Ben Devlin	Neuronal cytokine IL34 influences plaque pathology, microglia, and behavior in a mouse model of Alzheimer's disease.	Microglia-neuron crosstalk is critical for the proper functioning of the central nervous system. As the tissue resident macrophages in the brain, microglia serve essential functions including debris clearance, cytokine production, and the removal of unwanted or damaged synaptic connections and neurons in development. There is ample evidence that these microglial processes become overactive in diseases such as Alzheimer's, however the signals that properly regulate these processes remain elusive. Recent genome-wide association studies (GWAS), as well as empirical studies, have uncovered a link between the neuron-derived cytokine interleukin-34 (IL34), which signals through the colony stimulating factor 1 receptor (CSF1r) on microglia, and Alzheimer's disease progression. To investigate the mechanisms by which IL34 may influence neuroinflammatory outcomes, we crossed a mouse model of Alzheimer's disease (5XFAD) with IL34 heterozygous mice (IL34LacZ/+). We tested WT, 5XFAD, and 5XFAD/IL34 Het mice in open field, elevated plus maze, and Y maze behaviors at both 4 and 6 months. Additionally, we collected brains from these mice and performed histology to quantify amyloid beta plaque pathology along with microglia and synapse endpoints. Our preliminary data demonstrate that while there are no changes at the behavioral level in any of the genotypes tested at 4 months of age, there is a significant reduction in plaque numbers in the motor cortex of 5XFAD/IL34 mice compared to 5XFAD. Further, this change in plaque number coincided with an increase in plaque engulfment by microglia in 5XFAD/IL34 mice, suggesting that plaques are still forming in mice with the additional IL34 mutation, but the microglia are more effective at clearing them. At 6 months of age, we observed a significant increase in percent time spent in the open arms of the elevated plus maze only in 5XFAD/IL34 mice compared to WT mice, suggesting the additional mutation speeds up the progression of the behavioral pathologies seen in 5XFAD mice at older ages. In ongoing experiments, we are testing spatial memory deficits in these mice using the morris water maze and will assess neuroinflammatory endpoints including microglial engulfment of synapses. Furthermore, we will test the effects of virally overexpressing IL34 on older 5XFAD mice (8 months) on the same behavioral and molecular endpoints. In sum, this work points to a mechanistic link between IL34, microglia, and Alzheimer's disease and provides the foundation for investigating the therapeutic potential of targeting the IL34-CSF1r axis in Alzheimer's disease.
Debabrata Majumder	LentiSTARR-Seq: a high throughput assay to identify regulatory non-coding variants associated with multiple sclerosis in relevant cell types	Background: Recent genome-wide association studies for multiple sclerosis (MS)-a neurodegenerative disease have mapped many disease-associated single nucleotide polymorphisms (SNPs) to non-coding elements. While the role of inflammatory CD4 T cells, such as Th1 and Th17 cells, in the pathogenesis of MS is well documented, it remains a challenge to identify the bona fide SNP variants having gene regulatory activity within disease-relevant cell types in a high throughput manner. Considering these facts, this study aims to develop a high throughput reporter assay to identify the noncoding risk variants that alter the regulatory function of cis-elements controlling proinflammatory T cells. This strategy can potentially identify novel target genes involved in the etiology of MS. Methods: We developed a novel variation of self-transcribing active regulatory region sequencing (STARR-Seq) assay through which human Th1 and Th17 cells can be used as a platform to test the cis-regulatory activity of an MS-associated SNP library in a high throughput manner. The STARR-Seq cassette comprises a minimal promoter driving a mouse Thy1.1 reporter gene and a multiple cloning site in the 3' UTR for insertion of test cis region sequences. The STARR-Seq assay-specific cassette was cloned in the anti-sense orientation respective to that of the long terminal repeats (LTRs) into a lentiviral plasmid backbone. To mitigate RNA duplex formation in the virus producer 293FT cells, we generated a modified VSV-G-expressing plasmid carrying the Nodamura virus protein B for virus packaging. Finally, lentiviral particles harboring the STARR-Seq cassette were generated using mutated integrase to facilitate reporter assay in human Th1 and Th17 cells in an episomal manner. To validate the assay, we generated and tested control LentiSTARR-Seq viral constructs in which confirmed Th cell-specific enhancer and repressor elements were cloned into the 3' UTR region of the reporter. Following the validation Th1 and Th17 isolated from healthy donors were used to evaluate the regulatory activity of the autoimmune risk associated specifically MS-risk associated Th1 and Th17 specific SNP library having putative cis activity to identify the bona fide cis elements having altered regulatory activity. Result: The delivery of STARR-Seq plasmids into human Th1 and Th17 cells via nucleofection results in high rates of cell death. However, the lentiviral-based approach was successful in facilitating the STARR-seq assay in primary cells. The functional activity of the positive and negative lentiSTARR-Seq vector controls transduced into human Th1 and Th17 cells was analyzed by flow cytometry of Thy1.1 expression and RT-PCR and confirmed appropriate reporter readouts. We were able to identify a set of cis-elements having altered regulatory activity in the presence of MS-associated SNPs and the gene targets or the closest annotated genes of those SNPs are related to a variety of immune cell-specific functions ranging from cytokine production to intracellular signaling. Conclusion/Future goal: This novel adaptation of STARR-Seq has identified MS risk-associated non-coding SNP variants in relevant cell types for their altered cis activity. The set of regulatory risk variants identified will be used to perform CRISPR/Cas9-mediated genetic perturbations in human CD4 T cells to delineate bona fide regulatory gene targets.

