

## Abstracts for Oral Presentations, Saturday, Sept 29th

### Oral Session: Neuropathology, Neuroimaging and Diagnostics

8:30-8:50 – Melissa Murray (Mayo)

#### **The neuropathologic landscape of age and sex in Alzheimer's disease**

Women reportedly make up two-thirds of Alzheimer's disease (AD) dementia sufferers. Many estimates regarding AD, however, are based on clinical series lacking autopsy confirmation. The Florida Autopsied Multi-Ethnic (FLAME) cohort was queried for AD cases with a total of 1625 identified ranging in age from 53-102 years at death. Standard neuropathologic procedures were employed and clinical information was retrospectively collected. Clinicopathologic and genetic data were stratified by sex. Within the neuropathologically diagnosed AD cohort, the overall number of women and men did not differ. Men were younger at age onset, had a shorter disease duration, and more often had atypical (non-amnesic) clinical presentations. The frequency of autopsy-confirmed AD among women and men stratified by age at death revealed an inverse U-shaped curve in men and a U-shaped curve in women, with both curves having inflections at approximately 70 years of age. Regional densities of neurofibrillary tangles differed in women and men, especially when examined by age intervals. Amyloid- $\beta$  plaques were found to steadily decrease with advancing age across association cortices, primary cortices, and hippocampal regions. This pattern observed was similarly observed in men and women. Women had overall greater severity of tangle density compared to men, especially in the hippocampus. Men and women did not differ in frequency of *MAPT* haplotype or *APOE* genotype. Atypical clinical presentations, younger age onset and shorter disease duration were more frequent in men, suggesting that the lower reported frequency of AD in men may be due to more frequent atypical clinical presentations not recognized as AD. Our data suggest that neuropathologically confirmed AD has the same frequency in women and men, but their clinical presentations and ages at onset tend to differ.

8:50-9:10 – Zach Mceachin (Emory)

#### **RAN Translation of dipeptide repeats in SCA36**

Spinocerebellar ataxia 36 (SCA36) is characterized by an intronic TGGGCC repeat expansions with a striking similarity to the intronic GGGGCC repeat expansions in C9ORF72, which is the most common genetic cause of frontotemporal dementia and amyotrophic lateral sclerosis (c9FTD/ALS).

While little is presently known of the pathomechanisms by which the TGGGCC repeat expansion causes SCA36, toxicity resulting from the accumulation of repeat RNA is a likely culprit. RNA-mediated toxicity, a putative pathomechanism of many microsatellite expansion disorders, is believed to arise from the abnormal interaction between repeat expansion RNA and essential RNA-binding proteins, and the sequestration of these proteins into RNA foci. In addition to RNA-mediated toxicity, an atypical form of translation – repeat-associated non-ATG (RAN) translation – is thought to play a key role in various repeat expansion diseases. RAN translation of expanded repeats occurs in all reading frames and results in the production of potentially toxic proteins. The highly related GGGGCC repeat expansions in C9ORF72, are RAN translated into five dipeptide repeat (DPR) proteins that form aggregates in neurons.

This raised the question whether expanded TG3C2 repeats are similarly RAN translated in SCA36. Indeed, we detect the DPR protein, poly(GP), in SCA36 patient-derived cells and brain tissues. This implicates RAN translation as a potential new disease mechanism for SCA36. Our findings also suggest that DPR proteins present in patient biofluids can be used to diagnose and monitor disease activity, and to gauge target engagement for potential therapeutics that target UGGGCC repeat RNA.

9:10-9:30 – David Vaillancourt (UF)

**A paradigm shift for testing disease-modifying therapies in Parkinson's disease using imaging**

The past several decades have been witness to numerous disease-modifying clinical trials in Parkinson's disease (PD) that have fallen short of achieving their pre-defined outcomes. These failures are likely related to the therapeutics tested, but there is also a growing concern in the field that the failure is more likely due to the subjective clinical ratings used as outcome measures. This shortcoming of clinical trials in PD is leading to a paradigm shift such that in the near future PD disease-modifying therapies could be evaluated on other measures, such as an imaging marker, and there would be far fewer patients required to reach a primary outcome. Here, I will provide insight into how such a paradigm shift could emerge, and show evidence from single-site exploration, and multi-site validation studies that this paradigm shift could become a reality.

