Markesbery Symposium on Aging and Dementia

November 20 & 21, 2015
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November 20, 2015

Dear Conference Participants,

On behalf of the Sanders-Brown Center on Aging, UK HealthCare, and the symposium planning committee, I am pleased to welcome you to the 5th annual “Markesbery Symposium on Aging and Dementia.”

The symposium is named in honor and memory of the late William R. Markesbery, MD, founding Director of the Sanders-Brown Center on Aging and Alzheimer’s Disease Center at the University of Kentucky. Dr. Markesbery’s legacy of groundbreaking research at the Center on Aging has formed the bedrock for our quest to understand and treat Alzheimer’s disease and to improve the quality of life of the elderly. We have no doubt that Bill Markesbery’s work will live on for generations to come as we continue the work he started here almost four decades ago.

Over the next two days, in sessions for both the scientific and community audience, you will have the opportunity to hear clinicians and researchers from the University of Kentucky and other institutions share current findings, trends, and latest updates on dementia and aging disorders, particularly as related to Alzheimer’s disease.

In addition to the presentations conducted by some of the world’s leading scientists, we have invited investigators to display posters of their current research on aging and dementia. Please take some time to visit the research poster gallery on display in the atrium and discuss these ongoing studies with the researchers.

We are honored that so many of you have chosen to join us in seeking to expand our knowledge and friendships. I hope the symposium will be both scientifically rewarding and enjoyable.

Sincerely,

Linda J. Van Eldik, Ph.D.
Director, Sanders-Brown Center on Aging & Alzheimer’s Disease Center

Symposium Planning Committee:
Linda Van Eldik, PhD, Chair  Jose Abisambra, PhD  Steven Estus, PhD  Elizabeth Head, PhD
Derrick Hord  Sally H. Malley  Paula Thomason  Donna Wilcock, PhD

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THANK YOU TO OUR SYMPOSIUM SPONSORS!

The Sanders-Brown Center on Aging gratefully acknowledges the support of our sponsors. Their support enables us to provide the highest quality programming.
SCIENTIFIC SESSION AND POSTER PRESENTATIONS

Location: Auditorium and Atrium of the Albert B. Chandler Hospital, Pavilion A, 1000 S. Limestone, University of Kentucky Campus

10:00 am  Check-in begins: Receive poster assignment number, ID badge, & program

11:00 Welcome
Peter T. Nelson, MD, PhD
Professor of Pathology & Laboratory Medicine and Sanders-Brown Center on Aging
Linda J. Van Eldik, PhD
Director, Sanders-Brown Center on Aging and Alzheimer’s Disease Center
University of Kentucky

11:15 Biomarkers in the Community
Ronald C. Petersen, MD, PhD
Director, Mayo Clinic Alzheimer’s Disease Research Center & the Mayo Clinic Study on Aging, Professor of Neurology, Mayo Clinic College of Medicine
Rochester MN

12:15 Box Lunch and Poster Session (Atrium)

1:45 Research at the Sanders-Brown Center on Aging: an Update
Steven Estus, PhD  Translating Alzheimer’s Disease Genetics into Pharmacologic Agents
Mark Lovell, PhD  Blood-Based Biomarkers
Richard Kryscio, PhD  Subjective Memory Complaints
Elizabeth Head, PhD  A Canine Model of Alzheimer’s Disease

3:00 canceled  Current Concepts in Identifying Novel Therapeutic Targets in AD and CTE
Sam Gandy, MD, PhD
Director, Mount Sinai Center for Cognitive Health and NFL Neurological Center
Mount Sinai Endowed Chair in Alzheimer’s Research and Professor of Neurology and Psychiatry, Icahn School of Medicine at Mount Sinai, New York

4:00 Poster award presentations and closing remarks
Linda J. Van Eldik, PhD
“Biomarkers in the Community”

Ronald Petersen, MD, PhD
Director, Mayo Alzheimer’s Disease Research Center
and the Mayo Clinic Study of Aging
Professor of Neurology, Mayo Clinic College of Medicine

Dr. Ronald Petersen is a national leader in the field of Alzheimer’s research. He is the director of the Mayo Alzheimer’s Disease Research Center and the Mayo Clinic Study on Aging. He has authored over 550 peer-reviewed articles and edited five books on memory disorders, aging, and Alzheimer’s disease. Dr. Petersen received his Ph.D. in Experimental Psychology from the University of Minnesota and graduated from Mayo Medical School in 1980. He joined the staff of the Mayo Clinic in 1986. He became the Cora Kanow Professor of Alzheimer’s Disease Research in 2000, and was named the Mayo Clinic Distinguished Investigator in 2011.

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. He also received the inaugural Ronald and Nancy Reagan Research Institute Award in 2004 from the Alzheimer’s Association and the inaugural Leon Thal Prize of the Lou Ruvo Brain Institute in 2007. In 2012, he received the Khachaturian award of the Alzheimer’s Association and the Henry Wisniewski Lifetime Achievement Award in 2013.

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 Project Act and was appointed to the World Dementia Council in 2014 by UK Prime Minister David Cameron.
“Current Concepts in Identifying Novel Therapeutic Targets in AD and CTE”

Sam Gandy, MD, PhD
Director, Mount Sinai Center for Cognitive Health and NFL Neurological Care
Professor of Neurology and Psychiatry and Endowed Chair in Alzheimer’s Research
Icahn School of Medicine at Mount Sinai, New York NY
Chairman, National Medical and Scientific Advisory Council of the Alzheimer’s Association

Dr. Gandy is an international expert in the metabolism of the sticky substance called amyloid that clogs the brain in patients with Alzheimer’s. In 1989, Gandy and his team discovered the first drugs that could lower formation of amyloid. Dr. Gandy has written more than 250 original papers, chapters and reviews on this topic. Dr. Gandy has received continuous NIH funding for his research on amyloid metabolism since 1986.

Dr. Gandy is a member of the Faculty of 1000 Biology and serves as a Consulting Editor for The Journal of Clinical Investigation. He also serves on the Editorial Advisory Boards for the journals Public Library of Science-Medicine (PLoS), Neurodegenerative Diseases, and Current Alzheimer Research. He is Associate Editor of the journals Molecular Neurodegeneration and Alzheimer Disease and Associated Disorders. In 2009, Gandy was featured with other prominent research scientists as one of GQ's "Rockstars of Science" and featured in the international documentary film I Remember Better When I Paint which examines the phenomenon of how pathways to emotional parts of the brain of those with Alzheimer's are awakened through the creative arts.

Dr. Gandy received both his MD and PhD at the Medical University of South Carolina. He completed an internship in Internal Medicine at Columbia University College of Physicians & Surgeons and a residency in Neurology at Cornell University Medical College. Before assuming his current post as Mount Sinai Professor of Alzheimer's Disease Research at the Mount Sinai School of Medicine in 2007, he served as Paul C. Brucker, M.D., Professor of Neuroscience at Jefferson Medical College and Founding Director of the Farber Institute for Neurosciences from 2001 -2007.
Research at the Sanders-Brown Center on Aging: an Update

“Translating Alzheimer's Disease Genetics into Pharmacologic Agents”

Steven Estus, PhD
University of Kentucky

Dr. Estus is the Shih-Chung Wang Professor in the Department of Physiology and Sanders-Brown Center on Aging. Dr. Estus’ research seeks to elucidate the mechanisms underlying the actions of genetic polymorphisms that modulate the risk of disease, especially Alzheimer’s disease (AD). Since history has shown that drugs based upon genetic mechanisms are more likely to succeed in clinical trials, his goal is to translate these findings into novel approaches to prevent or treat AD.

“Blood-Based Biomarkers”

Mark Lovell, PhD
University of Kentucky

Dr. Lovell is the Jack and Linda Gill Arts and Sciences Research Excellence Fund Professor in the Department of Chemistry and the Sanders-Brown Center on Aging. His research focuses on the role of oxidative damage to DNA, RNA and lipids and the potential role of epigenetic changes in DNA in the pathogenesis of AD. In addition, he has been involved in the identification and characterization of blood based biomarkers of AD, stroke and traumatic brain injury (TBI) including the development of a point of care lateral flow device for rapid identification of mild TBI and stroke. Dr. Lovell is also involved in development and characterization of novel small molecule therapeutics for AD.

“Subjective Memory Complaints”

Richard Kryscio, PhD
University of Kentucky

Dr. Richard J. Kryscio is Professor in the Department of Statistics, College of Arts and Sciences and Chair, Department of Biostatistics, College of Public Health. He is the Leader of the Data Management and Biostatistics Core as well as Associate Director of the Sanders-Brown Center on Aging’s Alzheimer’s Disease Center. His research concerns many issues in Public Health including the clustering of disease in space and time, the spatial distribution of stroke and identification of risk factors for stroke, use of transvaginal sonography for the early detection of ovarian cancer, the mathematical theory of the spread of diseases, and statistical methodology used in Alzheimer's Disease (AD) research including clinical trials for preventing AD and Markov chains for modeling the flow of subjects through various cognitive health states with AD and death as a competing events.
“A Canine Model of Alzheimer’s Disease”

Elizabeth Head, PhD
University of Kentucky

Dr. Head is an Associate Professor in the Department of Pharmacology & Nutritional Sciences and the Sanders-Brown Center on Aging. Dr. Head has been working with a canine model of aging and Alzheimer’s disease for over 20 years and more recently, in collaboration with Dr. Donna Wilcock, has been interested in the role of cerebrovascular pathology on cognition in these animals. The goal of her research in the canine preclinical model is to identify and test interventions that can be translated to clinical trials to slow or prevent Alzheimer’s disease in adults with Down syndrome. In parallel, Dr. Head also co-leads a longitudinal aging study in adults with Down syndrome to assess cerebrovascular pathology and associated neuroinflammatory changes with her colleague Dr. Frederick Schmitt. Her research is currently funded by the National Institutes of Health.
Sleep apnea and dementia risk: Findings from the PREADViSE Alzheimer's disease prevention trial

Xiuhua Ding 1 • Richard Kryscio 2 • Joshua Turner 3 • Jicha Gregory 2 • Gregory Cooper 4 • Allison Caban-Holt 2 • Frederick Schmitt 2 • Erin Abner 2
1College of Public Health, University of Kentucky • 2Sanders-Brown Center on Aging • 3Counseling Psychology, New Mexico State University • 4Neurology, Baptist Health Medical Group

Student

Background
Sleep apnea is a common condition that has been demonstrated to have a direct impact on cognitive function. The impact of sleep apnea, and its interplay with other established risk factors, on the risk of incident dementia warrants exploration.

Objective
To investigate the association between baseline sleep apnea and risk of incident dementia in the Prevention of Alzheimer’s Disease with Vitamin E and Selenium (PREADViSE) study and explore whether the association depends on APOE ε4 allele status.

Design
Randomized controlled trial followed by exposure study with over 11 years of follow up.

Setting
Participants were assessed at 130 local clinical study sites during the clinical trial phase and later were followed by telephone from a centralized location.

Participants
7,547 subjects were enrolled in PREADViSE, and 4,271 of them consented to participate in the exposure study.

Measurements
Participants were interviewed at baseline for the sleep apnea. The Memory Impairment Screen (MIS) was administered to each participant annually. Subjects who failed to this initial screen were tested with more extensive secondary screening tests. Additional measures collected include medical history, medication use, and the AD8 dementia screening instrument.

Results
The effect of sleep apnea on dementia risk depends on APOE ε4 status. When the allele was absent, baseline sleep apnea was associated with a 66% higher risk of developing dementia (95% CI 2%-170%), while sleep apnea conferred no additional risk for participants with an ε4 allele.

