced the differential distribution of samples in the PCoA space, we employed statistical tests (depicted in Fig. 1k). Notably, the metabolome data were equally affected by these indicators, indicating the significance of these factors in shaping the metabolic profile of the subjects.

Detection of metabolic signatures in schizophrenia

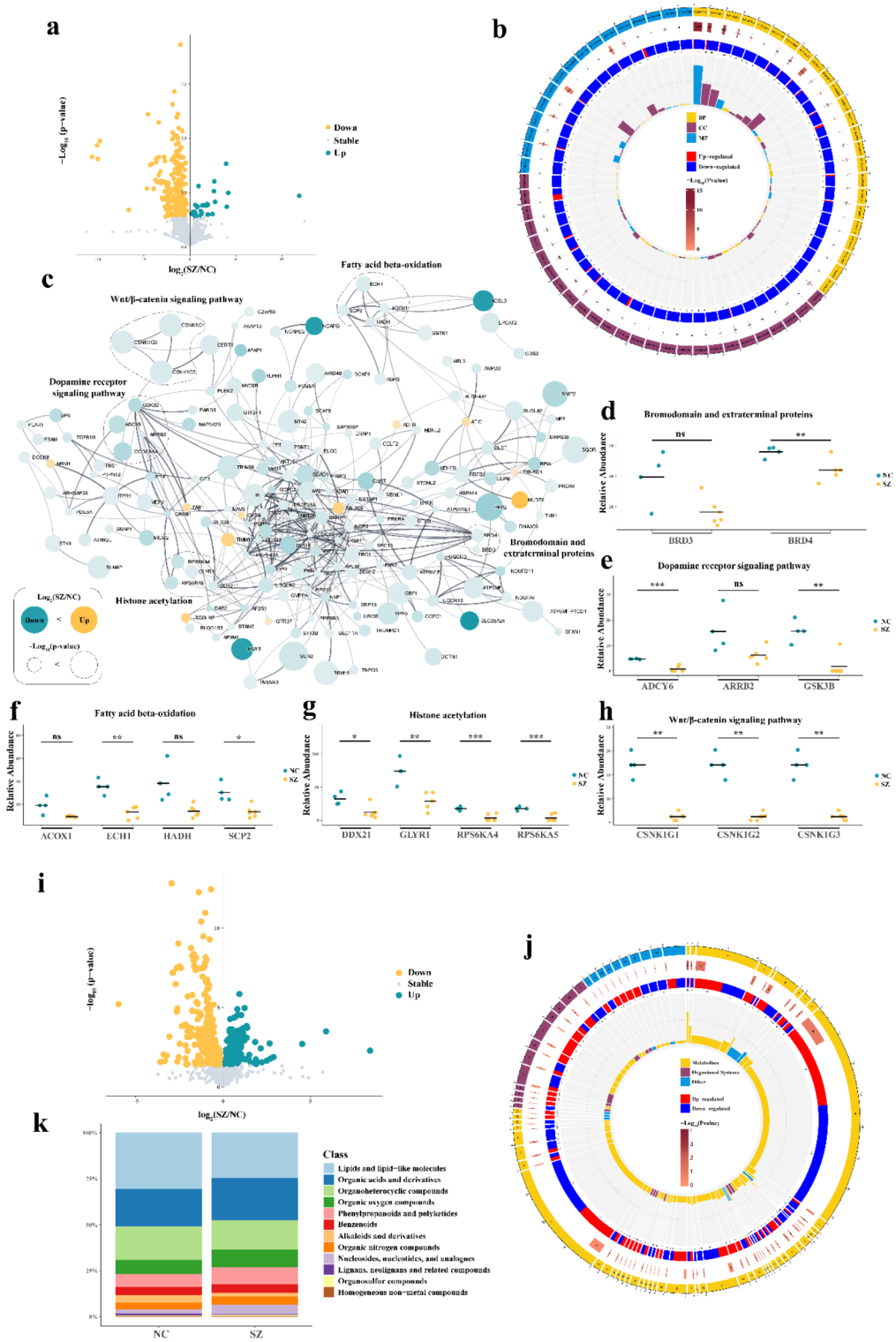
Our subsequent investigation aimed to delineate specific molecular characteristics linked to schizophrenia. Utilizing binary comparisons, we discerned distinct sets of proteins exhibiting elevated or reduced levels in individuals diagnosed with schizophrenia (Fig. 2a). Our analysis revealed 31 proteins with increased expression and 311 proteins with decreased expression in the comparison between patients with schizophrenia and nonpsychiatric controls. Pathway enrichment analysis revealed a signature of posttranslational modification (PTM) of protein metabolism in schizophrenia patients, characterized by terms such as positive regulation of histone acetylation, peptidyl-serine phosphorylation, histone H3-S28 phosphorylation, and histone H3-S10 phosphorylation. Furthermore, enrichment of the Wnt/β-catenin signaling pathway in neuroinflammation and the involvement of dopamine pathways in neurotransmitter signaling was identified (Fig. 2b, Supplement Table 3). To further understand the complex interconnections among proteins exhibiting changes in expression levels, a network analysis was performed on the altered protein pool via the knowledge-based software platform String-db (Fig. 2c). The analysis revealed a densely interconnected network of proteins related to various metabolic processes, such as fatty acid beta-oxidation, the Wnt/β-catenin signaling pathway, the dopamine receptor signaling pathway, histone acetylation, and bromodomain and extraterminal (BET) domains. The expression levels of the Wnt/β-catenin proteins casein kinase I isoform gamma (CSNK1G1), CSNK1G2, and CSNK1G3 were found to be decreased in individuals diagnosed with schizophrenia. This study also revealed a disturbance in metabolic pathways, specifically characterized by reductions in protein clusters related to dopamine, fatty acid metabolism, and histone acetylation (Fig. 2d–h).

