Cell suspensions were stained with fluorochrome-conjugated monoclonal antibodies for flow cytometry. Statistical significance was determined using a two-tailed Student t test or one way ANOVA multiple comparisons test. The minimal level of confidence deemed statistically significant was $p<0.05$. RESULTS/ANTICIPATED RESULTS: Preeclampsia resulted in lower body and heart masses in offspring. Although T cell populations in the thymus were not altered in preeclampsia offspring, total T cells, Thelper, and cytotoxic T cells were elevated. Total B and isotype-switched B cells were increased in offspring of preeclampsia. Total dendritic cell percentages were not changed in offspring of preeclampsia, however, total anti-inflammatory markers on dendritic cells were reduced. Lastly, offspring of preeclampsia had a reduction in microglia and astrocytes within the brain. DISCUSSION/SIGNIFICANCE: Our study could establish including in utero data in predicting future disease risk, addressing gaps in understanding rising rates of cardiovascular and behavioral diseases. It also uncovers the impact of preeclampsia on early immune programming and reduced glial cell populations, potentially affecting cognitive and behavioral development.

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Validation of a Novel CSF-Based Biomarker of Mitochondrial Function†

Dhanushki Abeykoon, Xiaowan Wang, Lesya Novikova, Amol Ranjan, Alexander Gabrielli and Russell Swerdlow
University of Kansas Medical Center

OBJECTIVES/GOALS: Determine the exosome mitochondrial DNA (mtDNA) copy number in cerebrospinal fluid (CSF) as a measure of neuronal mitochondrial integrity in patients with subarachnoid hemorrhage (SAH). Determine the patterns of beta amyloid and tau protein biology in CSF of SAH patients and correlate those measures with the clinical status of the SAH patients. METHODS/STUDY POPULATION: The CSF is collected from SAH patients undergoing ventriculostomy-based continuous CSF drainage. Adults from all ethnicities and sex are included in this study. The exosomes are isolated from CSF samples using a precipitation method and particle count and size are measured using NanoSight. The DNA is extracted using an exosomal DNA isolation kit (XCF kit). The CSF mtDNA copy number is measured using digital drop PCR with mitochondrial DNA primers. The levels of beta-amyloid (a-beta-40 and -42) and tau protein in CSF are measured using a sensitive ELISA-based assay. A quantitative evaluation of mitochondrial DNA copy number, clinical status of the SAH patients and beta amyloid, and tau protein levels will be conducted and reported. RESULTS/ANTICIPATED RESULTS: Preliminary results of four CSF samples showed similar patterns in CSF exosome particle number, particle size and exosomal mtDNA copy number in relation to samples from the admission day. Particle number decreased with time while particle size increased. More patient samples will be analyzed to confirm the patterns. We anticipate that mtDNA copy number will correlate with brain beta-amyloid and tau protein levels. Moreover, we anticipate that the clinical status of the SAH patients will associate with the mtDNA copy number. We specifically predict that higher mtDNA copy number levels will correlate with better clinical outcomes. DISCUSSION/SIGNIFICANCE: Mitochondrial function is critical to brain health, but we lack effective ways to monitor this parameter. Here we focus on a CSF based biomarker, exosome-derived mtDNA, which is intended to reflect the integrity of brain mitochondria. As bioenergetic metabolism influences beta amyloid and tau biology, predicting those levels are important.

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Identification of novel plasma protein biomarkers for diagnosis and prediction of Alzheimer's disease in African Americans*†

Lindsey Kuchenbecker, Joseph S Reddy and Minerva M Carrasquillo
Mayo Clinic Florida

OBJECTIVES/GOALS: To identify novel panel of plasma protein biomarkers to improve prediction and diagnosis of Alzheimer's disease (AD) for African Americans (AA), who are at greater risk of developing AD compared to non-Hispanic White individuals but are underrepresented in AD research. METHODS/STUDY POPULATION: Pre-existing plasma samples from 460 AA individuals with clinical diagnoses of AD, cognitively unimpaired (CU), mild cognitive impairment (MCI), or dementia with Lewy bodies (DLB) will undergo untargeted proteomics using the SomaScan assay, where modified single stranded DNA aptamers bind to protein targets which are quantified by DNA microarray. Protein expression levels will be compared between diagnostic groups to identify differentially expressed proteins. Additional clinical, genetic, and lifestyle factors will be compared with protein expression when available. Proteins of interest, identified by differential protein expression analysis results, will be included in receiver operating characteristic analyses to identify the optimal set of proteins for diagnostic classification. RESULTS/ANTICIPATED RESULTS: A pilot experiment utilizing plasma from 40 individuals identified multiple differentially expressed proteins (DEPs) between AD and non-AD groups. Eight proteins were nominated from the differential protein analysis into a receiver operating characteristic (ROC) analysis based on pvalue and previous implication in AD genome wide association studies. Proteins involved in microglial activation, neuronal adhesion, cell proliferation, and innate immunity were nominated. The ROC model achieved 100% classification accuracy of AD and CU groups using age, sex, and the eight nominated proteins. It is expected that there will be more significant associations when utilizing the full cohort of 460 AA and that DEPs between AD, CU, MCI, and DLB will be identified. DISCUSSION/SIGNIFICANCE: The nomination of a novel panel of plasma biomarkers developed from an AA cohort will directly serve the AA community by improving access to an early and accurate diagnosis of AD. Access to improved prediction and diagnosis will likely improve disease management, thus improving patient outcomes and decreasing burden on families and caregivers.

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Ischemic conditioning improves dynamic balance during treadmill walking in chronic stroke survivors

Stephanie Raab[1], Julia Athans[1], Zachary Kroll[2], Emilie Klevenow[1], Matthew Durand[3], Brian Schmit[2] and Allison Hyngstrom[1]
[1]Marquette University; [2]Marquette University and Medical College of Wisconsin and [3]Medical College of Wisconsin

OBJECTIVES/GOALS: Evaluate the use of IC to improve stroke survivors' capacity for reactive stepping and adapt their gait cycles in

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