9:50 am	Symposium Welcome Linda Van Eldik, PhD, Director, Sanders-Brown Center on Aging
10:00 am	***Keynote Speaker*** Is MCI Still a Viable Construct in 2020? Ronald Petersen, MD, PhD *Director, Mayo Alzheimer's Disease Research Center* *Professor of Neurology, Mayo Clinic College of Medicine, Rochester, MN*
10:45 am	Q&A with Dr. Petersen
11:00 am	Poster Session
12:55 pm	Poster Awards
1:00 pm	***Keynote Speaker*** Human Genetics Implicates Efferocytosis in Alzheimer's Disease Alison Goate, D.Phil. *Director, The Ronald M. Loeb Center for Alzheimer's Disease Willard T.C. Johnson Research Professor of Neurogenetics* *Professor of Neuroscience, Neurology and Genetics & Genomic Sciences* *Icahn School of Medicine at Mount Sinai, New York, NY*
1:45	Q&A with Dr. Goate
1:55	Closing Remarks Linda Van Eldik, PhD

Keynote Speaker

Is MCI Still a Viable Construct in 2020?

Ronald Petersen, MD, PhD

Professor of Neurology; Cora Kanow Professor of Alzheimer's Disease Research; Director, Mayo Alzheimer's Disease Research Center, Mayo Clinic College of Medicine Rochester, MN

Dr. Ronald C. Petersen received a Ph.D. in Experimental Psychology from the University of Minnesota and graduated from Mayo Medical School in 1980. He completed an internship in Medicine at Stanford University Medical Center and returned to the Mayo Clinic to complete a residency in Neurology. That was followed by a fellowship in Behavioral Neurology at Harvard University Medical School/Beth Israel Hospital in Boston, Massachusetts. Dr. Petersen joined the staff of the Mayo Clinic in 1986 and became a Professor of Neurology in 1996. In 2000 he was named the Cora Kanow Professor of Alzheimer's Disease Research and Mayo Clinic Distinguished Investigator in 2011. He is currently the Director of the Mayo Alzheimer's Disease Research Center and the Mayo Clinic Study of Aging and has authored over 990 peer-reviewed articles on memory disorders, aging, and Alzheimer's disease.

Dr. Petersen is one of the recipients of the 2004 MetLife Award for Medical Research in Alzheimer's Disease and the 2005 Potamkin Prize for Research in Picks, Alzheimer's and Related Disorders of the American Academy of Neurology. In 2012 he received the Khachaturian Award and the Henry Wisniewski Lifetime Achievement Award in 2013 from the Alzheimer's Association. In 2011 he was appointed by the Secretary of Health and Human Services to serve as the Chair of the Advisory Committee on Research, Care and Services for the National Alzheimer's Disease Plan, and in 2014, he was appointed to the World Dementia Council by the UK government. He received an Honorary Doctorate of Humane Letters from Hamline University in 2017.

Keynote Speaker

Human Genetics Implicates Efferocytosis in Alzheimer's Disease

Alison Goate, D.Phil.

Director, The Ronald M. Loeb Center for Alzheimer's Disease; Professor of Neurogenetics; Professor of Neuroscience, Neurology and Genetics & Genomic Sciences; Icahn School of Medicine at Mount Sinai, New York, NY

Dr. Alison Goate has worked on Alzheimer's disease genetics since 1987, when she joined Dr. John Hardy's lab, as a postdoctoral fellow at Imperial College, London and subsequently became an independent faculty member in the Dept. of Molecular Genetics. In 1992 she moved to Washington University in St. Louis, where she stayed until 2014, when she moved to the Icahn School of Medicine at Mount Sinai as the founding director of the Ronald M. Loeb Center for Alzheimer's disease. She has been part of many gene-finding teams that have successfully identified disease causing variants for both AD, FTD and ALS. Whilst working with Dr. Hardy she reported the first mutation to cause familial Alzheimer's disease and early studies at Washington University identified the mutation in the Colombian families that are now part of the API clinical trial. Her lab was also part of the team that first reported *MAPT* mutations in FTD.

Dr. Goate is also a leader in the study of late onset AD genetics using both GWAS and sequencing approaches. She has demonstrated that LOAD families can carry *PSEN* mutations with reduced penetrance. Her team led the identification of rare variants in *PLD3* as a risk factor for AD and collaborated with John Hardy in the identification of *Trem2* as an AD risk factor. More recently her work on common variants has highlighted the importance of microglial expressed genes in AD risk, identifying *SPI1*/Pu.1 as an important regulator of AD risk genes. Fine mapping of AD risk loci has identified causal genes/variants in many loci and further emphasized the importance of microglial gene expression and function to AD risk. Dr. Goate has received the Potamkin Award, the Khalid Iqbal Lifetime Achievement Award from the Alzheimer's Association and the MetLife Award for her research on AD. She was elected a fellow of AAAS in 2012 and a fellow of the National Academy of Medicine in 2016.

ABSTRACT TABLE OF CONTENTS

Lucille Yanckello [1] • Adam Bachstetter, PhD [2] • Ai-Ling Lin, PhD [3]
Department of Pharmacology and Nutritional Sciences University of Kentucky [1] • Department of Neuroscience University of Kentucky [2] • Department of Pharmacology and Nutritional Sciences, Department of Neuroscience University of Kentucky [3]

Effects of prebiotic supplementation on chronic mild traumatic brain injury recovery

Student

Background: Mild traumatic brain injury (mTBI) currently affects 1.6-3.8 million people in the US annually, including the hospitalization of 100-300 per 100,000 young adults. Recently, it was found that gut dysbiosis occurs acutely after traumatic brain injury and the literature suggests that manipulation of the gut microbiome may be actionable to reduce long term symptoms after mTBI. Prebiotic fibers are known to beneficially alter the gut microbiome and increase metabolites such as short chain fatty acids (SCFA) which play a role in metabolism and inflammation. It is also known that the gut microbiome plays a role in the regulations of brain vascular and metabolic integrity which are all important in recovery following mTBI.

Method: This study used all male mice and for the injury, at four months of age, a midline sagittal scalp incision was made. An electromagnetic impactor was used to deliver a single controlled mid-line cortical impact. A group of animals will undergo the procedure but will not receive the injury (sham). For the gut microbiome analysis, fecal samples were collected at 5 m.p.i. Shotgun metagenomic sequencing was done by CosmosID. Targeted metabolomics for SCFA analysis was done by Metabolon, Inc. Cerebral blood flow (CBF) was measured using MRI-based pCASL techniques. For brain structure analyses, diffusion tensor imaging (DTI) is used to characterize microstructural changes in the brain. MRI sequences were conducted at 9 months of age (5 m.p.i). Both inulin and cellulose diets were provided by TestDiet.

Results: For the gut microbiome, we see trends to indicate that inulin is increasing putatively beneficial bacteria such as *Bifidobacterium pseudolongum*, *Akkermansia muciniphila* and *Eubacterium spp.* We also see decreases in putatively harmful bacteria such as *Dorea sp. 5-2.* SCFA levels reflect these increases as *B. pseudolongum* and *A. muciniphila* both produce acetate, which we see higher levels of in the cecum and blood of mice fed inulin. Butyrate is also higher in the cecum of mice fed inulin and is produced by *Eubacterium spp* Inulin was also beneficial in the brain showing increased CBF in both the thalamus and hippocampus, and decreased Glycerophosphocholine (GPC) in the hippocampus.

Conclusion: The increase in beneficial bacteria and SCFA may tie back to the improved cerebral blood flow and reduced GPC. Butyrate is known to increase tight junction protein expression in the intestine and the blood brain barrier (BBB). This could be indicative of the higher CBF in inulin fed mice as a more intact BBB is protective of CBF levels. Inulin decreases the GPC levels this could be indicative of reduced glial proliferation. Inulin also is decreasing harmful bacteria such as *Dorea sp. 5-2* which has been positively correlated with intestinal permeability. It is clear that even when administered in the chronic phase of injury, inulin exerts beneficial effects that aid recovery. Zoom https://uky.zoom.us/j/81116226688

Nirmal Verma, PhD [1] • Larry B Goldstein, MD [2] • Florin Despa, PhD [3]
Pharmacology and Nutritional Sciences University of Kentucky [1] • Department of Neurology University of Kentucky [2] • Pharmacology and Nutritional Sciences University of Kentucky [3]

Amylin deposition in skin capillaries as a marker for cerebral small vessel disease

Staff

Objectives

Amylin is a β-cell hormone that forms pancreatic amyloid. Individuals with prediabetes, type-2 diabetes and obesity have aggregated amylin in pancreatic, brain, heart and renal microvessels. We previously showed that circulating aggregated amylin attaches to red blood cells and capillary endothelium, which induces hypoxia and microcirculatory disturbances. Given the amyloidogenicity of human amylin and its adverse effects on microvasculature, we hypothesized that amylin deposition in skin capillaries could be a marker for cerebral small vessel disease.

Methods/Results

Using rats with pancreatic overexpression of human amylin (HIP rats), we show that accumulation of human amylin in skin capillaries and brain microvasculature correlated with the development of cerebral small vessel disease and the activation of hypoxia signaling pathways. Co-staining for amylin and collagen IV, a component of the basement membrane structure, showed amylin deposition in skin and brain capillaries of HIP rats. Capillaries isolated from diabetic HIP brains showed elevated levels of incorporated aggregated amylin, and the accumulation of amylin in capillaries was associated with depletion of both caveolin-1 and collagen. The levels of claudin, occludin, and ZO adapter proteins were also lower in capillaries from HIP rats compared to WT littermates suggesting altered structural integrity of tight junctions in HIP rat capillaries. The immunoreactivity signal of HIF-1α was also higher in skin tissue from HIP compared to WT rats. Pharmacologically increased levels of endogenous epoxyeicosatrienoic acids (EETs) lowered amylin deposition in brain capillaries and improved capillary stability.

Conclusions

In conclusion, detection of amylin in a skin biopsy could be a biomarker of cerebral small vessels disease. A skin biopsy to detect capillary amylin deposition can complement brain and may provide a molecularly based approach for the identification of individuals at risk of cerebral small vessel disease. Blocking amylin dyshomeostasis may be a novel approach for reducing small vessel type ischemic brain injury.