Eileen Ma	Histone deacetylase 6 over-expression decreases leucine-rich repeat kinase 2 kinase activity	The aggregation of pathological forms of tau, namely hyperphosphorylated and acetylated tau, has been associated with numerous neurodegenerative diseases like Alzheimer's. Research has shown that this tau pathology also arises in leucine-rich repeat kinase 2 (LRRK2)-linked Parkinson's disease, which is associated with mutations like LRRK2G2019S that increase the protein's kinase activity. The inhibition of histone deacetylase 6 (HDAC6) has also been associated with a decrease in pathological tau levels although a protective function for the deacetylase has also been proposed. Interactions between LRRK2 and HDAC6 have previously been discovered in vitro, but the functional impacts of these protein interactions are not well-established. We proposed that LRRK2 was potentially phosphorylating HDAC6 in a kinase-dependent manner. To explore this further, HEK 293T cell lines were transfected with myc and/or HDAC6-myc, as well as FLAG, LRRK2WT-FLAG, and/or LRRK2G2019S-FLAG plasmids. Through Western blots (n = 4), we detected HDAC6 phosphorylation, LRRK2 autophosphorylation, and phosphorylation of the LRRK2 substrate Rab12. A complementary model system was then established in primary neurons, utilizing lentiviruses to transduce empty vector, LRRK2, LRRK2G2019S, and/or kinase dead LRRK2, as well as empty vector and/or HDAC6 in both the presence and absence of HDAC6 inhibitor tubastatin A. Due to size limitations, the LRRK2 lentiviruses contained only the catalytic core of LRRK2. Following these transductions, additional Western blots were performed, probing for the aforementioned proteins along with acetylated tubulin to confirm the inhibition of HDAC6 by tubastatin A. In the HEK lines, HDAC6 transfections significantly decreased the levels of both LRRK2 autophosphorylation and substrate phosphorylation in cells that overexpressed either LRRK2 allele. Meanwhile, LRRK2 transfections had no significant impact on the levels of phosphorylated HDAC6. In the primary neuron models, the LRRK2 lentiviruses failed to phosphorylate Rab12, likely due to the lentiviruses only packaging the catalytic core of LRRK2. However, tubastatin A was deemed a viable HDAC6 inhibitor as tubastatin A treatment rescued acetylated tubulin levels in cells overexpressing HDAC6. These findings in HEK cells support that HDAC6 transfection drives a decrease in LRRK2's kinase activity. In the future, wild-type LRRK2 and LRRK2G2019S primary neuron models will be developed and utilized to evaluate whether HDAC6 also dampens LRRK2 kinase activity in neurons and whether this effect can be reversed with a tubastatin A treatment.
Elizabeth Viverette	Nanoplastic Accumulation as a Promoting Factor for α-synuclein Aggregation in the Gut and Brain	Previous studies have suggested microplastic and nanoplastic (NP) contaminants impair neuronal lysosomes and may precipitate α-synuclein aggregation in neurons. Here we aim to study environmentally relevant NP particles and concentrations with a focus on NP exposure, biodistribution, and α-synuclein aggregation. To produce environmentally relevant particles similar to those NPs detected in human brain tissues, we developed a novel weathering chamber to simulate decades of environmental exposure and mechanical degradation to produce enough realistic NPs of different compositions for study in PD models. Combinations of multiple molecular dynamics simulations, surface plasmon resonance (SPR), zeta potential analysis, and real-time quaking induced conversion (RT-QUIC) seeding assays on multiple common NP types detected in human brain, including polyethylene, polypropylene, and polyacrylates, are being used to inform long-duration in vivo exposures studies to help understand NP accumulations on PD-relevant pathways both in the gastrointestinal system and brain. We are pioneering novel Fourier transform infrared spectroscopy (FTIR), pyrolysis gas chromatography/mass spectroscopy (PyGC/MS), and cryogenic electron tomography (Cryo-ET) to measure plastic contaminants in both the gut and brain. Preliminary RT-QUIC data indicates an increase in α-synuclein fibrillation when exposed to multiple types of NPs, with SPR showing a strong binding affinity between α-synuclein and a few different polymer types. Tissues of mice exposed to NPs through chow are being analyzed for both α-synuclein misfolding and misfolding propagation rates as a function of NP accumulations in different parts of the gut and brain. We aim to better understand the effects of environmentally relevant concentrations of realistic NPs on α-synuclein misfolding throughout the gut and brain, and their effect on PD-linked phenotypes.

Graham Wallace	CHIP Interaction Changes Driven by Isomerization, and the Implications for Spinocerebellar Ataxias	Regulation of protein quality control and the inflammatory stress response are essential processes for combatting the development and progression of neurodegenerative disorders, including the Spinocerebellar Ataxias (SCAs). The E3 ubiquitin ligase CHIP is canonically associated with protein quality control through its interaction with chaperones, but numerous findings point to a role in modulating immune and stress responses as well. A gap in knowledge exists in the understanding of how CHIP can serve these divergent roles and what factors facilitate the appropriate response. To better understand the factors that regulate CHIP my laboratory performed a proximity labeling experiment and found that CHIP interacts with a number of noncanonical substrates that compete with chaperones for binding to the CHIP TPR interaction domain. We also found that CHIP interacts with, and is a substrate of, the stress-responsive peptidyl prolyl isomerase FKBP51. My laboratory has found that FKBP51 unfolds the TPR domain of CHIP and thus is expected to regulate the affinity between CHIP and its substrates. Here I hypothesize that isomerization of CHIP by FKBP51 acts as a mechanism to mediate interactions between CHIP and its substrates. I will first further explore the stress-responsiveness of CHIP to inflammatory or stressful stimuli and begin to investigate the consequences of these stimuli in terms of changing CHIP interactions. I will next corroborate our existing in vitro findings for the CHIP-FKBP51 interaction, and resulting isomerization, in cell culture, as well as analyze the sufficiency of FKBP51 to drive the context-dependent changes seen with CHIP following stimulation. The completed and proposed work outlined in this poster demonstrates the potential for novel CHIP interactions and will help identify how CHIP protects against neurodegenerative diseases including the SCAs.
Griffin Carter	PVSRIPO Enhances Microglial Responses in GBM and increased Phagocytosis of Amyloid Beta	Alzheimer's disease (AD) remains an urgent challenge in neurodegenerative research, with no curative treatments available. A key pathological feature of AD is the dysregulation of microglial activation and responses. While microglia play a crucial role in maintaining homeostasis, their impaired function in AD contributes to disease progression. PVSRIPO, a modified poliovirus with immunostimulatory properties, has demonstrated the ability to engage the central nervous system (CNS) myeloid compartment and induce enhancement of microglial activation and function in the context of glioblastoma. However, its potential to modulate microglial activation in neurodegenerative diseases remains unexplored. I investigate the direct interaction between PVSRIPO and microglia, both in vitro and in vivo, to assess its potential as an therapeutic agent in AD. My findings reveal that PVSRIPO infection leads to sustained viral RNA replication in microglia, triggering a pronounced pro-inflammatory response. This response is characterized by upregulation of activation markers, increased phagocytosis of amyloid-beta aggregates, and induction of Type I IFN signaling pathways. Notably, PVSRIPO-treated microglia exhibit a shift toward a phagocytically active phenotype and morphology, suggesting a potential therapeutic avenue for modulating microglial function in AD. By elucidating the mechanisms underlying PVSRIPO-induced microglial activation, this research lays the groundwork for repurposing viral immunotherapies in neurodegenerative disease treatment. Further studies will be essential to determine its translational potential and long-term effects on neuroinflammation and cognitive outcomes in AD.
