**Title:** Analysis of Circulating Exosomes Reveals a Peripheral Signature of Astrocytic Pathology in Schizophrenia

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**Abstract**

There is considerable interest in identifying peripheral biomarkers of schizophrenia. Exosomes are a class of extracellular vesicles that are secreted into intracellular space and cross the blood brain barrier with their contents intact. Exosomes and their cargo, including lipids, proteins and genetic materials can be assayed peripherally. Studies of circulating exosomes have led to a greater understanding of their role as markers and mediators of pathology in neurodegenerative disorders, but there is scarce data in schizophrenia.

We examined neuropathology relevant protein biomarkers in circulating exosomes obtained from patients with schizophrenia and matched healthy controls. Nanoparticle tracking analysis was used to determine the size and concentration of exosomes. Exosomal membrane marker (CD9) and specific target cargo protein (glial fibrillary acid protein[GFAP], synaptophysin and $\alpha$-II-spectrin) immunopositivity was examined using Western blot analyses.

No group differences were observed in plasma exosomal concentration and size. Exosomal GFAP expression was significantly higher in schizophrenia compared to controls. In contrast, there were no group differences observed for exosomal synaptophysin and $\alpha$-II-spectrin.

Our results demonstrate for the first time, a differential pattern of exosomal protein expression in schizophrenia compared to matched healthy controls. Schizophrenia patients had greater exosomal GFAP expression, a finding that is consistent with the known astro-glial pathology in this disorder. This finding is particularly significant given the absence of group differences in the other exosomal markers and proteins assessed

in this study. These results warrant further examination of circulating exosomes as vehicles of novel peripheral biomarkers of disease in schizophrenia and other neuropsychiatric disorders.

## Introduction

Schizophrenia is a devastating illness affecting approximately 1% of the population, with onset of psychosis typically in adolescence or early adulthood, a heterogenous symptom profile and significant social and occupational impairments[1]. Most of what is known about the neuropathology of schizophrenia is derived from studies of postmortem tissues. In vivo studies of biomarkers that reflect central processes would be ideal to examine neuropathological changes over the course of the illness. However, the challenges in obtaining tissues that may be informative of neuropathology of the disorder in living patients remains a limitation. Thus, the search for peripheral biomarkers that reflect neuropathological changes in schizophrenia is being actively pursued, but results have remained elusive. The study of circulating extracellular vesicles assayed peripherally is one promising approach to identify putative peripheral biomarkers of schizophrenia.

Extracellular Vesicles (EVs) include several distinct types of cell-derived vesicles that are released into the extracellular environment and influence adjacent as well as remote physiological and pathological processes [2-5]. Exosomes refer to specific EVs that are typically 30-200nm in diameter and arise from late endosomes or multi-vesicular bodies (MVB) [6]. They are secreted into the extracellular space via a distinct secretory pathway and are detectable in biological fluids [3, 4].

Exosomes can be secreted from a wide range of cell types into the extracellular space and can be assayed from biological fluids including blood, cerebrospinal fluid (CSF) and urine [7]. In the central nervous system (CNS), exosomes may be secreted by neurons, astrocytes, microglia and oligodendrocytes and appear to be involved in

several physiological processes such as neuron-neuron or glial-neuron communication, regulation of myelination, regulation of stress and immune responses and modulation of synaptic plasticity [4, 5]. Exosomes have been shown to have the ability to cross the blood brain barrier, bidirectionally, in health and in illness, with their contents intact [2], that makes them particularly attractive biomarkers for neuropathology. Analysis of exosomal content provides information about the cellular source of the exosome (particularly valuable in peripherally isolated exosomes), the inflammatory state of the cellular milieu, and ongoing physiological or pathological processes. The nucleic acid content, in particular, the microRNA content may help elucidate downstream cellular targets and potentially, the functional impact of the exosomes. Post mortem studies have shed light on the role of exosomes in the pathogenesis and disease progression of several neurodegenerative disorders (reviewed in Levy 2017) [8]. In parallel, the ability to identify markers of neuropathology in exosomes obtained from blood or CSF offers great promise in the development of peripheral biomarkers of illness. The study of exosomes in neuropathology has largely been limited to disorders such as Alzheimer's disease, Parkinson's disease, Lewy body dementia and traumatic brain injury (TBI) [8-15]. The promising results of these studies has provided the impetus to search for similar biomarkers in psychiatric disorders such as schizophrenia.