Zoom: https://uky.zoom.us/j/86904684474

Cory Dungan, PhD [1] • Taylor Valentino, MS [2] • Ivan Vechetti Jr., PhD [2] • John McCarthy, PhD [2] • Charlotte Peterson, PhD [1]
Physical Therapy University of Kentucky [1] • Physiology University of Kentucky [2]

Modulation of hippocampal miRNA expression in female 3xTg mice following 20 weeks of progressive weighted wheel running (PoWeR)

Fellow

Changes to cerebral miRNA expression have been implicated in the progression of Alzheimer's disease (AD), as miRNAs that regulate the expression of genes involved in amyloid beta (Aβ) processing are dysregulated in those that suffer from AD. Exercise training improves cognitive function and reduces Aβ plaque burden, however, the mechanisms are not fully understood. **Objective:** Using our progressive weighted wheel running (PoWeR) exercise program, which utilizes an unbalanced running wheel to promote physiological adaptations similar to those seen in humans, we assessed the effect of 20 weeks of voluntary exercise training on changes to hippocampal miRNA expression in the 3xTg mouse model of AD. **Methods/Results:** Two month old female 3xTg mice were PoWeR trained for 20 weeks, running ~7km/day, while groups of female wild-type and 3xTg served as sedentary controls. At the end of the training period, the hippocampus was removed to assess miRNA abundance via Nanostring and gene expression with RT-qPCR. Exercise training had a significant effect on gene expression of the miRNA processing enzyme, Dicer. Specifically, Dicer mRNA was lower in sedentary 3xTg hippocampus, when compared to sedentary wild-type mice, but was robustly elevated following exercise. Correspondingly, ~45% of the miRNAs that were dysregulated in sedentary 3xTg mice were restored towards wild-type levels following exercise. Specifically, miR-29, which is a validated target of the APP processing protein, BACE1, was significantly higher in exercised 3xTg mice. Accordingly, BACE1 gene expression and detergent soluble $A\beta^{1-42}$ were lower in these mice. **Conclusions:** Based on these results, we conclude that exercise training alters cerebral miRNA expression via Dicer to affect APP processing. Therapies aimed at restoring Dicer expression could prove useful to slow AD pathology through modulation of miRNA abundance.

Support or Funding Information NIH AG049806 to J.J.M and C.A.P.. C.M.D. is supported by NIH T32 AG057461. Zoom: https://uky.zoom.us/j/88544701302

Eleanor Johnson [1] • Kendra Stenzel, PhD [1] • Sangderk Lee, PhD [1] • Eric Blalock, PhD [1]
Pharmacology and Nutritional Sciences University of Kentucky [1]

Reduced RNA itegrity indicates pathway-selective mRNA degradation in post-mortem human brain tissue

Student

Objectives

RNA degradation can be influenced by many factors (e.g., post-mortem interval, sample preparation, storage duration), and the degree to which RNA is degraded prior to quantification affects downstream measurements (e.g., situ hybridization, RT-PCR, transcriptional profiling). Agilent Technologies introduced the RNA integrity number (RIN; 1 being the worst to 10 being the best) to help quantify and standardize degradation across samples and labs. Recent studies have shown RIN influences mRNA levels, though relatively little work has been done to determine whether RNA is degraded across the genome or instead focused in certain biological pathways.

Methods/ Results

Using raw transcriptional data (accessed through the Gene Expression Omnibus) for multiple transcriptional profiling studies of post-mortem brain tissue from different individuals, we identified a robust and consistent expression profile of genes appearing more vulnerable to degradation. Importantly, we report that many of the pathways supported by these degradation-sensitive genes are neuronal, particularly vesicles, mRNA transport, synapses, and mitochondria. This indicates neuronal synaptic mRNA is particularly vulnerable to degradation. Using a mean subtraction method, we determined the most common templates a gene's RNA degradation followed and plotted the gene expression values with the template.

Conclusion

Our data found RNA degradation causes major drops in signal around a RIN of 6.7-7 and that there is no appreciable correlation between gene expression and RIN $\geq$ 8.3. Second, our data suggests this effect is pathway selective, possibly having important consequences for current bioinformatic RIN correcting procedures. Finally, this may have implications for neurodegeneration research like Alzheimer's disease itself (rather than technical issues associated with harvesting and storage), where the disease process may selectively degrade the mRNA in a pathway specific manner.

This research was funded by NIH NIA AG037868, NIH NIA AG057461, NIH UL1 TR001998. Zoom: https://uky.zoom.us/j/85292772226

Blaine Weiss, [1] • Jenna Gollihue, PhD [1] • N. Drew Farr, [2] • Pradoldej Sompol, PhD [1] • Irina A. Artiushin, MS [1] • Susan D. Kraner, PhD [1] • Malú Gamez Tansey, PhD [2]• Christopher M. Norris, PhD [3]

Sanders Brown Center on Aging University of Kentucky [1] • Department of Neuroscience and Center for Translational Research in Neurodegenerative Disease University of Florida [2] • Pharmacology and Nutritional Sciences University of Kentucky [3]

Synaptoprotective effects of the novel TNF inhibitor XPro1595 in 5xFAD mice: interactions between western diet and sex

Staff

Background: The western diet has been repeatedly shown to trigger inflammation and has been implicated as an environmental factor in cerebrovascular disease and Alzheimer's disease (AD) pathology. Our previous work has shown that inhibition of neuroinflammation by the selective anti-TNF biologic, XPro1595, ameliorates neurologic dysfunction in a mouse model of amyloid pathology. Unlike other TNF inhibitors, XPro1595 targets only the soluble form of TNF (sTNF), preserving the neuroprotective transmembrane TNF signaling pathways. Here, we investigate whether western diet-induced chronic inflammation accelerates AD-like pathology in a mouse with genetic predisposition for amyloid development and the extent to which this synergy is dependent on sTNF signaling. **Method:** Three-month-old 5xFAD mice received Teklad high fat and high fructose (HFHF) diet (TD 150111) or a balanced control diet (TD 150112) over a two-month period. At four-months-of-age and halfway through dietary intervention, subcutaneous injections of XPro1595 (10mg/kg, 2x/week) or saline were started and continued for another 4 weeks along with the dietary regimen. At endpoint, brain slices were prepared for electrophysiologic analyses of basal synaptic function and long-term potentiation (LTP) in hippocampal CA1. Synaptic parameters were compared across diet and drug treatment conditions (n= 9-14 mice/condition) using ANOVA and, where appropriate, Tukey's post hoc test. **Result:** Slices from XPro1595-treated mice exhibited an increase in both synaptic strength and LTP, regardless of diet. However, these effects also depended strongly on sex. LTP in males was similar across treatment groups (125-130% of baseline), but females treated with HFHF showed nearly no LTP. LTP deficits in HFHF-treated females were reversed by XPro1595 administration *in vivo*. In fact, HFHF females treated with XPro1595 showed the highest LTP levels (~140% of baseline) of all groups investigated. **Conclusion:** Aberration of sTNF pathways have a differential effect on synapse physiology depending on sex and diet. Protective effects of XPro1595 on synapses materialize predominantly in females under conditions of dietary stress, while males responded modestly to treatment regardless of diet conditions. Studies are underway to establish whether sex-specific differences reflect differences in amyloid burden between the sexes known to occur in this model, or are related to other differences unrelated to amyloid burden.

Zoom link: https://uky.zoom.us/j/81147763785

Elizabeth Rhodus, PhD [1] • Justin Barber, MS [1] • Erin Abner, PhD [1,2] • Shoshana Bardach, PhD [2] • Allison Gibson, PhD [3] • Gregory Jicha, MD, PhD [1]

Sanders-Brown Center on Aging University of Kentucky [1] • College of Public Health[2] • College of Social Work University of Kentucky [3]

Comparison of behaviors characteristic of autism spectrum disorder behaviors and behavioral and psychiatric symptoms of dementia

Fellow

Background
Similarities exist in behavioral expression of autism spectrum disorder (ASD) and Alzheimer's disease and related dementias (ADRD). The purpose of this study was to assess presence of behavioral and psychiatric symptoms of dementia (BPSD) and ASD-like behaviors in adults with ADRD.

Methods
Using a cross-sectional design, data from University of Kentucky Alzheimer's Disease Center participant cohort were used. Hierarchical linear regression was used to assess (1) the relationship between ASD-like behaviors (measured by the Gilliam Autism Rating Scale-Second Edition, GARS-2) and BPSD measured by the Neuropsychiatric Inventory (NPI), and (2) the relationship between ASD-like behaviors and dementia severity (measured by the Clinical Dementia Rating [CDR] sum of boxes), when controlling for BPSD.

Results
Complete data were available for 142 participants. Using α of 0.05, analyses identified ASD behaviors were significantly associated with BPSD severity ratings ($r=0.47$; $p<0.001$) and dementia severity ($r=0.46$; $p<0.001$). GARS-2 explained 6.1% ($p < 0.001$) of variance in CDR sum of boxes when controlling for NPI and other covariates.

Conclusions
There is significant overlap in behaviors characteristic of ASD and BPSD as assessed by the GARS-2 and NPI, despite the use of these instruments in disparate developmental vs. aging settings. ASD behaviors appear to not be solely present in early childhood as a manifestation of ASD but are also present in older adults with neurodegenerative cognitive impairment. Such associations warrant additional research into causation, assessment, and behavioral interventions to further enable new therapeutic approaches targeting ASD behaviors across the lifespan.

Funding: This study utilized University of Kentucky Alzheimer's Disease Cohort participants. Funding for the longitudinal cohort is provided by NIH/NIA P30 AG028383. The first author is funded by NIH T32 AG057461: "Training in Translational Research in Alzheimer's and Related Dementias (TRIAD)".

Zoom: https://uky.zoom.us/j/88477113618

N. Drew Farr [1] • Susan D. Kraner, PhD [1] • Peter T. Nelson, MD, PhD [1,2] • Christopher M. Norris, PhD [1,3] • Pradoldej Sompol, PhD [1]

Sanders-Brown Center on Aging University of Kentucky [1] • Pathology & Laboratory Medicine University of Kentucky [2] • Pharmacology & Nutritional Sciences University of Kentucky [3]

Fibronectin accumulation and oxidative modification in Alzheimer's disease

Student

Objectives: Oxidative stress in Alzheimer's disease (AD) alters the structure of brain proteins, complicating the pathophysiology of AD while suggesting potential drug targets. Fibronectin mediates cell attachment and function, in part by directing the reorganization of the extracellular matrix. High molecular forms of fibronectin appear more frequently and in higher amounts in AD while oxidative stress can cause nitration of fibronectin by 3-nitrotyrosine. Considering the role of fibronectin in maintaining vessel function, accumulation/oxidative modification of fibronectin may alter vessel function. Astrocytes are also damaged in AD, with significant physiologic consequences. In healthy brain, astrocytes maintain the integrity of the neurovascular unit by maintaining molecular, metabolic, and cellular homeostasis. In AD, phenotypically distinct astrocytes may play a role in neurovascular function. Combined with compromised astrocyte function, oxidative modification of fibronectin may further damage AD brains that are already less capable of repair. **Methods:** Astrocytic fibronectin and β-amyloid were quantified in WT and 5xFAD mice (n = 6-8 mice/group) using confocal microscopy and Western blot. GFAP, fibronectin, nitrotyrosine, and β-amyloid were also immunolabeled in postmortem human AD and age-matched control tissue and imaged using confocal microscopy. **Results:** We showed that fibronectin was increased in hippocampal astrocytes in 5xFAD vs. WT mice (p = 0.032). In 5xFAD brain homogenates, fibronectin was increased by 30% compared to WT. In human tissue, both fibronectin and nitrotyrosine were increased compared to control. Fibronectin also accumulated around β-amyloid plaques while fibronectin and nitrotyrosine colocalized in perivascular astrocytes, suggesting oxidative modification of fibronectin in human AD compared to control. We also observed morphologic changes in human AD astrocytes. AD white mater showed more fibronectin and nitrotyrosine in astrocytes compared to gray matter as well as more fibronectin in the extracellular space. Fibronectin was especially concentrated in the perivascular white matter. **Conclusions:** Oxidative changes to fibronectin in AD clustered in perivascular astrocytes, suggesting the central role of astrocytes in AD-related vascular dysfunction.

