Peripheral signature of altered synaptic integrity in young onset Cannabis Use Disorder: A pilot proteomic study of circulating extracellular vesicles

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Introduction

- Cannabis use often begins in adolescence or early adulthood - critical periods of neurodevelopment and neuromaturation
- ~30% of cannabis users may develop Cannabis Use Disorder (CUD)
- Chronic cannabis exposure results in neuropsychiatric and cognitive consequences
- Challenges in obtaining brain tissues from young persons with CUD – limits the study of molecular neuropathology
- Neuron Derived Extracellular vesicles (NDEs), assayed from the periphery may reveal markers of neuropathology in CUD
- Proteomic studies of NDEs cargo may provide some insights into molecular signatures of cellular milieu

Aims

1. To compare the proteomic profile of plasma derived NDEs in young onset CUD and matched healthy controls
2. To examine the enrichment of differentially abundant proteins to genes relevant to synaptic function

Methods

- Sample: 10 young-onset CUD and 10 age and gender matched Healthy Controls (HCs)
- Cannabis exposure characterized by Scale for Assessment of Lifetime Assessment of Cannabis Use
- NDEs enriched from plasma Extracellular Vesicle (EV) pool using ExoSORT (Figure 1A).
- NDE morphology and abundance characterized with electron microscopy and Nanoparticle Tracking Analysis (Figure 1B, 1C)
- Label Free Quantification (LFQ) analysis of NDE proteome with Liquid Chromatography, Tandem Mass Spectrometry (LC-MS/MS)
- Protein identity examined in Mascot search engine
- LFQ data processed in Progenesis QI software to derive differential abundance between CUD and HC
- Enrichment and pathway analysis with Panther and Ingenuity Pathway Analysis