Guillermina Rentsch	Increased Ciliation of CA3 Hippocampal Neurons in Alzheimer's Disease	Essential for memory encoding and retrieval, the CA3 subregion of the hippocampus produces brain derived neurotrophic factor in support of the dentate gyrus (DG), entorhinal cortex and the basal forebrain. In Alzheimer's disease (AD), hippocampal BDNF production is significantly reduced, however the mechanism underlying BDNF reduction remains elusive. We hypothesize that primary cilia, signaling organelles found on neurons, regulate BDNF production. To determine whether primary cilia dysfunction in AD is causing BDNF reduction by CA3 neurons, we first scored neuronal primary cilia in the CA3 of human postmortem AD brains compared to non-AD controls. We noted a significant increase in percent ciliation of MAP2+ neurons in the CA3 in post-mortem human AD brains compared to controls. Our preliminary data also suggest longer primary cilia in the CA3 neurons of AD brains compared to controls. Future studies will examine BDNF transcription in CA3 neurons as a function of ciliation. Because the remaining CA3 neurons are increasingly ciliated, our current data indicate that neuronal primary cilia may confer resilience to AD pathology.
Hailey Napier	Working towards a cross-species spatial multi-omics atlas of Purkinje cell subtypes	Cerebellar Purkinje cells include two major subtypes defined by striped expression of Zebrin II (ZII), both of which are integral for healthy cerebellum function. Recently, my lab found that dysregulation of the transcriptomic profiles maintaining striped ZII-defined Purkinje cell subtypes is associated with motor symptom onset in mouse models of spinocerebellar ataxias. Despite the relevance of spatially patterned Purkinje cell subtypes in mouse models of neurodegeneration, it remains unclear whether ZII Purkinje cell subtypes are transcriptionally conserved in primates, including humans. Consequently, it is uncertain whether Purkinje cell research in mouse models is relevant to human disease. To address this knowledge gap, I'm using novel spatial sequencing technology to generate the first spatial multi-omics atlas of the primate cerebellum and the first human multi-omics Purkinje cell atlas. These data will enable the identification of conserved gene-regulatory networks in Purkinje cells, illuminating mechanisms and therapeutic targets for spinocerebellar ataxias.

Ivana Barraza	Specificity in mitochondrial base lesions in Parkinson's disease	Parkinson's disease (PD) is the most common movement neurodegenerative disorder, affecting more than 10 million people worldwide. Mitochondrial dysfunction is one of the key contributors to PD pathogenesis. Defects to the mitochondria can alter mitochondrial DNA (mtDNA) and mitochondrial dynamics, resulting in neurodegeneration. One facet that results in mitochondrial dysfunction is the accumulation of mtDNA damage, a feature that is shared in both familial and idiopathic PD, suggesting that mitochondrial genome instability may play a role in PD pathogenesis. Studies from our laboratory have demonstrated brain region specific accumulation of mtDNA damage in PD models in vitro, in vivo, and in human postmortem brain tissue. Our lab has demonstrated that the PD-linked LRRK2 G2019S causes increased mtDNA damage and is LRRK2 kinase dependent. We have also found that levels of mtDNA damage are increased in blood derived from PD-linked LRRK2 G2019S (GS) mutation carriers, LRRK2 GS non-manifesting carriers, and idiopathic PD patients compared to aged matched healthy controls. The persistence of abasic sites and oxidative lesions in PD models suggests that deficient repair against oxidative lesions may play a role in the accumulation of mtDNA damage. The base excision repair (BER) pathway is the best characterized repair mechanism in the mitochondria that responds to oxidative lesions. I hypothesize that defects to mtDNA BER pathway contribute to the accumulation of oxidative base lesions in PD. To better understand the underlying mechanisms that are driving accumulation of mtDNA damage in PD, we have developed the Mito DNAOX, which is able to reveal the molecular identity of the type(s) of mtDNA base damage accumulating in models of PD in real-time. Using additional techniques such as RT-qPCR, western blot, and immunocytochemistry, the expression, levels, localization, and mtDNA repair capacity of select BER enzymes were investigated. Preliminarily, our lab has found selective accumulation of oxidative and uracil mtDNA base lesions in PD mutant LRRK2 G2019S knock-in (LRRK2G2019S/G2019S KI) HEK293 cells generated by CRISPR/Cas9 gene editing compared to HEK293 wild-type control cells. We have also observed small, but significant transcriptional changes in DNA polymerase enzymes in LRRK2G2019S/G2019S KI cells compared to wild- type. When evaluating the functional capacity of BER enzymes, we observed a significant increase in abasic sites and modest changes in ligase activity in LRRK2G2019S/G2019S KI cells compared to wild- type. Our data suggests selectivity for mitochondrial oxidative and uracil base lesions, which may be further attributed to defects in the ability to correctly process abasic sites and/or incorporate nucleotide bases in LRRK2G2019S/G2019S KI cells compared to wild-type. Understanding the role of mtDNA damage and repair can improve our understanding of mitochondrial dysfunction in PD and may identify a novel target for disease modifying therapies.
Jennifer Jenkins, PhD	Investigating the unique contributions of mitochondrial-derived vesicles in preserving mitochondrial integrity to combat neurodegeneration	Mitochondria are critical to neuronal survival and utilize a series of quality control mechanisms to maintain function and prevent neurodegeneration. Mitochondrial-derived vesicles (MDVs) are a novel mitochondrial quality control mechanism that favors the steady state and early response repair of oxidative damage. Previous research has primarily defined MDVs as delivery packages for oxidized mitochondrial components destined for degradation, inferred through static imaging. Additionally, MDV subtypes have historically been identified by limited established protein markers. However, recently reported roles for MDVs suggest a wealth of functional diversity, including inter-organelle communication and mitochondrial motility modulation. Furthermore, proteomic efforts suggest heterogeneity within vesicle populations and the potential for more robust vesicle markers. Thus, numerous undetected MDV populations likely have underappreciated roles in mitochondrial health and neuron physiology. We hypothesize that MDV subtypes have distinct but complementary roles in attenuating mitochondrial damage to maintain network function and neuronal health under oxidative stress to offset neurodegeneration. To model neurodegenerative disease-vulnerable neurons, we will use iPSC-derived motor neurons with a CRIPSR-edited familial amyotrophic lateral sclerosis-associated mutation (TBK1E696K). We will characterize the unique responses of two distinct MDV populations (i.e., TOMM20+ or MUL1+ vesicles) to physiologically relevant levels of oxidative stress in wildtype and TBK1E696K neurons. Using quantitative live imaging studies, we will track the behaviors of each subtype, capturing the dynamic nature of these vesicles. Additionally, to probe the stress-response loading of proteins into these MDV subtypes, we will analyze the proteomes of each subtype after the application of additional oxidative stressors, identifying cargoes differentially loaded in the disease background and screening cargos of interest as potential novel population markers. This presentation will show preliminary data on our developing model system and the generation of an MDV subtype-specific proteomics pipeline. Through our results, we will generate a framework to study MDVs in neurodegenerative diseases.