Conclusions
Sleep apnea may increase risk of dementia in the absence of APOE-ε. This may help inform prevention strategies for dementia or AD in older men with sleep apnea.
Reduced efficacy of anti-Aβ immunotherapy in a mouse model of amyloid deposition and vascular cognitive impairment co-morbidity.

*Erica Weekman • Carly Caverly • Tim Kopper • Tiffany Sudduth • Donna Wilcock, PhD*

Department of Physiology, University of Kentucky

*Student*

Alzheimer’s disease (AD) and vascular contributions to cognitive impairment and dementia (VCID) are the two most common forms of dementia yet they share similar pathologies. Both vasogenic edema and microhemorrhages occur in some cases of AD and some types of VCID and there are also significant adverse vascular events of anti-amyloid beta (Aβ) immunotherapy. Because it is estimated that 40% of AD patients also have VCID, it is imperative to determine the effect of anti-Aβ immunotherapy on cognition and vascular pathology when AD and VCID are co-morbid.

To model AD-VCID co-morbidity we use the APP/PS1 mouse model of amyloid deposition and induce hyperhomocysteinemia (HHcy) via diet, which models a form of VCID. We placed 9 month old wildtype or APP/PS1 mice on a control diet or a diet that induces HHcy. After 3 months on diet, when cerebrovascular pathology is induced by the HHcy, the mice received weekly intraperitoneal injections of IgG2a or 3D6 (N-terminal anti-Aβ antibody). Cognition was assessed with the two day radial arm water maze. During treatment, MRI for microhemorrhages and edema was performed. Matrix metalloproteinase (MMP) activation was measured by gelatin zymography and microhemorrhages were assessed by Prussian blue staining. Aβ levels were quantified using immunohistochemistry, Congo red staining and ELISA measurement. Neuroinflammation was assessed by qPCR for gene markers specific for peripheral macrophage phenotypes.

In block 10 of the radial arm water maze, APP/PS1 mice made more errors than wildtype mice and APP/PS1 mice with VCID on 3D6 treatment made more errors than APP/PS1 mice with VCID on IgG2a treatment. Imaging for microhemorrhages showed induction of bleeds in mice on the HHcy diet and on 3D6 treatment. Quantification of MMP expression and activation, Aβ levels, and the neuroinflammatory phenotype is in progress.

Overall, early assessment of the data indicates that anti-Aβ immunotherapy may not provide a clinical benefit in individuals with VCID co-morbidity.
Is chronic pain an initiator of tauopathy?

Karin Westlund High, PhD
Department of Physiology, University of Kentucky

Faculty

The hallmark of frontotemporal dementia (FTD) is the abundance of tau protein in specific vulnerable brain regions leading to progressive impairment in cognition, emotional and social behavior, language and executive function. Although progressive spread of tau neuropathology from frontal and hippocampal cortex is mapped in humans, animal models have not yet been reported that appropriately replicate tauopathy seen in humans. Understanding the basic mechanisms during the initial stages of tau pathology is fundamentally important to designing new strategies for early intervention. Our current novel findings are that chronic pain conditions produce early features of tauopathy which over time promote hippocampal stem cell and medial prefrontal cortical neuron loss. We are addressing the question of how chronic pain produces vulnerability for tauopathy by investigating the link between neuronal overactivation of the pain circuitry and intracellular stress responses that cause expression of dysfunctional tau proteins and result in neurotoxicity. Our time sequenced studies utilize animal models that allow study of the transition from persistent to chronic pain and associated behavioral changes. Hypersensitivity is later accompanied by anxiety- and depression-like behaviors. Neuropathological analysis at multiple time points over a 6 month period is providing a unique understanding that chronic pain is a generator causal in intracellular organelle stress. Chronic neuropathic pain induces expression of dysregulated endoplasmic reticulum (ER) stress sensor protein pPERK (phosphorylated promoter protein kinase R (PKR)-like ER kinase) and hyperphosphorylated PHF-1 tau protein within 3 weeks that accumulates over 6 months in wild type, genetically unmodified mice and rats. ER stress sensor pPERK is a suppressor of protein translation linked to tau pathogenesis. Hyperphosphorylated PHF-1 tau and pPERK signaling pathway proteins are indicative of cellular oxidative stress, neuronal damage and potential neurotoxicity. Treatment with a pPERK inhibitor reduces expression of PHF-1 tau and pPERK proteins, identifying pPERK pathway signaling as a potential therapeutic target for prevention of the long-term neuropathological consequences of chronic pain.

Our goal is comprehensive understanding of molecular and cellular mechanisms during the transition of chronic pain that produce risk for tauopathy to allow us to identify novel therapeutic targets. Relevance of this project is underscored by the fact that at present there are 100 million patients with chronic pain in the US at risk for tau pathology and dementia.
Age-related oxidative stress and neuroinflammation in spinal cord injury

Bei Zhang, PhD • William Bailey • Anna Mcvicar • John Gensel, PhD
Spinal Cord and Brain Injury Research Center and the Department of Physiology, University of Kentucky

Staff

There is an increasing incidence of spinal cord injury (SCI) in aged individuals. Previously, we demonstrated that aged mice exhibit worse functional deficits associated with differential macrophage activation following SCI. Reactive oxygen species (ROS)-mediated oxidative damage following CNS injury contributes to the secondary injury. It has been suggested that NADPH oxidase (NOX), the enzyme system primarily responsible for ROS production in phagocytic cells, plays an essential role in microglia/macrophage activation and subsequent inflammatory responses. We hypothesize that age increases oxidative damage in the injured spinal cord via increased NOX activity. In order to understand the mechanisms behind age-related differences in recovery, we compared oxidative stress associated with macrophage activation in 4-month-old (4 MO) and 14 MO mice after contusion SCI. By tracking oxidized dihydroethidine (ox-DHE), a marker for intracellular superoxide, we identified that overall superoxide generation is significantly higher in 14 MO mice than 4 MO controls at 3 days post injury (dpi). Notably, the expression of NOX2, which we primarily detected in ROS-producing macrophages, is also significantly increased in 14 MO mice. This suggests that during the acute phase of SCI, macrophages and NADPH oxidase are the major cellular and subcellular sources of oxidative stress and may potentiate secondary injury in older animals. Additionally, we observed increased activation of neurotoxic M1 macrophages (CD16/32-positive) in 4 MO SCI mice at 3 dpi. Although no difference between 4 and 14 MO mice in the level of protective M2 macrophage activation (Arginase-1-positive, ARG-1), we detected a larger proportion of ARG-1-positive macrophages produced ROS in the injured spinal cords of 14 vs. 4 MO mice. These data indicate that age plays an important role in the ability of macrophages to adopt different phenotypes after SCI. Further, in the aged injured spinal cord, normally protective M2 macrophages may potentiate secondary injury through superoxide generation.
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Reversal of aging-related neuronal Ca2+ dysregulation and cognitive impairment by adeno-associated viral delivery of FKBP1b (12.6) to hippocampus

John Gant, PhD 1 • Kuey-Chu Chen, PhD 1 • Inga Kadish, PhD 2 • Eric Blalock, PhD 1 • Olivier Thibault, PhD 1 • Nada Porter, PhD 1 • Philip Landfield, PhD 1

1Pharmacology and Nutritional Sciences, University of Kentucky • 2Cell Development and Integrative Biology, University of Alabama Birmingham

Staff

Brain Ca2+ regulatory processes are altered during aging, disrupting neuronal and cognitive functions. In hippocampal pyramidal neurons, the Ca2+-dependent slow after hyperpolarization (sAHP) exhibits an increase with aging, which correlates with memory impairment. The increased sAHP results from elevated L-type Ca2+ channel activity and ryanodine receptor (RyR)-mediated Ca2+ release, but underlying molecular mechanisms are poorly understood. Previously we found that expression of the gene encoding FK506-binding protein 12.6/1b (FKBP1b), a small immunophilin that stabilizes RyR-mediated Ca2+ release in cardiomyocytes, declines in hippocampus of aged rats and Alzheimer’s disease subjects. Additionally, knockdown/disruption of hippocampal FKBP1b in young rats augments neuronal Ca2+ responses. Here, we tested the hypothesis that declining FKBP1b underlies aging-related hippocampal Ca2+ dysregulation. Using microinjection of adeno-associated viral vector bearing a transgene encoding FKBP1b into the hippocampus of aged male rats, we assessed the critical prediction that overexpressing FKBP1b should reverse Ca2+-mediated manifestations of brain aging. Immunohistochemistry and qPCR confirmed hippocampal FKBP1b overexpression 4-6 weeks after injection. Compared to aged vector controls, aged rats overexpressing FKBP1b showed dramatic enhancement of spatial memory, which correlated with marked reduction of sAHP magnitude. Further, simultaneous electrophysiological recording and Ca2+ imaging in hippocampal neurons revealed that the sAHP reduction was associated with a decrease in parallel RyR-mediated Ca2+ transients. Thus, hippocampal FKBP1b overexpression reversed key aspects of Ca2+ dysregulation and cognitive impairment in aging rats, supporting the novel hypothesis that declining FKBP1b is a molecular mechanism underlying aging-related Ca2+ dysregulation and unhealthy brain aging and pointing to FKBP1b as a potential therapeutic target.
The elucidation of a spontaneous lactamization of L-lysine

Xiaodong Liu, PhD • Steven Van Lanen, PhD
College of Pharmacy, University of Kentucky

Fellow

Capuramycin-type nucleoside antibiotics are natural products endowed with excellent antimycobacterial activity. Most members of the family contain an L-α-amino-ε-caprolactam (L-ACL) moiety, which is also extensively found in other natural products like bengamide A, caprolactin A, nocardimicin A and mycobactins. (Figure A) The isotopic enrichment studies have indicated that the L-lysine is a direct biosynthetic precursor to L-ACL, which undergoes an amide bond formation reaction and is catalyzed by NRPS enzymes. The elucidation of molecular details behind this process will provide an opportunity for understanding this critical step during assembly of many natural products, and even help the enzyme-directed synthesis of new scaffolds.

Gene cluster analysis indicated that there is a NRPS enzyme, CapU, involved in the amide bond formation. CapU is bioinformatically predicted to consist with a condensation (C), adenylation (A), and thiolation (T) domains, while the condensation domain may lose the activity due to an important conservative His (H119) mutated to Gln. Amino acid sequence alignments revealed that CapV has a stand-alone C domain and might work with CapU together. The initially in vivo experiment was performed by double-crossover inactivation of capU and heterologous expression of capU and capV. The combination of these in vivo results suggest CapU and CapV orchestrate ACL biosynthesis. The experiments in vitro with reconstructed enzymes further proved that the ACL is from the lactamization of L-lysine, and the CapU is not only responsible for activation but also lactamization. The detailed reaction process was further explored by using pantetheine thioester activated L-lysine and recombinant CapU with only A and T domains. The combination of these results indicate that L-lysine is first activated by CapU and then forms ACL via a spontaneous cyclization, unlike other NPRS-derived peptide bond formation.
Mechanisms of insulin action on hippocampal neurons

Shaniya Maimaiti\textsuperscript{1} • Katie Anderson \textsuperscript{1} • Jelena Popovic \textsuperscript{2} • Lawrence Brewer \textsuperscript{1} • Zana Majeed \textsuperscript{2} • Hilaree Frazier \textsuperscript{1} • Nada Porter \textsuperscript{1} • Philip Landfield \textsuperscript{1} • Olivier Thibault \textsuperscript{1}

\textsuperscript{1}Pharmacology & Nutritional Sciences, University of Kentucky • \textsuperscript{2}Biology, University of Kentucky

Recent work from our lab found that intranasal insulin improves cognition in aged F344 rats. It is well documented that neurons in the brain are insulin sensitive. It has also been shown that brain insulin sensitivity may be reduced in aging and Alzheimer’s disease (AD). Further, clinical trials have repeatedly shown that intranasal insulin can significantly improve memory not only in AD patients, but also in younger healthy individuals. However, the mechanism whereby insulin can alter neuronal function is not clear. While prior work has highlighted changes in AMPA and NMDA receptors, very little work has focused on voltage-gated calcium channels/current (VGCCs) as a target of insulin action in the brain.