9:30-9:50 – Jeremy Herskowitz (UAB)

**Dendritic spine remodeling accompanies Alzheimer's disease pathology and genetic susceptibility in cognitively normal aging**

Subtle alterations in dendritic spine morphology can induce marked effects on connectivity patterns of neuronal circuits and subsequent cognitive behavior. Past studies of rodent and non-human primate aging revealed reductions in spine density with concomitant alterations in spine morphology among pyramidal neurons in the prefrontal cortex. We visualized and digitally reconstructed the three-dimensional morphology of dendritic spines from the dorsolateral prefrontal cortex in cognitively normal individuals aged 40-94 years. Linear models defined relationships between spines and age, Mini-Mental State Examination (MMSE), APOE  $\epsilon$ 4 allele status, and Alzheimer's disease (AD) pathology. Similar to findings in other mammals, spine density correlated negatively with human aging. Reduced spine head diameter associated with higher MMSE scores. Individuals harboring an APOE  $\epsilon$ 4 allele displayed greater numbers of dendritic filopodia and structural alterations in thin spines. The presence of AD pathology correlated with increased spine length, reduced thin spine head diameter, and increased filopodia density. Our study reveals how spine morphology in the prefrontal cortex changes in human aging and highlights key structural alterations in selective spine populations that may promote cognitively normal function despite harboring the APOE  $\epsilon$ 4 allele or AD pathology.

9:50-10:00 – Nkem Azu (Mayo)

**Identifying biomarker genes of selective hippocampal vulnerability in Alzheimer's disease variants by quantitative digital pathology and RNA-sequencing.**

Alzheimer's disease (AD) neuropathologic patterns of neurofibrillary tangles are used to classify AD subtypes, which we termed hippocampal sparing (HpSp), typical, and limbic predominant. Utilizing a translational neuropathologic approach, we assessed the genetic contribution to selective vulnerability of the hippocampus. The HpSp and limbic predominant AD cases were assessed as extreme phenotypes, which was complemented by comparison of controls and typical AD cases as enhanced phenotypes. RNA-sequencing was used to uncover gene expression changes associated with phenotype differences. To prioritize genes, we employed bioinformatics methods to examine differential expression, enrichment of cellular process networks, and known AD target genes. We validated our findings in a larger cohort using NanoString and examined relevance to hippocampal neuropathology. We quantified digital pathology measures of early tangles (CP13), mature tangles (Ab39), amyloid- $\beta$  burden (33.1.1), neuronal loss (H&E) and markers for microglia (CD68), endothelia (CD34), and astroglia (GFAP). Using a translational neuropathologic approach combined with deep learning based prediction models; the SLC38A2 gene was nominated in the top gene using a multi-way importance model. The SLC38A2 gene was originally prioritized in our RNA-sequencing data and found differentially expressed between controls and typical AD (FDR=0.002,  $p < 0.00001$ ). RNA-seq gene expression measures validated well with NanoString for SLC38A2 ( $R = 0.982$ ,  $p < 0.001$ ). Regression analysis was used to examine the contribution of demographics, tau and amyloid burden, neuronal loss and glial pathology. Early tau (Estimate=0.35,  $p = 0.03$ ) and late tau measures (Estimate=0.02,  $p = 0.009$ ) significantly predicted gene expression, but not amyloid measures or neuronal loss. Microgliosis approached significance (Estimate=0.35,  $p = 0.03$ ), with no contribution from endothelial cell burden or astroglia. Age approached significance as a predictor of SLC38A2 gene expression (Estimate=-0.11,  $p = 0.06$ ), but not sex (Estimate=0.03,  $p = 0.81$ ) or APOE  $\epsilon$ 4 (Estimate=0.17,  $p = 0.13$ ). Our data supports consideration of intra-disease divergence with regard to case stratification and may reveal genes previously masked by heterogeneity of cohorts.