Funding: NIH T32 AG057461 "Training in Translational Research in Alzheimer's and Related Dementias (TRIAD)," NIH RF1AG027297, NIH UL1TR001998, the Sylvia Mansbach Endowment, and The Hazel Embry Research Trust.

Zoom link: https://uky.zoom.us/j/7576990030?pwd=Wit2aElWbUF0NHV6VllpaEQ3Q1RIdz09

Password: 125075

Jenna Gollihue, PhD [1] • Pradoldej Sompol, PhD [1] • Susan Kraner, PhD [1] • Irina Artiushin [1] • Blaine Weiss [1] • Ryan Cloyd [1] • Shon Koren [1] • Grant Nation [1] • Jose Abisambra, PhD [2] • Orsolya Huzian [3] • Laszlo Hackler [3] • Laszlo Puskas, PhD [3] • Christopher Norris, PhD [1]

Sanders-Brown Center on Aging University of Kentucky [1] • Center for Translational Research in Neurodegenerative Disease University of Florida [2] • Avidin Ltd [3]

A novel NFAT inhibitor improves behavior and synapse function in APP/PS1 mice

Post Doc Scholar

Objectives: It has previously been shown that inhibition of calcineurin, a protein phosphatase, is beneficial in mice with Alzheimer's Disease (AD). However, there are concerns about off-target effects that have impacted the use of CN inhibitors in clinical trials. To circumvent these concerns, use of a more specific inhibitor of one of the CN substrates may be a more targeted approach that affords the same benefits as CN inhibition. A novel drug, Q134R, is a hydroxyquinoline drug that suppresses NFAT signaling downstream of CN. The goal of our study was to investigate the NFAT activity-dependent cognitive changes in an amyloidogenic mouse model of AD treated with Q134R.
Methods/Results: *In vitro* experiments confirmed Q134R as a calcinuerin-independent NFAT inhibitor in primary astrocytes challenged with ionomycin/phorbol ester or oligomeric Aβ. Next, transgenic APP/PS1 mice that were given Q134R for 5 days showed better cognitive behavioral outcomes compared to vehicle-treated animals. Chronic treatment paradigms (3 month duration) were also tested in the transgenic mice. Changes in NFAT4 expression were characterized, and it was found that astrocyte-specific NFAT expression was significantly increased in vehicle-treated APP/PS1 animals compared to their Q134R-treated counterparts. Interestingly, Q134R did not alter the amount of astrocyte reactivity as measured by GFAP expression, nor did treatment alter levels of Aβ plaque load. Electrophysiologic recordings showed a decrease in synaptic strength in the APP/PS1 animals compared to wild-type littermates that was rescued with chronic treatment with Q134R.
Conclusions: Q134R seems to have an effect on NFAT4 signaling in astrocytes independent of astrocyte activation and calcineurin activity. This shows promise for the drug as a therapeutic that acts more specifically than those working upstream on calcineurin activity. By inhibiting NFAT4 activation, we were able to restore synaptic function and cognitive behavioral outcomes in an APP/PS1 mouse model of Alzheimer's disease. Our studies focused on measurements of NFAT4, the isoform found in astrocytes. More testing of the drug is necessary to determine its effect on other isoforms of NFAT, including those found in neurons and microglia. Additionally, ongoing experiments are underway to determine the precise mechanism of NFAT inhibition by Q134R.

Funding: Work was supported by National Institutes of Health Grants (RF1AG027297, R21AG051945, T32AG057461), a UK CCT/UL1TR001998 grant, an Alzheimer's Drug Discovery Foundation grant, The Sylvia Mansbach Research Fund, and The Hazel Embry Research Trust.

Zoom link: https://uky.zoom.us/j/87994224295

Hilaree N. Frazier, PhD [1] • Adam O. Ghoweri [1] • Katie L. Anderson [1] • Ruei-Lung Lin, MS [1] • Olivier Thibault, PhD [1]

Pharmacology and Nutritional Sciences University of Kentucky [1]

P2X4 expression in the somatosensory cortex of F344 rats is age-dependent and may be altered by administration of intranasal insulin

Fellow

Objectives: Ca2+ is a major driver of neuronal physiology, cellular function, and learning and memory processes, and evidence shows that aging is associated with Ca2+ dysregulation, defined by altered Ca2+ levels, diminished synaptic plasticity, and reduced neuronal excitability. However, while Ca2+ dysregulation has been well-characterized in the aged brain, similar studies in the context of Alzheimer's disease (AD) have been somewhat limited. Recent data suggests the AD brain may not undergo the same alterations as the aged brain, and in fact, may even display increased, rather than decreased, neuronal excitability. Ionotropic purinergic receptors (P2XRs) are ligand-gated cation channels that are sensitive to ATP. Many P2XR subtypes are integral to synaptic transmission and intracellular Ca2+ release, and impaired P2XR function has been implicated in the pathogenesis of several neurological disorders, including AD. Here, we measured the expression of the P2XR subtype P2X4 in the somatosensory cortex (S1), as evidence has shown that this particular subtype is reduced in the AD brain and may also be modulated via insulin signaling.

Methods/Results: Using Western immunoblot techniques, we measured P2X4 levels in S1 of young and aged male F344 rats exposed to intranasal insulin (INI) or saline. Results revealed a main effect of age on P2X4 expression 30 min after treatment, with aged animals having reduced P2X4 levels compared to young across both treatment groups. A significant interaction term was also detected, with young animals exposed to INI having increased P2X4 expression compared to young saline controls, while the aged INI-treated animals had decreased expression compared to controls. Ongoing analyses of preliminary data obtained 120 min after treatment show a similar impact of both age and INI on P2X4 expression in S1, although these effects are somewhat reduced compared to those detected at the earlier timepoint.

Conclusions: Our results suggest that aging can impact P2X4 expression in F344 animals, and that downstream insulin receptor signaling conferred via exogenous ligand delivery can potentially mediate these receptors in an age-dependent manner. Future studies will generalize this work to animal models of AD by using similar techniques to measure P2X4 expression in 5xFAD mice. Additionally, this work will employ 2-photon imaging techniques to characterize network activity and Ca2+ dynamics in S1 following INI and administration of either a P2X4 positive allosteric receptor modulator (moxidectin) or a selective receptor antagonist (5-BDBD). Finally, by using tissue samples provided by the University of Kentucky's Alzheimer's Disease Research Center, we will also characterize the translatability of these measures to the clinic by testing whether aging and AD pathology can also modulate P2X4 expression.

This work is supported by the National Institutes of Health [R01AG033649 and T32AG057461].

Zoom: https://uky.zoom.us/j/89542752173

Zachary Winder [1] • Tiffany L. Sudduth [2] • David Fardo, PhD [3] • Qiang Cheng, PhD [4] • Larry B. Goldstein, MD [5] • Peter T. Nelson, MD, PhD [2] • Frederick A. Schmitt, PhD [6] • Gregory A. Jicha, MD, PhD [2] • Donna M. Wilcock, PhD [2]

Physiology University of Kentucky [1] • Sanders-Brown Center on Aging University of Kentucky [2] • Biostatistics University of Kentucky [3] • Biomedical Informatics University of Kentucky [4] • Neurology University of Kentucky [5] • Sanders-Brown Center on Aging University of Kentucky [6]

Associations between plasma biomarkers, chronic vascular pathology, and vascular cognitive impairment and dementia

Student

Background: Abnormalities of brain arterioles and capillaries (small vessel disease, SVD) can cause ischemic parenchymal injury potentially leading to Vascular Cognitive Impairment and Dementia (VCID) through hypothesized hypoxic and pro-inflammatory mechanisms. These mechanisms are further hypothesized to include up-regulation of angiogenic proteins, specifically Placental Growth Factor (PlGF) and Vascular Endothelial Growth Factor-A (VEGF-A), which may be biomarkers useful for the identification of patients with VCID. We evaluated the associations of PLGF and VEGF-A with VCID.

Methods: Participants with VCID and age-matched controls were selected from the University of Kentucky Alzheimer's Disease Center's clinical cohort. Plasma samples were analyzed using two digitized immunoassay techniques (Meso Scale Discovery and Quanterix Simoa) to determine levels of plasma biomarkers. Unsupervised machine learning was conducted to identify novel plasma biomarker profiles of VCID, whereas supervised machine learning was used to predict levels of chronic vascular pathology based on post-mortem neuropathological analysis.

Results: Participants with VCID-SVD had elevated levels of plasma PlGF ($p<0.01$) and a non-significant elevation in plasma VEGF-A compared to age-matched controls. This angiogenic profile was also identified with increased levels of inflammatory markers in a subset of patients with mild cognitive impairment (MCI). Using supervised classification analysis, severity of chronic vascular pathology could be predicted using both plasma angiogenic and inflammatory biomarkers.

Conclusion: Plasma angiogenic and inflammatory biomarkers are associated with the severity of brain vascular pathology and might be useful biomarkers for monitoring disease progression in patients at risk for VCID.

Funding: This work supported by NIA award 1UH2NS100606-01,NIH award 1T32 AG057461 "Training in Translational Research in Alzheimer's and Related Dementias (TRIAD) and the UK MD/PhD Program.

Zoom Link: https://uky.zoom.us/j/86889589967

Alexandria N Early [1,2] • Erica M Weekman, PhD [1,2] • Tiffany L Sudduth [1] • Brittani R Price, PhD [1] • Abigail E Woolums [1] • Danielle Hawthorne [1] • Charles E Seaks [1] • Donna M Wilcock, PhD [1,2]

Sanders Brown Center on Aging University of Kentucky [1] • Department of Physiology, University of Kentucky [2]

Time-course of pathological changes during progression of hyperhomocysteinemia induced cerebrovascular pathology.

Student

Objectives: Vascular contributions to cognitive impairment and dementia (VCID) is one of the leading causes of dementia. High levels of plasma homocysteine or hyperhomocysteinemia has been characterized as a risk factor for VCID however, the mechanism underlying the connection between hyperhomocysteinemia and development of VCID pathology remains elusive. I hypothesize that hyperhomocysteinemia initiates a pro-inflammatory cascade that increases the activity of MMP9 causing perivascular astrocytes to dissociate from their vessels, leading to blood brain barrier dysfunction and the progression toward VCID pathology.

Methods: For *in vivo* studies, C57BL6 WT mice were placed on a control diet or a diet deficient in folate and vitamins B6 and B12 and enriched in methionine to induce hyperhomocysteinemia for 6, 10, 14, or 18 weeks. For *in vitro,* experiments, astrocytes were treated with a 50μM homocysteine solution in serum free media for 24, 48, 72, or 96 hours. Immunohistochemistry and gene expression analysis were used to determine neuroinflammatory changes while histology was used to identify changes in astrocytic end-feet proteins and microhemorrhages. Gel zymography was used to assess proteinase activity of matrix metalloproteinases. Behavior was assessed using the 2-day radial arm water maze.

Results: After 6 weeks of diet administration in WT mice, we saw a significant increase in gene expression of TNFα, IL-1β, IL-6 and IL-12α. This was followed by increases in matrix metalloproteinase transcription and proteinase activity seen at 10 weeks on diet though this was the only timepoint at which this endpoint was quantified. Also, beginning at the 10-week time point, cognitive deficits became detectable, which coincided with significant disruptions between astrocytic end-feet and the cerebrovasculature. Finally, there was a significant increase in the number of microhemorrhages at 14 weeks on diet. *In vitro,* astrocytes showed decreased levels of several potassium channels and Aqp4 with up to 72 hours of homocysteine treatment and an increase in matrix metalloproteinase 9 after 48 hours of homocysteine treatment.

