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Comparing the proteome profiling of plasma-derived extracellular vesicles in individuals with schizophrenia and healthy controls: a pilot study

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ABSTRACT

Background: Studying extracellular vesicles (EVs)—tiny intercellular communicators with a diverse molecular cargo—may provide insight into the poorly understood neuropathology and pathophysiology of schizophrenia (SCZ). Here, we focused on the protein profile of plasma-derived EVs with the aim to detect differences between individuals with SCZ and healthy controls (HCs).

Methods: EVs were isolated from blood plasma of 73 individuals with SCZ and 77 HCs and evaluated by nanoparticle tracking analysis, electron microscopy, and Western blot analysis. EV proteins were identified by liquid chromatography coupled with mass spectrometry. Proteomic data were analyzed using logistic regression analyses.

Results: A total of 1659 proteins were identified, and analyses were performed with the 1021 proteins with quantitative data available for more than 50 % of the participants in each group (SCZ and HC). Comparing these proteins between the two groups showed no significant differences. An enrichment analysis based on the proteins with a noncorrected significant *p* value revealed a significant enrichment of pathways connected to complement system. Although comparison of complement components between the two groups did not show significant differences, but it demonstrated that antipsychotics and duration of illness may affect EV protein levels.

Conclusion: Overall, even as our findings highlight the importance of medication use and duration of illness on EV protein levels, they suggest that the complement system may be involved in the etiopathology of SCZ. Although, the results of this pilot study need to be replicated in larger research, they may help elucidate the mechanisms involved in the pathophysiology of SCZ.

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1. Introduction

Schizophrenia (SCZ) is a complex, highly heritable, and heterogeneous neuropsychiatric disorder with diverse clinical symptoms and manifestations, including hallucinations, delusions, disorganized thinking, and cognitive deficits (Mueser and McGurk, 2004). Although SCZ is one of the top 10 causes of long-term disability worldwide, with an estimated lifetime prevalence of around 1 % and a heritability rate of 80 %, its exact etiology is still unclear (Mueser and McGurk, 2004; Wang et al., 2022).

Currently, SCZ is diagnosed by assessing symptoms and signs through clinical interview-based tools and observation (Kumar et al., 2024; Wang et al., 2022). Because there are no reliable biomarkers for SCZ and some symptoms may overlap with those of other psychiatric disorders, the risk of misdiagnosis and inappropriate treatment is high (Kumar et al., 2024; Wang et al., 2022). To this end, discovering valid and robust molecular fingerprints of SCZ with high specificity and sensitivity may aid in the development of effective diagnostic and therapeutic strategies. In this context, technological advancements enabling multi-omics profiling approaches, such as genomics, proteomics, and metabolomics, have paved the way for defining biomarker panels for psychiatric disorders, including SCZ (Oraki Kohshour et al., 2023; Zorkina et al., 2024). For instance, liquid chromatography coupled with mass spectrometry (LC-MS) has enabled hypothesis-free identification and quantitative comparison of proteomics in different samples in a robust, high-throughput manner (Abramowicz et al., 2016; Shao et al., 2018).

Extracellular vesicles (EVs), a potential source of disease-related biomarkers, have emerged as an interesting field of study over the past decade (Abramowicz et al., 2016; Vassileff et al., 2020). EVs are membrane-enclosed nanoparticles that are released by many cell types and are present in various physiological fluids, such as plasma, cerebrospinal fluid, and urine (Mathew et al., 2021). They are thought to be involved in many biological processes, such as cellular communication, tissue homeostasis and regeneration, and immune response modulation (Cioanca et al., 2023; Mathew et al., 2021; Zhang et al., 2023). EVs with a diameter of less than 200 nm, referred to as small EVs, can cross the blood-brain barrier in both directions, so EV-mediated cell signaling has been hypothesized to regulate a range of physiological mechanisms by facilitating bidirectional communication between cells in the periphery and neuronal and glial cells in the central nervous system (CNS) (Cioanca et al., 2023; Théry et al., 2018; Tsilioni et al., 2014).