Thus far, there have been only a handful of published studies examining exosomes in schizophrenia. Banigan et al [16] analyzed exosomes extracted from frozen post mortem brain tissue of patients with schizophrenia and bipolar disorder and healthy controls and reported greater expression of disease specific exosomal miRNAs in patients, specifically miR-497 in schizophrenia and miR-29c in bipolar disorder. More

recently, Amoah et al [17] also examined exosomal microRNA in the orbitofrontal cortex of port mortem brains and reported increased expression of miR-223, that targets glutamate receptors, in schizophrenia and bipolar disorder compared to controls. Further, miR-223 was found to be enriched in astrocytes and sensitive to antipsychotic exposure in this study. Two recent studies have examined plasma exosomes in schizophrenia. Du et al validated several distinct miRNAs in serum exosomes, including 11 that could distinguish between the schizophrenia and the control groups [18]. Finally, Kapogiannis et al reported altered expression of proteins relevant to insulin signaling in plasma derived neuronal exosomes from drug naïve first episode schizophrenia patients, suggesting that altered insulin signaling precedes antipsychotic exposure in schizophrenia [19].

To our knowledge, there are no published studies in schizophrenia, examining neuropathology relevant protein cargo in circulating exosomes.

This cross-sectional study aimed to examine plasma exosomal protein content from patients with schizophrenia and matched healthy controls. Target proteins were identified based on the following criteria 1) cargo proteins previously identified in peripheral blood exosomal assays of disorders with significant neuropathology including dementias and TBI [20, 21] ; 2) proteins with relative cell specificity to the CNS neurons (axonal and synaptic) and glia (astrocytes); and 3) proteins that play vital roles in neurodevelopmental processes, perturbations of which result in Mendelian syndromes with severe neuropsychiatric sequalae. Based on these criteria, synaptophysin, (a neuronal synaptic vesicular membrane protein), $\alpha$-II -spectrin, (a non-erythrocytic neuronal cytoskeletal protein with prominent axonal expression), and Glial Fibrillary Acid

Protein, ([GFAP], an intermediate filament protein with predominant astrocytic expression in the CNS ), each associated with a neurodevelopmental phenotype (MIM-300802, MIM-613477, and MIM-203450 respectively) were examined as the target cargo proteins in our samples. We quantified exosomes in plasma and compared the normalized expression of target proteins as potential biomarkers in patients with schizophrenia and matched controls.

## Methods

Exosomal analysis was conducted in plasma obtained from blood samples from schizophrenia patients and matched controls participating in studies and conducted by the Schizophrenia Neuropharmacology Research Group at Yale (SNRGY). Study protocols adhered to the Declaration of Helsinki and was approved by the local Ethics Committee and were approved by Yale University Human Investigations Committee.

**Participant Evaluations:** All participants underwent detailed medical and psychiatric evaluations, physical examinations, electrocardiogram, laboratory assessments and a structured diagnostic interview (SCID-IV) as part of the standard evaluation after written informed consent was obtained. Subject demographics, information on duration of illness and antipsychotic medication (in patients) and substance use histories were systematically collected. Psychotic symptoms were assessed in patients using the Positive and Negative Syndrome Scale (PANSS). Substance use information was collected using a questionnaire and recent use of tobacco/nicotine and cannabis was assessed using the timeline follow back scale [22].

**Blood processing details:** Venous blood was drawn into EDTA-containing tubes and centrifuged at ~3000 rpm for 10-15 minutes. Resulting plasma was aliquoted (~0.5ml) and frozen to -80°C. De-identified plasma samples were shipped for exosomal analysis to the laboratory of Dr. T Mohanakumar under an MTA between Yale University and Dignity Health/St. Joseph's Hospital and Medical Center, Phoenix, AZ.

Plasma from a subset of schizophrenia subjects (n=8), was sampled twice, 4 weeks apart to examine the stability and reliability of the target protein signals identified in the exosomal isolates.

**EVs isolation and characterization**: Exosomes were isolated from plasma using a standard exosome isolation kit (4478360, Invitrogen; Thermo Fisher Scientific, Waltham, MA) followed by 0.2 micron filtration and size determination using NanoSight using standardized procedures previously described [23]. The exosome pellet of size from 40-200 nm was dissolved in PBS, the concentration of the exosomes was determined using the NanoSight and protein was measured using BCA kit. Exosomes were lysed with RIPA buffer; protein concentration was measured prior to immunoblots. Exosomal protein concentration was measured by BCA kit and an equal amount of protein (20 μg) was loaded per well.

**Nanoparticle tracking analysis ([NTA] NanoSight)**: Vesicles were analyzed by nanoparticle tracking using the NanoSight NS300 system (Malvern, Great Malvern, UK). Samples were administered and recorded under controlled flow, using the NanoSight syringe pump and script control system. For each sample, 5 videos of 60 seconds duration were recorded, with a 10-second delay between recordings, generating 5

replicate histograms that were averaged. Therefore, the typical number of completed tracks per sample was approximately 1200. The area under the curve was calculated using Prism-4 software version 4.03 (Graph Pad, San Diego, CA), to give average particle counts from these replicates. Particles ranging in size from 40-200 nm were considered exosomes consistent with previously published methods [24] (see sample image shown in Figure 1).