Conclusions: Collectively, our findings suggest that astrocytic MMP9 may play an integral role in the mechanism associating homocysteine induced neuroinflammation with vascular pathogenesis leading to VCID, highlighting this pathway as an important subject for future study.

Zoom Link

https://uky.zoom.us/j/85698565626

Ahmed Bahrani, PhD [1] • Weikai Kong, MS [2] • Yu Shang, PhD [3] • Chong Huang, PhD [2] • Charles Smith, MD [4] • David Powell, PhD [4] • Yang Jiang, PhD [5] • Abner Rayapati, MD [6] • Gregory Jicha, MD, PhD [1] • Guoqiang Yu, PhD [2]

Sanders-Brown Center on Aging University of Kentucky [1] • Biomedical Engineering University of Kentucky [2] • Key Laboratory of Instrumentation Science & Dynamic Measurement North University of China, Shanxi, China [3] • MRISC University of Kentucky [4] • Behavioral Science University of Kentucky [5] • Department of Psychiatry University of Kentucky [6]

Low-frequency oscillation method for evaluating the cerebral autoregulation in older adults using diffuse optical spectroscopy technique

Post Doc Scholar

Objective: Cerebrovascular disease (CVD) is the fifth most common cause of mortality in the United States. Diagnosis of CVD at early stages is essential for preventing sequential complications. CVD is often associated with abnormal brain microvasculature and tissue dysfunction, which may impact cerebral autoregulation (CA). This study aims to test whether the CA based on the low-frequency oscillation assessment of cerebral hemodynamics can be used as a biomarker to distinguish two groups of cognitively healthy elderly subjects with high or low risk for developing CVD. **Methods:** A novel hybrid near-infrared diffuse optical instrument and a finger plethysmograph were used to simultaneously detect low-frequency oscillations (LFOs) of cerebral blood flow (CBF), oxy-hemoglobin concentration ([HbO_2]), deoxy-hemoglobin concentration ([Hb]), and mean arterial pressure (MAP) in elderly subjects before, during, and after 70° head-up-tilting (HUT). LFOs were quantified in 4 frequency intervals: I (0.005-0.0095 Hz), II (0.0095-0.02 Hz), III (0.02-0.07 Hz), and IV (0.07-0.2 Hz). Interval-I and Interval-II reflect respectively nitric oxide dependent and independent endothelial metabolic activities. Interval-III and Interval-IV correspond respectively to neurogenic and myogenic related metabolic activities. The gains of the LFOs were determined by transfer function analyses with MAP as the input, and CBF, [HbO_2] and [Hb] as the outputs. In general, CAs correlate inversely with LFO gains. **Results:** At resting baseline, LFO gains in the high-risk group (n = 11, 84.2 ± 4.0 years) were relatively lower compared to the low-risk group (n = 13, 73.0 ± 4.3 years). The lower baseline gains in the high-risk group may attribute to compensatory mechanisms to maintain stronger steady state CAs. However, HUT resulted in smaller gain reductions at interval III and interval IV in the high-risk group compared to the low-risk group, suggesting weaker dynamic CAs. Also, the cerebrovascular risk seemed affecting CBF more than cerebral oxygenation. **Conclusions:** Cerebrovascular risk affects neurogenic and myogenic activities in intervals III and IV more than endothelial activities in other intervals. LFO gains are potentially valuable biomarkers for early diagnosis of CVD based on associations with CAs. In future studies, we will use our current developed technique with the LFO to assess the metabolic, endogenic, and myogenic activities of microvascular in different brain regions.

This work was partially supported by NIH, 5P30AG028383, 1R21HD091118-01A1, 3R21HD091118-02S1 1R21AR062356, and 1R01AG062480), AHA, 16GIA30820006 and NSF, EPSCoR1539068. The content is solely the responsibility of the authors and does not necessarily represent the official views of the HCED, NIH, AHA, or NSF.
https://uky.zoom.us/j/8846667221?pwd=WGxDcm9LQ1h1dDlsQWxyMlN3SUtPZz09
Password: 976046

David Braun, PhD [1] • Verda Davis, MS [1] • Linda Van Eldik, PhD [1]

Sanders-Brown Center on Aging University of Kentucky [1]

Knockout of microglial p38α MAPK in a mouse model of Alzheimer's disease

Fellow

Background: The p38a MAPK signaling pathway is a well-established regulator of neuroinflammation, and pharmacological inhibitors of this pathway can protect against cognitive impairment in animal models of Alzheimer's disease (AD). This protection by p38a inhibitors might be mediated via protective effects on neurons, anti-inflammatory effects on glia, or some combination thereof. To assess whether reduction of p38-dependent microglial proinflammatory responses is beneficial in this context, we generated AD model mice with microglial knockout (KO) of p38a.

Method: The APPswe/PS1dE9 (MMRRC #34832) mouse AD model was crossed with $p38a^{fl/fl}$ mice (Jax #031129) to generate AD model mice homozygous for the floxed p38a allele. These were subsequently crossed with $CX3CR1^{CreERT2}$ mice (Jax #020940) that express a tamoxifen-inducible promoter allowing for removal of p38a from microglia. This breeding scheme generated four groups of mice, used in a 2 x 2 study design: WT and AD mice with floxed p38a, with or without a copy of the myeloid-specific Cre allele. All mice were placed on tamoxifen diet (400 ppm) for 4 weeks beginning at 5 months of age, near the beginning of amyloid plaque deposition in this AD model. After administration of tamoxifen, mice were returned to normal chow for several months, allowing turnover of peripheral myeloid cells. Mice underwent behavioral testing in open field and novel spatial recognition y-maze (8 months of age), and radial arm water maze (11 months of age). Microglial isolation via fluorescence-activated cellular sorting and subsequent RNAseq analysis was performed on a subset of 4 mice per group.

Results: Microglial p38a KO had no effect on the hyperlocomotive phenotype associated with amyloid overexpression in this model; however, p38a KO increased errors in the RAWM test of spatial learning and memory in the AD model but not WT mice.

Conclusions: The p38a signaling pathway in microglia is important in restricting amyloid-associated cognitive decline. We are currently characterizing the effects of microglial p38 KO on amyloid pathology, neuroinflammation, and microglial gene expression.

This work was funded by a postdoctoral fellowship from the NIA, F32AG058456.

Zoom: https://uky.zoom.us/j/83510079406

Adam Dugan, MS [1] • Peter Nelson, MD, PhD [2] • David Fardo, PhD [1]

Biostatistics University of Kentucky [1] • Sanders-Brown Center on Aging and Alzheimer's Disease Research Center University of Kentucky [2]

Association between WWOX gene variants and neuropathologic endophenotypes

Student

Objectives: *WWOX* has been identified as an Alzheimer's disease (AD) gene. However, earlier research found *WWOX* to be associated with hippocampal sclerosis (HS). We hypothesized that WWOX may be preferentially associated with non-plaque- and non-tau-related neuropathological changes.

Methods/Results: Data from research participants with GWAS and autopsy measures from National Alzheimer's Coordinating Center's (NACC) and the Religious Orders Study and Memory and Aging Project (ROSMAP) were used. All variants within 250kb of *WWOX* were tested for association with several autopsy-confirmed endophenotypes and variant-level results were combined to obtain gene-level tests of association. *WWOX* was found to be associated with Limbic-predominant age-related TAR-DNA-binding protein-43 (TDP-43) encephalopathy (LATE), HS, neuritic plaques, intermediate/high likelihood of AD, and arteriolosclerosis. Adjustment for AD-related phenotypes did not affect results, which suggests that these associations are independent of AD.

Conclusions: *WWOX* may be independently associated with LATE, HS, and arteriolosclerosis.

Zoom Link: https://uky.zoom.us/j/87202192601

Holden Williams [1] • Elizabeth Allenger [1] • Adeline Walsh [1] • Lance Johnson, PhD [1]

Department of Physiology, University of Kentucky [1]

Investigating the effects of APOE on cerebral glucose metabolism using stable isotope resolved metabolomics

Student

Objectives: Apolipoprotein E (APOE) is present in humans as three main isoforms (E2, E3, and E4). E4 carriers face up to a 15-fold increased risk for developing Alzheimer's disease (AD), while E2 carriers are protected. Cerebral glucose hypometabolism, defined by decreased ^{18}FDG-PET signal, is present in AD and interestingly, a similar pattern is observed in E4 carriers, even when young and cognitively normal. While this phenomenon has been described for the past two decades, the cause remains unclear. We hypothesize that astrocytes are primarily responsible for the reduced glucose uptake associated with E4 due to mitochondrial impairments which influence intracellular glucose flux.

Methods/Results: Mitochondrial respiration was measured in immortalized astrocytes expressing human E2, E3, or E4 using Seahorse XF96 Glycolysis Stress Test. Glucose metabolism was also measured *in vivo* using stable isotope resolved metabolomics with a [U-^{13}C]glucose media. *In vivo* brain metabolism in human APOE mice (E2, E3, or E4) was examined using an oral gavage of [U-^{13}C] glucose. Metabolomics samples were analyzed by mass spectrometry.

Results: E4 astrocytes show a lower rate of oxygen consumption relative to E2 or E3. E4 astrocytes exhibit lower glucose flux into early-stage glycolysis with greater pentose phosphate pathway (PPP) activity as demonstrated by ^{13}C labeling patterns in these pathways. Conversely, glucose flux into pyruvate and lactate was increased in E4. The activity of pyruvate dehydrogenase (PDH) and pyruvate carboxylase (PC) enzymes, which govern entry of pyruvate to the TCA cycle, were decreased in E4, despite more abundant labeling in TCA cycle intermediates. Nucleotide biosynthesis was also increased, shown by ^{13}C labeling in purines, pyrimidines, and NADH. Finally, cortical tissue from E4 mice exhibit similar decreases in glycolytic flux and display greater TCA cycle enrichment.

Conclusions: Decreased mitochondrial respiration observed in E4 astrocytes may reflect a decreased ability to utilize glucose for energy. Attenuated PDH and PC would limit the oxidative capacity of the TCA cycle and thus decrease reducing equivalents for ATP synthesis. Oxidative phosphorylation might continue if other energy substrates were substituted, however the labeling in TCA intermediates suggests decreased glutamine oxidation. Increased PPP activity might compensate for the loss of reducing equivalents normally generated from TCA activity. Overall, these alterations in glucose utilization by E4 may be driven by decreased efficiency of the TCA cycle which is the subject of my next objective. My future directions are aimed at identifying whether there is an E4-specific preference for other energy substrates (glutamine or fatty acids), which may elucidate a mechanistic role by which APOE alters glucose metabolism which could illuminate specific enzymes as targets for therapeutics to mitigate the risk of AD for E4 carriers. Zoom link: https://uky.zoom.us/j/87653228026

Kevin Xie, • David Fardo, PhD

Department of Biostatistics, University of Kentucky

Exploration of race in Alzheimer's disease polygenic risk scores

Student

Objectives

To compare race in Alzheimer's Disease (AD) polygenic risk score (PRS) distributions.