EV abnormalities have been reported to play a role in the development of SCZ (Zhang et al., 2023). EVs contain bioactive molecules such as proteins, microRNAs, and lipids that are probably representative of the physiological state of the origin (parent) cells (Burgelman et al., 2022; Oraki Kohshour et al., 2023). There have been various studies on the composition of blood-derived EVs as possible biomarkers for SCZ. A lipidomic analysis of serum-derived EVs using LC-MS/MS has found 39 lipids that exhibit significant alterations in individuals with SCZ when compared to healthy controls (HCs) (Xu et al., 2025). Out of these 39 lipids, seven lipids were found by bioinformatics analysis to be highly effective in differentiating individuals with SCZ from HCs. This demonstrated that the serum-EV lipid composition may be improperly regulated in individuals with SCZ and that lipids might play a significant role in the pathophysiology of SCZ (Xu et al., 2025). In addition, a metabolomic study on serum-derived EVs from individuals with SCZ identified a panel of 25 metabolites that performed good to excellent in differentiating individuals with SCZ from HCs (Du et al., 2021). Also, differential expression profiles were found in the first genome-wide miRNA expression profiling of serum-derived EVs from individuals with SCZ. In this study, the expression of BDNF was found to be influenced by the most differentially expressed miRNA, miR-206, and its overexpression was proposed to cause BDNF dysfunction (Du et al., 2019).

The EV protein cargo may be engaged in a wide range of physiological processes (Mathew et al., 2021), and in the past years, numerous studies have examined the protein cargo of plasma- and brain-derived EVs of individuals with SCZ (Goetzl et al., 2021; Kapogiannis et al., 2019; Lee et al., 2020; Ranganathan et al., 2022; Wijtenburg et al., 2019). For example, one study on plasma-derived EVs found that individuals with SCZ had higher levels of glial fibrillary acid protein (GFAP) and lower levels of α-II-Spectrin than HCs (Ranganathan et al., 2022). In another study on astrocyte-derived EVs in plasma, higher levels of amyloid-beta 1–42 were reported in individuals with SCZ than in HCs (Lee et al., 2020). In 2021, Goetzl et al. showed that individuals with first-episode psychosis had abnormal amounts of mitochondrial proteins in both astrocyte- and neuron-derived EVs in plasma (Goetzl et al., 2021).

Accordingly, in this pilot study we used mass spectrometry-based proteomics to characterize the proteomic profile of plasma-derived EVs with the aim to explore differences in EV proteins between individuals with SCZ and HCs and to pave the way for the discovery of robust and reliable biomarkers.

2. Methods

2.1. Participants

This pilot study included 73 individuals with SCZ, diagnosed according to the International Classification of Diseases and Related Health Problems, tenth revision (ICD-10), and 77 HCs (Table 1). The participants are part of the Multimodal Imaging in Chronic Schizophrenia Study (MIMICSS), a subset of the PsyCourse Study, which was performed in Munich, Germany (Budde et al., 2019; Heilbronner et al., 2021). Written informed consent was obtained from each participant. The ethics committee of the University Hospital Munich approved the study (project number: 17-13), and the study was performed in compliance with the tenets of the Declaration of Helsinki.

2.2. Plasma samples and EV isolation

For our study, we used an aliquot of −80 °C frozen plasma samples obtained by centrifugation (1100*g* for 15 min at 4 °C) of whole blood in EDTA-treated tubes. After thawing on ice, 150 plasma samples were centrifuged at 3000 *g* for 10 min at 4 °C to precipitate cells and cellular debris. The supernatants were subsequently centrifuged at 12000*g* for 10 min at 4 °C to remove large vesicles. Then, size exclusion chromatography columns (qEV single gen2, IZON Company; https://izon.com) were used to separate EVs (according to EV size) from 150 µl of the supernatants. Freshly filtered (0.22 µm) phosphate-buffered saline was used as an elution buffer. According to the manufacturer's instructions,

Table 1
Demographic and psychopathological data of study participants.

	Group	
Data	SCZ[a]	HC
Participants, n	70	77
Sex, % female	21.5	40.3
Age, mean ± SD, y	35 ± 11.9	35.5 ± 13.9
Duration of illness, mean ± SD, y	4.26 ± 7.8	–
Number of antipsychotics per patient, mean	0.8	–
PANSS_Positive, mean ± SD	13.8 ± 5.4	–
PANSS_Negative, mean ± SD	16.5 ± 5.2	–
PANSS_General, mean ± SD	30.5 ± 8.2	–
BDI-II Sumscore, mean ± SD	13.8 ± 9.3	–
IDS-C_{30} Sumscore, mean ± SD	19.2 ± 9.7	–

[a] Individuals in this group were being treated as inpatients at the time of original study (MIMICSS).

BDI-II, Beck Depression Inventory; HC, healthy controls; IDS-C_{30}, Inventory of Depressive Symptomatology; PANSS, Positive and Negative Syndrome Scale; SCZ, schizophrenia

the majority of EVs usually elute in the 0.68 ml obtained after the buffer volume (0.87 ml), so collecting the first 0.51 ml is recommended for improved EV purity. However, to maximize purity, we collected only the first 0.4 ml after the buffer volume as an EV fraction.