Earlier studies from our lab looked at intracellular Ca\textsuperscript{2+} as a key neuronal molecular regulator of hippocampal-dependent memory. Elevated intracellular Ca\textsuperscript{2+} levels in hippocampal neurons in aged animals are associated with poor spatial memory. Recently, we showed that insulin reduces the Ca\textsuperscript{2+} dependent after hyperpolarization (AHP) in hippocampal neurons in both young and aged animals.

The goal of the present work is to test the hypothesis that insulin reduces VGCCs, thereby reducing AHP. We used whole cell patch clamping, Ca\textsuperscript{2+} imaging (Bis-Fura-2), and glucose imaging (2-NBDG) techniques to measure Ca\textsuperscript{2+} currents, intracellular Ca\textsuperscript{2+} concentration, and glucose utilization in 13-17 DIV mixed hippocampal neurons in culture. Active, boiled (10nM) glulisine insulin Apidra\textsuperscript{®} (rapid –acting, zinc-free form of insulin) and reconstituted insulin were tested for acute effects on neuronal VGCCs, Ca\textsuperscript{2+} levels, and glucose utilization. Results show that neurons treated with 10nM reconstituted insulin had significantly reduced Ca\textsuperscript{2+} current. This indicates the mechanism of insulin-mediated memory improvement could be due to reduced calcium flow through VGCCs and the ensuing reduction in the AHP).
Characterization of a truncated, constitutively active human insulin receptor signaling

Hilaree Frazier • Zana Majeed • Kaia Hampton • Shaniya Maimaiti • Katie Anderson • Jelena Popovic • Lawrence Brewer, PhD • Susan Kraner, PhD • Christopher Norris, PhD • Nada Porter, PhD • Rolf Craven, PhD • Olivier Thibault, PhD

Pharmacology and Nutritional Sciences, University of Kentucky • Biology, University of Kentucky • Sanders-Brown Center on Aging, University of Kentucky

Student

Insulin signaling is indispensable for key metabolic pathways in the periphery, and recently several studies have demonstrated that insulin signaling is also important for brain function. Early stage clinical trials report on the positive impact of intranasal insulin on memory recall in young subjects and in patients with mild cognitive decline or Alzheimer’s disease (AD). However, the underlying molecular mechanisms for the actions of insulin signaling on brain cognitive function are not well understood.

Here we sought to investigate the role of insulin in neuronal physiology by overexpressing a constitutively active human insulin receptor in rat pheochromocytoma (PC12) and HEK 293 embryonic kidney cells to obtain insights into the trafficking of the protein, as well as its activity and sensitivity to exogenous insulin. Cells were transfected by electroporation with pCI-ires-ds-red (a mammalian expression plasmid containing a gene encoding a red fluorescence protein) or pCI-HA-IRβ-ires-ds-red (the construct with a truncated human insulin receptor beta subunit (IRβ) transcript). The vector was originally tested in HEK293 cells. The expression of human IRβ receptor in PC12 cells was corroborated by the expression of red fluorescent protein (ds-red) since IRβ and ds-red were co-expressed under the control of the same promoter (CMV). The expression level and effect of IRβ receptor overexpression on insulin signaling was confirmed by performing immunoblots, using antibody against tagged IRβ, and measuring pAkt/Akt ratio, respectively, because Akt is one of the main downstream signaling molecule targets in the insulin receptor signaling pathway.

Our data show that overexpression of insulin receptor enhances neurite outgrowth in PC12 cells and increases the pAkt/Akt ratio. Experiments also revealed insertion of the protein in the plasma membrane. Further studies were performed to analyze the sensitivity of the truncated insulin receptor activation to acute insulin application. This initial characterization of the protein provides insights into future intervention approaches to combat reduced insulin signaling in AD and/or aging.
Monomeric Aβ40 and Aβ42 drive neurovascular leakage in Alzheimer’s disease

Ralf G Rempe, PhD 1 • Bjoern Bauer, PhD 1 • Anika Hartz, PhD 2
1Pharmaceutical Sciences, University of Kentucky • 2Sanders-Brown Center on Aging, University of Kentucky

Fellow

Abeta deposits in the brain of Alzheimer’s disease patients are considered to damage brain endothelial cells leading to blood-brain barrier leakage. We postulated that the Aβ isoforms Aβ40 and Aβ42 trigger barrier leakage through matrix metalloproteinases (MMPs). To test this hypothesis, we exposed isolated rat brain capillaries to 100 nM Aβ40, Aβ42, or their respective aggregated or reversed peptides and determined MMP-2 and MMP-9 protein expression and activity levels. We found that monomeric Aβ40 and Aβ42 peptides increased MMP-2 and MMP-9 protein and activity levels, whereas the aggregated and reversed forms of Aβ40 and Aβ42 had no effect. We also detected increased MMP-2 and MMP-9 protein and activity levels in isolated brain capillaries from a transgenic Alzheimer’s disease mouse model (Tg2576; human amyloid precursor protein [hAPP]-overproducing mice). To test if increased MMP levels are linked to barrier leakage, we determined expression levels of the blood-brain barrier tight junction proteins claudin-1, claudin-5, occludin, and ZO-1 in isolated brain capillaries from hAPP mice. Claudin-1 and claudin-5 protein levels were decreased in brain capillaries from hAPP mice compared to capillaries from control wild type mice, but we did not detect any difference in occludin and ZO-1 expression levels. To determine if reduced claudin-1 and claudin-5 levels resulted in blood-brain barrier leakage, we performed a permeability assay with brain capillaries isolated from hAPP mice. These experiments revealed barrier leakage in brain capillaries from hAPP mice compared to brain capillaries from wild type mice. Based on these data, we conclude that monomeric Aβ40 and Aβ42 increase MMP-2 and MMP-9 protein and activity levels in brain capillaries. MMPs, in turn, degrade capillary tight junction proteins, which results in barrier leakage.

Genetic variants and risk for Alzheimer’s disease: ABCA7

Jared B. Vasquez 1 • Steve Estus, PhD 1,2
1Department of Physiology, University of Kentucky • 2Sanders-Brown Center on Aging, University of Kentucky

Student

Genome-wide association studies (GWAS) identify single nucleotide polymorphisms (SNPs) that associate with Alzheimer’s disease (AD). Adenosine triphosphate binding cassette A7 (ABCA7) reached significance in several of these studies warranting investigation into this association. Our overarching hypothesis is that AD-associated SNPs modulate ABCA7 RNA expression and/or splicing to impact disease risk. Here, we analyzed ABCA7 expression in a set of human brain samples as a function of AD status and AD SNPs. We report that ABCA7 expression is increased in AD and in individuals that carry the protective rs3764650T allele. Additionally, rs200538373 has been reported to modulate ABCA7 splicing increasing AD risk. Elucidating the mechanism of action for this increased AD risk may help to target this pathway effectively with pharmacological agents. In preliminary results, we found that the minor allele of rs200538373 is associated with a 14 bp extension of exon 41 into intron 41, thereby altering the codon reading frame and introducing a premature stop codon. Additionally, we quantified expression of both isoforms using PCR to discern the extent that the atypical isoform is modulated by AD status, other SNPs, or cell type differences in the samples. Moving forward, we will use minigenes containing each allele to determine directly whether the AD-associated SNP modulates splicing. Currently our findings suggest a model that increased ABCA7 expression reduces AD risk and that the increase observed in AD reflects an inadequate compensatory change.
A translational approach to the innate immune responses following TBI: exploring the nexus of the post injury proinflammatory cytokine mediated response

Danielle Goulding \(^1\) • Linda Van Eldik, PhD \(^1\) • Scott Webster, PhD \(^1\) • D. Martin Watterson, PhD \(^2\) • Adam Bachstetter, PhD \(^1\)

\(^1\)Sanders-Brown Center on Aging, University of Kentucky • \(^2\)Pharmacology, Northwestern University

Closed head traumatic brain injury (TBI) triggers a broad innate immune and acute inflammation response that involves resident glia and other immune cells. Neurologic outcome is dependent on the essential balance between restoration of tissue homeostasis after the initial injury and resolution of the injury-induced innate immune responses, especially those involving the proinflammatory cytokines that are implicated in both aspects of the neurologic outcome balance. Natural resolution of the injury-induced proinflammatory cytokine response such that neurologic sequelae are attenuated is generally not successful. Therefore, therapeutic interventions during critical dosing time windows are needed in order to reduce the dysregulated inflammation that is causally linked to the neuropathologic sequelae. Previous work has generated a causal link between the p38\(\alpha\) mitogen-activated protein kinase (MAPK) mediated intracellular signaling pathway and the injurious proinflammatory cytokine response in neurodegenerative animal models of disease. The recent availability of highly specific \textit{in vivo} molecular probes for p38\(\alpha\) MAPK inhibition allows a more refined \textit{in vivo} analysis of this intracellular signaling pathway and its link to dysregulated glia function and neuroinflammation in TBI with its more rapid pathology progression kinetics. We have recently explored these processes in TBI through the combined use of these \textit{in vivo} p38\(\alpha\) MAPK dynamic molecular probes and genetics based \textit{in vivo} tools, such as targeted knockdown of p38\(\alpha\) MAPK in specific inflammatory cell types. We found that genetic suppression of p38\(\alpha\) MAPK in myeloid cells resulted in less TBI induced deficits in a running wheel behavioral task and cognitive deficits as measured by the radial arm water maze. Suppression of p38\(\alpha\) MAPK activity through selective pharmacological action or through reduction of p38\(\alpha\) MAPK protein levels generated reduction of injury-induced cytokine levels in the brain. The congruence of outcomes from genetic and pharmacological approaches provides a unique battery of outcomes consistent with p38\(\alpha\) MAPK as a potential therapeutic target in TBI.
Addressing innovation in CNS drug discovery: functional or single molecular target approaches to novel compounds that attenuate synaptic dysfunction

Linda Van Eldik, PhD • Ottavio Arancio • Saktimayee Roy • D Martin Watterson, PhD 3
1Sanders-Brown Center on Aging, University of Kentucky • 2Taub Institute, Columbia University • 3Pharmacology, Northwestern University

Faculty

Diverse CNS disorders display common pathophysiology themes. Ongoing efforts to develop disease-modifying therapeutics reflect the increasing appreciation of these core mechanisms. Major challenges include identification of disease progression time windows amenable to intervention, the linkage of disease related phenotypes to druggable molecular targets, and the need for clinical landmarks. Retrospective analyses of new molecular entity drug approvals reveal the differing impact of phenotypic vs single molecular target drug discovery approaches, dependent on the state of knowledge about druggable disease progression mechanisms. The less biased phenotypic approach allows probing for interventions in the absence of established mechanisms in areas of critical unmet medical need, whereas single molecular target approaches provide exceptional efficiencies in areas of critical unmet medical need.