## Oral Session: Therapeutics II

10:15-10:35 – Erik Roberson (UAB)

### **Preclinical development of Tau-SH3 interaction inhibitors for the treatment of Alzheimer's disease**

Current treatments for Alzheimer's disease (AD) provide only modest benefits and there is a strong need for disease-modifying therapies. Targeting tau is attractive because of the early and robust tau abnormalities that are universal in AD. Progress in developing such tau-directed therapies, however, has been slowed by uncertainty about how tau mediates neuronal dysfunction/death in AD and how best to target these processes. Considerable effort has been devoted to therapies aimed at tau phosphorylation, other tau post-translational modifications, tau aggregation, tau-dependent effects on microtubule stability, & intercellular tau propagation, but none has yet shown clinical efficacy and there is a strong need for new paradigms for targeting tau. We have been developing a novel approach to tau therapeutics by targeting tau's interactions with proteins containing Src homology 3 (SH3) domains. Tau's central proline-rich domain contains seven PXXP motifs that mediate binding to SH3 domain-containing proteins, including the Src-family tyrosine kinase, Fyn, which is strongly implicated in AD and is the canonical tau-interacting SH3 protein. We developed a peptide inhibitor of tau-SH3 interactions as a tool compound and found that this inhibitor reduced A $\beta$  toxicity in primary neurons. We developed high-throughput screening-compatible assays for the tau-Fyn interaction and used them to screen libraries of small molecules. We identified several series of tractable structures with good drug-like properties and ability to inhibit tau-fyn interactions, and found that these compounds also reduce A $\beta$  toxicity. These data support the idea of targeting tau-SH3 interactions as a novel therapeutic strategy for AD & related disorders.

10:35-10:55 – Sara Burke (UF)

### **Metabolic Interventions for Enhancing Cognitive Resilience in Aging and Alzheimer's Disease**

By the year 2020 the number of Americans over the age of 65 is projected to reach 55 million, occupying a larger portion of our population aging demographics than ever recorded in history. It is therefore imperative that the ability of these individuals to live independently is preserved for reasons of personal dignity as well as the financial and public-health consequences that result from the necessity of long-term care. Unfortunately, a large proportion of older adults experience memory loss or other types cognitive decline that interfere with quality of life. To date, therapeutic options for mitigating cognitive dysfunction in aging and Alzheimer's disease are limited. A barrier to advancing treatments for cognitive loss in aging is that the brain regions critical for higher cognition show distinct neurobiological mechanisms of dysfunction in old age. One factor that changes ubiquitously across the brain in old age, however, is a reduced ability to use glucose for energy production. Importantly, neurons can utilize ketone bodies for metabolism. Circulating levels of ketone bodies can be elevated by nutritional ketosis in which the macronutrient composition shifts to a higher proportion of fat relative to carbohydrates and protein. While ketogenic diets were initially introduced to suppress seizures, they are garnering attention for their potential to treat a myriad of neurodegenerative and metabolic disorders that are associated with advanced age. This talk will review recent data regarding the physical, biochemical and cognitive impacts of a long-term ketogenic diet in young and aged rats. Moreover, potential mechanisms for how ketosis normalizes aberrant neural activity in aging and the early stages of Alzheimer's disease will be discussed.

10:55-11:15 – Andrew Arrant (UAB)

### **Progranulin Gene Therapy Improves Pathology and Reverses Social Deficits in Mouse Models of Frontotemporal Dementia and Neuronal Ceroid Lipofuscinosis Due to Progranulin Mutations**