Subsequent analysis focused on identifying altered metabolites in schizophrenia patients (Fig. 2i), resulting in the identification of 242 upregulated and 302 downregulated metabolites when individuals with schizophrenia were compared with nonpsychiatric controls. The metabolites linked to schizophrenia and those of nonpsychiatric controls were systematically categorized into separate functional groups (Fig. 2j). Notably, a significant portion of the modified metabolites fell within the categories of carboxylic acids, as well as fatty acyls, suggesting a potential association between schizophrenia and perturbed circulating amino acid and fatty acid profiles (Fig. 2k).

Unravelling the molecular determinants influencing age-related disease risk via a machine learning approach

Considering the profound risk of mortality associated with schizophrenia and the profound influence of aging on our proteomic profiles, we conducted a comprehensive evaluation of age-specific patterns in proteome abundance. Participants were categorically divided into three distinct age groups: young (under 31 years), middle- age (31–50 years), and advanced-age (over 50 years). This stratification was applied uniformly to both schizophrenia patients and nonpsychiatric controls. To validate the robustness of this age-based classification system, we employed hierarchical clustering analysis on the average protein abundances (Fig. 3a). Intriguingly, the proteomic profiles of nonpsychiatric controls over 50 years of age clustered closely with those of schizophrenia patients, whereas younger nonpsychiatric controls exhibited distinct clustering patterns. This finding implies a striking similarity in the serum proteome between schizophrenia patients of all ages and the advanced-age subset of nonpsychiatric controls. In order to further verify the differences in protein and metabolites expression among different age groups, we conducted a relative expression analysis based on the protein and metabolites expression levels of each age group, and the results showed that there were differences in the expression proteins at the three ages (Fig. 4).

To gain insights into age-related trends in our dataset, K-means clustering was performed on the average protein abundance from each of the eight distinct groups (Fig. 3b, Supplementary Table 4). Of particular interest was cluster 7, which exhibited a decline in average abundance values not only in the schizophrenia group but also in the nonpsychiatric control groups. We postulate that this cluster comprises a subset of associated proteins that may be linked to neurodegeneration and the premature emergence of age-related illness in schizophrenia patients. This hypothesis is based on the observation that these proteins exhibited reduced abundance levels among both older nonpsychiatric controls and patients with schizophrenia. The proteins within this cluster were



subjected to functional analysis. Peptidyl-serine phosphorylation, the Wnt signaling pathway, endocytosis, fatty acid β-oxidation, the histone phosphorylation pathway with protein kinase as the catalytic substrate and histone ubiquitination were the most significantly enriched terms (Fig. 5a).

Additionally, we evaluated the relationship between age and disease status on the basis of metabolic data. The metabolites were categorized via K-means clustering analysis on the basis of age division to visualize the relationships between metabolites and age. The metabolites in cluster 2 met the scope of our search, and enrichment analysis was subsequently performed on these metabolites (Fig. 6, supplementary Table 5). Purine metabolism, steroid hormone biosynthesis, nicotinate and nicotinamide metabolism and biosynthesis of unsaturated fatty acids were the most significantly enriched terms (Fig. 5b).

◀**Fig. 2**. Identification of characteristic profiles in the context of schizophrenia. (**a**) Volcano plot depicting binary comparisons of proteome data: The log10(SZ/NC) ratios highlight upregulation patterns in patients with schizophrenia (positive) versus nonpsychiatric controls (negative). Proteins marked blue or yellow represent significant differentially abundant proteins (p value < 0.05). (**b**) A comparative analysis of the molecular function Gene Ontology (GO) terms between upregulated and downregulated proteins in patients with schizophrenia with nonpsychiatric controls. (**c**) String-db network of dysregulated proteins in schizophrenia patients: significant alterations compared with those in nonpsychiatric controls (assessed by log10(SZ/NC) ratios), which include protein categories of fatty acid beta-oxidation, the Wnt/β-catenin signaling pathway, the dopamine receptor signaling pathway, and histone acetylation. Protein abundance profiles of (**d**) bromodomain and extrinsic proteins, (**e**) the dopamine receptor signaling pathway, (**f**) fatty acid beta-oxidation, (**g**) histone acetylation, and (**h**) the Wnt/β-catenin signaling pathway in patients with schizophrenia and nonpsychiatric controls. (**i**) Volcano plot depicting a binary comparison of metabolome data: log10(SZ/NC) ratios highlight upregulation patterns in patients with schizophrenia (positive) versus nonpsychiatric controls (negative). Proteins marked blue or yellow represent significant differentially abundant proteins (p value < 0.05). (**j**) Comparative analysis of the molecular function Gene Ontology (GO) terms between upregulated and downregulated proteins in patients with schizophrenia compared with nonpsychiatric controls. (**k**) Classification distribution of metabolites in nonpsychiatric control and schizophrenia subjects. There are distinct variations in the proportional distributions of identified lipids and lipid-like molecules, organic acids and derivatives, organic heterocyclic compounds, organic oxygen compounds and phenylpropanoids and polyketides. *SZ* schizophrenia patients, *NC* nonpsychiatric controls.