As part of a multi-site collaboratorium focused on innate immunity and synaptic dysfunction as a common pathophysiology progression theme, we explored the potential for novel small molecule drug candidate discovery using these two distinct approaches and a common molecular scaffold or fragment. Specifically, the functional approach focused on early stage overproduction of proinflammatory cytokines causally linked to synaptic dysfunction, and the single molecular target was brain p38αMAPK, an established regulator of innate immunity in glia and an intracellular neuronal kinase up-regulated in stress responses.

Our goal was to start with synthesis of a restricted set of novel small molecules compliant with chemoinformatic and pharmacoinformatic based considerations for brain penetrance and risk reduction and subjected to either:

1. an in vivo screening approach based on disease-relevant pharmacodynamic endpoints (functional approach; up-regulation of proinflammatory cytokine production), or
2. an activity screening based approach based on target structure-assisted design (p38αMAPK).

An aminoarylpypidazine molecular fragment was the common core. Deliverables from each approach used a common secondary phase, pharmacology driven medicinal chemistry refinement. Novel small molecule deliverables from each approach showed efficacy in diverse preclinical animal models. Both showed in vivo attenuation of innate immunity related pathologies and behavioral deficits.

Novel candidates are in early clinical trial stage or IND-enabling preclinical drug development.
Microglia heterogeneity in the hippocampus of Alzheimer’s disease, dementia with Lewy bodies, and hippocampal sclerosis of aging

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Faculty

There is an increasing support for the hypothesis that microglia participate in Alzheimer’s disease (AD) pathogenesis. Despite the extensive neuropathological, genetic, and biochemical characterization of microglia in AD little is known about microglial morphology in other common forms of age-related dementia: particularly, dementia with Lewy bodies (DLB) and hippocampal sclerosis of aging (HS-Aging). The clinical disease formerly referred to simply as “Alzheimer’s disease” is, at the population level, a complex manifestation of many different brain conditions. These age-related brain pathologies include AD (characterized by amyloid plaques and neurofibrillary tangles), as well as cerebrovascular disease, DLB, and HS-Aging. Here we studied cases with pathologically-confirmed AD (n=7), HS-Aging (n=7), AD + HS-Aging (n=4), DLB (n=12), and normal (cognitively intact) controls (NC) (n=9) from the University of Kentucky Alzheimer’s Disease Center autopsy cohort. The Aperio ScanScope digital neuropathological tool was used along with two well-known microglial markers: IBA1 (a marker for both resting and activated microglia) and CD68 (a lysosomal marker in macrophages/microglia associated with phagocytic cells). Hippocampal staining analyses included studies of subregions within the hippocampal formation and nearby white matter. In addition, we defined and quantified, in the CA1 region, five microglia morphological phenotypes in the autopsy samples: ramified, hypertrophic, dystrophic, rod-shaped, and amoeboid. Using these tools and methods, we describe variation in microglial characteristics that show some degree of disease specificity, including, (1) increased microglia density and number in HS-Aging and AD + HS-Aging; (2) low microglia density in DLB; (3) increased number of dystrophic microglia in HS-Aging; and (4) increased proportion of dystrophic to all microglia in DLB. We conclude that variations in morphologies among microglial cells, and cells of macrophage lineage, can help guide future work connecting neuroinflammatory mechanisms with specific neurodegenerative disease subtypes.
Preventing P-glycoprotein degradation lowers amyloid-beta brain levels in an Alzheimer’s disease mouse model

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Failure to clear Aβ from the brain is in part responsible for Aβ brain accumulation in Alzheimer’s disease (AD). A critical protein for clearing Aβ across the blood-brain barrier is the efflux transporter P-glycoprotein (P-gp) in the luminal plasma membrane of the brain capillary endothelium. In AD, P-gp is reduced at the blood-brain barrier, which contributes to Aβ brain accumulation. However, the mechanism responsible for P-gp reduction is poorly understood.

Here we focused on identifying critical mechanistic steps that mediate P-gp reduction in AD. We exposed isolated brain capillaries to 100 nM Aβ40, Aβ42, and their respective reversed peptides. Only Aβ40 triggered reduction of P-gp protein expression and transport activity levels, which occurred in a dose and time-dependent manner. To identify the steps involved in Aβ-mediated P-gp reduction, we inhibited protein ubiquitination, protein trafficking, and the ubiquitin-proteasome system, and monitored P-gp protein expression, transport activity, and P-gp-ubiquitin levels. We observed that exposing brain capillaries to Aβ40 triggered ubiquitination, internalization, and proteasomal degradation of P-gp. To verify our findings in vivo, we used a transgenic Alzheimer’s disease mouse model (Tg2576; human Abeta-overproducing mice) and demonstrated that P-glycoprotein expression and transport activity were substantially reduced in brain capillaries of these mice. Using this Alzheimer’s disease mouse model, we also show in vivo that inhibiting both cellular trafficking and the proteasome prevented P-gp degradation and significantly reduced Abeta brain levels.

Together, our findings provide for the first time a plausible mechanism that mediates degradation of P-gp in AD. Further, they imply a pernicious positive feedback loop where reduced P-gp levels lead to increased Aβ brain accumulation, which in turn drives further P-gp degradation, leading to even greater increases in Aβ brain levels and eventually AD pathology. This scenario could contribute to the progressive nature of AD. In this regard, our data may provide potential therapeutic avenues within the blood-brain barrier to limit P-gp degradation in AD, improve Aβ40 brain clearance, and delay or prevent cognitive impairment.
Preventing P-glycoprotein degradation in an Alzheimer's disease mouse model decreases Aβ accumulation

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Fellow

Decreased accumulation of amyloid-β (Aβ) in the brain is in part responsible for Aβ brain accumulation in Alzheimer's disease (AD). A critical protein for clearing Aβ across the blood-brain barrier is the efflux transporter P-glycoprotein (P-gp), which is expressed on the luminal surface of brain capillary endothelial cells. P-gp expression is reduced at the BBB in AD patients and in AD mouse models, suggesting P-gp could potentially be a therapeutic target in AD.

Our work focuses on two independent strategies: (1) Protecting and (2) restoring P-gp to reduce Aβ brain levels in a transgenic AD mouse model in vivo.

Strategy 1: We targeted the ubiquitin-proteasome degradation pathway to protect brain capillary P-gp from degradation. Dosing hAPP mice with the ubiquitin ligase inhibitor PYR-41 (2 mg/kg, i.p, every 3 days for 2 weeks) restored P-gp protein expression and activity levels, increased P-gp-mediated Aβ transport, and reduced Aβ levels in isolated brain capillaries from hAPP mice to levels observed in wild type mice. These results suggest that inhibiting Aβ-mediated P-gp ubiquitination to limit P-gp degradation is a potential therapeutic strategy to improve Aβ clearance in AD.

Strategy 2: We are currently testing if restoring brain capillary P-gp will delay and improve cognitive impairment in a transgenic AD mouse model in vivo.

Together, these findings may provide two novel strategies for better AD treatment. It remains to be seen if restoring brain capillary P-gp will improve cognitive impairment in AD.
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Photoaffinity probes of the Pittsburgh compound B (PIB) site on Aβ(1-40) fibrils

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Objective
The introduction of the PET imaging agent Pittsburgh Compound B (PIB) in 2004 revolutionized the diagnosis of Alzheimer's disease (AD) by enabling detection of early stage fibrillar β-amyloid brain pathology before clinical symptoms are evident. Both PIB binding and AD are only found in humans, not in animal models of Aβ amyloid disease. Elucidation of the molecular basis for this difference in PIB binding is important to relate human AD Aβ pathology to model systems. We designed and synthesized heteromultifunctional small molecule photo-activated ligands of the PIB binding site to covalently attach to the PIB binding complex. Our molecules contain moieties for crosslinking to nearby components and affinity purification of the adducts.

Methods
The benzothiazole aniline scaffold incorporating a tetrafluoro-aryl azide and substitution on a chalcone scaffold optimized to incorporate an alkyne for click chemistry applications for affinity purification of adducts and a diazirine for photocrosslinking were prepared. A filtration-based 3H-PIB binding competition assay was used to determine the apparent affinity of compounds for human AD tissue and synthetic Aβ fibrils. The photoprobes were prebound to Aβ(1-40) fibrils and their aryl azide or diazirine photochemistry activated by UV light. After removing non-covalently bound ligand with 90% v/v acetone the fibrils were dissociated in 70% v/v formic acid, the formic acid vacuum evaporated, and the peptide separated by 12% acrylamide MES SDS-NuPAGE. Fibril-incorporated ligand was quantified by its intrinsic fluorescence (PIB analog or chalcone) in a fluorimeter or following SDS-PAGE with a Typhoon fluorescence imager (chalcone).

Results
Substitutions on the benzothiazole aniline core of PIB to construct the tetrafluoro-photoreactive azide and the modified chalcone reduced their affinity somewhat for Aβ fibrils and AD brain. The probes covalently incorporated into synthetic Aβ(1-40) fibrils when exposed to UV light. Chalcone titration of Aβ(1-40) fibrils incorporated the photoprobe at the same Aβ:chalcone stoichiometry determined by Scatchard analysis of 3H-PIB binding.

Conclusions
Benzothiazole aniline and chalcone scaffolds that compete for 3H-PIB binding to Aβ can be modified with photoactivatable moieties and covalently incorporated into Aβ(1-40) fibrils in a UV-light-dependent manner with a stoichiometry consistent with that determined by 3H-PIB binding.

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Human ATP-binding cassette transporters in Alzheimer’s disease

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Staff

Alzheimer’s disease (AD) is characterized by accumulation of neurotoxic amyloid-β (Aβ) in the brain, which contributes to neurodegeneration leading to dementia. Recent reports suggest that the blood-brain barrier transporter, P-glycoprotein (P-gp), is involved in clearing Aβ from brain to blood. Our findings suggest that Aβ brain accumulation is, at least in part, due to reduced brain capillary P-gp levels and that upregulating P-gp will enhance Aβ clearance on animal studies. However, it is little known about changes in ATP-Binding Cassette (ABC) transporters expression levels at the human blood-brain barrier during AD. We have established a protocol to isolate brain capillaries from human tissue that can be combined with biochemical and molecular techniques to investigate changes of transporters at the blood-brain barrier in clinical samples with Aβ brain burden, and/or with cognitive decline in AD patients. Our data indicate that protein expression levels of P-gp and breast cancer resistance protein (BCRP) in isolated human brain capillary membranes from AD patients are lower than in those from healthy control individuals. Our primary data suggest that ABC transporters change at the blood-brain barrier in AD patients, which provides a first step in translating our findings from mouse to human.
Amylin vasculopathy: a novel mechanism of cerebrovascular injury and neurologic deficits in diabetes

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Objectives
Human amylin is an amyloidogenic hormone that forms toxic oligomers that kill the insulin-producing β-cells in the pancreas of patients with type-2 diabetes. We recently showed that the pancreatic amylin pathology is also linked with cerebrovascular dementia and diabetic heart disease by increased circulating levels of toxic oligomerized amylin. Here, we tested the hypothesis that the cerebrovascular accumulation of oligomerized amylin injures the brain, leading to neurologic deficits independently of hyperglycemia.