Loss-of-function mutations in progranulin (*GRN*) are a major autosomal dominant cause of Frontotemporal Dementia (FTD). *GRN* mutations exhibit a gene-dose effect, with homozygous *GRN* mutations causing the lysosomal storage disorder Neuronal Ceroid Lipofuscinosis (NCL). All known disease-causing *GRN* mutations are loss-of-function mutations, most of which cause progranulin haploinsufficiency. Boosting progranulin levels is therefore a promising approach to treatment. We generated an AAV2/1-progranulin vector (AAV-*Grn*) to test whether restoration of progranulin could correct FTD- and NCL-relevant phenotypes in progranulin-insufficient mice. AAV-*Grn* or an AAV-GFP vector were infused into the medial prefrontal cortex (mPFC) of 10–12 month-old wild-type, *Grn*<sup>+/-</sup>, and *Grn*<sup>-/-</sup> mice. *Grn*<sup>-/-</sup> mice were euthanized for assessment of pathology 8–10 weeks later, and *Grn*<sup>+/-</sup> mice were assessed for social behavior 4–6 weeks later. AAV-*Grn* reduced lipofuscinosis and normalized cathepsin D activity in *Grn*<sup>-/-</sup> mice. AAV-*Grn* reduced microgliosis in *Grn*<sup>-/-</sup> mice in brain regions away from the AAV injection site, but increased inflammation at the AAV injection site due to an apparent non-self reaction to progranulin. This reaction was not observed in wild-type or *Grn*<sup>+/-</sup> mice, and is unlikely to occur in FTD-*GRN* patients, who maintain at least 25% of normal progranulin levels. AAV-*Grn* reversed social deficits and normalized markers of lysosomal dysfunction in brains of *Grn*<sup>+/-</sup> mice. These data show that restoration of progranulin to progranulin-insufficient mice reduces FTD/NCL-like pathology, normalizes markers of lysosomal dysfunction, and reverses deficits in social behavior. Our AAV-*Grn* vector expressed progranulin with a C-terminal tag that disrupted binding of progranulin to sortilin, showing that sortilin is not required for these beneficial effects of progranulin. These data provide support for the potential efficacy of progranulin-boosting therapies in *GRN* mutation carriers.

11:15-11:35 – Christopher Holler (Emory)

### **The progranulin cleavage products, granulins, are able to rescue lysosomal defects in a cellular model of progranulin deficiency**

Frontotemporal dementia (FTD) is the second most common neurodegenerative disease in people <65 years old. Heterozygous mutations in the progranulin gene (*GRN*) leading to progranulin (PGRN) haploinsufficiency are a major cause of familial and sporadic FTD. Interestingly, homozygous *GRN* mutations cause an early onset lysosomal storage disease called neuronal ceroid lipofuscinosis (NCL), indicating that PGRN plays an important role in lysosomal homeostasis. NCL-like pathologies have also been reported in *Grn*-deficient mouse models and FTD-*GRN* patients. Recently, we reported that intracellular full-length PGRN (~80 kDa) is proteolytically processed into stable granulin (GRN) proteins (~6-12 kDa) in the lysosome and these GRNs are haploinsufficient in FTD-*GRN* patient tissues (Holler et al, eNeuro 2017; 4(4)). To address whether GRNs have functional activity in the lysosome, we transiently transfected or exogenously added recombinant GRNs to cultured *Grn*<sup>-/-</sup> fibroblasts, which display characteristic markers of lysosomal dysfunction (eg. increased cathepsin D protein). Interestingly, we found that specific GRN proteins were endocytosed, trafficked to the lysosome, and rescued lysosomal deficits associated with loss of PGRN. Disruption of conserved disulfide bonds in GRNs results in an unstable protein unable to rescue the lysosomal deficits. These results indicate that GRNs are the functional units of PGRN in the lysosome and carry out critical metabolic roles related to lysosome homeostasis. Ongoing experiments will test GRN function and rescue in *GRN* deficient human iPSC and derived neurons. Future experiments will focus on identifying GRN-interacting partners in the lysosome and testing whether GRNs can rescue pathologies *in vivo* in *Grn*-deficient mouse models.