Integrated pathway and network analysis

A total of 230 metabolites in cluster 2 and 431 proteins in cluster 7 were submitted to MetaboAnalyst for canonical analysis. Combining the results of the canonical analysis with the result of the differentially expressed proteins enriched in fatty acid beta-oxidation, the Wnt/β-catenin signaling pathway, the dopamine receptor signaling pathway, histone acetylation and bromodomain and extrinsic proteins, the final five pathways, including the peroxisome, tryptophan metabolism, dopaminergic synapse, peroxisome proliferator-activated receptor (PPAR) signaling pathway and the mitogen-activated protein kinase (MAPK) signaling pathway were listed in Table 1. Additionally, in this integrated pathway and network analysis, 5-methoxytryptamine, 6-hydroxymelatonin, and homovanillic acid were identified described as robust biomarkers of schizophrenia (Fig. 5c-e).

disease risk biomarkers identified in the integrated analysis of proteins and metabolites.

Discussion

Schizophrenia is a chronic disease with an unknown cause that significantly disrupts to daily life; however, schizophrenia is also associated with physiological comorbidities thought to contribute to a shortened lifespan[18]. The course of the disease is generally prolonged, with repeated attacks, aggravation or deterioration, and the condition of some patients eventually decline to mental disability; however, some patients remain cured or near cured after drug treatment and psychological treatment[19]. The aetiology of schizophrenia is intricate and involves genetic, psychological, social and environmental factors, as well as brain structural and functional abnormalities. The investigation of the molecular mechanisms underlying schizophrenia is of paramount importance in improving our understanding of the metabolic disruptions experienced by patients with schizophrenia. In this study, metabolomic and proteomic analyses were employed to elucidate the comprehensive metabolic and proteomic alterations in schizophrenia patients, and metabolic pathways modified during schizophrenia development were identified. We subsequently constructed and screened metabolites and pathways related to age to determine the effect of age on the occurrence of schizophrenia. The sample size of the cohort (29 schizophrenia patients and 30 nonpsychiatric controls for metabolomics, and 6 schizophrenia patients and 4 nonpsychiatric controls for proteomics analysis) was determined, and dynamic changes were captured, resulting in the extraction of dependable differential information. Multivariate statistical analysis revealed a distinct separation between the schizophrenia group and the nonpsychiatric group. The k-means clustering algorithm was used to screen the profiles that coincided with the fluctuations in age. The potential biomarkers contributed to the separation of the schizophrenia group and the nonpsychiatric group. Our investigation revealed a significant alteration in approximately 544 serum proteins associated with fatty acid beta-oxidation, positive regulation of histones, histone ubiquitination, histone phosphorylation and dopamine receptor signaling. The identified proteins encompassed dopamine and histone mediators, indicating disorders of neurotransmitters and posttranslational modifications (PTMs) of histones. While the identification of these diagnostic proteins from a limited sample size is promising, extensive experimentation is needed to assess the reliability of the findings. The enriched pathways identified in schizophrenia, largely overlap with those associated with accelerated aging in schizophrenia, therefore providing circumstantial evidence for the relationship between aging and schizophrenia.

PTMs of the histone metabolic pathway may play a significant role in the pathogenesis of schizophrenia, with proteins associated with this pathway reflecting the molecular alterations observed. PTMs of histones involve the addition or removal of various chemical groups or proteins after their synthesis, altering their characteristics and activities. Histones serve as fundamental chromatin proteins within cells. In conjunction with DNA, histones contribute to the formation of the nucleosome structure and participate in the regulation of gene expression. Modifications to histones can impact the interaction between nucleosomes and DNA, subsequently influencing chromatin organization, nucleosome integrity, and chromosome confirmation[20]. These modifications have the capacity to convert chromatin to an active transcriptional state or repress gene expression. The patterns of