Methods/Results
A diabetic rat model overexpressing amyloidogenic human amylin in the pancreas (the HIP rat) and appropriate controls were used to investigate mechanistically cerebrovascular effects of amylin accumulation. As controls, we employed wildtype (WT) littermates and age- and glucose-matched diabetic rats expressing only non-amyloidogenic WT amylin, which does not accumulate in pancreas or other organs. Compared to controls, HIP rats showed reduced exploratory drive, vestibulomotor performance and recognition memory. Cortical arteries isolated from HIP rats displayed a ~40% higher myogenic tone (P<0.05), which correlates with an increased mean arterial blood pressure by ~20% (P<0.05). We also found elevated lipid peroxidation (by 18±3%; P<0.05) and activated Ca2+-mediated hypertrophy signaling in cortical smooth muscle cells from HIP rats compared to control rats. Serial staining with the ED1 antibody and amylin antibody indicates possible activated microglia/macrophages which are clustering in blood vessel areas positive for amylin infiltration. Multiple inflammatory markers are expressed in HIP rat brains compared to control rats, confirming that amylin deposition induces an inflammatory response.

Conclusions
Overall, our data suggest that cerebrovascular amylin deposition is associated with neurologic deficits via mechanisms of vascular dysfunction, oxidative stress and neuroinflammation.

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Acute intranasal insulin in young and aged F344 rats: signaling and MRI brain changes

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Staff

Objectives
In an attempt to combat decreased insulin signaling in the brain of Alzheimer’s disease (AD) patients, several groups have used intranasal insulin in clinical and pre-clinical settings. Results show improved memory in young volunteers, patients with mild cognitive impairment, as well as in animal models of aging, diabetes or AD. It is not clear how intranasal insulin alters functional communication, improves learning, or facilitates memory retrieval, particularly in animal model of aging.

Methods/Results
Here we characterized the impact of acute (single dose) intranasal insulin across different brain regions at different times following delivery. We tested the impact of Apidra® on 8 different brain regions in young (3-4 months) and aged (21-24 months) F344 rats (n=12/age group). Animals received a unilateral dose of Apidra® (0.0715 IU/rat) or saline. Olfactory bulbs and brains were removed at 30, 60, or 120 min after delivery. Insulin activity was quantified with a focus on the canonical Akt and pAkt protein expression pathway with Western blots. The pAkt/Akt ratio significantly increased at 30m in the right olfactory bulb, which was exposed to insulin, and went down to normal levels by 120 min. Dorsal and ventral signaling increases were noted at 60 and 120 min post-delivery.

Acute Apidra® was also tested on memory retrieval on the Morris water maze in young and aged F344 (n=20/age group) following a single dose. Saline delivery lasted for 8 days with MWM training starting on the fifth day. On the 9th day, half the animals received Apidra®. In a subset of these animals, neurotransmitter levels were determined by MR spectroscopy (MRS) and cerebral blood flow was measured by MRI.

Conclusions
Our work highlights new details on acute intranasal insulin delivery, and in particular its’ impact on memory retrieval compared to acquisition. Surprisingly, chronic low dose Apidra® reduced performance in aged F344 rats. Neither acute nor chronic intranasal delivery of Apidra® improved recall. Because Apidra® is a zinc-free insulin formulation, this may unmask the actions of zinc on memory. This result appears to be well aligned with recent clinical trials showing no memory improvement or impact on cognition. Our test of whether intranasal insulin could increase insulin signaling in the brain of young and aged animals showed increases in the pAkt/Akt ratio approximately 2 hr following delivery. This signaling was particularly more pronounced in the aged animals and to a greater extent, in the ventral rather than the dorsal regions of the brain. Using MRS techniques, we were able to identify potential mechanisms by which chronic intranasal insulin was able to reduce learning in aged animals. Those included a large decrease in N-acetylaspartate signal and a trend for a decrease in myo-Inositol, which are markers of neuronal integrity and glial over activation (inflammation), respectively.

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Amylin proteotoxicity links type-2 diabetes to neuroinflammation

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Potential complications in patients with type-2 diabetes include neuroinflammation and cognitive decline. Proteotoxicity caused by aggregated or damaged proteins and organelles appears as an important contributor in some of the pathogenic pathways for both these complications. However, the possibility of a direct molecular link remained unexplored. Using human tissues, transgenic animals and cell models, we demonstrate that elevated blood levels of amylin, an amyloidogenic hormone that injures the insulin-producing β-cells in type-2 diabetes, promotes accumulation of aggregated amylin in the brain leading to neuron membrane damage, intracellular oxidative stress and IL-1β activation. Thus, we have identified the accumulation of aggregated amylin as a direct and potentially treatable cause of oxidative stress and inflammation in brains of diabetic patients.

Synaptosomes isolated from brain tissue patients with AD Bind the Aβ-specific ligand Pittsburgh Compound B (PIB)

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Objective: Alzheimer’s disease (AD) diagnosis was revolutionized in 2004 by introduction of the PET imaging agent Pittsburgh Compound B (PIB) that enabled detection in vivo fibrillar β-amyloid brain pathology prior to clinical symptoms. AD is only found in humans; no other animal or animal model of Aβ amyloid disease develops AD-like neuronal death and/or dementia. High affinity PIB binding to Aβ pathology is only seen in human brain. Elucidation of the molecular basis for this difference in PIB binding is important for understanding human AD Aβ pathology and absence of this pathology in animal systems.

Recently we observed two distinct species of PIB binding complexes in autopsy AD frontal cortex that can be differentiated by their sensitivity to SDS treatment. While a large proportion of PIB binding in AD brain is SDS-resistant and is correlated with Aβ plaque pathology, a significant fraction of PIB binding remains unaccounted for. Since oligomeric Aβ can interact with synaptic endings and disrupt neuronal synaptic function, we hypothesized that accumulation of Aβ in the synaptic regions of neurons might induce formation of non-plaque PIB binding Aβ complexes.

Methods: Autopsy AD and normal age-matched frozen frontal cortex tissue (6 cases each) obtained through the UKADC Brain Bank was homogenized and fractionated by differential centrifugation and synaptosomes prepared by sucrose density gradient centrifugation. A filtration-based 3H-PIB binding assay was used to quantify PIB binding. Flow cytometry was used to analyze the distribution of 6-CN-PIB (a fluorescent analog of PIB) binding and fluorescently-labeled antibodies for markers of synaptosomes, neuronal types, and glial cell fragments on the individual particles. Displacement of 6-CN-PIB by the non-fluorescent competitor and non-immune IgG were used to control for autofluorescence.

Results: We determined that a substantial fraction of synaptosomal particles isolated from AD autopsy brain tissue bind radioactive 3H-PIB and that 6-CN-PIB efficiently displaced 3H-PIB with high affinity from these particles. Flow cytometry distinguished populations of synaptosomes with different properties, including a substantial fraction of particles that bound 6-CN-PIB colocalized with the synaptosome marker SNAP-25 and the VGlut1 presynaptic glutamate vesicular transporter. A marker of glial cells, GFAP, was absent from these particles.

Conclusions: Synaptosomes from AD brain can be prepared from frozen autopsy AD brain tissue and are suitable for flow cytometry analysis of high affinity fluorescent ligands. A proportion of frontal cortical neuronal synaptosomes from AD brain colocalize 6-CN-PIB binding with VGlut1 vesicular glutamate transporter. CN-PIB-labeled synaptosomes are candidates for flow sorting to purify the PIB-positive and PIB-negative particles of different neuronal types from the mixed population for further analysis.
Influence of chronic restraint (psychosocial stress) on young and aged rats

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Student

Psychosocial stress is a non-painful stimulus associated in humans with major life changes, such as loss of a job or spouse or social isolation, and strongly influences multiple systems (e.g., corticosterone level, body temperature regulation, sleep and cognition). There is an increased likelihood of experiencing chronic psychosocial stress with age, and the negative consequences to that exposure are more severe. Despite this, little work has investigated mechanistic changes with age in the chronic psychosocial stress response. We hypothesize that, compared to young, aged subjects will have worsened outcomes. To test this, young (3 mos) and aged (19 mos) male Fischer 344 rats were assigned to control or psychosocial stress groups and implanted with wireless telemetry from DSI (Data Sciences International) to monitor sleep architecture and body temperature. Chronic psychosocial stress (restraint, 3 h/ day, 4 days/ week, 4 weeks) effects on Morris water maze and body temperature are reported.

White matter microstructure in a tractography-derived fornix template is associated with neuropathological markers of Alzheimer's disease

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Alzheimer’s disease (AD) has a prolonged preclinical stage, during which pathological proteins accumulate and alterations to brain structure and function begin to appear. Recent evidence suggests that, along with amyloid deposition, decline in white matter microstructure in the fornix may be an early preclinical process. The fornix is the primary white matter tract responsible for hippocampal output, and declines in white matter microstructure in this tract appear to precede hippocampal atrophy. However, widely available atlases of the fornix include only subsections of the tract, typically in the form of multiple submasks, limiting the ability and simplicity of assessing white matter microstructure in the fornix as a continuous structure. In the present study, a new fornix template was developed using probabilistic tractography in adults ranging from 25-77 years old and then applied to an independent cohort of cognitively normal older adults (ages 65-92) to investigate the relationship between white matter microstructure and cerebrospinal fluid (CSF) biomarkers of preclinical AD. Ninety-five adults ranging from 25-77 years old underwent diffusion tensor imaging, which was used to perform probabilistic tractography. Tractography used hippocampal seeds and waypoint masks based on the current JHU-ICBM White Matter Labels Atlas to develop a continuous fornix from the fimbria through the body. This fornix template was applied to a separate cohort of 34 cognitively normal older adults for whom CSF levels of β-amyloid (Aβ42), total tau, and phosphorylated tau (p-tau181) were available. Correlation analyses revealed that fractional anisotropy (FA) in the fornix template was positively correlated with CSF Aβ42 (r = 0.46, p = 0.008), negatively correlated with CSF p-tau181/Aβ42 ratio (r = -0.38, p = .03), and marginally negatively correlated with CSF total tau (r = -0.30, p = 0.09) when controlling for age and sex. In contrast, FA in the current (JHU-ICBM) labels atlas had only marginal positive correlation with CSF Aβ42 levels (r = 0.32, p = 0.07) and had no correlation with other CSF markers (p > 0.22) when controlling for age and sex. Importantly, a global FA measure had no relationship with any CSF marker (p > 0.14). These findings indicate that there is a relationship between lower white matter microstructure in the fornix and early markers of Alzheimer’s disease, particularly increasing Aβ42 burden. The new fornix template provides a valid and sensitive tool for measuring age-related alterations to white matter microstructure. The new fornix mask may also provide an additional neuroimaging biomarker of preclinical AD pathology for use in future research.
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Acute upregulation of α5β1 Integrin destabilizes the blood-brain barrier after stroke
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Stroke is the fifth leading cause of death in the U.S., with a person dying of stroke every 4 minutes. Repeated opening of the blood-brain barrier (BBB) after stroke significantly contributes to and exacerbates injury. As the β1 integrin receptor family has recently been linked to changes in BBB permeability via changes to tight junction protein expression and function, we hypothesized that one particular β1 integrin subtype, α5β1, a pro-angiogenic fibronectin receptor that is predominantly expressed in brain vasculature during brain development, is acutely upregulated after ischemic stroke and contributes to BBB breakdown via affects on the localization and expression of the tight junction protein, claudin-5. First, α5β1 brain immunohistochemistry after transient middle cerebral artery occlusion in mice demonstrated a luminal increase in core infarct and peri-infarct vascular expression of α5β1 as early as 24 hours after stroke that became even more prominent by post stroke day 2. Next, in vitro studies where stroke was simulated using oxygen and glucose deprivation or TNF-α treatment demonstrated an increase in barrier permeability, as measured by trans-endothelial cell electrical resistance, FITC-dextran permeability, and immunocytochemistry, that correlated with an increase in α5β1 expression and changes in claudin-5 expression and localization away from the cell-surface (i.e. away from tight junction complexes). Importantly, this increased permeability could be completely prevented by addition of the α5β1 inhibitor ATN-161. Furthermore, ATN-161 treatment significantly reduced infarct volume in wild-type mice, and mice with α5 integrin selectively knocked out in endothelial cells had little to no infarct with an intact BBB. Collectively, our results demonstrate that endothelial cell α5β1 integrin expression is increased acutely after stroke, may contribute to BBB breakdown and subsequent expansion of brain injury, and therefore could represent a novel stroke therapeutic target worthy of further investigation.