Following the EV isolation, we used electron microscopy, nanoparticle tracking, and western blot analyses to confirm the characteristics of EVs using five randomly selected EV-isolated samples.

2.3. Transmission electron microscopy

EV morphology and size were investigated by transmission electron microscopy (TEM). In brief, 5 μl of the EV samples were applied to carbon-coated copper grids, washed twice with double-distilled water, stained for 20 s with 1 % uranyl acetate that also contained 0.1 % glucose, blotted on a filter paper, and air dried. TEM of negatively stained samples was performed at 80 kV with a Zeiss electron microscope EM912 (Zeiss, Oberkochen, Germany) in combination with a 2 k × 2 k slow-scan charge-coupled device camera (TRS Tröndle Restlichtverstärkersysteme, Moorenweis, Germany).

2.4. Nanoparticle tracking analysis

Nanoparticle tracking analysis (NTA), which assesses particles by detecting the light they scatter, was performed to determine the size distribution and concentration of the isolated EVs. We used a Zetaview twin nanoparticle tracking analyzer (Particle Metrix, Germany; https://particl-metrix.com/zetaview). The EV samples were thawed individually and kept on ice. Then, they were illuminated by laser (520 nm; sensitivity, 85; shutter, 100) and the movement of the nanoparticles due to Brownian motion was recorded (other parameters: particle size, 5–1000 nm; minimum brightness, 30; minimal area of particles, 30; minimal tracking length, 15 frames). Data were analyzed with the ZetaView analysis software, version 8.05.16.

2.5. Western blot

Western blot (WB) analysis was used to determine the surface markers of the isolated EVs. To do this, the EV samples were lysed in RIPA buffer (1:1) previously mixed with 1 % protease inhibitor (Halt Protease and Phosphatase Inhibitor Cocktail, ThermoFisher) and 5 mM EDTA for 15 min on ice. Lysates were centrifuged at 14,000*g* for 12 min at 4 °C, and proteins in the supernatants were quantified by detergent compatible protein assay (Bio-Rad). A volume of 21 μl from each EV sample was incubated in 7 μl of 4× Lämmli buffer (Bio-Rad) and then separated on stain-free 4 % to 20 % Criterion gels through sodium dodecyl-sulfate polyacrylamide gel electrophoresis with TGS buffer (250 mM Tris, 1.92 M glycine, 0.1 % sodium dodecyl-sulfate, pH 8.3; 50 V for 15 min and then 200 V for 40 min). After that, proteins were transferred onto polyvinylidene fluoride membranes (Immuno-Blot Low Fluorescence PVDF Membrane, Bio-Rad). The membranes were blocked for 1 h in Intercept Blocking Buffer (LI-COR) and then incubated overnight with one of the primary antibodies, i.e., anti-CD9 (1:1000, MCA469GA, Bio-Rad, USA), anti-flotilin-2 (1:1000, VPA00740, Bio-Rad, USA), or anti-Calnexin (1:1000, 2433S, Cell Signaling Technology, USA), at 4 °C. Then, the membranes were incubated with the fluorescent secondary antibodies Starbright Blue 520 and 700 Goat-anti mouse or Goat-anti rabbit (Bio-Rad) at a concentration of 1:10000 for 1 h. Each step was followed by five washes in Tris-buffered saline with Tween20 for 5 min. Blots were visualized with the ChemiDoc Imaging System (Bio-Rad).

2.6. Mass spectrometry sample preparation and measurement

Sodium deoxycholate (SDC) buffer (6 % SDC [*w/v*], 100 mM Tris pH 8.5) was added to approximately 100 μL of the samples to reach a final concentration of 2 % SDC, and samples were then incubated at 95 °C, 1000 rpm, for 10 min. Subsequently, samples were sonicated on a Covaris E220 focused ultra-sonicator (2 min, peak incident power 175 W, 200 cycles per burst, 10 % duty factor, 20 °C), and centrifuged (3220 *g*, 20 °C, 10 min). A bicinchoninic acid assay (Pierce 23225) was performed on the supernatants to determine the protein concentration, and, if possible, the protocol was continued with 1 μg protein per sample. Samples were filled up to 100 μL with 1 % SDC (*w/v*), 10 mM Tris (2-carboxyethyl) phosphine, 40 mM 2-chloroacetamide in 100 mM Tris pH 8.5 and were incubated at 95 °C, 1000 rpm, for 10 min in a ThermoMixer C (Eppendorf) for reduction and alkylation.