**Western blot analysis**: Western blotting was performed on the total protein extracts from exosome samples. RIPA lyses buffer (150 mM NaCl, 50 mM Tris, pH 8.0, 5 mM EDTA, 0.5% sodium deoxycholate, 0.1% SDS, 1.0% Nonidet P-40) with protease and phosphatase inhibitor cocktails (Sigma-Aldrich, St. Louis, MO, USA) was used for the total protein fraction. Protein concentrations in cell extracts as well as exosomes were determined using the BCA protein assay (Thermo Fisher). 20 µg of total lysates were diluted 1:1 in RIPA SDS-PAGE sample buffer, loaded onto polyacrylamide gels, and blotted onto polyvinylidene difluoride membranes (Bio-Rad). Membranes were blocked with 5% non-fat milk in PBS, pH 7.6, 0.2% Tween-20 for 1 hour and then incubated with mouse monoclonal anti-CD9 (1:1000, BD Pharmingan), mouse polyclonal anti-Synaptophysin (1:1000, Invitrogen), rabbit polyclonal anti-GFAP (1:1000, Invitrogen), rabbit polyclonal anti-$\alpha$-II -spectrin (1:1000, Invitrogen), that binds $\alpha$-II-spectrin and its break down products, and rabbit polyclonal anti-Calnexin (1:1000, Abcom) primary antibodies overnight. After washing in PBS-T, pH 7.6, 0.2% Tween-20, the membranes were incubated with a horseradish peroxidase-conjugated goat anti-rabbit antibody (1:2000, Cell Signaling) or anti-mouse antibody (1:2000, Cell signaling), and the immunoblots were visualized using ECL detection kits, (Pierce; Rockford, IL,

USA). The band intensity was quantified using ImageJ software (National Institutes of Health, Bethesda, MD). CD9 is a tetra-spanin exosomal membrane protein marker that is typically absent in exosomes derived from B cells, NK cells and mesenchymal stromal cells and was used to confirm the identity of exosomes in the sample analyzed [7, 25]. Calnexin, a chaperone protein predominantly expressed in the endoplasmic reticulum, was included to demonstrate purity of the isolate from intracellular contaminants, specifically from the secretory pathway [7, 26].

**Statistical Analysis:** The demographic variables (age, gender and ethnicity) and substance use measures (nicotine and cannabis use) were summarized and compared for differences between the schizophrenia and the control groups. The distributions of exosome concentrations and modal exosomal sizes between the two groups were examined with boxplots and compared with Wilcoxon rank sum test. The proportion of samples in the schizophrenia and the control groups that tested positive on the immunoblot assays for each of the three target proteins were calculated. The sample proportions between groups were compared with Fisher's exact test of independence owing to the small number of samples examined in the study. Bonferroni correction was applied to adjust the p values for the independent comparisons of the three protein markers. Confidence intervals (95%) for the sample proportions were estimated using 'exact' method (Clopper-Pearson interval) due to the extreme the proportions noted in the sample [27]. Data analysis was carried out in R version 3.6.1 [28] and the figures were generated with the packages tidyverse [29] and ggpubr [30].

## Results

**Sample characteristics (Table 1):** The sample consisted of 24 (20 males;4 females) individuals with schizophrenia and 12 (8 males; 4 females) age matched healthy controls. Demographic details and illness variables including the details of nicotine and cannabis use are presented in Table 1. The schizophrenia and the control groups were comparable in age, gender and ethnicity. A significantly higher proportion of participants with schizophrenia compared to controls had cannabis and tobacco use. None of the control participants had received a diagnosis of any major psychiatric disorder or were currently receiving treatment for a chronic medical condition. Three healthy controls had lifetime history of exposure to cannabis, but none were current users of tobacco or cannabis. Schizophrenia patients were ill for a median (IQR) duration of 2.65 (5) years and ~80% of these participants were within the first six years of their illness. They were moderately to severely ill at the time of recruitment with a mean PANSS total score of 79.1 [31]. The PANSS total and subscale scores for schizophrenia participants is provided in Table 1. All except two patients were on oral or depot antipsychotic medications at the time of sample collection.