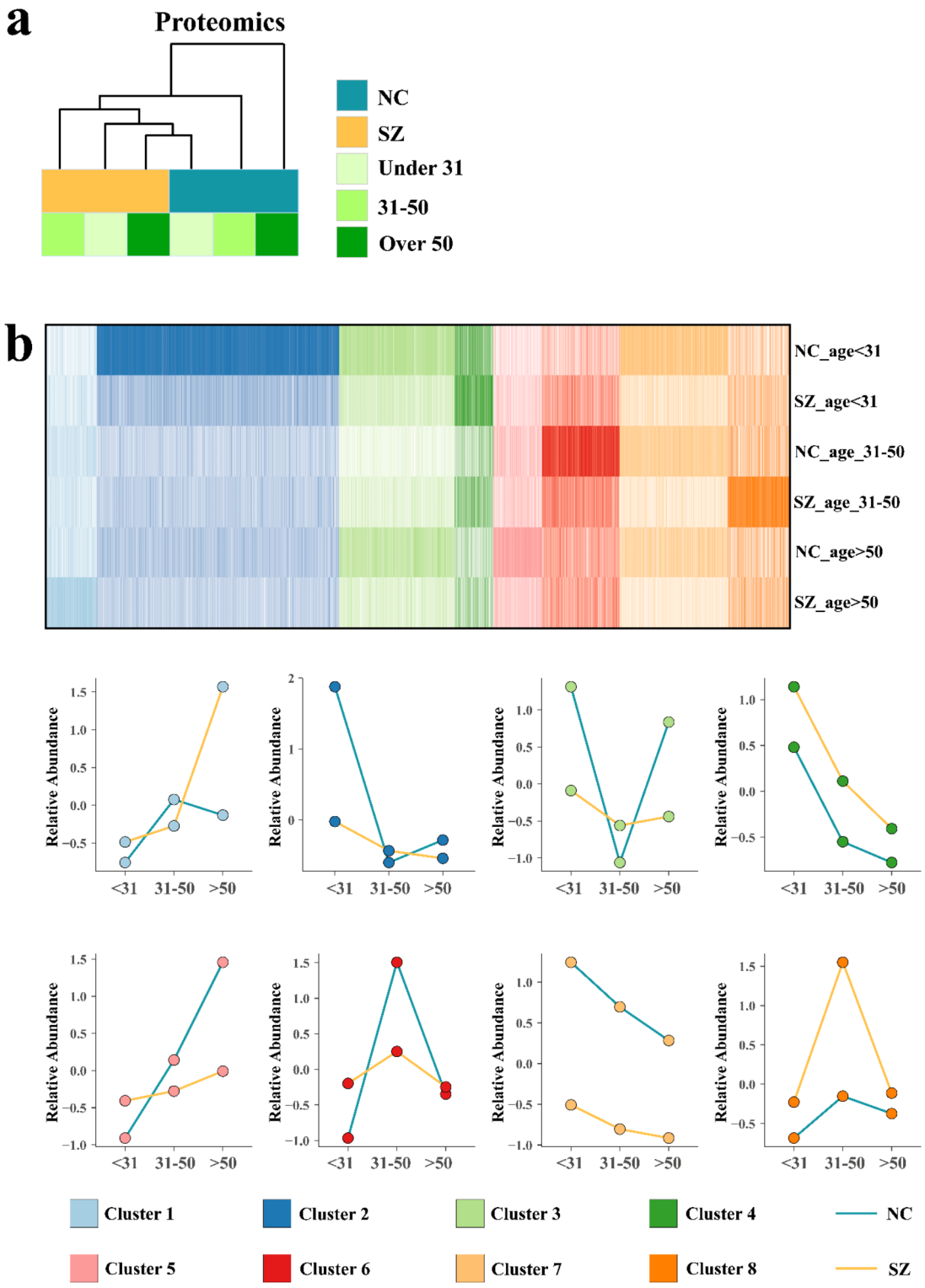


Fig. 3. Elucidating the molecular underpinnings of age-related disease through a machine learning-driven strategy. (**a**) Hierarchical clustering analysis of proteomic profiles in schizophrenia patients and nonpsychiatric controls across age groups revealed distinct clusters defined by disease status and age. Yellow: schizophrenia; blue–green: nonpsychiatric control; green: age over 50 years; fluorescent green: age 31–50 years; pale green: age under 31 years. (**b**) Stratification of differentially expressed protein clusters in schizophrenia (SZ) patients versus nonpsychiatric control subjects by age. The heatmap depicts protein expression levels, with white representing the relative minimum value per protein, while cluster-specific colors highlight the relative maxima for distinct clusters 1–8.