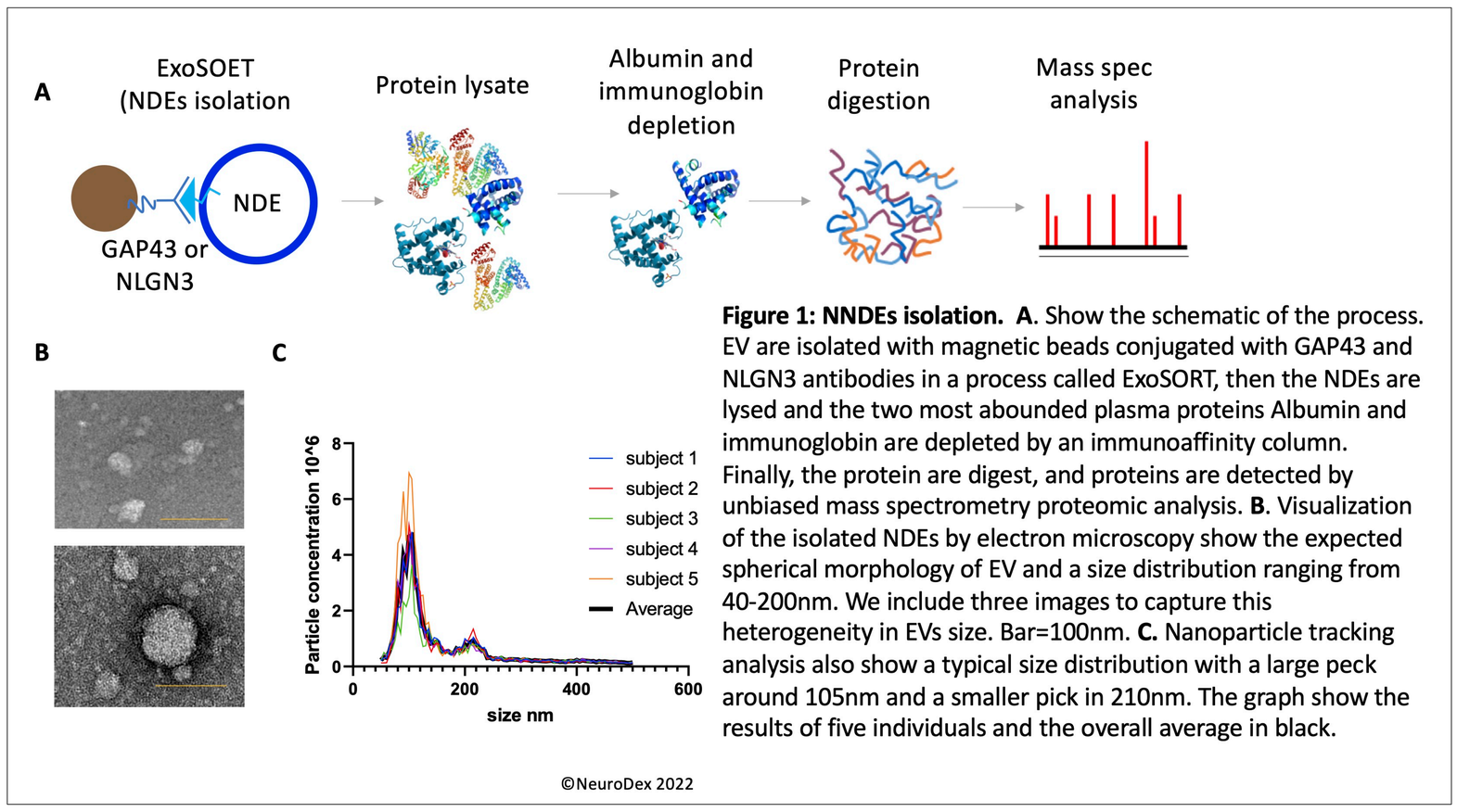


Figure 1: NNDEs isolation. A. Show the schematic of the process. EV are isolated with magnetic beads conjugated with GAP43 and NLGN3 antibodies in a process called ExoSORT, then the NDEs are lysed and the two most abounded plasma proteins Albumin and immunoglobin are depleted by an immunoaffinity column. Finally, the protein are digest, and proteins are detected by unbiased mass spectrometry proteomic analysis. **B**. Visualization of the isolated NDEs by electron microscopy show the expected spherical morphology of EV and a size distribution ranging from 40-200nm. We include three images to capture this heterogeneity in EVs size. Bar=100nm. **C.** Nanoparticle tracking analysis also show a typical size distribution with a large peck around 105nm and a smaller pick in 210nm. The graph show the results of five individuals and the overall average in black.