**Exosome characteristics and concentration (Figure 1 and 2):** Transmission electron microscopy (TEM) confirmed the morphology of the exosomes as spherical membrane bound vesicles with an approximate diameter of 100 nm (Figure 1a). All 36 samples of exosome isolates stained positively on immunoblotting with anti CD9 antibody. Additionally, none of the samples were positive with anti-calnexin antibody, demonstrating sample purity (Figure 1b) [7]. The median (IQR) concentration of the

exosomes in the schizophrenia samples was 6.49 $*10^8$ ($7.12*10^8$) which was lower than the concentration, 8.02 $*10^8$ ($3.4*10^8$) in the control samples, but this difference was not statistically significant (Wilcoxon p value = 0.072) (Figure 2). The modal size for exosomes derived from schizophrenia subjects was larger than that of control samples - median (IQR) size in nanometers 85 (118) vs 51.5 (4.25), although this was not statistically significant (Wilcoxon p value = 0.077). A greater variability was noted in the exosome concentration and the size in the samples from the schizophrenia group. The exosome concentrations and size distribution in individual samples from controls and schizophrenia subjects were within the ranges reported in the literature for circulating exosomes [32].

**Astrocytic and neuronal protein cargo in peripheral exosomes (Figure 3 and 4):** All 24 (100%, 95%CI: 85.8% - 100%) samples from participants with schizophrenia demonstrated positive immunostaining with polyclonal anti-GFAP antibody in the Western blot analysis. In contrast only 2 (16.67%, 95%CI: 2.1% - 48.4%) of the control samples showed GFAP immunopositivity. The difference in the sample proportions was statistically significant with Fisher exact OR > 12.64, $p = 2.59*10^{-7}$, $p_{adj} = 7.79*10^{-7}$ (Figures 3 and 4). It may be noted from the Western Blot image for GFAP (Figure 3, panel A), that the positive staining noted with the control samples yielded a very low intensity signal. However, owing to the inherent challenges in quantifying protein concentrations from Western blot signal intensities [33], we did not formally quantify the protein concentrations in our analyses.

Synaptophysin immunopositivity was noted in in all 24 samples from participants with schizophrenia (100%, 95%CI: 85.8% - 100%) and in 10 out of 12 (83.3%, 95%CI: 51.6%-97.9%) samples from control participants (Figures 3 and 4). The difference in the proportion of participant samples within each group that tested positive for this synaptic vesicular protein was small and not statistically significant.

$\alpha$-II Spectrin immunopositivity was detected in all samples from schizophrenia (100%, 95%CI: 85.8% - 100% and control participants (100%, 95%CI: 73.5% - 100%) (Figures 3 and 4).

Finally, in the subset of 8 out of 24 (33.33%) schizophrenia participants in whom a second plasma sample (~4 weeks after initial sampling) was examined, the results of exosomal analyses remained unchanged from the previous timepoint and all samples tested positive for GFAP, Synaptophysin and $\alpha$-II Spectrin.

**Discussion**

Our results demonstrate for the first time, a differential pattern of exosomal protein expression in schizophrenia compared to matched healthy controls. The ability to extract exosomes from plasma and identify neuropathology relevant signatures represents a significant advance in the quest for peripheral biomarkers of schizophrenia.

Specifically, we demonstrate expression of GFAP, a well-recognized marker of astrocytic pathology [34], in plasma exosomes of all schizophrenia participants assessed unlike its absence in the majority of healthy control participants. This finding is particularly significant given the absence of group differences in the other markers and

target proteins assessed in this study. All schizophrenia and control group samples tested positive for CD9, an exosomal membrane protein, and negative for Calnexin, an endoplasmic reticulum protein demonstrating purity of the samples. Furthermore, both groups were similar in the exosomal expression of synaptophysin and $\alpha$-II -Spectrin, suggesting the presence of neuronally derived exosomes (NDE) in our samples of plasma exosomes irrespective of the disease status. Thus, the nearly exclusive detection of exosomal GFAP in schizophrenia is suggestive of *selective* enrichment of exosomal protein of astrocytic origin only in patient samples.

These findings are consistent with the neuroinflammatory hypothesis and the role of astro-glial pathology in schizophrenia [34, 35]. GFAP is highly expressed in astrocytes in the CNS, in a cell type specific fashion with minimal expression noted outside the CNS [36] and elevated GFAP expression is associated with increased astrocytic activation and inflammation [34]. Post mortem studies in schizophrenia demonstrate that GFAP is upregulated in the pre-frontal cortex in schizophrenia although results from other brain regions remain mixed [37-41]. These mixed results may be related to region specific differences in GFAP expression, diversity of astrocytes with respect to GFAP positivity and heterogeneity in schizophrenia or subsets within the disorder [34]. For instance, Catts et al reported that elevated levels of brain GFAP and astro-gliosis was observed only in a subset of schizophrenia patients and associated with greater neuroinflammation [42]. Our study did not assess for peripheral or neural markers of inflammation and further studies are needed to determine if distinct patterns of exosomal GFAP expression are detectable in subsets of patients with schizophrenia. To our knowledge, there has been only one published study of free (non-exosomal) GFAP in CSF and serum samples

schizophrenia, with no differences reported between first episode psychosis patients and controls [43]. However, exosomal analyses may offer greater reliability in the identification of neuropathology relevant proteins given the cell-of-origin specificity of exosomes, and the enrichment and stability of their cargo protein.