After samples had cooled down, 40 ng of Trypsin/Lys-C mix (Promega V5073) was added and protein digestion was performed at 37 °C, 350 rpm, in a ThermoMixer C (Eppendorf) for 7 h. Samples were acidified to 1 % TFA to stop digestion. After centrifugation (3220 *g*, 20 °C, 30 min) to remove precipitations, 50 μL of the supernatant were directly loaded on Evosep One tips for injection into the LC device according to the manufacturer's instructions. Briefly, the C18 material in Evotips was washed with ACN/0.1 % FA, conditioned in 2-propanol, and equilibrated with 0.1 % FA before loading the sample; the Evotips were washed twice with 0.1 % FA. Peptides were injected onto an Evosep One LC (Evosep) directly coupled to an Exploris 480 mass spectrometer (Thermo Scientific). The 30 samples per day method with a 15 cm column (PepSep, 15 cm × 150 μm, 1.5 μm ReproSil C18) was used on the LC device, and the mass spectrometer was operated in data-independent acquisition (DIA) mode. In addition to the samples, replicate injections were measured to monitor LC-MS performance and pool samples that were digested on the plates. Raw files were searched with Spectronaut version 19.5.241126.62635 (Biognosys) with direct DIA+ by using the human fasta file downloaded in the software (uniprot_sprot_2022-01-07, 20375 entries) and cross-run local normalization, with a Qvalue cutoff of 1 %, and a peptide-spectrum match, peptide, and protein false discovery rate of 1 % with carbamidomethyl (C) as fixed and acetyl (protein N-terminus) and oxidation (M) as variable modifications.

2.7. Statistical analysis

Data were normalized by applying the inverse transformation method to the protein levels, and only the proteins that were identified in at least 50 % of the samples in each group were kept for further analyses. Logistic regression models in R version 4.3.0 (https://www.R-project.org/) were used to investigate the association of plasma-derived EV-protein levels with diagnostic status. The proportion of explained variance (both R2 and Nagelkerke's pseudo-R2) was calculated by subtracting the effects of the covariates from the full model. The dimensionality of the EV-proteome dataset was reduced by principal component analysis (PCA), an unsupervised feature transformation method that checks the difference in principal components between cases and controls. The analyses included age, sex, duration of illness, and antipsychotics (i.e., number of antipsychotics taken by each individual at the time of sampling) as covariates. The "sensemakr" package in R was used to conduct sensitivity analysis of the covariates in our study in order to statistically check the fragility of putative causal estimations (Cinelli and Hazlett, 2020; "R package sensemakr"). The results were considered statistically significant if p value was less than 0.05 after adjusting for multiple comparisons by the stringent Bonferroni correction.