Jennifer Liu	PARP1 activation caused by the Parkinson's disease-linked G2019S LRRK2 mutation	Parkinson's disease (PD) is the most common movement neurodegenerative disorder, with over 10 million cases worldwide. Mutations in leucine-rich repeat kinase-2 (LRRK2) are a common cause of PD. The most frequent pathogenic LRRK2 mutation is the G2019S variant, which results in increased kinase activity. The molecular mechanisms by which G2019S LRRK2 causes PD are not well understood. The significance of DNA damage and genome integrity is emerging in PD; however, the pathogenic role of DNA damage and repair in PD is not completely understood. Consistent with the PD-linked mutant impacting the cell's ability to process specific types of DNA damage, LRRK2G2019S/G2019S KI cells exhibit increased sensitivity to hydrogen peroxide and the alkylating agent MMS in comparison to wild-type. Lesions resulting from these genotoxic agents activate PARP1 and lead to the generation of PAR polymers. A recent study reported that PARP1 activation contributes to PD pathology and neurodegeneration. Preliminarily, we found that G2019S LRRK2 causes a nearly 250 percent increase in PAR levels from the wild-type control. Treatment with olaparib and veliparib (potent inhibitors of PARP1 and PARP2) abrogates LRRK2 mediated and basal levels of PAR accumulation, indicating that the PAR increase is PARP1/2 dependent. Consistent with our in vitro findings, preliminary data demonstrate increased PAR levels in midbrain lysates from 4-6 month old Lrrk2 G2019S KI (GKI) mice compared to wild-type controls. To understand the effect of inhibiting PARP1/2 in a LRRK2 model, inhibition of PARP1/2 caused increased apoptosis of LRRK2G2019S/G2019S KI cells compared to wild-type, suggesting PARP1 function is critical for viability. To better understand the underlying mechanisms of PARP1 activation caused by G2019S LRRK2, we performed miniTurbo proximity labeling followed by mass spectrometry in wild-type and LRRK2G2019S/G2019S cells with and without exposure to DNA damage. Future and ongoing work is focused on evaluating the DNA damage landscape and elucidating PARP1 localization and interactors to understand downstream cellular consequences in LRRK2 PD. Findings from these studies may provide increased understanding of the molecular mechanisms that drive PD pathogenesis.
Kashyap Sreeram	Personalized Spatiotemporal Modeling of the Alzhiemer's Disease Biomarker Cascade	Spatiotemporal modeling of the Alzheimer's Disease (AD) Biomarker Cascade (ADBC) could play a significant role in development of personalized therapies for AD. A previous computational model by Petrella et al. 2024 utilized an interdependent system of ordinary differential equations to model longitudinal changes in beta-amyloid (A-beta), phosphorylated-tau (p-tau), and neuronal dysfunction/loss in an AD patient cohort with high accuracy. This work extends the previous model through the addition of a spatial component, represented by a system of partial differential equations (PDEs) that characterizes the temporal within-region and between-region spread of these biomarkers via white matter tracts. Fractional anisotropy data based on the Desikan-Killiany atlas were aggregated and thresholded to produce a Laplacian matrix representing the rate of change of a biomarker at a given spatial region. Roughly, the formulation of our model is of the form dx/dt - DxLx = f(x,p), where Dxrepresents the diffusion coefficient across brain regions for each biomarker, L is the graph laplacian for each biomarker, and f(x,p) refers to the mathematical formulation of biomarker aggregation. We used available longitudinal demographic and Positron Emission Tomography (PET) imaging biomarker data in 223 subjects across the cognitive spectrum from the Alzheimer's Disease Neuroimaging Initiative, including participants that were cognitively normal, had mild cognitive impairment, or were diagnosed with probable AD dementia. Personalization of the ADBC to model the spread of A-beta with individual PET biomarker data yielded four personalized parameters in each subject corresponding with physiologically meaningful characteristics, including growth rate, carrying capacity, and A-beta diffusion between regions. Performance was very high, with 98.21% of subjects demonstrating loss values below 0.2 on a normalized loss scale between 0 and 1. Visualizations reveal region- and disease-specific trajectories for A-beta, supporting the feasibility of personalizing mechanistic models. Thus, we present a computational platform to model patient-specific aggregation of AD biomarkers like A-beta and predict individual response to theoretical prevention.