Methods/Results

PRS were calculated for individuals in Alzheimer's Disease Genetics Consortium (ADGC) with Haplotype Reference Consortium (HRC) imputed data using PRSice-2. These scores were derived using summary statistics from a recent genome-wide association study (GWAS). When using all available SNPs, the distributions of PRS's were unimodal. However, the distribution when using the significant SNPs selected from the GWAS was multimodal. As expected, the PRS values are significantly different between AD cases and controls when including either all SNPs ($p < 2*10^{-16}$) or only significant SNPs ($p < 2*10^{-16}$). Additionally, when observing a subset of the sample in ADNI-Stage 1, the PRS varied significantly between races ($p < 0.004$). In contrast, PRS differences between cases and controls of White participants of this subset did not reach significance ($p = 0.284$).

Conclusions

When considering PRS of AD for diverse populations, the risk scores may be more a reflection of race or ancestry rather than AD susceptibility if not handled appropriately. PRS computed based on European ancestry has been shown to be inaccurate when applied to individuals of non-European ancestry. Furthermore, a distinction between social factors (race) and biological factors (ancestry) should be made clear in the analysis of PRS. Ancestry-specific PRS models are being actively pursued which could improve AD PRS calculations for all populations.

Zoom: https://zoom.us/j/9796390393?pwd=UEdldzhraXBPM1h3MG5LbnI2Rk0xdz09

Passcode: kWZb6m

Chao Ma, MS [1,3] • Jerry Hunt [.1] • Andrii Kovalenko [3] • Huimin Liang, MS [1] • Marcia Gordon, PhD [3] • Dave Morgan, PhD [4] • Paula Bickford, PhD 5 • Daniel Lee, PhD [1,2]

Sanders-Brown Center on Aging University of Kentucky [1] Department of Neuroscience University of Kentucky [2] • Pharmaceutical Sciences University of South Florida [3] • Translational Neuroscience Michigan State University [4] • Neurosurgery and Brain Repair University of South Florida [5]

Arginase 1 deficiency in brain myeloid cells activates amyloid-β plaque associated glial genes in a mouse model of Alzheimer's disease

Visiting Student

Objectives: Brain myeloid cells, including infiltrating macrophages and resident microglia, play an important role in responding to and inducing of neurodegenerative diseases, such as Alzheimer's disease (AD). Genome-wide association studies (GWAS) implicate many AD casual and risk genes enriched in brain myeloid cells. Altered arginine metabolism has been recently proposed as a promising biomarker for AD. Coordinated arginine metabolism through arginase 1 (Arg1) is critical for brain myeloid cells to perform biological functions, whereas dysregulated arginine metabolism disrupts. We previously reported Arg1 deficiency in myeloid cells exacerbated amyloidosis related neuropathology and behavioral impairment. However, it remains unclear how Arg1 deficient myeloid cells impact the whole brain to promote amyloidosis. Herein, we aim to determine how myeloid Arg1 deficiency during amyloidosis affect fundamental neurodegeneration pathways at the transcriptome level.

Methods/Results: We extracted posterior cortex mRNA from mouse brains of *nTg/Arg1+/+/LysMcreTg/+*, *nTg/Arg1fl/+/LysMcreTg/+*, *APP+/-/Arg1+/+/LysMcreTg/+* and *APP+/-/Arg1fl/+/LysMcreTg/+*. Then we performed a transcriptomic profiling analysis by applying the mRNA samples in nCounter mouse neuropathology panel (770 genes, NanoString Technologies, Inc). From several bioinformatic analyses, we found amyloid-β (Aβ) stimulated autophagy pathway and increased inflammatory response of myeloid cells, whereas myeloid Arg1 deficiency during Aβ stimulation promoted lipid metabolism and myelination pathways and increased migration of myeloid cells. Focusing on neurodegenerative disease-associated glial transcriptomic signatures, we found myeloid Arg1 deficiency up-regulated glial gene transcripts that positively correlated with Aβ plaque burden. We also observed Aβ preferentially activated disease-associated microglial signatures that were phagocytic, while myeloid Arg1 deficiency selectively promoted homeostatic and non-phagocytic microglial signatures.

Conclusions: These novel findings suggest that proper arginine metabolism regulated by Arg1 in brain myeloid cells is critical for performing phagocytosis to restrict amyloidosis and neuroinflammation and thus can be a key therapeutic target in AD.

Grant Support: NIH/NIA R21AG055996; Alzheimer's Association (AARG); Florida Department of Health Ed and Ethel Moore Alzheimer's disease

Zoom Link: https://uky.zoom.us/j/5975994792

Pradoldej Sompol, PhD [1] • Stephanie Edelmann [1] • Ruei-Lung Lin [2] • Christopher Gant, PhD [2] • Susan Kraner, PhD [1] • Blaine Weiss [1] • Jenna Gollihue, PhD [1] • Nathan Farr [1] • Olivier Thibault, PhD [2] • Donna Wilcock, PhD [1] • Christopher Norris, PhD [1]

Sanders-Brown Center on Aging University of Kentucky [1] • Department of Pharmacology and Nutritional Sciences University of Kentucky [2]

Astrocyte activation and neurovascular function in a diet-based model of vascular contributions to cognitive impairment and dementia (VCID)

Faculty

Objective:
Vascular contributions to cognitive impairment and dementia (VCID) are highly comorbid with Alzheimer's disease (AD) pathology and may accelerate the progression of dementia and/or reduce the clinical efficacy of anti-AD treatments. Neurotoxic astrocyte activation linked to aberrant calcineurin/NFAT signaling is a shared mechanism between VCID and AD. Recently, hyperhomocysteinemia, a major risk factor for VCID and a variety of other vascular-related diseases, was linked to astrocyte dysfunction and calcineurin/NFAT hyperactivation. However, causal relationships between astrocytes and neurovascular dysfunction under hyperhomocysteine conditions has yet to be determined. Here, we investigate the hypothesis that diet-induced hyperhomocysteinemia disrupts cerebrovascular function, in part, through an astrocyte-based calcineurin/NFAT mechanism.
Method:
Wild type mice (2-3 months-of-age) received intracortical injections of adeno associated virus (AAV) equipped with an astrocyte-specific promoter to target astrocytes with EGFP (control) or VIVIT-EGFP, to inhibit the calcineruin/NFAT pathway. A chronic, glass cranial window was then installed for longitudinal multiphoton imaging of astrocytes and cerebrovascular parameters in barrel cortex. Three weeks post-surgery, mice were fed with control or hyperhomocysteinemia-inducing diet (low levels of folate, vitamins B6 and B12 and enriched with methionine) for 12 weeks. Astrocytic EGFP volume, microvessel leakage, and functional hyperemia (during whisker stimulation) were measured in the same brain region before and after 12 weeks of diet treatment.
Results:
Upregulation of astrocytic EGFP volume developed during hyperhomocysteinemia consistent with astrocyte activation. This change was attenuated by CN/NFAT inhibition with VIVIT, similar to previous observations in amyloidogenic mice. Hyperemic capillary responses to whisker stimulation in barrel cortex were also reduced with the development of hyperhomocysteinemia, suggesting an impairment of cerebrovascular function. Microvessel integrity (leakage) and the role of astrocytic NFATs are currently under investigation.
Conclusion:
The results indicate that hyperhomocysteinemia, a major risk factor for VCID, may trigger a neurotoxic activated astrocyte phenotype leading to neurovascular injury, and ultimately cognitive decline. Zoom: https://uky.zoom.us/j/93685748084

Susan Kraner, PhD [1] • Jenna Gollihue, PhD [1] • Blaine Weiss [1] • Irina Artiushin [1] • Pradoldej Sompol, PhD [1] • Chris Norris, PhD [1]

Sanders Brown Center on Aging University of Kentucky [1]

Development of monoclonal antibodies specific for the calpain-generated Δ48 kDa calcineurin fragment, a marker of distressed astrocytes

Staff

Background/Objective: Calcineurin (CN) is a Ca2+/calmodulin-dependent protein phosphatase expressed at high levels in brain. In healthy tissue, CN exists mainly as a full-length (~60 kDa) highly-regulated protein involved in essential cellular functions. However, in diseased or injured tissue, CN is proteolytically converted to a constitutively active fragment that has been causatively-linked to numerous pathophysiologic processes. The 48 kDa CN fragment (DCN) appears at high levels in human brain at early stages of cognitive decline associated with Alzheimer's disease. DCN tends to show-up in regions of frank amyloid and cerebrovascular pathology, especially in select subsets of astrocytes, both in humans and in animal models. Our goal was to develop monoclonal antibodies to DCN for use in neuropathology research.

Method: A peptide encompassing the calpain sensitive region of the CN carboxyl terminus was used for antibody generation. Antibodies were screened in ELISAs against the immunizing peptide, but ***decision-making screens*** were carried out as a Western analysis of 5XFAD mouse brain extracts, which express high levels of the DCN fragment.

Result: We identified 2 monoclonals, one of which (17E1) was highly reactive to DCN in Western blots, but not in ELISAs, of 5xFAD brain extracts. In contrast, the second antibody (26A6) reacted well against the peptide in ELISAs, but worked only modestly in Westerns. Importantly, neither antibody reacted with full-length (60 kDa) CN, nor did antibodies detect DCN in extracts from healthy WT mice. In immunostaining, 17E1 strongly labeled subsets of GFAP-positive astrocytes in 5xFAD mice with a high signal-to-background. Consistent with Westerns, there was no antibody reactivity to WT mouse brain sections.

Conclusion: New monoclonals are highly selective for the 48 kDa CN proteolytic fragment and label subsets of astrocytes, and possibly other cell types, under pathological conditions. These antibodies could be a useful tool for marking insidious brain pathology and identifying novel astrocyte phenotypes.

Acknowledgements:

Work supported by awards from the NIH (AG027297), the Kentucky Spinal Cord and Head Injury Research Trust (12-10A), and The Hazel Embry Research Fund to CMN.

Zoom: https://uky.zoom.us/j/89913019751

Tyler Hammond [1] • Arnold Stromberg, PhD [2] • Lumy Sawaki, MD, PhD [3] • Ai-Ling Lin, PhD [4]

Neuroscience University of Kentucky [1] • Statistics University of Kentucky [2] • Physical Medicine & Rehabilitation University of Kentucky [3] • Pharmacology and Nutritional Sciences University of Kentucky [4]

Evaluating the microbiome to boost recovery from stroke: the EMBRS study

Student

Over 795,000 people suffer a stroke every year in the United States alone, and post-stroke dementia may affect up to one third of survivors. Recent advances in acute stroke therapies have lowered stroke mortality, but survivors are often left severely impaired. Accumulating evidence from animal studies suggests that gut microbes modulate brain plasticity via the bidirectional gut-brain axis and may play a role in stroke rehabilitation. A severely imbalanced microbial community, or dysbiosis, has been shown to occur following stroke, causing a systemic flood of neuro- and immunomodulatory substances due to increased gut permeability and decreased gut motility[5]. These substances can impact neuroinflammation as commensal bacteria invade the bloodstream and as intestinal lymphocytes migrate from gut-associated lymphoid tissue to the brain[6]. Currently, no human studies have been performed to analyze changes in the microbiome over the first three-month course of stroke rehabilitation and whether these longitudinal changes correlate with gut permeability and subsequent recovery as measured by neuroimaging and functional testing, making it difficult to confirm whether the microbiome could be a therapeutic target in stroke rehabilitation. Here we measure post-stroke increased gut dysbiosis over the first three months of rehabilitation in humans.