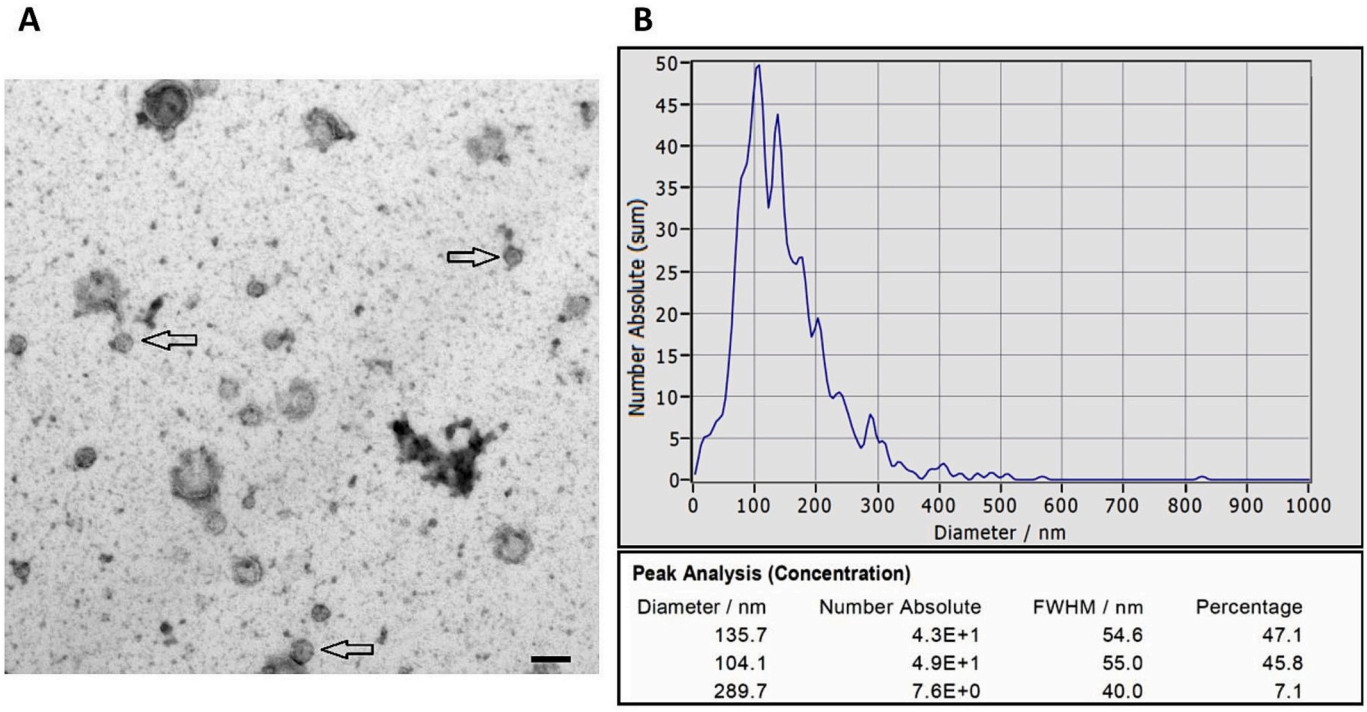
3. Results

LC-MS analysis identified a total of 1659 proteins in the samples. After normalization and selection of proteins for which quantitative data were available for more than 50 % of participants in each group (SCZ and HCs), 147 individuals (101 men, 46 women; age [mean ± SD], 35.3 ± 12.9 years) and 1021 proteins remained for analyses. The two groups did not significantly differ in age (F value = 0.044; $p = 0.833$), but the

SCZ group had a significantly lower proportion of women than the HC group (χ squared = 5.203; p = 0.022). The demographic and psychopathological information of study participants is presented in Table 1.

3.1. Characterization of EVs

TEM analysis of our samples revealed the presence of spherical-shaped vesicles surrounded by a membrane (Fig. 1A), and NTA