Keerthana Surabhi	Identification of actin mutants with neurodegenerative disease-like phenotypes via mutagenesis of the actin-ATP interface	Identification of actin mutants with neurodegenerative disease-like phenotypes via mutagenesis of the actin-ATP interface Authors Keerthana Surabhi1, Maelee Becton1, Lizzie Phipps1, Noah Mann2, Robert Hughes2 and Erzsebet M. Szatmari1 Affiliations 1 Department of Physical Therapy, College of Allied Health Sciences, East Carolina University, Greenville, NC 2 Department of Chemistry, East Carolina University, Greenville, NC INTRODUCTION AND OBJECTIVES: Abnormal neuronal cytoskeleton dynamics is a common feature of neurodegenerative disorders including Alzheimer's disease (AD). Hirano bodies (composed of filamentous actin and actin-binding proteins) are neuronal inclusions associated with aging and universally present in the AD brain. This study focuses on understanding the involvement of actin-ATP interactions in cytoskeletal anomalies and their role in neuronal inclusion formation. MATERIALS AND METHODS: DNA constructs: Point mutations (G158L, S14V, K18A, and D154A) were introduced into Actin in a pNic28 plasmid using site-directed mutagenesis. Primary neuron cultures and transfections: Dissociated cortical neurons were prepared from E18 mouse embryos (CD1) and cultured in 6-well plates with coverslips at 500K/ml density. Neurons were transfected with 5 µg plasmid/well on DIV5 using Lipofectamine LTX reagent. Fixed neuron experiments: 48-72 hours post-transfection neurons were immunostained for MAP2 or tau. Imaging: Confocal images were obtained on a Zeiss LSM 800 microscope with Airyscan. Particle analysis: Images were analyzed using FIJI, which was equipped with the BioFormats package. RESULTS Distinct cytoskeletal phenotypes were associated with specific mutants. G158L and S14V lead to cofilin-actin rod phenotype with structural destabilization reminiscent of pathological actin-cofilin rods, while K18A and D154A exhibited large cluster phenotypes, similar to Hirano bodies. These results highlight the critical role of the actin-nucleotide binding pocket in regulating actin function. CONCLUSIONS This work provides insights into the role of actin-ATP interactions in cytoskeletal anomalies observed in neurodegenerative diseases and lays the groundwork for further
Kiwoon Sung		
Kylie Azizzadeh	Effects of APOE4 on the Metabolome in Human NOS2 Mice	Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive decline and memory loss. In addition to reversible risk factors that include obesity, hypertension, and deficits in early education, AD also has genetic predispositions including the apolipoprotein E (APOE) gene. Specifically, the APOE ε4 allele is the most potent genetic risk factor for AD. The protein encoded by the APOE4 gene is correlated to the accumulation of extracellular amyloid plaques and intracellular tangles of tau found in brains with clinical AD. The human NOS2 gene (HN) is responsible for production of nitric oxide (NO), which has prolonged generation in AD and, at high concentrations, is cytotoxic. In AD, immune dysregulation is pivotal in causing AD onset and supporting progression, due to factors such as microglia dysfunction, T cell infiltration, and chronic inflammation from pro-inflammatory cytokines and chemokines. Both APOE4 and HN genes are thought to modulate the innate immune response, potentially affecting microglial activation and the inflammatory milieu within the brain. The goal of our study is to gain insights to how metabolism supports the immune response in AD, by completing a computational analysis of metabolic data from APOE4 and HN mice. Studying metabolomic data associated with immune response modulation from mice of these genotypes will give insight to how these genetic factors regulate the immune system.
Laura Wang	Computerized crossword puzzle training in MCI: Rationale and design considerations for a multisite clinical trial (COGIT-2)	MCI represents a high-risk group for progression to Alzheimer's disease (AD). Computerized crossword puzzles offer promise to potentially slow the progression of MCI through neuroplasticity. COGIT-2 will be the first controlled long-term trial to evaluate the effects of home-based, digital, crossword puzzle training on clinical and biomarker outcomes. This 78-week randomized trial will randomize 240 patients with MCI, across 4 sites, to crossword puzzle training or an active control condition. This presentation will discuss the rationale and design considerations in this study. Positive results from this trial will accelerate the scaling of low-risk, low-cost, home-based digital cognitive training interventions for MCI.

Lauren Shiell	Engineering well-characterized and environmentally-relevant nano and micro plastics for experimental mouse models	The ingestion of plastics presents a significant concern for human health as the mass of plastic estimated to be ingested by individuals is increasing every year. Some studies suggest that there is the relative consumption of plastic found in 50 shopping bags per person per year. However, the implications for human health are complex due to the diverse nature of polymer structure and surface charges which could significantly vary biodistribution and interactions with other molecules like proteins, lipids, and nucleic acids. Current studies often rely on pristine engineered nanobeads, usually polystyrene which is relatively rare in the environment, to understand nanoplastic biology. However, these nanobeads do not mimic environmentally relevant particles in both polymer compositions, surface chemistries and morphologies. To generate particles for research purposes that better mimic what we are exposed to in daily activities, we have developed a methodology for the production of particles with differing surface charges and shapes from the most common types of plastic pollutants. Sourced recycled starting plastics were obtained from different manufacturers and cryomilled to micro and nano mixtures, and exposed to a novel environmental chamber to simulate exposures in the marine environment in years- to decades ranges. Particles are characterized through orthogonal informative suites of approaches that will include empirically measured ZetaPotential for surface charges, and shapes defined by light-scattering and Cryo-EM. Bioavailability of these particles will be studied in young and aged mice, and in mouse models of disease. The goal is to understand the chemical requirements of plastic pollutants to access different compartments in mammals and what their contribution might be to disease risk and progression.