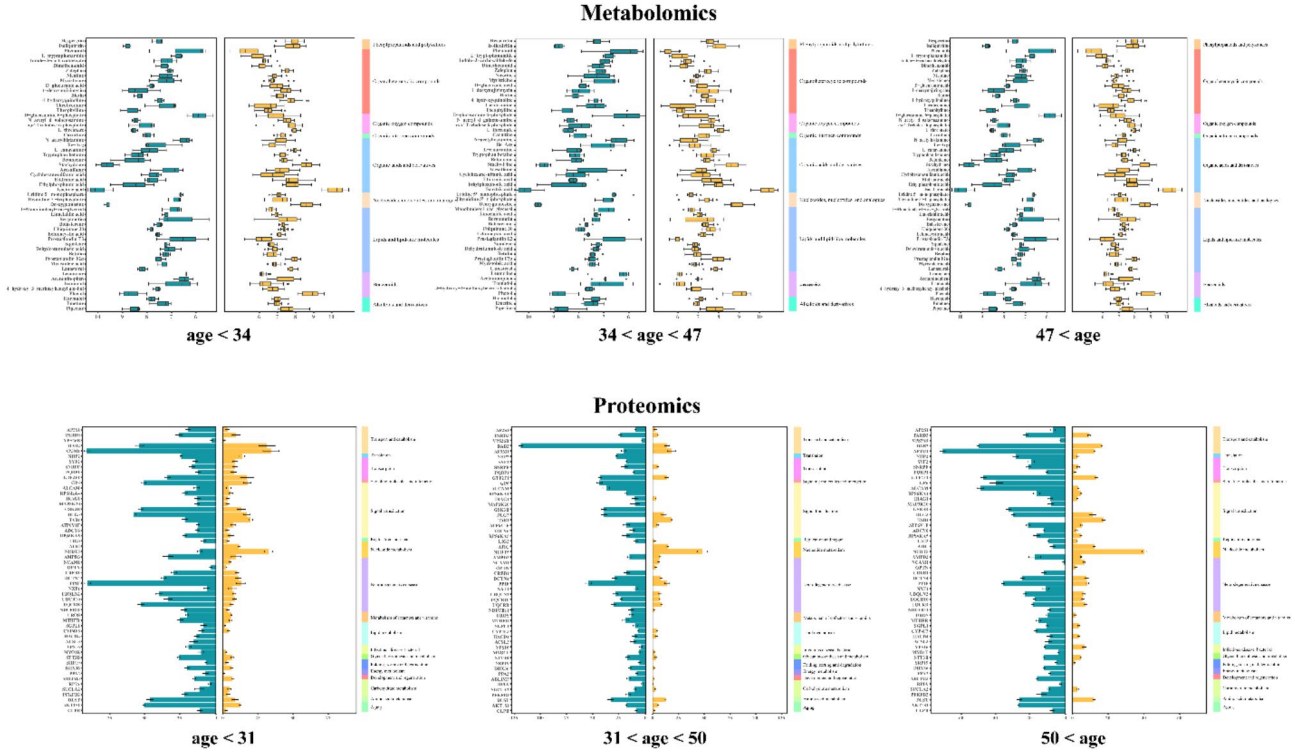


Fig. 4. Discriminating proteins and metabolites in schizophrenia patients and nonpsychiatric controls. Depiction of relative content of proteins and metabolites for distinguishing schizophrenia patients from nonpsychiatric controls in three age groups.

PTMs on histones establish a distinctive 'histone code' that is interpreted by specific reader proteins, such as the bromodomain and extraterminal (BET) family proteins. These readers exhibit a high affinity for altered histones, leading to the recruitment of effector complexes and the coordination of chromatin remodeling processes[21,22]. Through protein–protein interaction (PPI) analysis, the pivotal node proteins bromodomain-containing protein 3 (BRD3) and BRD4 were identified. BRD4, with its two bromo domains, can recognize and bind to histone N-ε-acetyl lysine (Kac) residues, thereby contributing to transcriptional regulation and chromatin remodeling[23]. The regulation of the therapeutically essential gene regulatory network is achieved through the recruitment of transcription factors leading to mediator complexes, the phosphorylation of RNA polymerase II, and intrinsic histone acetyltransferase activity[24]. Additionally, BRD4 affects the pathogenesis of various diseases and conditions, such as cancer, viral infections, inflammation, and neurological disorders[25].The downregulation of BRD4 expression in schizophrenia patients highlights the impact of transcriptional regulation on relevant genes, thereby influencing the progression of schizophrenia. Accumulating evidence has confirmed the connections between various changes in histone modifications and neurodegenerative diseases[26]. Proteins enriched in this pathway include ribosomal protein S6 kinase polypeptide 4 (RPS6KA4), RPS6KA5, and DExD-box helicase 21(DDX21), with RPS6KA4 and RPS6KA5 belonging to the ribosomal protein S6 kinases family, which is involved in diverse biological processes such as cell signaling and regulation[27]. PRS6KA4, which acts as a crucial threonine protein kinase, plays a key role in regulating diverse neural functions in the brain and is recognized as a memory-related molecule that is essential for synaptic plasticity, learning and memory[28]. Previous research findings have indicated that the cognitive impairments observed in schizophrenia may result from disruptions in kinase activity, and these disruptions affect complex intracellular signaling pathways. The activation of neurotransmitter receptors is intricately linked to cyclic adenosine monophosphate (cAMP)-mediated pathways through the enzymatic action of protein kinases[29]. Upon activation by cAMP, protein kinases phosphorylate downstream target molecules, leading to the modulation of essential cellular responses[30]. PRS6KA4, a kinase capable of phosphorylating various substrates, such as transcription factors and regulatory proteins, plays a role in influencing gene expression in neuropsychiatric disorders[31]. Furthermore, as a downstream effector of the MAPK pathway, extracellular signal-regulated kinase (ERK) can be activated by upstream MAPKs. ERK, a critical element of the signal transduction pathway, is involved in the regulation of cell proliferation, and differentiation, behavioural responses, and cognitive functions in organisms. Activation of the ERK pathway leads to the coordinated involvement of various complex components in a precise regulatory cascade. The duration and intensity of signal activation, as well as the dynamic subcellular redistribution of pathway components, in conjunction with complex chaperone–scaffold protein interactions and intricate communication with other signaling networks, collectively govern the stability, phosphorylation status, and subcellular localization of gene-encoded proteins[32]. These intricate response mechanisms also drive the extensive expression of downstream proteins, thereby playing crucial roles in the regulatory network of cellular physiology.

