# EVs in Diseases of the Nervous System
# Chairs: Eva Maria Albers; Tine Hiorth Schøyen
# Location: Exhibit Hall

17:15–18:30

## PF07.01

### Extracellular vesicles as part of the search for Alzheimer's disease blood-based biomarkers

Jessica Wahlgren; Kina Höglund; Henrik Zetterberg; Kaj Blennow

Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, University of Gothenburg, Mölndal, Sweden

**Background**: To support the clinical diagnosis of Alzheimer's disease (AD), there is a need for blood-based biomarkers to facilitate sampling and analysis. Several obstacles need to be overcome including development of sensitive methods and evaluation of pre-analytical factors. Here we investigate the potential use of extracellular vesicles from blood as biomarkers to improve the diagnostic utility of already established cerebrospinal fluid (CSF) AD biomarkers in blood and to thereby improve the diagnosis of AD at an early stage.
**Methods**: Extracellular vesicles were isolated from paired plasma and serum samples using an established immunoprecipitation method enriching for neural cell adhesion molecules (L1CAM) by capturing positive vesicles on L1CAM-coated beads. Quantification and size determination of extracellular vesicles was performed using nanoparticle tracking analysis (NTA). Detection of exosome and AD marker proteins was done using Western blot and ELISA. Comparative studies between AD and controls using exosomes isolated from paired serum and plasma samples were performed using ELISA kit for total tau, phosphorylated tau and amyloid beta protein.
**Results**: L1CAM-positive vesicles from both serum and plasma were positive for amyloid beta and tau, including phosphorylated tau protein. There were no significant differences between AD and control in serum for any of the AD markers. However, in plasma a small difference was detected for total and phosphorylated tau. Negative control beads, i.e. not coated with antibody yielded no positive signal. Interestingly, NTA showed particles of considerable amounts present in these isolates.
**Summary/Conclusion**: There is an L1CAM-positive subpopulation of extracellular vesicles in the blood from AD as well as healthy control subjects. Unspecific binding of extracellular vesicles that are not L1CAM positive to the streptavidin-coated resin beads seems to occur of similar count as beads incubated with EVs stained with L1CAM antibody. All three established CSF biomarkers in AD were detectable with ELISA, but no differences between AD and controls were seen in exosome isolates from serum. However, a modest difference was observed in exosome isolates from plasma for total tau and phosphorylated tau.

## PF07.02

### Processing of the amyloid precursor protein in the exosomal pathway: propagation of Alzheimer's disease pathology

Rocio Perez-Gonzalez[1]; Efrat Levy[2]

[1]Center for Dementia Research, Nathan S. Kline Institute for Psychiatric Research, Orangeburg, NY, USA; [2]Departments of Psychiatry, Biochemistry & Molecular Pharmacology, and the Neuroscience Institute, NYU Langone Medical Center, New York, NY, USA

**Background**: The main component of the amyloid deposited in the brain of Alzheimer's disease patients is β-amyloid (Aβ), a proteolytic product of the amyloid β precursor protein (APP). Mature APP undergoes proteolytic cleavage by α- and β-secretases to produce C-terminal fragments (APP-CTFs). β-APP-CTF is a neurotoxic protein that is also the source of Aβ following cleavage by γ-secretase. It was previously shown that amyloidogenic APP processing mainly occurs in endosomes and that exosomes contain APP, APP-CTFs, a minute fraction of Aβ, and the secretases involved in APP metabolism, but the exosomal contribution to amyloid pathology remains unknown. We have investigated whether APP processing occurs in the exosomal pathway.
**Methods**: Exosomes were isolated from postmortem human and mouse brains, and from the culture media of human fibroblasts and of the neuroblastoma cell line SH-SY5Y. The content of APP, APP metabolites and APP secretases in exosomes was analysed by Western blot and compared with the content in the brain or cell homogenates.
**Results**: We found that exosomes isolated from human and mouse brains as well as exosomes secreted by cells *in vitro* are enriched in APP-CTFs. All three APP secretases were detected in the exosome preparations and interestingly, β-secretase 1 (BACE1) and the mature form of the -secretase ADAM10 were also enriched in exosomes, whereas the γ-secretase subunit Nicastrin was not. Our data also show that exosomal β- and α- secretases are active, based on the observation of continuous generation of APP-CTFs in isolated exosomes.
**Summary/Conclusion**: Our data show that APP processing continues in exosomes following their release into the extracellular space from the endosomal multivesicular bodies, implicating exosomes as carriers and generation sites of the neurotoxic β-APP-CTF and an extracellular source of Aβ. Given the stability of exosomes, this may propagate amyloid pathogenicity throughout the brain.
**Funding**: This work was supported by the NIH (P01 AG017617 and R01 AG057517) and the Alzheimer's Association (NIRG-14-316622).

## PF07.03

### To study anti-tau antibody loading and neuronal uptake efficiency of human bone marrow mesenchymal stem cells-derived extracellular vesicles

Azadeh Amini[1]; Hamid Akbari Javar[2]; Faezeh Shekari[3]; Koorosh Shahpasand[3]; Hossein Baharvand[3]

[1]Department of Pharmaceutical Biomaterials and Medical Biomaterial Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran; [2]Department of Pharmacutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran; [3]Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, Tehran, Iran

**Background**: Despite significant progress in drug delivery issue, efficient central nervous system (CNS) delivery of neuro therapeutics remains challenging. Extracellular vesicles (EVs), part of normal cell-to-cell communication, were introduced recently as a transporter that can overcome biological barriers against CNS delivery. So these natural nanoliposomes are promising tools for delivery systems design, especially for CNS. In present study, we examine the efficiency of mesenchymal stem cell (MSC)-derived EVs for drug loading and neuronal uptake.
**Methods**: We isolated human bone marrow-derived mesenchymal stem cells (hBMSCs)-EVs by differential ultracentrifugation coupled to density gradient technique. The protein content of harvested vesicles was measured using a BCA Protein Assay Kit. Then vesicles were characterized by performing dynamic light scattering, transmission electron microscopy and Western blotting. We examined different drug loading methods (incubation, freeze and thaw and sonication) and comprise the loading efficiency using an ELISA procedure. Neuronal uptake of vesicles also was studied using PKH-26-labeled vesicles.