GFAP expression may be affected by age [42, 44] and antipsychotic medications [45]. GFAP expression detectable in brain tissue, CSF and serum increases with age [44], with very low levels in healthy individuals till around 40 years of age. The absence of detectable GFAP amongst our control subjects (mean age 27.16 years) is consistent with this literature. Thus, the GFAP positivity in our *age-matched* schizophrenia samples is particularly noteworthy. Further studies are needed in larger samples to fully understand the impact of age and gender on exosomal GFAP in schizophrenia. Amongst patients, there was no difference in GFAP expression noted with duration of illness, illness severity, substance use or exposure to antipsychotics. All the patients in this study had been exposed to antipsychotic medications, although two were not currently treated with antipsychotics. There is mixed data on the impact of antipsychotic medications on GFAP expression [45]. Larger, and ideally, longitudinal studies are needed to determine whether circulating exosomal GFAP expression varies by stage or duration of illness, symptom domains, antipsychotic medications, or other clinical factors including concomitant substance use. Interestingly, both the healthy controls participants whose exosome samples tested weakly positive for GFAP reported a history of lifetime cannabis use although none reported current cannabis or other substance use.

Synaptophysin expression was observed in exosomal samples from both schizophrenia and healthy control participants in our study. Synaptophysin is a synaptic vesicle membrane protein expressed in neurons and neuroendocrine cells where it plays a critical role in the formation and regulation of synaptic vesicles [46]. Several studies have demonstrated reduced synaptophysin mRNA and protein expression in post mortem tissue of schizophrenia patients consistent with reduced synaptic vesicle density and function in schizophrenia as previously reviewed [47, 48]. Exosomal synaptophysin has also been examined in several neurodegenerative disorders such as Alzheimer's disease[12] and TBI[49]. Goetzl et al reported lower levels of synaptophysin in NDE in frontotemporal dementia and Alzheimer's disease and the reductions in synaptophysin significantly predated the cognitive decline[12]. Our study did not isolate NDE in plasma samples, although synaptophysin positivity in our samples is indicative of neuronal derived exosomal content in our analytes.

α-II Spectrin expression was observed in all exosomal samples in this study. α-II Spectrin is a cytoskeletal protein expressed in non-erythroid tissue and enriched in the brain where it is present abundantly in neurons, particularly in axons and synaptic cytoskeleton [50]. α-II Spectrin and its break down products are detected in the cerebrospinal fluid as freely soluble proteins as well as in exosomes in patients with TBI and sub-arachnoid hemorrhage[49-51] suggestive of neuronal loss. Similar to synaptophysin, α-II Spectrin expression in all samples may be also indicate the presence of NDE in our analytes.

While, our understanding of exosomal biology, cargo selection and function are still emerging, it is well recognized that exosomes play critical roles in physiological and

pathological processes in the CNS [4]. Thus, the ubiquity of exosomal synaptophysin/ α-II Spectrin expression in both groups in our study may be the consequence of the physiological role of NDE regardless of disease state. Further studies may be needed to quantify exosomal synaptophysin/ α-II Spectrin to determine whether levels of expression of these proteins are altered in schizophrenia. Our results may also be reflective of the expression of these proteins in non-neuronal tissues, and future studies should specifically examine their expression in circulating NDE in schizophrenia.

**Strengths and Limitations:** These data represent the very first study examining neuropathology relevant protein biomarkers in circulating plasma derived exosomes in schizophrenia. The analytical methods employed here were consistent with the recommended guidelines included in the "Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines" for studies on exosomes [7]. As recommended in the MISEV2018 guidelines, exosomes were extracted from plasma samples with a standardized protocol using exosome separation kits. The morphology of the exosomes was characterized with both TEM and NTA. NTA was also used to determine the concentration per ml of plasma sample. Exosomal confirmation included assessment of transmembrane CD9 protein by Western blot. Further, the purity of the isolate was demonstrated with the absence of calnexin protein in the sample thus precluding contamination from other particles in the secretory pathway such as endoplasmic reticulum. Finally, participants were well characterized, including demographics, details of illness, symptomatology and medication history amongst

patients. Additionally, the repeat analyses of a small number of plasma samples from schizophrenia patients supports the stability of our results.

We did not specifically extract NDE from plasma samples in this study. Further, the lack of a second patient group prevents our ability to account for the effect of chronic illness or stress. Finally, we did not study antipsychotic naïve patients in order to examine the effect of antipsychotic medications on exosomal protein expression. These limitations notwithstanding, these data strongly support the potential of exosomal proteins as peripheral biomarkers of neuropathology in schizophrenia.