Nada Khairy Abdelnaby Elbarbary	Sulfonamides as a broad class of protein aggregation inhibitors	[redacted] Nada Elbarbary,1,2 Sabin Khatiwada,2 Nailya Gilyazova,1 Lindsey Costantini,3 Ling Wu,1,4 Bin Xu,1,2,4 1Biomanufacturing Research Institute & Technology Enterprise (BRITE), 2Department of Pharmaceutical Sciences, 3Department of Biological and Biomedical Sciences, North Carolina Central University, Durham, NC 27707, USA. 4Affiliated Member, Duke/UNC Alzheimer's Disease Research Center, Durham, NC 27710, USA. ABSTRACT Protein misfolding and aggregation into cytotoxic amyloid plays an important role in the pathogenesis of various neurodegenerative diseases including Alzheimer's Disease. AD is a neurodegenerative disorder characterized by loss of memory and related cognitive dysfunctions. However, it has no cure or prevention to date. In an effort to prevent or treat related amyloidogenesis, we identified sulfonamides as a broad class of protein aggregation inhibitors based on literature search and library screenings. Literature search identifies twenty individual repurposing sulfonamides as potential amyloid inhibitors. In library screening with a 2500-compound Selleckchem repurposing drug library, we used engineered human tau variants (4RCF and 3RCF) as target proteins to identify sulfonamide-based tau aggregation inhibitor hits. Results of library screenings include eight identified sulfonamide hits from 4RCF tau screening and twelve sulfonamide hits from 3RCF tau screening. We further validated and characterized selected potent hits against three amyloidogenic protein targets, amylin, 2N4R tau, and Aβ42. Our results showed glyburide, zafirlukast, sulfasalazine, and amsacrine have strong in vitro inhibitory activities. We used both qualitative ThT fluorescence-based aggregation, remodeling assays, and quantitative IC50 dose dependent inhibition potency measurements. Our results showed four novel inhibitors, glyburide, zafirlukast, sulfasalazine, and amsacrine from in vitro assays with IC50 varying from 15-30 μM, which are close to a well-known strong inhibitor EGCG (4 μM). We further demonstrated using Transmission Electron Microscopy (TEM) imaging that glyburide, an anti-diabetic drug and zafirlukast, an anti-asthma drug, both significantly remodeled amylin fibrils from regular fibrillar morphology to globular beads like aggregates, similar to the effect of EGCG. (Sponsored in part by NIH grants R01AG067607, R03AG085058, and NC Biotechnology Center

Natalie Dzikowski	Profiling DNA Methylation and Gene Regulation in Alzheimer's Disease Using Long-Read and Single-Cell Approaches	Increasing evidence suggests that epigenetic mechanisms, such as DNA methylation, may contribute to neurodegenerative diseases like Alzheimer's disease (AD). While both genetic and non-genetic risk factors for AD have been studied, the molecular mechanisms that drive disease pathology remain incompletely understood. The human brain exhibits AD-associated variation in DNA methylation, and much of this variation is cell-type specific. However, analyses of AD-associated DNA methylation have been limited to subsets of CpG sites, leaving a large fraction of the methylome unexplored. Additionally, it remains unclear whether these methylation differences are causal. Does AD-associated variation in DNA methylation impact gene regulation and contribute to disease pathology, or is it a consequence of AD neuropathology? To address these gaps, we are applying long-read DNA sequencing to characterize AD-associated methylation across major cell types in the human brain. This approach will enable us to capture cell-type specific methylation differences at complex regions of the genome, such as telomeres or structural variants, that may have been missed in previous analyses. Furthermore, we are developing a single-cell, methylation-specific massively parallel reporter assay (sc-mSTARRseq) to functionally test whether AD-associated methylation differences have downstream impacts on gene regulatory activity. These approaches combined will provide new insights into the contributions of different cell types to Alzheimer's disease and the mechanisms by which epigenetic dysregulation may drive neurodegeneration.
Nicole Scott-Hewitt	RNA-dependent neuroimmune interactions impact protein homeostasis in the aging brain	The contribution of neuroimmune mechanisms to disease-associated cellular and cognitive dysfunction remains an area of active investigation, with mounting genetic, molecular, and clinical evidence implicating these in Alzheimer's Disease (AD) pathology. The innate immune complement cascade is one such pathway. Our recent work has uncovered a unique role of the microglial-secreted complement protein C1q in critical intraneuronal processes; demonstrating that C1q undergoes RNA-dependent liquid-liquid phase separation (LLPS), interacts with neuronal ribonucleoprotein (RNP) complexes in an age- and RNA-dependent manner, and alters neuronal protein homeostasis in vivo in the adult brain. We will continue to build off this work to explore how AD-associated proteotoxicity alters intraneuronal RNA-mediated neuroimmune interactions, and whether these contribute to AD-related pathologies including neuronal and synapse loss, plaque deposition, glial dysfunction, and neuroinflammation.
Nirali Patel	Evaluation of novel patient-derived α-synuclein fibril conformations in Parkinson's disease models	Recent efforts towards uncovering structural compositions of α-synuclein fibrils from postmortem brain samples revealed a presence of multiple conformations of fibrils. A prevalent hypothesis suggests that the structural composition within fibril variants holds a crucial functional component, encoded on conformational levels by different strains, that may contribute to disease manifestation and severity. Cryo-electron microscopy (cryo-EM) analysis of patient-amplified conformations revealed different structural properties of fibrils amplified from CSF between the patients. In humanized α-synuclein mice, these fibrils had distinct properties of pathobiology, despite only minor structural variation. The research supports the hypothesis that different patient α-synuclein conformers may convey specific functional characteristics to disease endpoints and associated pathological features.

Paula Mendez de Inza	Connectome-Based Brain Age Prediction Model as a Marker for Alzheimer's Disease	Connectome-Based Brain Age Prediction Model as a Marker for Alzheimer's Disease Paula Mendez de Inza, Hae Sol Moon, Robert J Anderson, Alexandra Badea Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by amyloid and tau proteinopathies and neurodegeneration. Early diagnosis is critical to enable timely intervention, as neurodegeneration begins years before clinical symptoms appear. Brain network analyses hold promise for early detection. This study explores the use of structural brain connectomes derived from high resolution diffusion MRI, to estimate brain age in cognitively normal subjects, providing a potential marker for Late-Onset Alzheimer's disease (LOAD) risk. A predictive model was developed using Graph Neural Networks (GNNs) on data from 86 subjects, each with an 84x84 structural connectome based on the Desikan Killiany labeling and the Illinois Institute of Technology atlas. Besides connectomes, our model uses as features metadata including demographic, cognitive and APOE genotype information. Connectome matrices were transformed into graphs, where nodes correspond to brain regions and edges represent connectivity strength. Various strategies were applied to improve the model's performance. Logarithmic transformations were explored to stabilize connectome values. Clustering coefficients were added as features, and we used thresholding to reduce graph noise. Regularization techniques including dropout, batch normalization, weight decay, learning rate scheduling, and early stopping were incorporated to enhance generalization. A permutation-based edge ablation analysis was conducted to identify the most influential connections. Preliminary results revealed moderate predictive accuracy (MAE ~ 8.5 years). Future work will include data normalization and data augmentation. This approach highlights the potential of brain connectivity patterns as non-invasive tools for late onset Alzheimer's disease risk prediction.
Ran Ming	Identification of the TPR domains of co-chaperones as FKBP51 substrates	Chaperone complexes are dynamic with tetratricopeptide (TPR) domain containing co-chaperones competing for interaction with the C-terminus of chaperones to regulate assemblies and dictate client outcomes. Currently, factors that regulate the affinity between chaperones and co-chaperones are poorly defined. Here we identify TPR domain-containing co-chaperones as substrates of FK506 Binding Protein 51 (FKBP51). Using BioID, Nuclear Magnetic Resonance (NMR) and in vitro biochemical assays, we find that FKBP51 induces the unfolding of co-chaperone TPR domains in a manner dependent upon its catalytic activity and upon the presence of its TPR domain. We further show that thermodynamic instability is a key feature of FKBP51 substrates and that stabilization of these TPR domains prevents structural changes induced by FKBP51. Finally, we show that mutations to the TPR domain of CHIP that cause Spinocerebellar Ataxia Type 48 (SCA48) reduce the thermal stability of CHIP and induce structural changes to the TPR domain of CHIP that resemble those induced by FKBP51. Strikingly, increasing the thermodynamic stability of the TPR domain of SCA48 mutant CHIP is able to overcome this structural defect suggesting the therapeutic potential of small molecules that stabilize SCA48 mutant CHIP. Together these findings identify the first structurally validated substrates for FKBP51 and suggest a role for FKBP51 in regulating chaperone assemblies.