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Quantification of global and loci-specific cytosine modifications in Alzheimer’s disease
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Student

DNA methylation of the fifth position of cytosine is a key epigenetic modification regulating gene transcription. Although epigenetic modifications to cytosine have been extensively studied in embryonic development and cancer, there has been little study of epigenetics as it relates to neurodegeneration, particularly Alzheimer’s disease (AD). Analysis of global cytosine modifications, as well as loci-specific levels of cytosine, 5-methylcytosine (5-mC), and 5-hydroxymethylcytosine (5-hmC), could provide a more comprehensive picture of epigenetic modification as it relates to AD. To determine global cytosine modification profiles in the brain of AD and age-matched normal control (NC) subjects, a GC/MS method using stable labeled standards of cytosine, 5-mC, and 5-hmC was developed. Global levels of epigenetic marks were quantified in vulnerable (superior and middle temporal gyrus (SMTG), hippocampus/parahippocampal gyrus (HPG), and inferior parietal (IP)) and non-vulnerable (cerebellum (CER)) brain regions of AD and NC subjects, expressed as percent of control values. Loci-specific levels of cytosine, 5-mC, and 5-hmC modifications in Exon 4 of PS1 were determined using a glucosylation assay coupled with quantitative polymerase chain reaction (qPCR) in nuclear DNA samples isolated from the HPG of AD and NC subjects. To correlate epigenetic modification to transcriptional regulation, changes in protein levels were determined using Western blot analysis. Our data show significantly altered loci-specific levels of epigenetic marks in PS1 in the HPG of AD subjects compared to NC subjects, as well as altered global levels of 5-mC in the SMTG region in AD vs NC. Collectively, these studies suggest epigenetic modifications to cytosine may be associated with AD and potentially play a role in neurodegeneration and dysfunction.
Diabetes and the brain; a comparative test of brain amylin levels in diabetic patients with late-onset AD vs. non-diabetics with early-onset AD

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Background
We recently suggested that type-2 diabetes may accelerate the development of Alzheimer’s disease (AD) by promoting brain deposition of amylin, a pancreatic hormone co-secreted with insulin that has amyloidogenic properties similar to β-amyloid (Aβ). However, aging may independently contribute to the brain amylin accumulation in humans. We, therefore, expected that individuals suffering from early-onset (familial) AD will show no significant brain accumulation of amylin.

Methods
Amylin was measured in supernatant fraction of temporal cortex from healthy individuals (controls; Ctl group; N=12), presenilin or amyloid precursor protein mutation carriers without diabetes (early-onset familial AD; F-AD group; N=6), non-diabetics with late-onset AD (NDi-De group; N=20), and diabetic patients with late-onset AD (Di-De group; N=23) by ELISA and western blot. Tissue pellets were treated with formic acid, freeze dried and then the resulting powders were re-suspended in guanidine hydrochloride for dot blot analysis.

Results
Brains in F-AD and Ctl groups showed comparable levels of soluble fractions of amylin by ELISA. Amylin oligomer size distributions were also similar in F-AD and Ctl groups. Only the molecular weight band at ~36 kDa appeared elevated (by ~70%; P = 0.07) in the F-AD group. Compared to controls, the total soluble fraction of amylin was significantly increased in brains from the Di-De group (by ~145%; P < 0.001). The result correlated with significantly elevated amylin oligomer levels in the 8 kDa ÷ 50 kDa domain. In contrast, brains from the NDi-De group displayed only slightly increased amylin level (by ~32%; P = 0.11) versus controls. The amylin oligomer at ~24 kDa (amylin hexamer) appears significantly elevated (by ~40%; P < 0.05) in the NDi-De group versus Ctl group. Dot blot analysis showed more than 300% (P<0.001) increased amylin levels in post-treatment vs. pre-treatment samples in all groups. The dot blot test suggests that large amylin aggregates fragmented into small oligomers that were recognized by the anti-amylin antibody.

Conclusions
Diabetes is the most significant source of amylin deposition in the brain. Further analysis is required to elucidate whether aging may contribute to a steady accumulation of amylin in the brain.
Perlecan LG3 is neuroprotective when administered intra-arterially as an acute treatment after stroke

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Despite recent advances in stroke therapy, ischemic stroke remains a leading cause of morbidity and mortality. We have previously demonstrated the therapeutic potential of exploiting the brain’s endogenous protection and repair processes following ischemic stroke. Specifically, we reported that the LG3 protein fragment of the heparan sulfate proteoglycan perlecan is neuroprotective and proangiogenic in vitro. Therefore, we now hypothesize that LG3 could be therapeutic in experimental stroke. However, as previous in vivo LG3 studies suggest that it may not readily target stroke-affected brain upon systemic administration, we administered LG3 in a super selective intra-arterial (IA) model following our transient focal ischemic model of stroke in mice. 3-month-old male C57/Bl6 mice underwent transient CCA and MCA occlusion for one hour. Upon reperfusion the mice received LG3 or Vehicle controls via IP or IA administration. All vitals were closely monitored via the MouseOx system and showed no difference between the treatment groups. We observed that the mice receiving LG3 in either route of administration had a smaller infarct than those receiving the vehicle. Importantly, those mice that received the LG3 IA, had significantly smaller strokes and performed significantly better in forced movement and in free movement settings than all other treatment conditions. Currently, our lab is expanding upon this novel observation by determining the mechanism by which it occurs. We believe that by administering this endogenous protein super-selectively, we can mitigate infarct expansion and jumpstart the brain’s natural recovery processes.
Listeria monocytogenes: how specific defects in innate immunity influence susceptibility in vulnerable populations

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Microbiology, Immunology, and Molecular Genetics, University of Kentucky

Student

Background
Listeria monocytogenes (Lm) is a foodborne pathogen and the causative agent of listeriosis, a disease that may present with symptoms ranging from mild gastrointestinal distress to septicemia and meningitis. Although any person with some level of immune deficit is at risk, nearly 60% of all Lm infections occur in adults age 65 and older. It remains to be determined, however, whether it is a lack of specific immune function or an inappropriate immune response that contributes to the susceptibility of aged individuals to Lm infection.

Objectives
The objective of these studies is to discern how differences in innate immunity contribute to susceptibility to Lm infection. Interferon (IFN) α/β signaling has been proposed to shift immunity during Lm infection toward a quiescent state. Prostaglandin E2 (PGE2), which naturally increases in humans with age, is also thought to play an immunosuppressive role in the response to Lm. The contribution of both to the establishment of Lm infection was tested.

Methods
To study the IFNα/β response to infection, mice were fed Lm. IFNα/β secretion and the downstream effects of IFNα/β signaling were monitored in the spleen.

Secretion of PGE2 by cells recruited to the liver following infection was examined by isolating the mononuclear cell fraction from the livers of infected BALBcBy/J and C57BL/6 mice. These cells were cultured with and without exogenous stimulation to allow for secretion of PGE2 into the supernatant.

Results
IFNα/β secretion following foodborne Lm infection was not sufficient to induce a loss of TCRβ+ lymphocytes, increases in IL-10, or modulation of neutrophil recruitment to the spleen. Contradicting previous reports, expression of IFNGR1 was not dependent on IFNα/β signaling.

Lm infection induced a significant monocyte infiltrate in the livers of both strains of mice used. Cultured mononuclear cells obtained from BALBcBy/J mice secreted significantly more PGE2 into the supernatant following stimulation than did cultured cells from C57BL/6 mice.

Conclusions
IFNα/β secretion in the spleen is unlikely to play a significant role in overall susceptibility to Lm infection. The cellular infiltrate in the livers of susceptible BALBcBy/J mice appears to be primed to secrete more PGE2 than resistant C57BL/6 mice in response to stimulation. This may prevent the activation of immune cells responsible for killing Lm and contribute to differences in susceptibility to infection.

This work was supported by National Institutes of Health grant AI101373 (to S.E.F.D.)
Alzheimer’s biomarkers correlate with brain connectivity differentially during rest and task but not with cognition in older adults

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Facility

β-amyloid (Aβ) plaques and tau-related neurodegeneration are pathologic hallmarks of Alzheimer’s disease (AD). Thus far, the utility of AD biomarkers, including those measured in cerebrospinal fluid (CSF), in predicting future AD risk and cognitive decline is still being refined. By examining the complex relationship between cortical default-mode network (DMN) connectivity, age, CSF biomarkers (Aβ42 and pTau181), and cognitive status in older adults, we tested the hypothesis that DMN connectivity in resting- and task-driven states detect differential network alterations due to subclinical AD pathology. A novel time series based measure of functional connectivity, i.e., Total Interdependence, was also applied. In our sample of 27 cognitively intact older adults with cognitive status within normal limits, no significant associations were found between levels of Aβ42 or pTau181, and cognitive scores or regional brain volumes. Assessing functional connectivity among the cortical regions in DMN during both resting-state and a short-term memory task, we report several new findings. First, increased connectivity of bilateral anterior middle temporal gyri is positively correlated with higher level of CSF Aβ42 and Aβ42/pTau181 ratio during rest or task, indicating lower β-Amyloid deposition. Second, increased bilateral parietal connectivity during the short-term memory task, but not during rest, correlates with higher level of CSF pTau181, indicating increased risk of neurodegeneration. Third, increased connectivity between left middle temporal and left parietal cortices during task is associated with decreased global cognitive status, but not linked to with CSF biomarkers. Lastly, we found that our new method was more sensitive to the CSF Aβ42-connectivity relationship, while the traditional cross correlation method is more significant to levels of CSF pTau181 and cognitive status. These findings indicate that resting-state and task-related connectivity can be used as complementary neuroimaging measures as differential non-invasive neuroimaging markers that correlate with Aβ burden, neurodegeneration in the brain, and cognitive status in cognitively normal older individuals.
From bench to bedside, the re-evaluation of verapamil as a therapeutic option for acute ischemic stroke

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Student

As thrombectomy is now a standard of care for emergent large vessel occlusion (ELVO), there is a new opportunity to explore potential adjunctive neuroprotective compounds that can be administered following recanalization. Verapamil, a calcium channel blocker, has a well-documented history of being used as a vasodilator intracranially, but more recent data suggests a neuroprotective effect as well. We evaluated the efficacy of verapamil administration following ELVO in a bench to bedside approach. We have incorporated two models of stroke in conjunction with a Phase I/II clinical trial; *in vitro* oxygen glucose deprivation (OGD), *in vivo* tandem transient middle cerebral common carotid artery occlusion (MCAo) and Phase I/II clinical trials to determine verapamil’s neuroprotective effects. Starting *in vitro*, with OGD studies we exposed C57 endothelial cells, differentiated PC-12 and neurospheres to OGD (1, 2, 3, 4, 5 and 8 hour) conditions followed by 15 minute reperfusion with verapamil (0.15, 0.20, 0.25, 0.32, and 0.5µg/ml) treatment followed by 24 hour reperfusion. Results from cell viability assays (MTT/Trypan Blue) showed a significant (P < 0.05) increase in cellular activity for those cells treated with verapamil when compared to control (media only).