We followed 4 individuals with stroke and 21 control individuals over three months. We measured the gut microbiome by performing whole shotgun sequencing on stool samples collected at admission, discharge, and three-month follow up. We correlated microbiome findings with imaging data (structural imaging, cerebral blood flow, and white matter integrity) and cognitive assessments (memory testing, emotional and social support questionnaires).

There was increased bacterial diversity in the context of stroke increasing from 100.762 to 113.000 on the Chao index ($p=0.02261$). Proteobacteria (the major phylum to which Escherichia coli belongs) was inversely associated with gray matter volume (slope=-1919620 mm3/unit increase in relative abundance, $p=0.0176$). Gray matter volume was positively associated with performance on the list sorting task (slope = 0.0001283 points/mm3, $p=0.0065$)

We found that microbial communities are disrupted in a stroke population, showing an increase in alpha diversity. The dysbiosis was associated with changes in imaging markers and cognitive assessments. This is the first microbiome study to be conducted with a longitudinal/prospective component in human patients recovering from stroke. This preparatory study will *improve scientific knowledge* of the bidirectional microbiome-brain axis in humans and lay the foundation for understanding its link to recovery and post-stroke dementia by elucidating whether the effects seen in animals are replicated in humans.

Funding: NIH 1T32AG057461-01 and NIH RF1AG062480-01

Zoom: https://uky.zoom.us/j/87843900672

Benjamin Shaw,[1] Yuriko Katsumata, PhD [1] • Dave Fardo, PhD [1] • Steve Estus, PhD [2]

Biostatistics University of Kentucky [1] • Physiology, Sanders-Brown Center on Aging University of Kentucky [2]

CD33 molecular genetics in Alzheimer's disease risk

Student

Objectives: Genome-wide association studies have established *CD33* as a risk factor for late-onset Alzheimer's Disease (LOAD) *CD33* is an inhibitory receptor which contains polymorphisms which decrease risk of LOAD. The primary polymorphism associated with reduced risk of LOAD is a proxy for a functional SNP, rs12459419, which reduces splicing efficiency in exon 2 (*D2-CD33*). This exclusion of exon 2 in the mature transcript is translated to an in-frame protein without a ligand binding domain and thought to be a loss of function. Recently, however, we have established that a complete loss of cell surface expression of CD33 protein through a 4 bp insertion/deletion (indel), rs201074739, in exon 3 which results in a premature stop codon, before the transmembrane domain, is not associated with AD risk. Since a bona fide loss of function is not associated with risk, we now hypothesize that *D2-CD33* may represent a novel gain of function. The objective of this study is to determine whether D2-CD33 confers AD risk protection via activating microglia rather than loss of function or inhibitory signaling.

Methods and Results: Using data from the most recent International Genomics of Alzheimer's Project analysis, we examined the *CD33* exon 2 splicing SNP, rs12459419, and found a significant association with AD risk (OR = 0.92, 95% CI: 0.90 - 0.95, $p = 4.5 \times 10^{-7}$). This was a robust sample set of 21,982 AD cases and 41,944 non-AD cases. However, the 4 bp indel rs201074739 was not significantly associated with AD risk in these same data (p = 0.1337, OR = 0.90, 95% CI: 0.79 - 1.03). We next validated cell surface expression of the D2-CD33 protein through overexpression in a human monocyte cell line U937 and murine microglial cell line BV-2. In both cell lines, we observed strong cell surface labeling by confocal microscopy. Finally, we used biotinylated, tyrosine-phosphorylated peptides corresponding to the cytosolic domain of CD33 in bait-capture immunoprecipitation experiments followed by discovery-phase proteomics to uncover novel CD33 protein-protein interactors which may support the gain-of-function hypothesis. We observed weak associations with many proteins involved in phagocytosis and myeloid activation such as spleen tyrosine kinase (Syk).

Conclusions: The indel polymorphism, resulting in a complete loss of CD33 cell surface expression, is not associated with AD, and this calls into question the loss-of-function assumption for D2-CD33 mechanism of action. We show here that the D2-CD33 protein is stable on the cell surface in an overexpression paradigm, and that it is capable of interacting with activating and phagocytic proteins, supporting our hypothesis that D2-CD33 has a gain of function to confer risk protection from AD. Future work will strive to elucidate the mechanism by which this novel isoform acts.

Funding: This work was funded by R21AG068370-01 and RF1AG059717-01 to SE.

Zoom link: https://uky.zoom.us/j/88198385433

Charles Seaks [1] • Courtney Kloske [1] • Kathleen Salmeron, PhD [2] • Adam Dugan, MS [3] • Peter Nelson, MD, PhD [1] • Donna Wilcock, PhD [1]

Sanders-Brown Center on Aging University of Kentucky [1] • Department of Neuroscience University of Kentucky [2] • Department of Statistics University of Kentucky [3]

RNA expression patterns of Alzheimer's disease and vascular dementia patients infected with common herpesviruses

Student

Background: Alzheimer's disease (AD) is the leading cause of dementia worldwide followed by vascular contributions to cognitive impairment and dementia (VCID). Studies have connected multiple herpesviruses to the development and pathogenesis of AD, though the connection remains controversial. Mixed pathologies are common, with ~60% of AD cases presenting with a VCID co-morbidity. One important question associated with herpesvirus-dementia is how such ubiquitous viruses affect relatively few individuals neurologically. The goal of this study is to examine differences in RNA expression of neuroinflammatory genes that might account for differences in susceptibility to recurrent herpesvirus infection and secondary effects in the brain. We hypothesize that AD cases with multiple herpesviruses present will have suppressed inflammatory gene expression compared to vascular and control cases, thereby permitting enhanced herpesvirus replication and spread.

Method: Using droplet-based PCR, we quantified the prevalence of four herpesviruses (herpes simplex virus 1, herpes simplex virus 2, cytomegalovirus, and human herpes virus 6) in a subset of brain autopsy tissue obtained from the Sanders-Brown Center on Aging. RNA and genomic DNA were extracted from the superior medial temporal gyrus and cerebellum of 49 cases (n=18 control, n=10 VCID, n=20 AD, n=1 mixed AD+VCID). Genomic DNA was used to determine the presence of latent virus and cDNA to determine actively replicating virus. The RNA from these regions was assayed using the NanoString Human Neuroinflammatory Panel which quantifies expression of over 700 immune-related genes, ranging from neuroinflammatory markers to cell specific functional genes.

Result: There was a significant increase in the number of latent viral populations in the AD brain relative to control and VCID. NanoString analysis supported our initial hypothesis regarding gene regulation relating to inflammation, though the suppression was most evident in tumor necrosis factor family gene expression in the superior medial temporal gyrus region. In addition, we observed differential expression of B and T cells maturation factors, which may suggest peripheral immune infiltration in these cases.

Conclusion: Our results demonstrate differences in inflammatory gene expression in AD cases with multiple herpesviruses that suggest either underlying differences in these infected patients or alterations due to the infection.

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Zoom link: https://uky.zoom.us/j/88984955816

Kathryn Van Pelt, PhD [1] • David Powell, PhD [2] • Elizabeth Head, PhD [3] • Frederick Schmitt, PhD [1,4]

Sanders-Brown Center on Aging University of Kentucky [1] • Magnetic Resonance Imaging and Spectroscopy Center University of Kentucky [2] • Department of Pathology & Laboratory Medicine, Institute for Memory Impairments and Neurological Disorders University of California Irvine [3] • Department of Neurology University of Kentucky [4]

Perivascular space volume and the association with age and cognition in an aging Down syndrome population

Post Doc Scholar

Objectives: The brain's glymphatic system is composed of interconnected perivascular spaces (PVS) which allow the exchange of cerebral spinal fluid and interstitial fluid. The glymphatic system is important for energy metabolism and clearance of amyloid-β (Aβ) and tau. Glymphatic system dysfunction is associated with enlarged PVS and has been observed in Alzheimer's Disease (AD) and associated with Aβ and tau burden. However, there has been little research on PVS in individuals with Down Syndrome (DS), who are particularly vulnerable to AD. The current study's objectives were to quantify PVS in an aging DS cohort and determine the associations between PVS, age, and dementia.
Methods: The current study examined anatomical (T1) brain images from an ongoing study of AD in DS. Participant's T1 images were processed according to the method outlined by Sepehrband et al 2019. This process involved non-local means filtering, Frangi filter, followed by robust thresholding. Examination of PVS was limited to the white matter. The volume of PVS was converted into a fraction of white matter volume (WMV) in order to account for between-subject differences in WMV. Linear regression examined the relationship between age and PVS volume and also modeled the relationships between PVS volume and cognitive assessments. All analyses were conducted using R 4.0.2.
Results: Twenty-four (50% male) participants were included in the current analysis. Participants ranged in age from 25-60 (mean = 38.39; SD = 9.37) and 25% (n = 6) were diagnosed with possible/probable dementia. There were no differences in PVS volume by sex ($p = 0.98$), level of intellectual disability ($p = 0.64$), or dementia diagnosis ($p = 0.20$). Increasing age was significantly associated with PVS volume, where there was an exponential increase in PVS volume with increasing age ($F(2,21) = 6.64$; $p = 006$; $R^2_{adj} = 0.33$). PVS volume was also associated with the Dementia Questionnaire for People with Learning Disabilities (DLD) scores ($F(2,11) = 4.10$; $p = 0.047$; $R^2_{adj} = 0.37$), controlling for age and intellectual disability level. DLD scores increased exponentially with increasing PVS volume, indicating greater cognitive impairment with increasing PVS volume.
Conclusions: The current study demonstrates the feasibility of quantifying PVS *in vivo* in an aging cohort of people with DS. PVS increases exponentially after age 40, consistent with other studies demonstrating rapid accumulation of AD neuropathology after age 40. Furthermore, increased PVS volume was associated with greater dementia symptoms.

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Zoom Link: https://uky.zoom.us/j/89677189932

Lincoln Shade[1], Yuriko Katsumata[1,2], Peter Nelson[2,3,4], David Fardo[1,2]

[1]Department of Biostatistics, University of Kentucky; [2]Sanders-Brown Center on Aging, University of Kentucky; [3]Division of Neuropathology, University of Kentucky; [4]Department of Pathology, University of Kentucky

Colocalization analysis of genome-wide association and QTL signals detects target genes for brain arteriolosclerosis

Student

Objectives: Brain arteriolosclerosis (B-ASC) is a cerebrovascular pathology characterized by dysmorphic arteriolar wall thickening. B-ASC is common in elderly people and is observed in over 80% of autopsied individuals over 80 years of age. B-ASC pathology is associated with other neuropathologies, including Alzheimer's disease and limbic-predominant age-related TDP-43 encephalopathy (LATE), and is independently associated with cognitive decline. However, despite its frequency and clinical importance, B-ASC remains understudied. In this study, we perform the first genome-wide association study of B-ASC. We then perform colocalization analysis using quantitative trait loci (QTL) data to locate gene targets for B-ASC pathology to investigate potential molecular functional pathways by which identified variants may affect B-ASC risk.