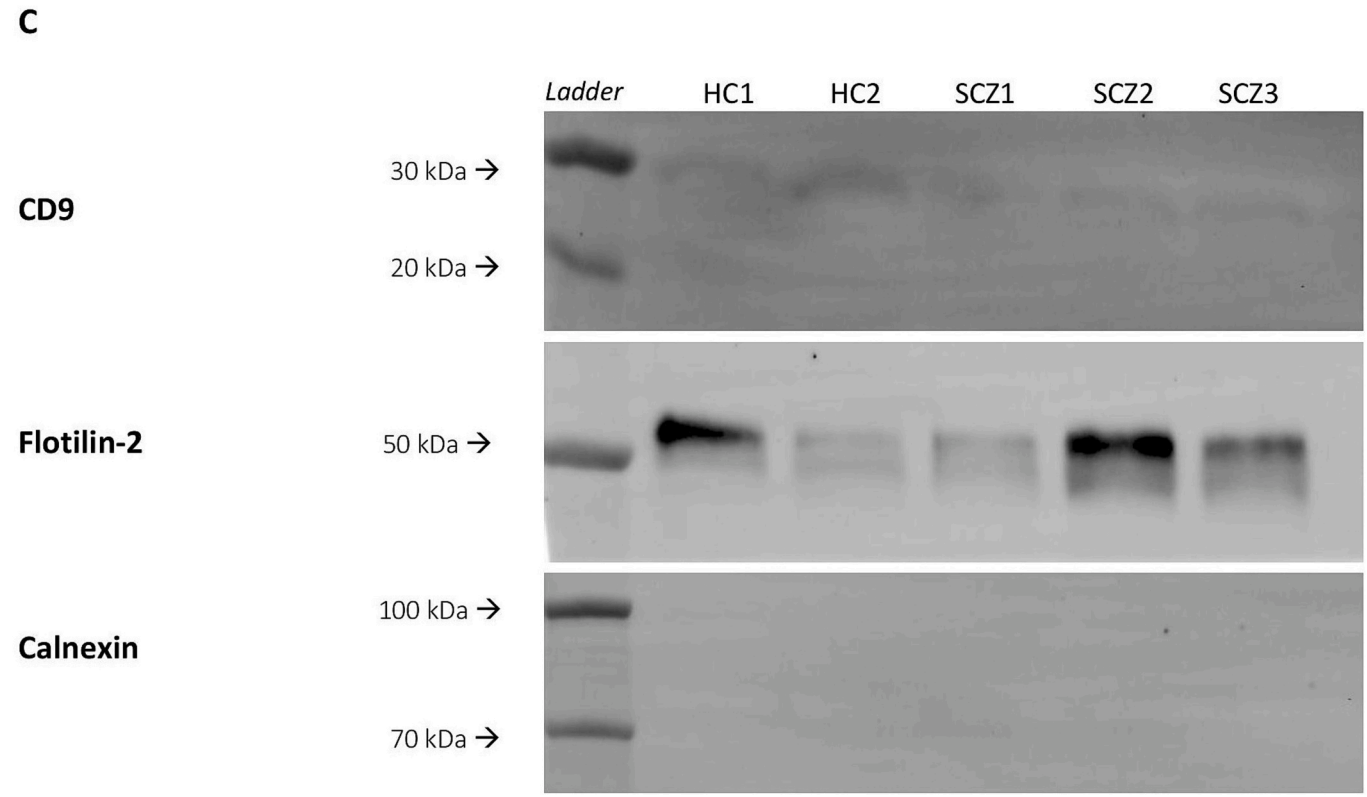


Fig. 1. Characterization of extracellular vesicles. A. The morphology of isolated extracellular vesicles (EVs) was evaluated by transmission electron microscopy (scale bar, 100 nm). The arrows point to some of the isolated EVs. B. The size distribution of the isolated EVs was determined by nanoparticle tracking analysis. C. Western blot analysis of two positive EV markers (CD9 and flotilin-2) and a negative marker (calnexin).
SCZ, schizophrenia; HC, healthy control.

showed that the isolated EVs mostly ranged in size from 50 to 200 nm (Fig. 1B). Moreover, WB analysis revealed that EV markers (i.e., CD9 and flotilin-2) were present in our EV samples and that calnexin, which generally shows contamination by intracellular proteins, was absent (Fig. 1C). Altogether, these findings confirmed that we successfully isolated EVs from the plasma samples.

3.2. Mass spectrometry-based protein analysis of plasma-derived EVs

Logistic regression analysis revealed that the proteome quantitative data from the two groups did not differ significantly when all the proteins analyzed in our study ($n = 1021$) were compared (*Supplementary Table S1*). The PCA with a dataset that included 106 individuals with non-missing data for 312 proteins also showed that the case and control groups could not be efficiently separated (Fig. 2); the demographic and clinical features of this subsample were similar to those of the entire sample.

Based on the proteins with a non-corrected p value of less than 0.05 ($n = 104$ proteins), we performed an enrichment analysis using Enrichr (https://maayanlab.cloud/Enrichr). The findings revealed a significant enrichment of pathways connected to complement system (*Supplementary Table S2*). Therefore, we compared the complement components between the SCZ and HC groups, and the results showed no significant association (*Supplementary Table S3*). According to the results of sensitivity analyses for the available covariates, antipsychotics with a robustness value (RV) of 0.6 and a $R^2Y \sim D|X$ of 0.48 had a relatively robust effect, and sex with a RV of 0.05 and a $R^2Y \sim D|X$ of 0.003 had a relatively low impact on the EV protein levels analyzed in this study (*Fig. S1; Supplementary Table S4*). When we ran a regression analysis without controlling for antipsychotics and duration of illness (the covariates with RV greater than 20 %), the levels of complement C4A (Bonferroni-adjusted $p = 0.019$, odds ratio [OR] = 1.91, 95 % CI = 1.28–2.85) and complement C9 (Bonferroni-adjusted $p = 0.025$, OR = 1.92, 95 % CI = 1.27–2.9) were found to differ significantly between the SCZ and HC groups (*Fig. S2; Supplementary Table S5*).

4. Discussion

EVs are a potential biomarker source for a variety of diseases, including neuropsychiatric disorders. The difference in plasma-derived EVs profile could point to the role and importance of circulating EVs and indicate that they may represent an effective platform for interfering with the pathological mechanisms of SCZ. In this study, we used LC-MS to analyze the protein cargo of plasma-derived EVs from 73 individuals with SCZ and 77 HCs. There were no significant differences when all the proteins analyzed in our study were compared and according to the PCA on the protein subset, the case and control groups could not be effectively differentiated. However, enrichment analysis on the proteins with nominal significance showed a significant enrichment of complement-related pathways. When comparing the complement components without controlling for the effects of antipsychotics and duration of illness, significant overexpression of C4A and C9 was found in individuals with SCZ compared to HCs; however, these findings disappeared when the effects of these covariates were taken into account. These results indicate that duration of illness and in particular antipsychotics may have an impact on EV protein levels. They also may reflect in part the significance of complement system in differentiating between individuals with SCZ and HCs.