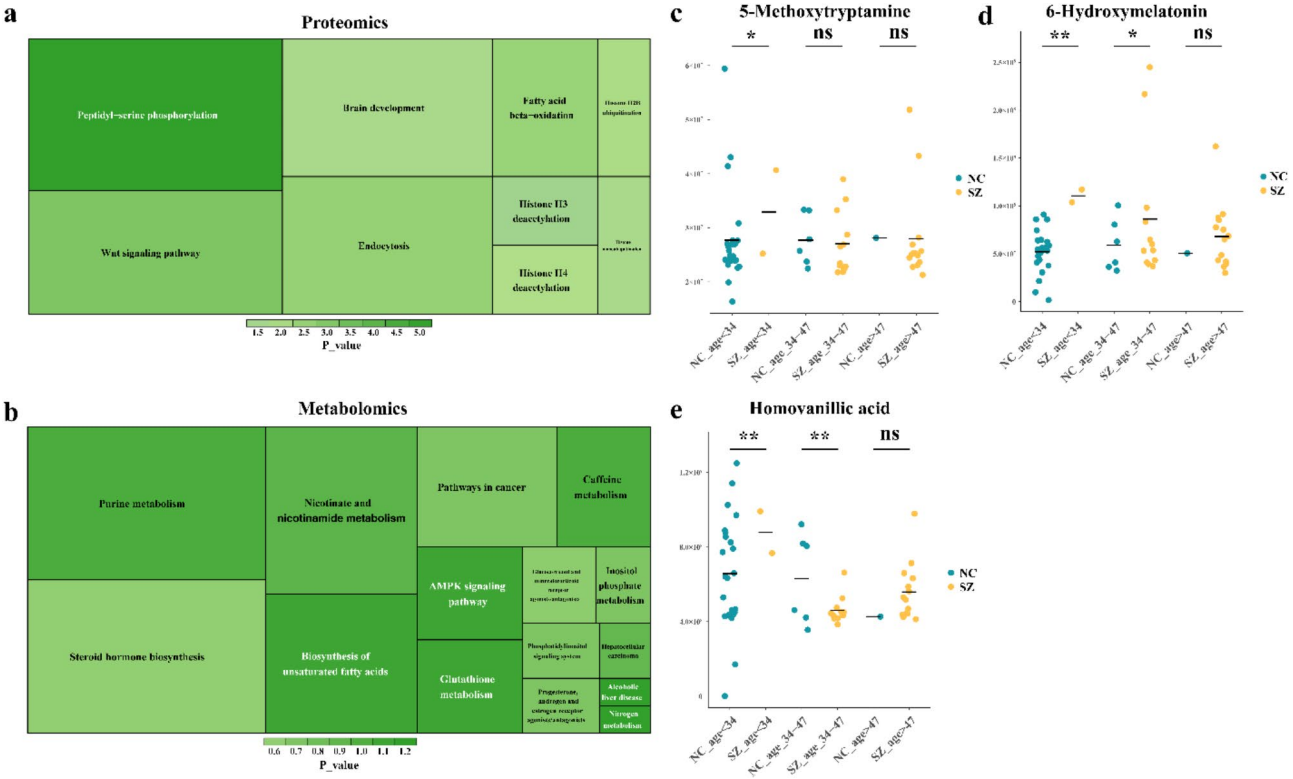


Fig. 5. Identifiable and targetable factors contributing to early morbidity in schizophrenia: key determinants for intervention strategies. (**a**) Reactome-based functional analysis of proteins belonging to cluster 7, visualized in a treemap for intuitive understanding of their biological roles and interactions. (**b**) Reactome-based functional analysis of metabolites belonging to cluster 2, visualized in a treemap for intuitive understanding of their biological roles and interactions. (**c**–**e**) Representative.

This study revealed enrichment of the Wnt/β-catenin signaling pathway, which plays a crucial role in various aspects of brain development, such as hippocampal development, neuronal proliferation and migration, brain regionalization, and synapse formation[33]. Within the Wnt/β-catenin signaling pathway, the Wnt family proteins act as ligands, secretory glycoproteins that bind to receptors on the cell membrane to facilitate signal transduction[34]. β-catenin, upon translocating to the nucleus after interacting with cadherins at cell junctions to form adherens junctions, regulates the expression of downstream genes in this process. A disrupted Wnt/β-catenin pathway has been implicated in the neuroinflammation associated with neurodegenerative diseases, depression, and autism spectrum disorder[35,36]. Our study revealed a significant decrease in the expression of CSNK1G1, CSNK1G2, and CSNK1G3, members of the casein kinase family, in patients with schizophrenia. This finding aligns with previous research by Wang, highlighting the role of the pleiotropic gene CSNK1G1 in immune-related signal transduction and pathways related to cerebral disorders[37]. The casein kinase family genes, while not directly involved in the Wnt/β-catenin signaling pathway, demonstrate extensive substrate specificity and the ability to phosphorylate various proteins, thereby impacting the development of schizophrenia.

Dopamine neurotransmission is crucial in modulating intricate cognitive and behavioural functions such as motivation and movement[38]. Dopamine, a monoamine neurotransmitter, that is located primarily in the substantia nigra compacta and ventral tegmental area of the midbrain, plays a crucial role in modulating various behaviours through the formation of neural circuits with neurons in other brain regions. Dopamine receptors can engage in indirect allosteric interactions with other receptor proteins, resulting in the formation of receptor complexes that enable the modulation of signal processing at the membrane level, resulting in distinct pharmacological properties[38]. In the realm of signal transduction, there is a prevailing consensus that receptors primarily facilitate the activation of the second messenger cAMP through interaction with the Gs protein[39]. The expression of key proteins within this pathway, such as β-arrest in 2 (ARRB2), adenylyl Cyclase 6 (ADCY6), and glycogen synthase kinase 3 Beta (GSK3B), tends to decrease. ARRB2, specifically, plays a crucial role in modulating the desensitization, internalization, and signal transduction of G-protein-coupled receptors. Zaïmia's research was focused primarily on elucidating the regulatory role of ARRB2 in modulating the signaling of G-protein-coupled receptors, specifically the glucagon-like peptide-1 receptor (GLP-1R) and glucose-dependent insulinotropic polypeptide receptor (GIPR), within the framework of diabetes[40]. While direct research linking ARRB2 to schizophrenia is scarce, its pivotal involvement in GPCR signaling pathways and the association of dopamine receptors with this disorder suggest a potential contribution of ARRB2 to certain aspects of schizophrenia. GSK3B is a serine/threonine kinase implicated in diverse pathophysiological mechanisms within the nervous system, including transcriptional activation, cell proliferation, and cell