Methods/Results: We used neuropathology data from participants in the National Alzheimer's Coordinating Center (NACC) Neuropathology Dataset linked to genotype data in the Alzheimer's Disease Genetics Consortium (ADGC) for our GWAS. We used a case-control logistic regression model, assigning participants with no or minor B-ASC as controls and those with moderate or severe B-ASC as cases, and an ordinal regression model. Variants exceeding a p-value threshold of 10^{-5} in either model were compared to expression QTL (eQTL) and splicing QTL (sQTL) data from the Genotype-Tissue Expression Project (GTEx). We performed colocalization analysis using a Bayesian-based approach to compare shared loci identified in GWAS and QTL analyses, designating a posterior probability (PP) of at least 50% as evidence of colocalization.
In total, 3318 participants and 4.9 million variants passed QC and were included in GWAS. One locus on chromosome six achieved the genome-wide significance p-value threshold of $5x10^{-8}$ (rs2603462, odds ratio = 1.5, p-value = $1.4x10^{-8}$). Of 19 independent loci meeting the suggestive threshold, four were also significant QTL in GTEx. Three of these loci colocalize with at least one QTL with a posterior probability of 50%, indicating that these GWAS loci share signals with QTL. rs2603462 colocalizes with *ELOVL4* expression with a PP of 93.3%; rs2352974 colocalizes with *DALRD3, FAM212A,* and *MST1R* expression as well as multiple *RNF123* sQTL with PP > 50%; and rs34349961 colocalizes with *SPRED2* expression with PP of 66%.

Conclusions: Our study employed the first GWAS of autopsy-proven B-ASC. We further investigated our initial GWAS findings with colocalization analysis, a technique that can identify shared genetic association signals between phenotypes. We identified one genome-wide significant locus that colocalizes with *ELOVL4* gene expression in the brain and several other suggestive loci that colocalize in GTEx. These findings provide gene targets for future studies of B-ASC pathophysiology.

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Zoom Link: https://uky.zoom.us/j/3654278422

Christopher Bauer, PhD [1] • Valentinos Zachariou, PhD [1] • Elayna Seago, MS [1] • Brian Gold, PhD [1]

Neuroscience University of Kentucky [1]

White matter hyperintensity volume and location: associations with WM microstructure, brain iron and cerebral perfusion

Post Doc Scholar

Objectives: Explore associations between three potential early markers of cerebrovascular disease (cSVD) and white matter hyperintensity (WMH) volume. Specifically, the unique variance in total and regional WMH volumes accounted for by white matter microstructure, brain iron concentration and cerebral blood flow (CBF) was assessed. Regional volumes explored were periventricular (PV) and deep (peripheral) regions. **Methods:** Eighty healthy older adults (ages 60-86) were scanned at 3 Tesla MRI using fluid attenuated inversion recovery (FLAIR), diffusion tensor imaging (DTI), multi-echo gradient-recalled echo (GRE) and pseudo-continuous arterial spin labeling (pCASL) sequences. **Results:** In a stepwise regression model, DTI-based radial diffusivity (DR) accounted for significant variance in total WMH volume (adjusted R^2 change=0.136). In contrast, iron concentration (adjusted R^2 change=0.043) and CBF (adjusted R^2 change=0.027) made more modest improvements to the variance accounted for in total WMH volume. However, there was an interaction between iron concentration and location on WMH volume such that QSM predicted peripheral (p=0.034) but not PV (p=0.414) WMH volume. **Conclusions:** Our results suggest that WM microstructure may be a better predictor of WMH volume than either brain iron or CBF but also draws attention to the possibility that some early cSVD markers may be location-specific.

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Poster Session Zoom link: https://uky.zoom.us/j/84040885652

Deepak Kotiya, PhD [1] • Nirmal Verma, PhD [1] • Florin Despa, PhD [1]

Pharmacology and Nutritional Sciences University of Kentucky [1]

Detection of amylin-Aβ oligomer as a potential biomarker of Alzheimer's Disease

Post Doc Scholar

Introduction: Elevated levels of blood amylin, an amyloidogenic hormone secreted by the pancreas, is common in individuals with prediabetic insulin resistance and is associated with Alzheimer's disease (AD) via formation of mixed amylin-Aβ deposits in the brain.

Hypothesis: Amylin-Aβ oligomers may form in blood and CSF and could be associated with early AD processes.

Methods: We have developed a novel sandwich ELISA to detect amylin-Aβ oligomers by using a commercially available anti-Aβ antibody (mouse monoclonal, Biolegend) and an "in house" generated polyclonal anti-amylin antibody (raised in rabbits; against N-terminal of human amylin). The novel amylin-Aβ ELISA was tested on human AD brain homogenates and blood, CSF and brain tissues from APP/PS1 rats transgenic for human amylin in the pancreas (APP/PS1/HIP).

Results: We detected amylin-Aβ oligomers in the blood, CSF and brain tissue homogenates of APP/PS1/HIP rats. Soluble amylin-Aβ oligomers were also identified in brain tissues from patients with AD.

Discussion: It has been established that the cerebral mixed amylin-Aβ deposits contribute to AD pathology. Detection of Amylin-Aβ oligomers in AD could be a potential biomarker of Alzheimer's Disease.

Conclusion: Additional experiments are needed to test the efficiency of the novel amylin-Aβ ELISA on human tissues and laboratory models of AD.

Zoom link: https://uky.zoom.us/j/87893290995

Geetika Nehra, PhD [1] • Ruedeemars Yubolphan, MS [2] • Jason Zheng [1] • Samantha Mullins [1] • Pornpun Vivithanaporn, PhD [2] • Bjoern Bauer, PhD [3] • Anika MS Hartz, PhD [1,4]

Sanders-Brown Center on Aging University of Kentucky[1] • Department of Pharmacology Mahidol University, Thailand [2] • Department of Pharmaceutical Sciences, College of Pharmacy University of Kentucky [3] • Department of Pharmacology and Nutritional Sciences, College of Medicine University of Kentucky 4

Implications of blood-brain barrier leakage on cognition in 5xFAD mice

Post Doc Scholar

Objective: Blood-brain barrier leakage in Alzheimer's disease (AD) contributes to AD pathology and cognitive decline in patients. The onset, extent and underlying mechanisms leading to blood-brain barrier leakage, however, remain to be defined. These metrics have the potential to help in developing novel intervention strategies that can repair barrier leakage and slow the rate of cognitive decline in AD. In this study, we evaluated aspects of barrier leakage in 5xFAD mice that features AD pathology consisting of early, aggressive Ab accumulation, neuronal loss, gliosis, synaptic loss and cognitive impairment.
Methods: We first evaluated spatial memory acquisition in young (3-month-old) and adult (12-month-old) wild-type mice and 5xFAD mice using the Y-Maze and Morris Water Maze tests. Next, we installed chronic cranial windows to determine vascular changes in these mice. Specifically, we injected fluorescent-labeled dextrans (3 kDa), acquired time-lapse Z-stacks using via two-photon microscopy, and assessed changes in vascular area, branch point density, vessel length etc. using Fiji/ImageJ and AngioTool. We then isolated brain capillaries to assess extent of capillary leakage using Texas Red® dye.
Results: Behavioral tests show that spatial memory is not significantly different in young 5xFAD mice compared to young wild-type mice. In contrast, spatial memory is significantly reduced in adult 5xFAD mice compared to adult wild-type mice. We also observed that the extent of capillary leakage of Texas Red® (625 Da) is significantly elevated in isolated capillaries from young 5xFAD mice compared to age-matched wild type mice. *In vivo* two-photon imaging revealed that young 5xFAD mice show a trend towards increased vascular area, branch points, average vessel length, and reduced lacunarity.
Conclusion. Our findings provide key insights into the role of barrier leakage prior to the onset of spatial memory impairment in the 5xFAD mouse model of AD. We are now investigating the extent of barrier leakage in 5xFAD mice after the onset of spatial memory impairment. We are also identifying the mechanisms that lead to barrier dysfunction in 5xFAD mice and if these mechanisms are altered at the human blood-brain barrier. These findings will allow us to identify factors that contribute to barrier leakage in AD patients and age-matched controls based on postmortem brain tissue analysis.
Grant Support. Restoring Blood-Brain Function to Improve Cognition in Alzheimer's Disease (AD; NIH 2R01AG039621) Zoom: https://uky.zoom.us/j/86762176309

Jenny Lutshumba, PhD [1] • Barbara Nikolajczyk, PhD [2] • Adam Bachstetter, PhD [3]

Sanders-Brown Center on Aging University of Kentucky [1] • Pharmacology and Nutritional Sciences University of Kentucky [2] • Spinal Cord and Brain Injury Research Center University of Kentucky [3]

Immune profiling towards new tools to impact dementia

Fellow

Objectives: The vascular contribution to cognitive impairment and Dementia (VCID) are a significant contributor to age-related cognitive decline and neurodegenerative disease. Inflammation and/or systemic immune cell dysfunction is a common etiologic factor associated with VCID. Inflammation is a complex process that involves different cells of the innate immune system (e.g., macrophages), and adaptive immune system (e.g., T Cells), as well as endothelial cells, and in the brain, glia. The goal of this project is to use a cutting-edge bioinformatics to define source of inflammation associated with cognitive decline and VCID risk-factors. We will test the hypothesis that precise inflammatory profiles predict an immune profile for cognitive impairment that will be unique in those people with VCID risk-factors compared to those people without VCID risk-factors. Results from this study will indicate biomarkers and targets for anti-inflammatory treatments that will effectively alleviate systemic immune dysfunction in people at risk for cognitive decline associated with VCID risk-factors

Methods:

Purified peripheral blood mononuclear cells will be stimulated with T cell- or Myeloid (i.e. macrophage)- targeted stimuli (anti CD3/CD28 or E. coli LPS, respectively) and measure supernatant cytokines with a multiplex protein (Luminex-based) protocol. We will then use bioinformatics and statistical tools for dimension reduction and pathway analysis, which will be used to compare immune cell status between groups. The four experimental groups that will be used to address our aims are: 1. No cognitive impairment (Clinical Dementia Rating (CDR) = 0) with no more than one VCID risk factors. 2. No cognitive impairment (CDR = 0) with 2-to-3 VCID risk factors. 3. Mild Cognitive impairment (MCI) or early dementia (CDR= 0.5 to 1) with no more than one VCID risk factors. 4. MCI or early dementia (CDR=0.5 to 1) with 2-to-3 VCID risk factors

Conclusion: We anticipate to identify the dominant source of inflammation among individuals with the risk factors associated with VCID. This source of inflammation will be the focus for future studies and targets for drugs to prevent and/or alleviate cognitive impairment of vascular etiology.

This work is supported by TRIAD "Training in Translation Research in Alzheimer's and Related Dementia" T32 Grant#: 5T32AG057461

Zoom Link: https://uky.zoom.us/j/87392048096

Patricia V. Rocha-Rangel[1], • Zainuddin Quadri[1], • Dale Chaput[3], •Daniel C. Lee [1,2], • Maj-Linda B. Selenica[1]

Sanders-Brown Center on Aging and Department of Molecular and Cellular Biochemistry, University of Kentucky [1] • Department of Neuroscience, University of Kentucky [2] • Proteomics and Mass Spectrometry Core Facility, Florida Center of Excellence for Drug Discovery and Innovation (CDDI), University of South Florida [3]

Citrullination a potential post-translational modification linked to TDP-43 pathology

Staff

Objectives: The objective of this study was to investigate the role of citrullination, a novel PTM of TDP-43, in TDP-43 pathology. Citrullination is catalyzed by peptidyl arginine deiminases (PADs), and this study aimed to understand the mechanism of PAD4 in progression of pathology in animal models.