Research suggests that antipsychotics may have an impact on the content of EVs. These studies often explore EVs as potential biomarkers for psychiatric disorders and treatment response. In a study that used proteome profiling to identify differentiating EV-based biomarkers for SCZ and predict antipsychotic treatment response, the authors established personalized discrimination scores based on the identified

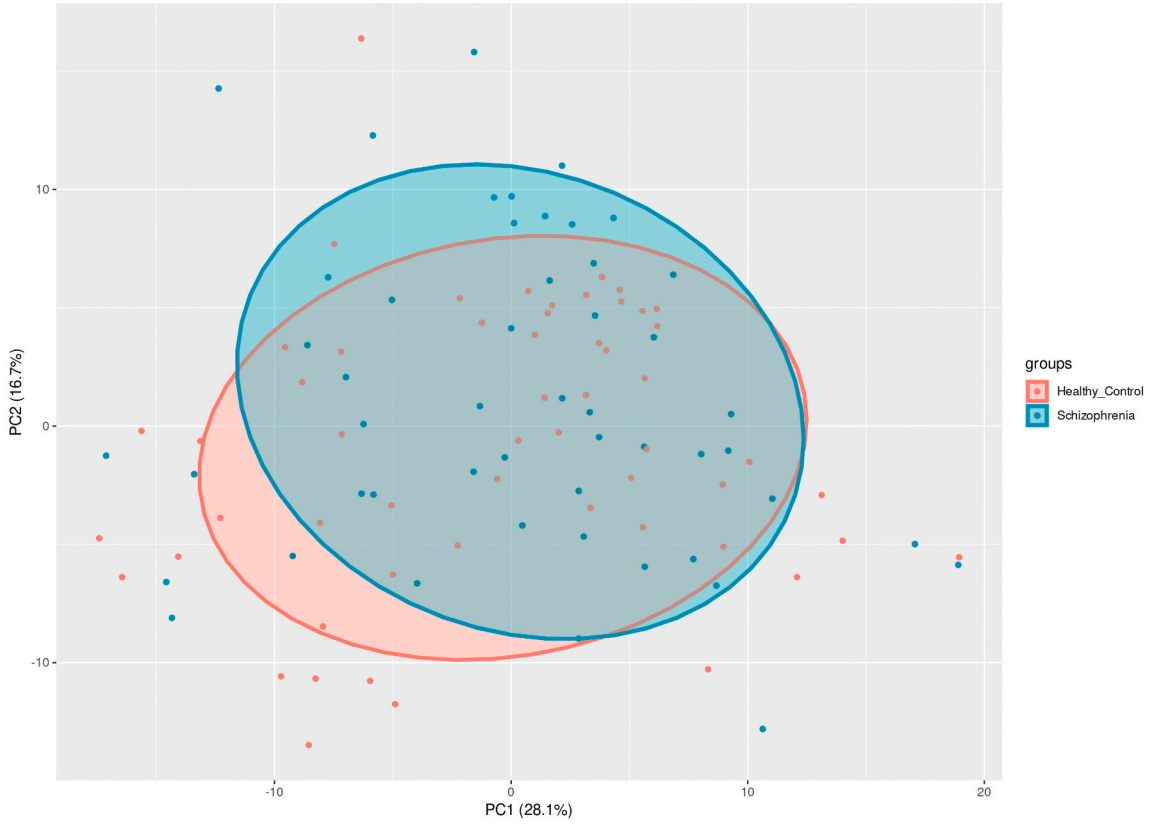


Fig. 2. Principal component analysis plot. Levels of plasma-derived EV-proteins in individuals visualized by diagnosis (number of individuals = 106 [50 SCZ; 56 HC]; number of proteins = 312).

differential biomarker panel (which was mainly composed of complement components) that was found to be significantly associated with response to antipsychotics (Xue et al., 2024). Another study investigated how antipsychotics modulate microglial function through the EVs released by astrocytes. The authors suggested that antipsychotics could change the content of astrocyte-derived EVs, which in turn influences microglia biology and glial cell communication (Yeh et al., 2025).

In the past few decades, researchers have paid increasing attention to the possible contribution of the complement system to the etiopathology of SCZ, and several studies have indicated that SCZ may be a neuroimmune disorder caused by changes in complement system activity and inflammatory processes in the CNS (Woo et al., 2020; Xue et al., 2024). According to Xue et al. (2024), the majority of the EV-derived biomarkers they found in their study are components of the complement system, and they revealed that individuals with SCZ had higher levels of complement activation that was mediated by EVs. The complement system—a group of plasma proteins that are part of the innate immune system—has several functions, including mediating inflammation and bridging innate and adaptive immune responses (Markiewski and Lambris, 2007; Woo et al., 2020). Complement proteins can be present in EVs and are linked to a variety of neuroinflammatory disorders (Burgelman et al., 2022). In individuals with SCZ, reduced cortical synaptic densities may suggest a role for excessive synaptic elimination in the disorder (Hogenaar and van Bokhoven, 2021). The complement system is thought to be one of the main pathways that causes synaptic elimination (Hogenaar and van Bokhoven, 2021). It should be noted that the complement system is required for the body's immune system to deal with a variety of pathogens and diseases. Even though novel complement-targeted treatments, including monoclonal antibodies, fusion proteins, and peptidomimetics, have shown promise in treating brain disorders, further investigation is needed to fully comprehend the exact mechanisms by which the complement system causes excessive pruning (Dalakas et al., 2020; Hogenaar and van Bokhoven, 2021).