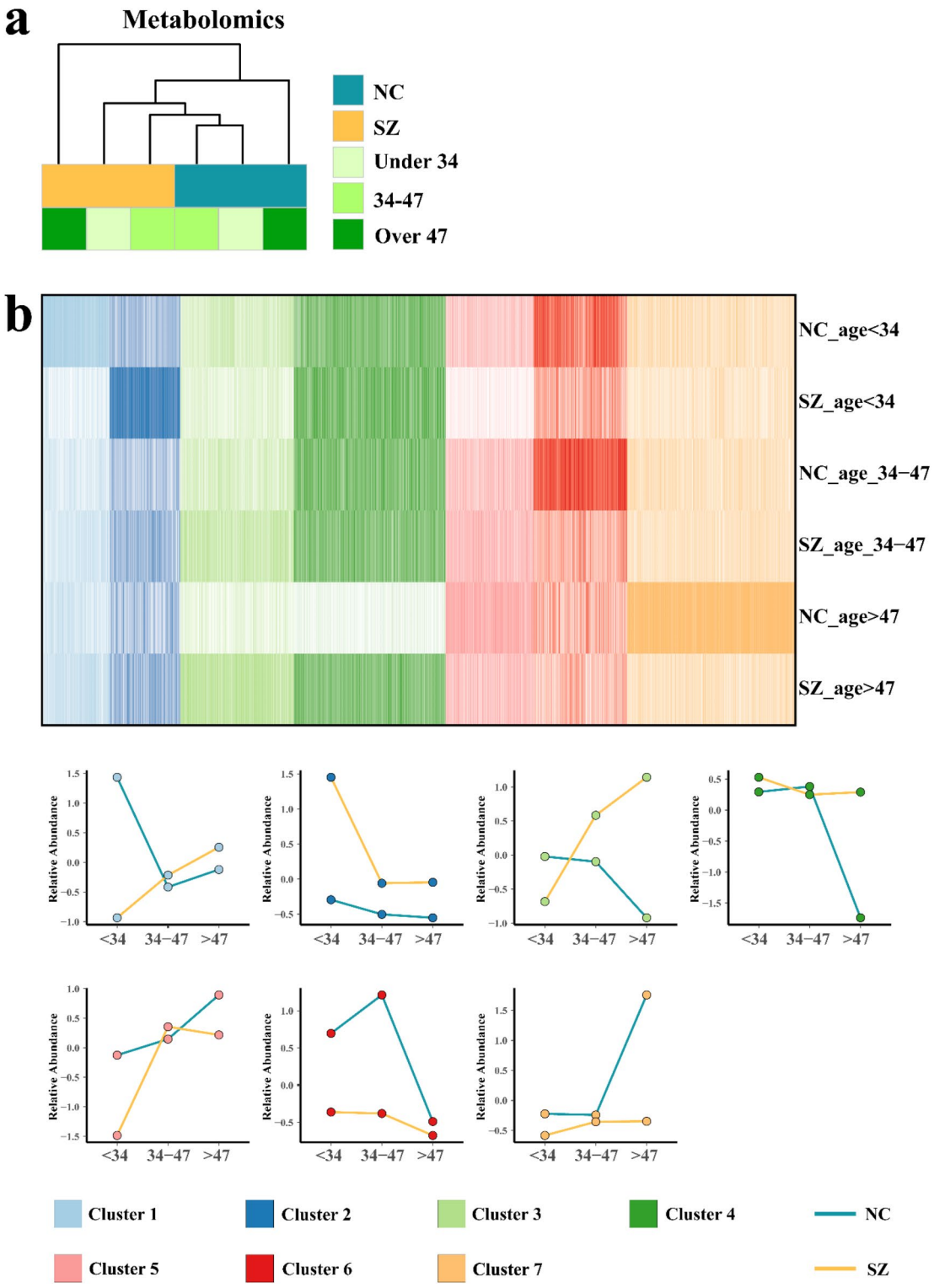


Fig. 6. Elucidating the molecular underpinnings of age-related disease through a machine learning-driven strategy. (**a**) Hierarchical clustering of metabolomic profiles in schizophrenia patients and nonpsychiatric controls across age groups revealed distinct clusters defined by disease status and age. Yellow: schizophrenia; blue–green: nonpsychiatric control; green: over 47 years; fluorescent green: 34–47 years; pale green: under 34 years. (**b**) Stratification of differentially expressed protein clusters in schizophrenia (SZ) patients versus nonpsychiatric controls subjects by age. The heatmap depicts protein expression levels, with white representing the relative minimum value per protein, while cluster-specific colors highlight the relative maxima for distinct clusters 1–7.