**Future Directions:**

These initial results provide the impetus to examine a wider array of exosomal derived proteomic signatures in schizophrenia. Whether the observed differences are also present in other psychiatric disorders remains to be examined. Finally, studies are also needed to determine whether peripheral exosomal biomarkers represent trait markers of the disorder or vary by stage of illness, antipsychotic treatment, or other clinical factors given the heterogeneity of the schizophrenia.

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**Conflicts of Interest:** The authors have no relevant conflicts of interest to disclose.

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**Table 1: Participant Demographics**

| | **Schizophrenia (n = 24)** | **Control (n = 12)** | **Statistic** | **P value*** |
|---|---|---|---|---|
| Age – mean (SD) | 28.29 (9.23) | 27.16 (4.76) | t = 0.48 | 0.63 |
| Gender – F:M | 4:20 | 4:8 | OR (CI) = 2.43 (0.36 – 16.79) | 0.39 |
| Ancestry | | | * | 0.58 |
| Caucasian | 7 (29.17%) | 5 (41.67%) | | |
| African American | 11 (45.83%) | 6 (50.0%) | | |
| Other | 6 (25.0%) | 1 (8.33%) | | |
| Current smoking | 13 (54.2%) | 0 (0%) | OR > 2.44 | 0.002 |
| Current cannabis use | 7 (29.2%) | 0 (0%) | OR > 0.81 | 0.07 |
| Lifetime cannabis use | 16 (66.7%) | 3 (25%) | OR (CI) = 5.68 (1.04 – 41.95) | 0.03 |
| Duration of illness in years – median (IQR) | 2.65 (5) | NA | | |
| PANSS positive - mean (SD) | 20 (3.43) | NA | | |
| PANSS negative - mean (SD) | 19.7 (4.12) | NA | | |
| PANSS general - mean (SD) | 39.4 (5.87) | NA | | |
| PANSS total - mean (SD) | 79.1 (9.03) | NA | | |

*Difference in age between the groups was examined with t-test. Gender, ancestry, smoking and cannabis use were compared with Fisher's exact test of independence.

**Figure legends**

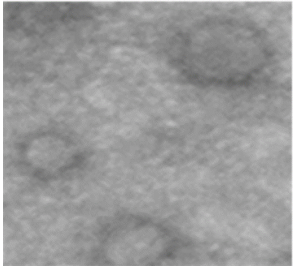
**Figure 1** – Characterization of plasma derived circulating exosomes with Transmission Electron Microscopy and Western blotting. **a)** Transmission Electron Microscopy image of a representative sample demonstrating that the isolates contained vesicles in the expected size range. **b)** Western blot analysis with anti-CD 9 and Calnexin antibodies in 5 representative from each group. Top panel: CD9 immunopositivity demonstrates the presence of this constitutive exosomal membrane protein in all samples from schizophrenia and matched controls. Bottom Panel: Absence of Calnexin immunopositivity in all samples demonstrates the purity of the exosomal isolate.

**Figure 2** – Characterization of plasma derived circulating exosomes with NanoSight: Nano-particle Tracking Analysis. **a)** Boxplot with jitter representing the distribution of plasma exosome concentration per milliliter: Schizophrenia samples had a lower median plasma concentration of exosomes compared to control samples and a higher variance in their distribution. The group difference examined with Wilcoxon rank sum test for non-parametric data was not statistically significant with $p = 0.072$. **b)** Boxplot with jitter representing the distribution of modal size of exosomes in schizophrenia and control samples. Exosomes derived from schizophrenia samples had a higher modal diameter and a greater variance in their distribution. The difference between the two groups was not statistically significant (Wilcoxon test p value = 0.078). **c)** The size distribution curves for one representative sample each from schizophrenia and control groups. Both samples had maximal distribution of the isolated particles between 30-200 nm.

**Figure 3** – Western blot images of five representative samples from the schizophrenia and control groups for three target proteins examined. Glial Fibrillary Acidic Protein (GFAP) was present in the exosomes derived from every sample in the schizophrenia group but was weakly positive in 2 of the 12 control subjects tested (weak positive result for C1 shown in the image). Synaptophysin and $\alpha$-II-spectrin (with its breakdown products) were detectable in samples from both schizophrenia and control groups.