11:35-11:55 – Paramita Chakrabarty (UF)

### **Harnessing innate immune response as potential therapy in neurodegenerative diseases**

Innate immune activation is an underlying pathological sequelae across the spectrum of neurodegenerative dementias. Systems biology approaches utilizing genetics, transcriptomics and functional data have demonstrated a close interrelationship between immune dysfunction and protein homeostasis failure in these diseases, a phenomenon that we have termed immunoproteostasis. The misfolded proteins that underlie proteostasis in these diseases can stimulate the innate immune system as damage associated molecular patterns (DAMP), much like pathogens that similarly activate innate immunity. Guided by our ongoing systems level studies in mouse models of Alzheimer's and Parkinson's disease, we have explored whether decoy immune receptors, that typically interact or respond to DAMP signaling, could have the potential of targeting immunoproteostasis for therapeutic benefit. Here, we will present data demonstrating the efficacy of two different classes of decoy receptors as safe A $\beta$ -selective biotherapies and further discuss preliminary data showing that this principle of therapeutic targeting of immunoproteostasis can be extended to other models of neurodegenerative diseases. Such first generation preclinically validated biotherapy reagents have the potential to be further optimized and utilized for effective targeting of multiple types of proteinopathies characterized by immune activation.

11:55-12:05 – Demetrius Maraganore (UF)

### **Design and implementation of pragmatic clinical trials using the electronic medical record and an adaptive design: the example of mild cognitive impairment and nootropic drugs**

**Objectives:** To demonstrate the feasibility of pragmatic clinical trials comparing the effectiveness of treatments using the electronic medical record (EMR) and an adaptive assignment design.

**Methods:** We have designed and are implementing pragmatic trials at the point-of-care using custom-designed structured clinical documentation support and clinical decision support tools within physician's typical EMR workflow. We are applying a subgroup based adaptive design (SUBA) that enriches treatment assignments based on baseline characteristics and prior outcomes. SUBA uses information from a randomization phase (phase 1, equal randomization, 120 patients), to adaptively assign treatments to the remaining participants (at least 300 additional patients total) based on a Bayesian hierarchical model. Enrollment in phase 1 is underway in our neurology clinical practices for 3 separate trials using this method, for migraine, mild cognitive impairment (MCI), and epilepsy.

**Results:** We are successfully collecting structured data, in the context of the providers' clinical workflow, necessary to conduct our trials. We are currently enrolling patients in 3 point-of-care trials of non-inferior treatments. As of September, 2018, we have enrolled 63% of eligible patients into our MCI study (the focus of this presentation). Enrollment is ongoing and validation of outcomes has begun.

**Discussion:** This presentation demonstrates the feasibility of conducting pragmatic trials using the EMR and an adaptive design.

**Conclusion:** The demonstration of successful pragmatic clinical trials based on a customized EMR and adaptive design is an important next step in achieving personalized medicine and provides a framework for future studies of comparative effectiveness.

## Oral Session: Hot Topics

2:00-2:10 – Zach Wallen (UAB)

### **Replicable and robust associations between gut microbiota and Parkinson Disease**

Background: Many disorders, including PD, exhibit altered gut microbiome (GM). The evidence for GM dysbiosis is robust and has been consistently reproduced across studies, but attempts to identify the individual microorganisms have produced vastly varied lists. This is true even for gastrointestinal diseases, such as Crohn's disease. The inconsistent findings may be due to differences in study populations (as GM is highly influenced by medications, diet and environmental factors), differences in methods for detection and analysis, small sample sizes, etc.. More disconcerting is the possibility that they are chance findings and false positives. Aim: Is it possible to detect disease-taxa associations that are replicable across datasets, and robust to differing analytical methods? Methods: We sequenced 16S rRNA amplicons in two datasets (201 PD, 132 controls, and 319 PD, 184 controls), and processed the data using DADA2 bioinformatics pipeline. We tested abundances of 163 genera in PD vs. controls, using three different analytical methods (DESeq2, Kruskal-Wallis, and zero-inflated negative-binomial GLM), correcting for multiple testing. Results: Seven genera were detected as being significantly altered in PD by all three methods, and in both datasets, all at  $FDR < 0.05$ . Bifidobacterium, Lactobacillus, Hungatella, Porphyromonas and Methanobrevibacter were elevated in PD, Roseburia and Agathobacter were reduced in PD. Conclusion: It is possible to detect replicable and robust associations between disease and gut microbiota. This study did not have resolution below genus level; shotgun metagenomics sequencing is underway. The study design was very stringent to minimize false positives; thus, many true associations may have been missed. While the functional and clinical significance of the 7 genera is being studied, we caution against the use of probiotics to self-medicate because the main commercial probiotics, Bifidobacterium and Lactobacillus, are already abnormally high in PD.