Ryan Rodriguez	The CHIP/CHMP2B E3 ubiquitin ligase complex regulates CHMP4B ubiquitination and endo-lysosomal dynamics	CHIP is a key E3 ligase that counters aging and neurodegeneration. The E3 ligase CHIP recruits misfolded proteins by forming a carboxy clamp with the C-terminal aspartic acid residue of chaperones. Using a Bio-ID screen we found that CHIP is in proximity to proteins that have a C-terminal Asp Residue (CAspR). We find that one CAspR, CHMP2B, binds to the TPR domain of CHIP and that a CHIPCHMP2B E3 complex ubiquitinates CHMP4B. We further show that mutations in CHMP2B that cause FTD/ALS, and mutations in CHIP that cause SCA48, disrupt the formation of the CHIPCHMP2B complex and impair ubiquitination of CHMP4B, disrupting endosomal dynamics. Finally, we show that restoring the targeting of FTD/ALS mutant CHMP2B to CHIP restores CHMP4B ubiquitination and is sufficient to prevent defects in endosomal dynamics. Together, our studies identify the CAspR CHMP2B as substrate adaptor for CHIP and provide insight into cellular pathways disrupted by mutations in CHIP and CHMP2B that cause neurodegeneration.

Samuel Strader	LRRK2 in peripheral leukocytes mediates dopaminergic neurodegeneration in mouse models of Parkinson's disease.	LRRK2-targeting therapeutics have advanced into clinical trials in Parkinson's disease (PD). Several pre-clinical studies, mostly in mice and rats, have demonstrated that hyperactivation of LRRK2 kinase activity tends to exacerbate the damaging effects of PD-associated α-synuclein, whereas LRRK2 repression or ablation may curtail α-synuclein neurotoxicity. With LRRK2 expression and activity in both brain resident cells and high LRRK2 expression and activity in peripheral pro-inflammatory immune cells that include monocytes, macrophages, and neutrophils, it has been unclear whether LRRK2 primarily exerts a modifying effect for α-synuclein neurotoxicity via expression in cells in the brain or potentially contributions from immune cells originating from the periphery. A better knowledge of the origins and mechanisms of LRRK2 neurotoxicity could have implications in therapeutic strategies, since LRRK2-targeting antisense technology in clinical trials may not affect peripheral LRRK2 activity in the same manner as small molecules that distribute across the body. To help address this knowledge gap, we use a complementation strategy to create mosaic mice that remove LRRK2 expression only in peripheral immune cells, or the reverse with LRRK2 knocked out or overexpressed only in brain-resident cells with intact expression of LRRK2 in peripheral immune cells. After reconstitution of the marrow and a return of the mice to immunological homeostasis, the mice received an intracranial injection with AAV-A53T α-synuclein into the SNpc to initiate progressive dopaminergic neurodegeneration known to be accompanied by neuroinflammation. Neurotoxicity and the infiltration of peripheral immune cells were evaluated in the striatum and SNpc at both one- and three-months post-injection. Results from these studies, which are ongoing, will help clarify the role of both peripheral and central LRRK2 expression relevant to α-synuclein-related neurodegeneration in mice. We continue to evaluate the molecular pathways altered by LRRK2 activity in the CNS and in resident and peripheral immune cells to help elucidate the relationship between LRRK2 activity and α-synuclein-related neurodegeneration.
Seneca Oxendine	Neuron-microglia interactions via interleukin-34 are protective against inflammatory challenge in mouse models of aging and Alzheimer's disease	Neuroimmune interactions are critical for physiological brain function, and these interactions are implicated in the prevention and promotion of many neurodegenerative diseases, including Alzheimer's disease (AD). Microglia, the resident macrophages of the central nervous system, play key roles in these interactions and have diverse functions, including synaptic remodeling, injury repair, and response to immune challenge. Immune challenges, including viral infections associated with AD, can cause changes in microglial function that result in future dysfunctional responses. Multiple recent genome-wide association studies have linked mutations in the human interleukin-34 gene, which encodes for a neuron-derived cytokine that signals through the microglial colony-stimulating factor 1 receptor (CSF1R), to increased risk of AD, and additional studies have found decreased IL34 expression and protein levels in postmortem analysis of human brains. Recent studies from our lab have established that neuronal IL34 signaling acts as a homeostatic regulator of microglia during cortical development; however, the contributions of IL34 signaling to microglial response to inflammatory challenge and the pathogenesis of neurodegenerative disease remain unknown. This project leverages acute postoperative and viral inflammatory challenges in the 5XFAD mouse model of AD and aged wild type mice, along with in vivo viral overexpression of IL34 in hippocampal neurons, to identify behavioral, cellular, and molecular outcomes associated with microglial response to inflammation. Our preliminary data shows that hippocampal IL34 overexpression increases regional homeostatic microglia, is associated with improved postoperative cognitive outcomes in aged mice, and influences microglial response to amyloid plaques in 5XFAD mice. In addition, our preliminary data illustrates the impact of a viral inflammatory challenge (influenza infection) on behavioral symptoms and microglial function in the acute and recovery stages of disease, and links IL34 signaling to these outcomes. Ultimately, this project aims to determine the role of IL34 signaling in infection-induced microglial changes and long-term neuroprotection, which will provide insight into the role of microglia in the pathogenesis of neurodegenerative disease.