Second, *in vivo* studies of C57/Bl6 male mice undergoing MCAo for 60 minutes followed by intra-arterial (IA) administration of verapamil (2.5mg/kg at a dose of 2.5µl/min and volume of 10µl) demonstrated significant (P < 0.05) reduction in infarct volume, astrocyte activation and apoptosis with a significant (P < 0.05) increase in mature neuron survival. Functional measures of forced motor and free roam movement showed significant (P < 0.05) differences on post-stroke day (PSD) 1, 3 and 5 for rotor rod and PSD 7 for open field between treated and control.

Finally, we recently concluded enrollment on a Phase I safety study of IA verapamil immediately following thrombectomy in 11 ELVO patients. The primary outcome measure of safety (no significant intracranial hemorrhage) was achieved. Therefore, intra-arterial verapamil represents a feasible, safe, and possibly therapeutic adjunct to thrombectomy in stroke. Further translational evaluation including dose-response and toxicity studies in this modality are planned and underway.
Objective
No treatments exist for the impairments in cognition that occur in many patients with Parkinson’s disease (PD). A loss of cholinergic cell function in the basal forebrain, specifically the nucleus basalis of Meynert (NBM) in PD is associated with cognitive decline, gait dysfunction, and increased incidence of falls. Results from multiple previous non-clinical, pre-clinical, and early clinical trials show that neurotrophic factors help restore cell function. While direct delivery of neurotrophic factor(s) is complicated by complex technical and regulatory issues, a potential alternative source of neurotrophic factors is the Schwann cell from the peripheral nervous system. After injury, Schwann cells release several growth factors including NGF, NT-3, BDNF, and GDNF. We hypothesize neurotrophic factors may help slow the degeneration and/or restore function in the cholinergic cells of the NBM. A Phase I clinical trial [NCT01833364] involving delivery of peripheral nerve tissue to the substantia nigra in conjunction with deep brain stimulation (DBS) therapy to patients with PD has shown the treatment to be safe and feasible. We plan to complete a Phase I clinical trial [NCT02369003] to assess the safety and feasibility of simultaneous bilateral DBS of the globus pallidus interna (GPI) and unilateral peripheral nerve grafting to the NBM.

Methods/Results
Six participants diagnosed with PD for at least 5 years and who have been selected for and consented to DBS of the GPI will receive an autologous, sural nerve graft implanted unilaterally into the NBM during DBS surgery. DBS will be programmed to optimize treatment of PD symptoms, and adverse events will be continuously monitored. Evaluations for clinical efficacy will be based on neuropsychological evaluations, gait analysis, UPDRS evaluations, as well as non-motor symptom and quality of life assessments. These will also be measured pre-operatively and at 6 and 12 months after surgery. Our first study participant has demonstrated marked improvement in executive function, language, and psychomotor speed at his first follow up assessment 3 months after surgery. No adverse effects due to graft implantation have been noted thus far, and we are continuing to optimize his DBS programming.

Conclusions
Initial results from our first study participant are promising. Five other participants will also be enrolled in the trial and are currently undergoing pre-operative evaluations. Anticipated positive outcomes from this study will provide the foundation for a Phase II study to determine the benefit to patients with PD who have cognitive decline, gait disturbances, or increased incidences of falls.

Grant acknowledgments/Disclosures
Support provided by gifts to the Brain Restoration Center, Tom Dupree for Parkinson's disease research, University of Kentucky start-up funds (CVH), National Center for Advancing Translational Sciences through grant UL1TR000117, and educational support grants from Medtronic (CVH).
Examining hippocampal sclerosis of aging genes: ABCC9, GRN, KCNMB2 and TMEM106B

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1 Biostatistics, University of Kentucky • 2 Biomedical Informatics, University of Kentucky • 3 Pathology, University of Kentucky

Student

Objectives
Hippocampal sclerosis of aging (HS-aging), a common disease among aged individuals, leads to dementia similar to Alzheimer’s disease, but exhibits distinct neuropathological properties. Recent studies have implicated four risk genes for HS-aging: KCNMB2, TMEM106B, ABCC9 and GRN. To learn more about genetic association with HS-aging, we conducted gene-based association testing for those risk genes. We also applied haplotype analysis to the identified intronic region of the ABCC9 gene for the SNPs with smaller p-values.

Methods
Genetic data were obtained from Alzheimer’s Disease Genetics Consortium (ADGC) database, and neuropathological data were obtained from National Alzheimer’s Coordinating Center (NACC) database. 3,730 subjects with genotype and autopsy information were available. After applying inclusion and exclusion criteria and quality control filtering, data from 3,253 subjects were used in the analyses. To generate SNP-based association p-values, we used logistic regression analysis assuming an additive model of inheritance adjusted for age at death, sex and the top 3 principal components. Gene-based association analysis was subsequently conducted by combining the p-values of SNPs within each gene using the gene-based association test using extended Simes procedure (GATES). In the haplotype analysis for the intronic region on the ABCC9 gene, we phased haplotypes using an EM algorithm and performed haplotype score test adjusted for the same covariates.

Results
Of 3,253 subjects, 267 (8.2%) were HS-aging cases, and 1,663 (51.1%) were females. The mean age at death was 80.8 ± 8.9 years (84.7 ± 8.3 in cases and 80.4 ± 8.8 in controls). The highest association signal came from a SNP on the ABCC9 gene (rs829080; p=2.86 × 10^-5). Three of the four genes had significant gene-based association with HS-aging (KCNMB2: p=0.005, TMEM106B: p=0.003, ABCC9: p=0.002). For 6 tag SNPs in the ABCC9 region, the most frequent haplotypes were CCAGCC (36.8% overall, 44.0% in cases, and 36.2% in controls), TAGGTT (23.1% overall, 20.3% in cases, and 23.4% in controls), TAGATT (17.0% overall, 11.3% in cases, and 17.5% in controls). The first and third common haplotypes were significantly associated with HS-aging (p<0.001), but the second one was not (p=0.110).

Conclusion
The KCNMB2, ABCC9, and TMEM106B genes were detected to have gene-based associations with HS-aging. In the haplotype analysis for the region of the ABCC9 gene, we found that the haplotype CCAGCCG had risk effect and TAGATTG had protective effect on HS-aging. The p-value of global score test was very similar with the p-value of gene-based association test for the ABCC9 gene, suggesting the haplotype association in the region would capture the gene-based association with HS-aging. In the future studies, we need to elucidate the biological roles of these genes and the region on the ABCC9 gene.

This research was partially supported by K25 AG043546, P30 AG028383.
Pathological tau promotes neuronal damage by impairing ribosomal function and decreasing protein synthesis

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Student

One of the most common symptoms of Alzheimer’s disease (AD) and other related tauopathies is memory loss. The exact mechanisms leading to memory loss in tauopathies are not yet known; however, decreased translation due to ribosomal dysfunction has been implicated as a part of this process. Here we use a proteomics approach that incorporates sub-cellular fractionation and co-immunoprecipitation of tau from human AD and non-demented control brains. We show that ribosomes associate more closely with tau in AD than with tau in control brains, and that this abnormal association leads to a decrease in RNA translation. The aberrant tau-ribosome association also impaired synthesis of the synaptic protein PSD-95, suggesting that this phenomenon contributes to synaptic dysfunction. These findings provide novel information about tau-protein interactions in human brains, and they describe, for the first time, a dysfunctional consequence of tau-ribosome associations that directly alters protein synthesis.

Chemotherapy induced early-onset dementia: concerns underlying myth

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Staff

Hypothesis: We hypothesize that the combination of lowering cognitive reserve from chemotherapy treatment with usual risks associated with advanced age will result in early onset dementia in cancer survivors. This will be a significant burden to the society and their family. The field is actively engaged in assessing if there is cognitive decline during chemotherapy, producing ‘chemo-brain’ in the short-term, with little investigation on the long-term effects of treatment.

Background: Administration of cytotoxic chemotherapy produces many immediate side effects that are commonly associated with cancer treatment and include fatigue, alopecia, nausea and vomiting. Long term sequelae from chemotherapy are both less known and investigated. As the cancer survivor population continues to grow, one of the worrisome side-effects emerging is cognitive decline. In the short-term, patients may experience ‘chemo-brain’ or ‘chemo-fog’; this side effect is not unexpected as numerous chemotherapy agents cross the blood-brain-barrier (BBB).

Epidemiology: In the United States, the 65 years and older age group population is estimated to double to greater than 70 million in 2030. This population is susceptible to both cancer (the over 65 age group has 50% of all cancers and 70% of cancer mortality) and dementia (prevalence of dementia among those over 71 years of age is estimated to be 14% in the United States in 2007 and is expected to rise).

According to the American Cancer Society, the estimated 5-year relative survival rate for all cancers diagnosed between 2004 and 2010 was 68%, an increase from 55% in 1987-1989 (ACS). According to the ACS in collaboration with the National Cancer Institute (NCI) there are 14.5 million cancer survivors alive today and the number is expected to rise to 19 million by the year 2024.

Conclusions: Wide adoption of chemotherapy for adjuvant therapy is thought to be a major cause of this increase in cancer survival. Chemotherapy treatment frequently produces pleiotropic side-effects, and the brain is often sensitive to toxicity. Although chemotherapy treatment may initially be clinically silent, it may lower cognitive reserve and with advancing age result in early-onset dementia. Patient advocacy groups must engage their physicians on these concerns to spur more research.
Brain region specific changes in gene expression following environmental enrichment in aged female rats

Katherine Murray, MS • Jenne Westberry, PhD • Melinda Wilson, PhD
Physiology, University of Kentucky

Staff

Objectives
Estrogen plays an important role in maintaining cognitive function as females age. After menopause there is a steep drop in the level of estrogen and alterations in the expression of estrogen receptors in some areas of the brain. Hormone replacement therapy (HRT) has mixed results in alleviating the symptoms of menopause, along with having a variety of negative side effects. Alternatively, exposure to enriched conditions can increase spatial reference memory in rats, and lowers the risk of dementia in the elderly. DNA methyltransferases (DNMTs) are responsible for maintaining and creating new DNA methylation of promoters in mammalian cells and regulating gene expression. By using environmental enrichment we hypothesized we could alter the levels of DNMTs in order to allow for the maintenance of estrogen receptor expression in the brain.

Methods and Results
Three brain areas were chosen for study: Prefrontal cortex (PFC), hippocampus, and cerebellum. 12-14 month old female Sprague-Dawley rats were singly housed (n=6) kept in standard housing (2/cage; n = 6) or housed in an enriched environment (2/cage; n = 14) for 11 weeks. Toys were rotated out every other weekday and rats were handled every weekday afternoon. After 11 weeks the rats were sacrificed and brains removed and flash frozen to examine gene expression. Real time PCR was done to measure gene changes in DNMT1, DNMT3a, ERalpha, ERbeta and BDNF mRNA. There was a significant decrease in ERbeta (P = 0.043), and an increase in both DNMT1 (P=0.001), and DNMT3a (P=0.001) in the cerebellum with enrichment. There was also a significant increase in BDNF (P=0.018). The hippocampus exhibited no significant changes. The PFC showed a significant decrease in ERbeta as well (P=0.042), with only a trend in DNMT1 towards a significant decrease (P=0.066).