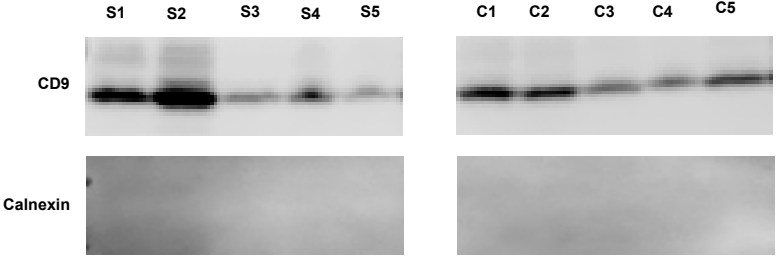
**Figure 4** – Bar graphs representing the differences in the proportions of plasma derived exosome samples testing positive for the three target proteins. Error bars represent 95% confidence intervals for sample proportions calculated with exact method for binomial distribution. The sample proportions for presence of GFAP was significantly higher in the schizophrenia group compared to controls (Fisher exact test $p = 2.59e-7$). There were no group differences in the proportions positive for synaptophysin and $\alpha$-II-spectrin.

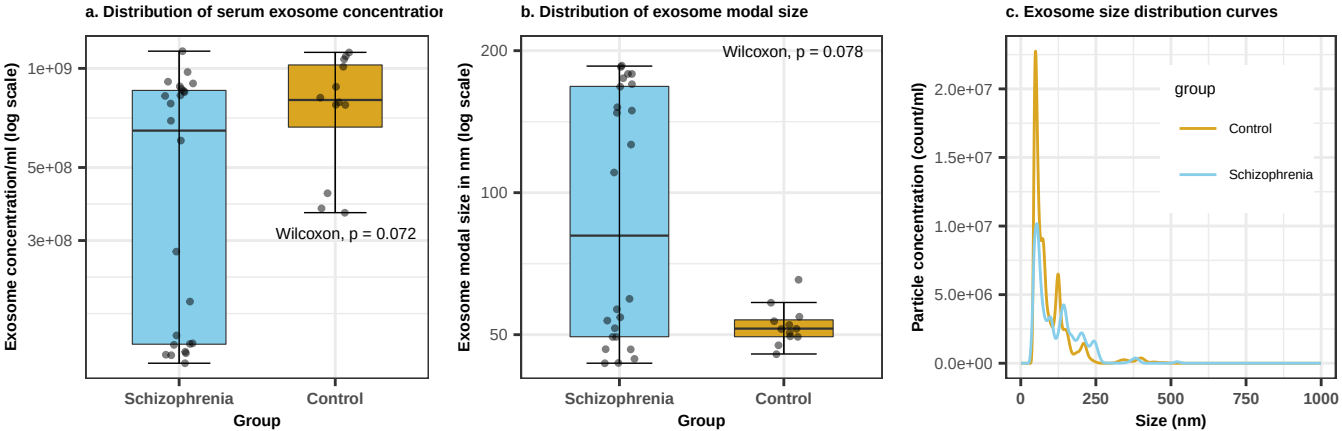
**a. Representative TEM image of exosome**