Our study has several limitations, the most important of which may be the small sample size, particularly because we compared a high number of EV proteins. Another important limitation is that we investigated plasma-derived EVs in general and did not focus on brain-derived EVs in plasma. In the search for biomarkers for brain disorders, EVs enriched for CNS origin provide a more sensitive and reliable approach (Mustapic et al., 2017). Regarding the EV purity of our samples, we should note that they were probably not 100 % pure because they likely contained contaminants with comparable characteristics and because of the limitations of the isolation methods. Others have even suggested that more than 70 % of the particles isolated from plasma may not be EVs, with lipoproteins and platelets being the main impurities (Waury et al., 2024). Preparation of peptides for LC-MS analysis from the isolated EV samples resulted in polymers being visible in the raw data, which could impact the identification and quantification of EV proteins. Another limitation is the difference in the sex ratio between the SCZ and HC groups, which may have affected the results, although sensitivity analysis results showed that this effect was low. Furthermore, the plasma samples of cases and controls were stored in the freezer (at −80 °C throughout the storage period, which is the recommended condition for long-term storage of EVs (Wu et al., 2021)) for an extended period of time (about 8 years), which may have affected the concentration, integrity, and levels of proteins in our EVs samples because fresh plasma has been shown to have more and purer EVs than stored plasma (Jin et al., 2016).

5. Conclusion

Overall, although our study's findings emphasize that EV content may be influenced by medication use and the duration of illness, they also might partially explain the importance of complement components in differentiating between individuals with SCZ and HCs. The complement components may aid to find more accurate diagnostic and treatment options for SCZ because the complement system is believed to be an immunological mediator of SCZ (Woo et al., 2020). Despite the increasing amount of research on EV cargo over the past decade, the role of EV-derived bioactive molecules, especially proteins, in psychiatric disorders such as SCZ is not well characterized (Xue et al., 2024). However, focusing on small EVs, which can move between the CNS and the peripheral circulation (meaning that brain-derived small EVs to be accessed in the periphery; an approach referred to as EV-based brain liquid biopsy (Kano et al., 2021)) may provide additional and useful information regarding SCZ pathology. To this end, it may be worthwhile to employ a larger sample size, applying a combination of EV isolation methods, and focusing specifically on brain-derived EVs to identify significant differences in EV cargo levels across diagnostic groups and define unique EV fingerprints. This approach may help to determine diagnostic and prognostic biomarkers, find connections between reliable biomarkers and the pathophysiological mechanisms of psychiatric disorders, and lead to biomarker-targeted therapeutic strategies.

CRediT authorship contribution statement

Mojtaba Oraki Kohshour: Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Teresa K. Barth:** Writing – review & editing, Resources, Methodology, Formal analysis, Data curation. **Chiara Huber:** Writing – review & editing, Resources, Methodology, Conceptualization. **Andreas Klingl:** Writing – review & editing, Methodology, Conceptualization. **Jennifer Grünert:** Writing – review & editing, Methodology. **Urs Heilbronner:** Writing – review & editing, Investigation, Conceptualization. **Monika Budde:** Writing – review & editing, Investigation, Conceptualization. **Monika Rübekeil:** Writing – review & editing, Methodology. **Anton Eberharter:** Writing – review & editing, Methodology. **Thorsten Müller:** Writing – review & editing, Supervision, Conceptualization. **Moritz J. Rossner:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Andrea Schmitt:** Writing – review & editing, Data curation, Conceptualization. **Peter Falkai:** Writing – review & editing, Data curation, Conceptualization. **Florian J. Raabe:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Sergi Papiol:** Writing – review & editing, Supervision, Investigation, Formal analysis, Data curation, Conceptualization. **Axel Imhof:** Writing – review & editing, Resources, Methodology, Investigation, Data curation, Conceptualization. **Thomas G. Schulze:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that there are no conflicts of interest in relation to the subject of this study. General declaration of potential conflict of interests: Moritz J. Rossner is a shareholder and consultant of Systasy Bioscence GmbH; Peter Falkai received research support/honoraria for lectures or advisory activities from Boehringer-Ingelheim, Janssen, Lundbeck, Otsuka, Recordati, and Richter.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.schres.2025.09.008.

Data availability

Data generated or analyzed during this study are available from the corresponding author upon reasonable request.

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