Methods/Results: Through mass spectrometric analyses, we identified that PAD4 induced citrullination of recombinant TDP-43 protein in 11 out of 20 TDP-43 arginine (R) residues. We developed several anti-citrullinated arginine (citR) antibodies, two of which were further validated in cellular and animal models of TDP-43. We demonstrated antibody validation using TDP-43 transgenic animals that develop gene dose-dependent TDP-43 pathology (TAR mice). Western blot analysis on 15-month-old TAR4 and 19-day old TAR4/4 cortical homogenate revealed high-molecular weight TDP-43 oligomers, confirming protein oligomerization and antibody specificity to citrullinated protein. Anterior cortex and hippocampus from TAR mice and non-transgenic (ntg) littermates were immunohistochemically and biochemically analyzed with PAD4, citR83 TDP-43 and citR268/272 TDP-43 antibodies. We found that PAD4 expression is increased followed by induced levels of citR as TDP-43 pathology progressed. Further, PAD4 level expression significantly increased in the neuronal cytoplasm of TAR4/4 mice compared to TAR4 and ntg mice. Citrullination of TDP-43 was significantly increased within pathological TDP-43 phenotypes, and followed the toxic pattern of neuronal cytoplasmic accumulation.

Conclusions: From our findings, it is plausible that citrullination may be a factor responsible for TDP-43 aggregation in neuronal cytoplasm. We hypothesized that PAD4-induced citR unfolds TDP-43, leading to an accumulation of unfolded TDP-43 oligomers and soluble aggregates. Evidence of increased PAD4 levels, combined with increased citR TDP-43 levels, suggests that citrullination is a novel PTM and an attractive therapeutic target for treating TDP-43 proteinopathy. Additionally, PAD4 and both citR antibodies are especially relevant to TDP-43 proteinopathies, such as LATE and AD, as these proteins expressed higher abundancy within the anterior cortex of diseased brain. Zoom: https://uky.zoom.us/j/81831403942

Brandi Carey [1] • Justin Barber, MS [2] • Gregory Jicha, MD [2] • Peter Nelson, PhD [2] • Jordan Harp, PhD [2]

Morehead State University [1] • Sanders-Brown Center on Aging University of Kentucky [2]

Early life ADHD symptoms are not related to Lewy body pathology at autopsy

Student

Objective: The objective of this study was to assess the possible relationship between early life ADHD symptoms and Lewy body pathology (LBP) at autopsy in older adults with and without dementia.
Background: ADHD and the Dementia with Lewy body (DLB) clinical presentations are characterized by similar deficits in attention and executive function. Prior research by others has suggested that early life ADHD symptoms may be overrepresented in those with DLB as compared to those with normal cognition or late life cognitive decline associated with a non-DLB clinical phenotype.
Methods: Retrospective ADHD symptomatology in early life was assessed using the validated Wender-Utah Rating Scale (WURS) which was completed by 310 UK-ADRC participants with normal cognition, mild cognitive impairment (MCI), or dementia in 2009. ADHD symptoms were considered the mean of all items responded to on the WURS, and higher values reflect more symptoms. For this analysis, we obtained the ADHD data for participants who had come to autopsy as of July 2020. We classified LBP using the methodology adopted by the third report of the DLB consortium guidelines; none, brain-stem only, basal forebrain/limbic only, or neocortical. We compared age at death, sex, and other pathologies between LBP groups to check for covariates, and participants were split into two groups by Braak Alzheimer's staging: Braak stages 0-1 or Braak stages ≥ 2, based on the former having significantly higher WURS. This split Braak variable was a covariate. Chi-squared tests and ANOVA were used for analysis.
Results: In total, 97 participants with ADHD data had come to autopsy. The sample was 61% female (n=59), with an overall mean age at death of 87y (*SD* = 6.8) and a mean education of 16.6y (*SD*= 2.5). The number of participants in each LBP group was 67 for none, 11 for brain-stem, 13 for basal forebrain/limbic, and 6 for neocortical. Age of death, sex, Limbic-Predominant Age-Related TDP-43 Encephalopathy (LATE), Age-Related Tau-Astrogliopathy (ARTAG), Primary Age-Related Tauopathy (PART), Braak stage, Thal amyloid phase, total infarcts, cerebral amyloid angiopathy, severity of arteriosclerosis, and brain weight were not significantly different between the LBP groups. There was no main effect of LBP group on WURS score, $F(3,90) = 1.201$, $p = 0.31$, with or without Braak stage as a covariate ($p = 0.35$ without), and no pairwise differences between (all ps > 0.29). Braak stage 0-1 group had higher WURS compared to Braak stage ≥ 2 group, $F(1,88) = 9.09$, $p < 0.001$.
Conclusion: In contrast with previous reports of an association between early life ADHD and late life DLB clinical phenotypes, the present analysis did not find a relationship between ADHD and autopsy-confirmed LBP. Unexpectedly, there was a significant relationship between Braak stages 0-1 and higher mean WURS score. The relationship between ADHD and tauopathy in AD deserves further exploration. Zoom: https://uky.zoom.us/j/86566812866

Courtney Kloske [1,4] • Adam Dugan, MS [2] • Erica Weekman, PhD [1,4] • Zachary Winder [1] • Josh Morganti, PhD [1,3] • Peter Nelson, MD, PhD [1] • David Fardo, PhD [2] • Donna Wilcock, PhD [1.4]

Sanders-Brown Center on Aging [1] • Department of Biostatistics [2] •Department of Neuroscience [3] •Department of Physiology[4] University of Kentucky

Assessing the neuroinflammatory profile associated with Apolipoprotein E4 in Alzheimer's disease

Student

Background: There are many genetic risk factors that impact the risk of Alzheimer's disease (AD). One of the most influential risk factors is Apolipoprotein E (APOE), a cholesterol and lipid transporter in the brain.There are three common isoforms: APOE-e3 is considered the "control" phenotype, APOE-e2 protects against AD, and APOE-e4 confers an increased risk for AD. Studies show that ApoE isoforms have differing effects on AD pathologies. ApoE has also been shown to impact microglial activation and the neuroinflammatory response to AD pathologies in animal models. **Objectives**: 1) Perform an unbiased inflammatory panel to determine the impact of ApoE isoforms on the neuroinflammatory response with AD pathology; 2)Validate RNA findings, with NanoString digital spatial profiling to determine protein expression on a separate group of patients. **Method:** We used two methods to determine the impact of APOE-e4 in human autopsy tissue. First, the Human Neuroinflammation NanoString panel identified the neuroinflammatory RNA profile in the superior medial temporal gyrus and cerebellar regions of age and sex matched individuals with the following genotypes and pathology: APOE-e3/3-AD; APOE-e4/4-AD; and APOE-e3/3-control. Results were analyzed with NanoStringDiff followed by additional statistical analysis. In a separate cohort, we used NanoStringDigital Spatial Profiling (DSP) to investigate protein levels of inflammatory markers on fixed tissue surrounding AD pathology in both APOE-e3 and APOE-e4 subjects. **Result:** Significant gene expression changes were found in pathways associated with AD in APOE-e3individuals. We then examined differences between APOE-e4/4-AD and APOE-e3/3-control individuals to determine AD-associated effects with APOE-e4. These results showed no significant differences, suggesting that while APOE-e4/4-AD individuals have AD pathology, they potentially have an impaired inflammatory response. Further, we compared APOE-e3/3-AD; and APOE-e4/4-AD results to identify APOE isoform-associated effects with AD pathology. We found significant changes; however, the differences paralleled those between APOE-e3/3-AD and APOE-e3/3-control, suggesting that APOE-e4/4-AD brains have an impaired response to the pathology. Finally, we used the DSP data to verify these findings and the data also suggests that APOE-e4 individuals have an altered inflammatory phenotype. **Conclusion:** Our results suggest APOE-e3 individuals respond to AD pathology compared to non-AD individuals. Our results indicate that APOE-e4 individuals have an impaired inflammatory response and cannot produce an appropriate response to the pathology. These findings highlight the importance of accounting for APOE isoforms in studies when investigating inflammatory mechanisms of AD.

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Zoom: https://uky.zoom.us/j/88389892403

Fengpei Yuan, MS [1] • Xiaopeng Zhao, PhD, D.Sc [1]

Department of Mechanical, Aerospace and Biomedical Engineering University of Tennessee [1]

Explore of adaptive strategy in robot dialogue system for simulated people with dementia

Student

Objectives: People with Alzheimer's disease and related dementias (ADRD) often show symptom of repetitive questioning, which brings great burden on patients and their caregivers. The application of a conversational social robot may relieve such burdens. However, a strategy is needed to enable a robot to answer the repetitive questions from patients and engage patients in other topics of daily conversation.

Methods: The technique of reinforcement learning (RL) is applied to explore such strategy for robot dialogue system. The RL agent learns from experience (i.e. patient-robot conversation) under the context of repetitive questioning and optimize the policy towards the goals. An action for a robot includes the probability of asking a follow-up question (i.e., follow-up rate, P_f) and the difficulty level of the follow-up question (i.e., question difficulty, *QD*). There are five potential states based on the patient-robot interaction/dialogue. The reward function is defined based on the patient's response to the robot's follow-up question. We start the exploration of strategy with simulated persons with ADRD, each of whom is modelled as probabilities of providing relevant, irrelevant and no response to a follow-up question. These three probabilities are influenced by individual's cognitive capability and engagement level. There are four basic users during our study, User 1, 2, 3, and 4, corresponding to older adults without cognitive impairment, with mild cognitive impairment, moderate dementia, and severe dementia, respectively. The RL problem is solved using Q-learning.

Results: Our model showed converged performance in learning. More specifically, the RL agent suggested adaptive optimal policy, [P_f, *QD*] for patients with different cognitive capability and engagement level. In terms of cognitive capability, the optimal policy suggested for User 1 is P_f = 1.0, *QD* = difficult, that is, always asking difficult following-up questions. Comparatively, the optimal policy for User 4 is P_f = 0.1, *QD* = Easy. For the same user with different engagement level, for example, the optimal policy towards User 3 with high, medium, and low engagement, is P_f = 0.1, *QD* = Difficulty, P_f = 1.0, *QD* =moderately difficulty, and P_f = 1.0, *QD* = Easy, respectively.

Conclusions: We applied the technique of reinforcement learning to explore the strategy for the robot dialogue system in the context of repetitive questioning by people with ADRD. With simulated users, the RL agent is capable of adapting the policy (i.e., follow-up rate and question difficulty) for a social robot to patients with different cognitive capabilities and engagement level. Our model might be useful to relieve the burden of repetitive questioning on patients' caregivers, distract patients from their repetitive questioning, and engage patients in conversation with robots. This study is meaningful to the application of social robots to Alzheimer's care. Zoom**:** https://tennessee.zoom.us/j/93852000890