Pathway	Raw-*p*	Proteins	Metabolites
Peroxisome	0.002	ACOX1; SCP2; ECH1	
Tryptophan metabolism	0.006	HADH	5-Methoxytryptamine; 6-Hydroxymelatonin
Dopaminergic synapse	0.01	GSK3B; ARRB2	Homovanillate
PPAR signaling pathway	0.02	SCP2; ACOX1	
MAPK signaling pathway	0.06	RPS6KA4; RPS6KA5; ARRB2	

Table 1. Top canonical pathways identified via metaboanalyst of integrated proteomic and metabolomic data.

differentiation. GSK3B activation plays a crucial role in the aberrant aggregation of tau proteins, a key factor in neuronal death and a primary driver of Alzheimer's disease progression[41,42]. Emamian's findings indicate a decrease in the phosphorylation levels of AKT1 and GSK3B in the brains and peripheral lymphocytes of individuals with schizophrenia, which aligns with our own research findings[43]. Recent research has highlighted the pivotal role of the GSK3B signaling pathway in modulating inflammatory responses, which have been implicated as contributing factors in the development of neurological disorders[44].

Lipid mobilization via fatty acid β-oxidation is crucial for energy production to support biological functions[45]. Fatty acid metabolism plays a crucial role in the complex network of energy metabolism, involving the synthesis, transport, and oxidative degradation of fatty acids. Furthermore, increased β-oxidation has been shown to significantly increase active oxygen species (ROS) and oxidative stress levels[46]. The augmented generation of ROS and the presence of antioxidant defence mechanisms indicate that oxidative stress is involved in the pathophysiology of schizophrenia[47]. Elevated oxidative stress has been detected in the central nervous system of individuals with schizophrenia[48] which is consistent with our findings. The suppression of the proteins hydroxyacyl-coenzyme A dehydrogenase (HADH) and enoyl-CoA hydratase 1(ECH1) identified in this study may indirectly impact neuronal energy provision and metabolic equilibrium by regulating the efficacy of fatty acid β-oxidation, thus significantly affecting normal neuronal function. Sterol carrier protein 2 (SCP2) plays a crucial role in the transportation and equilibrium of cholesterol and fatty acids during fatty acid metabolism.

Aberrations in nicotinate and nicotinamide metabolism have been consistently reported in various studies on schizophrenia[49]. Research has shown that antipsychotic drugs can potentially improve cognitive function in individuals with schizophrenia by improving energy metabolism[50]. Nicotinamide adenine dinucleotide (NAD+) plays crucial roles in energy metabolism. Studies have indicated that NAD + levels are notably lower in individuals with schizophrenia than in those without the disorder[51]. Supplementation with NAD + or its precursors, such as nicotinamide mononucleotide, has been shown to improve learning and memory, indicating that increasing NAD + levels may help restore brain energy metabolism and mitigate cognitive deficits[52]. AMP-activated protein kinase (AMPK) functions as a crucial cellular energy sensor and metabolic regulator, playing a pivotal role in maintaining energy homeostasis[53,54]. Through the phosphorylation of various transcription factors and metabolic enzymes, AMPK modulates their activity in response to fluctuations in energy metabolism[55]. In conjunction with cholesterol, steroid hormones play a significant role in the synthesis of neurosteroids, which are progesterone metabolites, in various brain regions to regulate neuronal development, regeneration, and neurotransmission[56].

Age-based stratification analysis revealed that specific molecular features are associated with an increased risk of age-related comorbidities and mortality in individuals with schizophrenia, as determined through canonical pathway analysis. The identification of tryptophan metabolism and the dopaminergic synapse pathway through this analysis suggests that molecules that function in these pathways could be targets for the prevention of comorbidities in younger patients with schizophrenia. The dysregulation of tryptophan metabolism can result in the accumulation of neuroactive metabolites with potential neurotoxic properties, potentially influencing the pathophysiological mechanisms of schizophrenia[56]. Additionally, specific metabolites produced through tryptophan metabolism have the ability to impact the activity and expression of dopamine receptors, consequently influencing the efficacy of dopaminergic synaptic transmission[57]. This phenomenon can be explained by the physiological changes that occur in individuals as they age, including alterations in metabolic rates and characteristics that impact the number and sensitivity of dopamine receptors. These changes subsequently influence the transmission rate of synapses, thereby affecting the overall function of the nervous system. Additionally, evidence suggests that interactions between tryptophan metabolism and the immune system may play a role in the development of schizophrenia[58].

Conclusions

In this study, a total of 342 metabolites and 544 proteins were identified through univariate and multivariate statistical analyses. Pathway enrichment analysis revealed that the differentially abundant metabolites primarily participated in processes such as fatty acid β-oxidation, the dopamine receptor signaling pathway, the Wnt/β-catenin signaling pathway and histone acetylation. Additionally, age-related modules were developed using multi-omic data to provide a detailed understanding of the initiation and progression of schizophrenia. The results of this study may help elucidate the potential mechanism involved in the pathogenesis of schizophrenia. Nevertheless, limitations such as the small sample size and absence of verification analysis were factors in the current study. Thus, validation of these findings through a study of utilizing a larger, external sample is necessary to substantiate the findings of this research.

Data availability

Data available within the article or its supplementary materials. All raw data were deposited in Mass Spectrometry Interactive Virtual Environment under accession number MSV000095241 for proteomics data and the direct link is ftp://massive.ucsd.edu/v08/MSV000095241/ and MSV000095242 for metabolomics data and the direct link is ftp://massive.ucsd.edu/v08/MSV000095242/.

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Dong Dong Qi and Peng Liu wrote the main manuscript, all authors reviewed the manuscript.

Declarations

Competing interests
The authors declare no competing interests.

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