Figure 2: Differentially abundant NDE proteins between CUD and matched controls

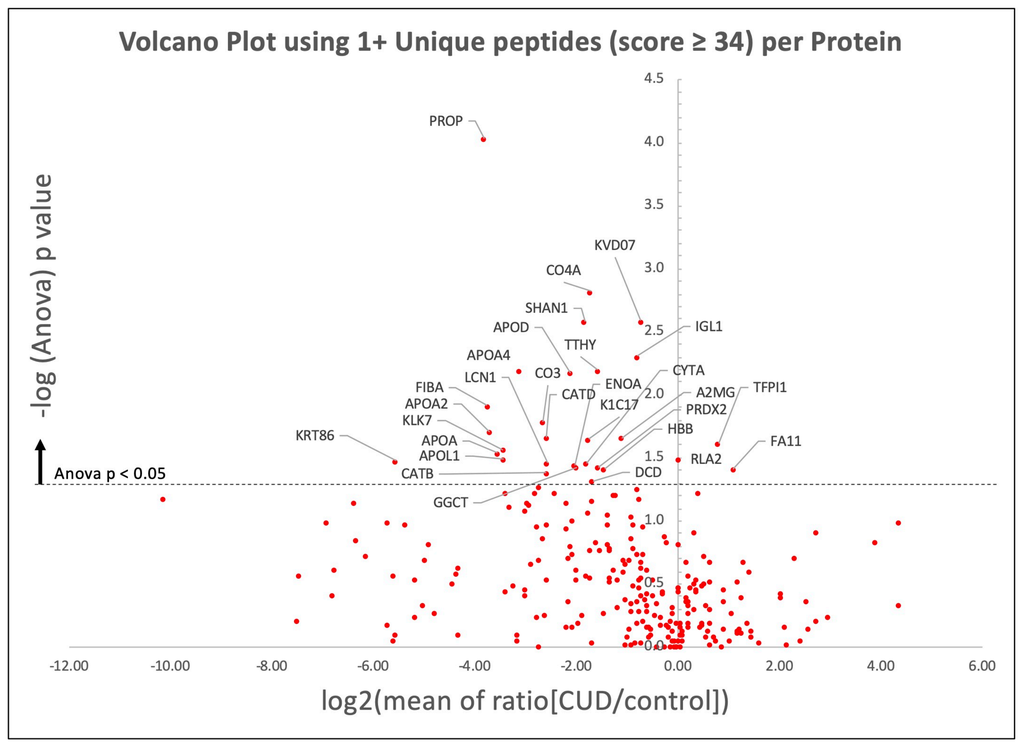
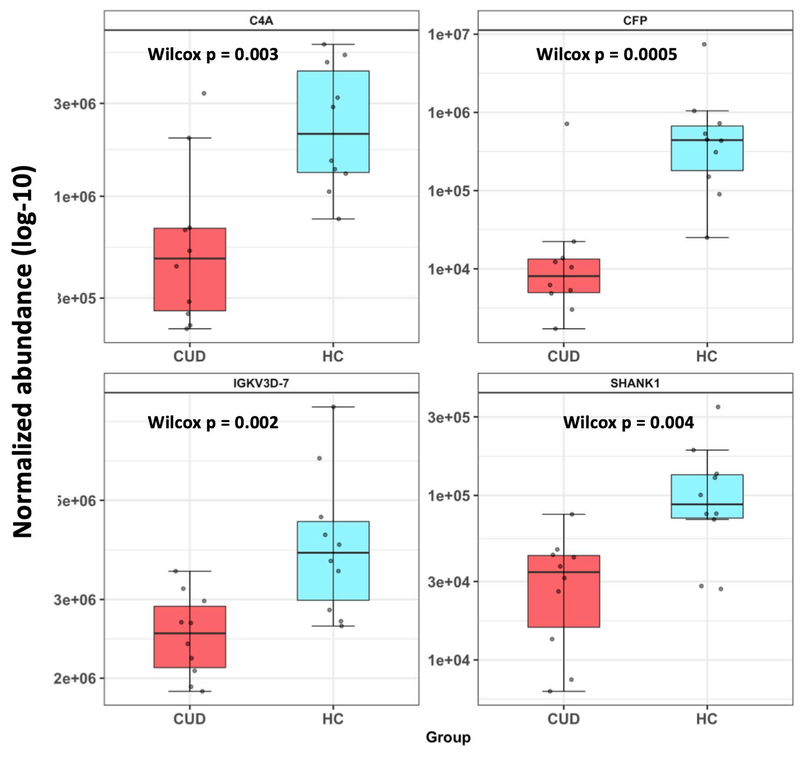


Figure 3: Top four differentially regulated proteins



Results

- Total of 231 (+/-10) unique proteins were identified in NDE preparations in 20 samples
- 28 proteins differentially abundant between CUD and controls
- Difference in properdin (CFP) - statistically significant after correction (Figure 2)
- Nominally significant proteins included SHANK1 a synaptic protein and proteins in complement and coagulation cascade (Figure 3)

Discussion

- Reduced abundance of SHANK1 in CUD - protein involved in the structural and functional integrity of glutamatergic post-synapse
- Differential abundance of proteins in complement, coagulation and immune cascades – possible role of neuroimmune axis in chronic cannabis exposure
- LFQ analysis of NDEs from plasma – potential insights into neuropathology of CUD and other neurodevelopmental disorders
- Results warrant validation in future studies in larger samples

References

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