2:10-2:20 – Chao Ma (USF)

### **Tauopathies Reveal Uncoupling of Arginine Sensing Pathways and mTORC1 Activation**

Tauopathies consists of a group of neurodegenerative diseases characterized by pathogenic protein inclusions formed by abnormal accumulation of microtubule-associated protein tau. The aggregation of tau remains a central target for drug discovery, however, no disease-modifying treatments exist. One pathway that governs cellular proteostasis includes the mechanistic target of rapamycin (mTOR) pathway, which can be stimulated by amino acids like arginine. Our preliminary work has uncovered a unique interaction between tau biology and mTORC1 signaling linked to arginine metabolism. Human solute carrier family 38, member 9 (SLC38A9) and cellular arginine sensor for mTOR complex 1 (mTORC1) subunit 1 (CASTOR1) were the first two arginine sensors identified to modulate mTORC1 signaling within the lysosome and cytoplasm, respectively. Other key studies revealed that recently deorphanized G protein coupled receptor (GPCR) family C, group 6, member A (GPC6A), also binds to arginine. We observed dysregulation of arginine metabolism and arginine-sensing pathways in human Alzheimer's disease (AD) brains as well as two mouse models of tauopathy. We performed mRNA microarray and discovered that arginine producing enzymes/ sensors, and mTORC1 complexes were increased in the hippocampus of AD patients compared to age matched controls. High protein levels of arginine producing enzymes/ sensors, and mTORC1 activation substrates have been identified in two tauopathy mouse models. Mass spectrometry analysis indicated that tau transgenic mice show increased arginine levels in both total brain homogenate and brain interstitial fluid microdialysate compared to non-transgenic littermates. Furthermore, genetic repression of GPCR6A through targeted siRNA inhibited mTORC1 signaling, activated autophagy and reduced total tau level in tau overexpressing cell lines. Overall, we identified tau overexpression leads to hyper-mTORC1 activation through uncoupling of arginine-sensing pathways in human AD brains and mice with tauopathy. Therefore, manipulation of the putative extracellular arginine sensor GPCR6A may serve as a novel therapeutic target to treat patients with tauopathies.

2:20-2:30 – Issac Bishof (Emory)

**RNA-binding proteins with basic–acidic dipeptide (BAD) domains self-assemble and aggregate in Alzheimer's disease**

U1 small nuclear ribonucleoprotein 70 kDa (U1-70K) and other RNA-binding proteins (RBPs) are mislocalized to cytoplasmic neurofibrillary tau aggregates in Alzheimer's disease (AD), yet the co-aggregation mechanisms are incompletely understood. U1-70K harbors two disordered low-complexity domains (LC1 and LC2) that are necessary for aggregation in AD brain extracts. The LC1 domain contains highly repetitive basic (R/K) and acidic (D/E) residues, referred to as a basic–acidic dipeptide (BAD) domain. We report here that this domain shares many of the properties of the Q/N-rich LC domains in RBPs that also aggregate in neurodegenerative disease. These properties included self-assembly into oligomers and localization to nuclear granules. Co-immunoprecipitations of recombinant U1-70K and deletions lacking the LC domain(s) followed by quantitative proteomic analyses were used to resolve functional classes of U1-70K–interacting proteins that depend on the BAD domain for their interaction. Within this interaction network, we identified a class of RBPs with BAD domains nearly identical to that found in U1-70K. Two members of this class, LUC7L3 and RBM25, required their respective BAD domains for reciprocal interactions with U1-70K and nuclear granule localization. Strikingly, a significant proportion of RBPs with BAD domains had elevated insolubility in the AD brain proteome. Furthermore, we show that the BAD domain of U1-70K can interact with tau from AD brains, but not from other tauopathies. These findings highlight a mechanistic role for BAD domains in stabilizing RBP interactions and in potentially mediating co-aggregation with pathological, AD-specific tau isoforms.