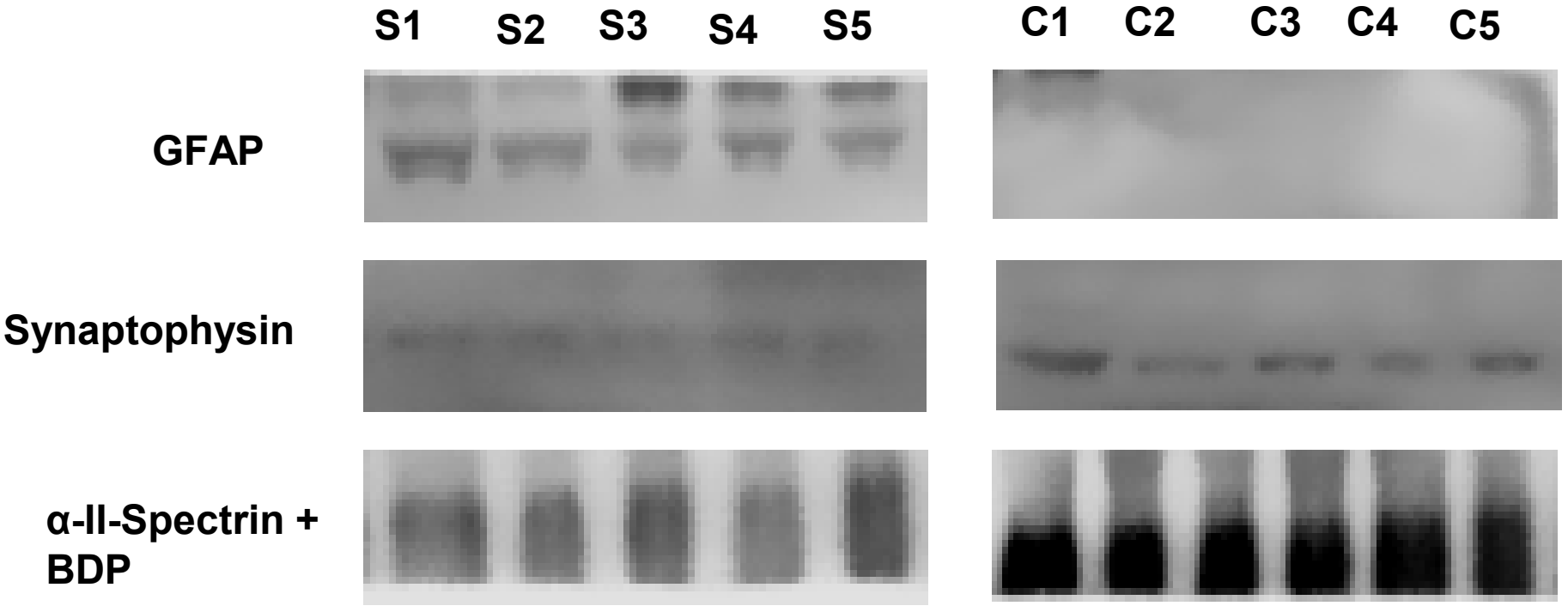


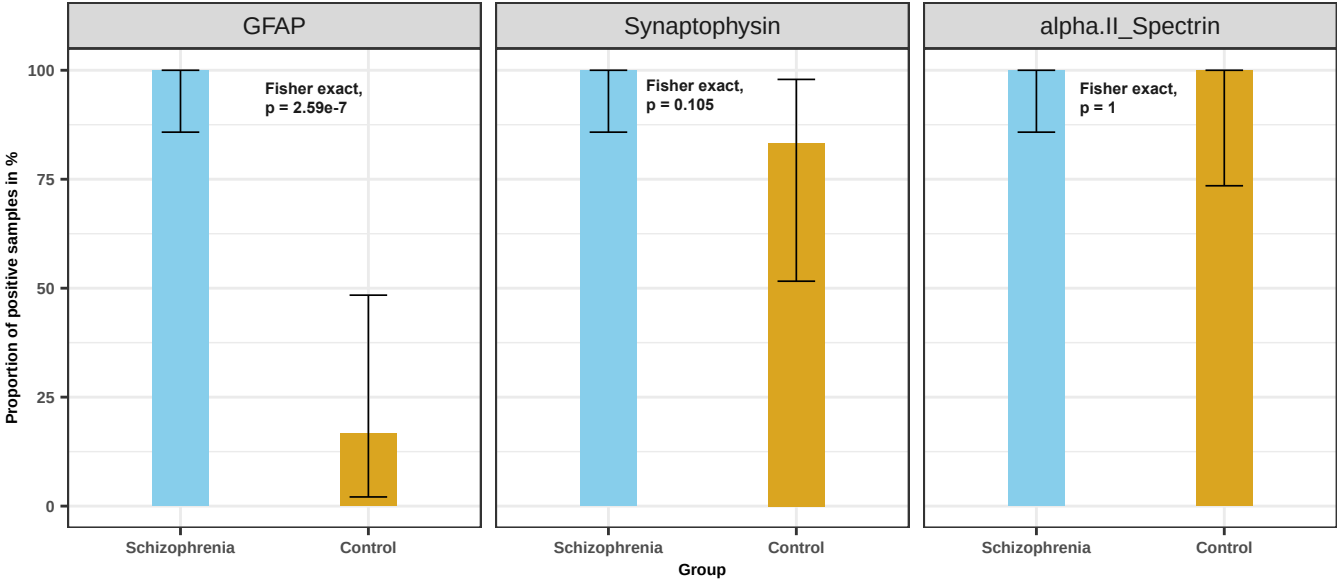
100 nm

**b. Western blot for CD9 and calnexin**




a. Distribution of serum exosome concentratio
Exosome concentration/ml (log scale)
1e+09
5e+08
3e+08
Wilcoxon, p = 0.072
Schizophrenia
Control
Group
b. Distribution of exosome modal size
Exosome modal size in nm (log scale)
200
100
50
Wilcoxon, p = 0.078
Schizophrenia
Control
Group
c. Exosome size distribution curves
Particle concentration (count/ml)
2.0e+07
1.5e+07
1.0e+07
5.0e+06
0.0e+00
group
Control
Schizophrenia
0
250
500
750
1000
Size (nm)


S1
S2
S3
S4
S5
C1
C2
C3
C4
C5
GFAP
Synaptophysin
α-II-Spectrin +
BDP


GFAP
Synaptophysin
alpha.II_Spectrin
Fisher exact,
p = 2.59e-7
Fisher exact,
p = 0.105
Fisher exact,
p = 1
Proportion of positive samples in %
100
75
50
25
0
Schizophrenia
Control
Group