Conclusions
Our findings show that environmental enrichment does cause changes in gene expression in the brain. The changes are also region specific. Our hypothesis was disproven, showing that estrogen receptors instead decreased after exposure to environmental enrichment despite a preliminary experiment indicating otherwise in estrogen receptors. A possible explanation could be a matter of timing. An experiment containing both shorter and longer time trials could be performed. Our study shows with just small daily changes over a period of time, dramatic changes in gene expression occur in genes that are involved in regulating many other genes. Theoretically this implementation of environmental enrichment could provide a simple intervention for post-menopausal females who choose not to have, or cannot have, HRT.

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38 ORE CORE
COMMUNITY SESSION

Location: Bluegrass Ballroom, Lexington Convention Center, 430 W Vine, Lexington, KY

8:30 am  Check-in and Continental Breakfast Buffet

9:00  Welcome and Introductions
Linda J. Van Eldik, PhD
Director, Sanders-Brown Center on Aging and Alzheimer’s Disease Center
University of Kentucky

9:15  How Early Can We Diagnose Alzheimer’s Disease?
Ronald C. Petersen, MD, PhD
Director, Mayo Clinic Alzheimer’s Disease Research Center &
the Mayo Clinic Study on Aging, Professor of Neurology, Mayo Clinic College of Medicine,
Rochester MN

10:15  Break

10:30  Sanders-Brown Center on Aging Faculty Research Highlights and Audience Q & A
Frederick Schmitt, PhD  Moderator
Erin Abner, PhD  PREADVISE Trial
Donna Wilcock, PhD  CVD and Mixed Dementias
Gregory Jicha, MD, PhD  Clinical Prevention Trials
Stephen Scheff, PhD  Early Synaptic Changes

12:00  Closing Remarks
Linda J. Van Eldik, PhD
Ronald Petersen, MD, PhD
Director, Mayo Alzheimer’s Disease Research Center
and the Mayo Clinic Study of Aging
Professor of Neurology, Mayo Clinic College of Medicine

Dr. Ronald Petersen is a national leader in the field of Alzheimer’s research. He is the director of the Mayo Alzheimer’s Disease Research Center and the Mayo Clinic Study on Aging. He has authored over 550 peer-reviewed articles and edited five books on memory disorders, aging, and Alzheimer’s disease. Dr. Petersen received his Ph.D. in Experimental Psychology from the University of Minnesota and graduated from Mayo Medical School in 1980. He joined the staff of the Mayo Clinic in 1986. He became the Cora Kanow Professor of Alzheimer’s Disease Research in 2000, and was named the Mayo Clinic Distinguished Investigator in 2011.

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. He also received the inaugural Ronald and Nancy Reagan Research Institute Award in 2004 from the Alzheimer’s Association and the inaugural Leon Thal Prize of the Lou Ruvo Brain Institute in 2007. In 2012, he received the Khachaturian award of the Alzheimer’s Association and the Henry Wisniewski Lifetime Achievement Award in 2013.

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 Project Act and was appointed to the World Dementia Council in 2014 by UK Prime Minister David Cameron.
Fred Schmitt is a Professor of Neurology, Psychiatry, Psychology, Behavioral Sciences, Spinal Cord and Brain Injury Research Center, Neurosurgery, and Sanders-Brown Center on Aging. Dr. Schmitt is the Neuropsychology section chief in the Department of Neurology and Kentucky Neurosciences Institute. Throughout his career, Dr. Schmitt has developed and evaluated statistical and cognitive methods for the early detection of Alzheimer’s disease and other dementias — and for the statistical assessment of various treatment interventions. A prolific applied researcher with over 200 publications. Dr. Schmitt’s research interests are in the areas of HIV dementia, Alzheimer's disease, Down syndrome, life-span neurocognition, clinical trial assessments and biostatistics. His current NIH-funded research includes studies of dementia prevention, statistical models of dementia risk and associated neuropathology, and the evolution of Alzheimer’s disease in Down syndrome.

**“PREADVISE Trial”**

Dr. Abner is an Assistant Professor in the Department of Epidemiology in the College of Public Health, the Sanders-Brown Center on Aging, and in the Graduate Center for Gerontology. Dr. Abner’s current research interests include clinicopathological correlations, the use of Markov chains in longitudinal data analysis, practice effects in cognitive screening instruments, and the relationship between cognitive impairment and self-rated quality of life. She focuses in the clinical area of Alzheimer’s disease and associated disorders and has a general interest in aging research.

**“CVD and Mixed Dementias”**

Donna M. Wilcock, PhD is the Sweeney-Nelms Endowed Professor in the Department of Physiology and Sanders-Brown Center on Aging. Dr. Wilcock has an active research program focusing on the role of inflammation in Alzheimer’s disease and vascular dementia. Her research is focused in vascular dementia; the second most common cause of dementia behind Alzheimer's disease. In addition to being a major cause of dementia, Alzheimer's disease patients commonly have vascular dementia as a co-morbidity. She has projects to examine the molecular mechanisms of vascular dementia, focusing primarily on inflammatory processes. Dr. Wilcock also has projects that determine the influence vascular dementia has on the progression and severity of Alzheimer's disease, as well as how vascular dementia affects response to Alzheimer's disease targeted therapeutics. In addition, Wilcock has a project in collaboration with Elizabeth Head of Pharmacology & Nutritional Sciences and Frederick Schmitt of Neurology to assess neuroinflammatory changes in Down syndrome. Her research is currently funded by the National Institutes of Health and the Alzheimer’s Association.
“Clinical Prevention Trials”

**Gregory Jicha, MD, PhD**  
University of Kentucky

Greg Jicha is an Associate Professor in the Department of Neurology and Sanders-Brown Center on Aging. Dr. Jicha serves on the Executive Committee and is the Director of the Clinical Core of the NIA-funded UK Alzheimer’s Disease Center. He also directs the Telemedicine Cognitive Clinic at UK, designed to reach out to rural populations across KY for both clinical and research-related activities in the area of Alzheimer’s disease (AD) and related disorders.

Dr. Jicha holds the Robert T & Nyles Y McCowan Endowed Chair in Alzheimer’s Research at UK. His current research interests lie in the areas of mild cognitive impairment, clinico-pathological correlations in early preclinical disease states, and clinical trials of disease-modifying therapies for AD. He is the principal investigator at UK for the National Alzheimer’s Disease Cooperative Study Group and also serves on the Clinical Task Force and Steering Committee for the National Institute of Aging Alzheimer’s Disease Center Program.

“Early Synaptic Changes”

**Stephen Scheff, PhD**  
University of Kentucky

Dr. Scheff is a Professor of Anatomy and Neurobiology and Associate Director of the Sanders-Brown Center on Aging. He holds the Mansbach Endowed Chair in Alzheimer’s Disease. Dr. Scheff is involved in numerous projects which relate to normal aging and Alzheimer’s disease. His research involves studying changes in brain connectivity in the very early stages of the disease process and how this relates to oxidative stress. In addition, his laboratory studies the capacity of the brain to respond to traumatic injury. By employing animal models of cortical contusion, it is possible to assess specific cellular and molecular events which can be manipulated to improve functional outcome. These experiments are multidisciplinary in their approach and utilize numerous morphologic, neurochemical, cellular and molecular techniques to probe some of the mechanisms behind the compensatory process.
The Sanders-Brown Center on Aging (SBCoA) was established in 1979, and received funding as one of the original ten National Institutes of Health Alzheimer’s Disease Centers in 1985. Internationally acclaimed, the SBCoA is recognized for its contributions to the fight against brain diseases that are associated with aging.

Our vision: The University of Kentucky Sanders-Brown Center on Aging will be recognized locally and nationally as a premier, vitally productive and innovative aging center that effectively translates research findings into interventions and information that will benefit older adults.

“I spent more than 50 years in health care and know the difference that research has made in our lives.” – Mrs. Doris Engles (with her husband Morris), one of our healthy research volunteers, describes why she supports the Sanders-Brown Center on Aging, through active research involvement.

ALZHEIMER’S DISEASE FACTS

- Someone in the US develops Alzheimer’s disease every 67 seconds.
- Alzheimer’s disease is the 6th leading cause of death across all ages in the USA, and the 5th leading cause of death for those aged 65 and older.
- In 2015, 68,000 people (age 65 or older) in Kentucky were living with Alzheimer’s disease.
- An estimated 5.3 million persons in the U.S. have Alzheimer’s disease.
- By 2050, as many as 16 million Americans will have Alzheimer’s disease, and a new case will be diagnosed every 33 seconds.
More than 100 faculty and staff pursuing the following areas of research:

- Basic and clinical research in Alzheimer’s disease
- Neurodegenerative disorders
- Stroke
- Normal brain aging

A global pioneer in Alzheimer’s disease research, the Center has over thirty years of published work and 800 study volunteers (some with the disease and some without). These individuals are studied over time and plan to donate their brains upon death. Our cutting-edge research focuses on identifying problems as early as possible, before memory loss develops, so that Alzheimer’s disease can be prevented or delayed.

The ultimate goal of the Center on Aging is to catalyze innovative and outstanding brain research while ensuring a more rapid rate of progress toward new therapies to delay or prevent age-related brain diseases such as Alzheimer’s disease, so that our volunteers, patients and caregivers become the beneficiaries of our advances in knowledge.

Unless science finds a way to slow the progression of this devastating disease, the United States will see a nearly 50 percent increase in the number of victims by 2030. In addition to the direct impact on the patient, Alzheimer’s disease also affects the lives of family members and friends.

The Center is directed by Linda J. Van Eldik, PhD, Professor, Department of Anatomy and Neurobiology. Associate Director is Stephen W. Scheff, PhD, Professor, Department of Anatomy and Neurobiology.

- Alzheimer’s disease is the leading cause of dementia, and affects 1 in 9 people aged 65 and older.
- In 2014, Americans provided unpaid care for a person with Alzheimer’s or other dementias—care valued at > $220 billion
- No cure or preventive measure currently exists for Alzheimer’s disease, but a number of promising therapies are being developed and tested, including several at the University of Kentucky.
- By investing in the development of therapies now, we can save billions of dollars and heartache in the future. You can help through financial donations, or by participating in one of our research programs.

From the 2015 Alzheimer’s Association Facts and Figures publication.

Please help us today in our fight against Alzheimer’s disease. For more information on research, clinical trials and ways to get involved, contact us at 859-323-6040 or visit our website www.centeronaging.uky.edu.
WILLIAM R. MARKESBERY, MD (1932-2010)

The Markesbery Symposium on Aging and Dementia is named in honor of William R. Markesbery, MD, a gifted scientist and internationally recognized neurologist and neuropathologist. Dr. Markesbery’s creativity and commitment to aging research provided the impetus for the University of Kentucky to establish the Sanders-Brown Center on Aging in 1979 and name him as the first director. He held that position until his death in January 2010.

In 1985, Bill Markesbery became the director of the Alzheimer’s Disease Research Center, one of the original 10 National Institute on Aging (NIA)-funded centers in the United States, with a primary focus on neuropathology. After more than 30 years, the Alzheimer’s Disease Center continues to be funded by NIA, a remarkable achievement that demonstrates the strength and caliber of this program. During his academic career, Dr. Markesbery published more than 400 scientific papers and was one of the world’s leading experts on Alzheimer’s disease and oxidative stress. He will always be remembered as a compassionate and caring physician, a brilliant researcher, and an inspirational leader.