Seth Tart	Examining Astrocyte Primary Cilia Dysfunction in Alzheimer's disease	Primary cilia are vital signaling organelles that regulate cell function. Nearly all astrocytes in the adult brain are ciliated, and dysfunction of primary cilia is associated with Alzheimer's disease (AD). Importantly, however, no studies to date have characterized astrocyte primary cilia in human AD brains. To determine whether AD-related primary cilia dysfunction is recapitulated in humans, we quantified the percentage of ciliated astrocytes in human postmortem AD brains and controls. We found that both AD and non-AD brains possess ciliated astrocytes in various subregions of the hippocampus, specifically the hilus, CA1, CA3, and entorhinal cortex (EC). Astrocytes in the hilus and CA3 of AD brains have significantly fewer primary cilia. To gain molecular insight into astrocyte primary cilia loss, we also induced pluripotent stem cells (iPSCs) directly toward astrocyte differentiation. We confirmed that induced astrocytes (iAstrocytes) form primary cilia, and we transfected iAstrocytes with wild type (WT) or mutant amyloid precursor protein (APP) or tau. Early analysis indicates that cultured cells transfected with mutant tau exhibit reduced percent ciliation compared to controls. This work represents the first characterization of primary cilia in post-mortem human AD brains and establishes a method for studying the primary cilia of AD-like human astrocytes in vitro. Ultimately, this will provide a framework for understanding astrocyte primary cilia dysfunction in AD.
Shiyi Wang	Astrocytic LRRK2 Controls Synaptic Connectivity via Regulation of ERM Phosphorylation	Astrocytes regulate synaptic connectivity. However, whether astrocyte dysfunction causes synaptic pathologies in disorders such as Parkinson's Disease is unknown. Here, we investigated how the most common Parkinsonism gene mutation, LRRK2 G2019S, impacts structure and synaptogenic function of cortical astrocytes. In human and mouse cortex, the LRRK2 G2019S mutation caused astrocyte morphology deficits and enhanced the phosphorylation of the ERM proteins (Ezrin, Radixin, and Moesin), components of the perisynaptic astrocyte processes. Reducing ERM phosphorylation in LRRK2 G2019S mouse astrocytes restored astrocyte morphology and corrected excitatory synaptic deficits. Using an in vivo BioID proteomic approach, we found astrocytic Ezrin interacts with Atg7, a master regulator of autophagy. The Ezrin/Atg7 interaction is inhibited by Ezrin phosphorylation, thus diminished in LRRK2 G2019S astrocytes. Importantly, Atg7 function is required to maintain proper astrocyte morphology. These studies reveal an astrocytic molecular mechanism that could serve as a therapeutic target for synaptic pathologies seen in PD.
Silas A. Buck	Leucine-rich repeat kinase 2 activation in progressive tauopathies	Alzheimer's disease (AD) and primary tauopathies are characterized by pathological tau aggregation, in the form of neurofibrillary tangles and dystrophic neurites. Recent evidence suggests tau pathology is also observed in synucleinopathies, including Parkinson's disease (PD) and Dementia with Lewy Bodies (DLB). Interestingly, mutations in the leucine-rich repeat kinase 2 (LRRK2) gene cause autosomal dominant PD, and both alpha-synuclein and tau pathology are observed in LRRK2 PD. To date, however, direct evidence linking LRRK2 to alpha-synuclein or tau pathology in human neurodegenerative diseases has not been described. Since PD-causing LRRK2 variants display increased kinase activity, we investigated whether levels of LRRK2 kinase activity markers correlate with pathology by performing western blot and immunohistochemical/immunofluorescent labeling in brains from subjects with AD, DLB, idiopathic PD (iPD), and LRRK2 PD, as well as unaffected age-matched controls. We used phospho-Rab12 S106 (pRab12) and phospho-Rab10 T73 (pRab10) as markers of LRRK2 kinase activity. Follow-up studies were performed in PS19 mice, which overexpress human pathogenic mutant tau in neurons, and primary rat cortical neurons transduced with P301L/S320F double-pathogenic mutant tau lentivirus. We found that Rab12 phosphorylation is increased in parallel with advancement of AD co-pathology stage in DLB patient hippocampus and temporal cortex, suggesting LRRK2 is activated by AD co-pathology in DLB. We also discovered that pRab12 localizes to tau aggregates in AD and both tau aggregates and Lewy bodies in DLB, iPD and LRRK2 PD. Double-labeling in DLB suggests pRab12 labels a subset of pathological tau aggregates. In addition, we observed labeling of granulovacuolar degeneration bodies (GVBs) in AD, DLB, iPD and LRRK2 PD by both pRab12 and pRab10, which we will herein term Phospho-Rab-Associated Vesicles (PRAVs). We replicated this pRab12 PRAV labeling in PS19 mice, demonstrating that pathological tau triggers pRab12-positive PRAVs. Further, we found that lysosomal marker LAMP1 labels an outer membrane surrounding this pRab12-positive dense core, confirming reports that PRAVs/GVBs are lysosomal structures. Finally, in rat primary cortical neurons transduced with P301L/S320F tau, we observed accumulation of pRab12-positive PRAVs. In conclusion, LRRK2 phosphorylation of Rab12 is activated in tauopathies, and this activation may play a role in pathological tau and PRAV/GVB accumulation across neurodegenerative diseases.

Tara Richbourg	Investigating Rab family proteins as mediators of mitochondrial dysfunction in Parkinson's disease	Mutations in leucine-rich repeat kinase (LRRK2) are associated with the onset of late-autosomal dominant familial Parkinson's disease (PD). One widely accepted pathogenic contributor to dopaminergic cell death in PD is mitochondrial dysfunction, with several abnormalities in the mitochondria having been reported in association with dopaminergic neurodegeneration in patients, animal models, and cellular systems representative of PD. The overall goal of this work is to elucidate potential players in the mechanisms underlying the accumulation of mitochondrial dysfunction in PD, contributing to a wider understanding of PD etiology which is vital for the pursuit of disease-modifying therapeutics. We have observed mitochondrial defects in models of the PD-causing LRRK2 G2019S mutation, including defects in mitophagy and a loss in integrity of mitochondrial DNA. PD-linked mutations in LRRK2 lead to an increase in LRRK2 protein kinase activity and hyperphosphorylation of its substrates, including the Rab GTPase family of proteins. Our preliminary evidence implicates that Rab proteins are involved in mediating PD-associated mitochondrial defects. Knockout models of certain Rab proteins exhibited impaired mitochondrial depolarization-induced mitophagy in a manner similar to models of LRRK2 PD, leading us to hypothesize that select Rab protein family members are critical for mitophagy, and that their dysregulation may contribute to mitophagic defects downstream to the LRRK2 mutation. To test this hypothesis, we performed a high-throughput siRNA-based knockdown screen of the entire Rab family of proteins in a HeLa cell line expressing mKeima and YFP-Parkin. mKeima/YFP-Parkin HeLa were plated with pre-stamped siRNAs for each Rab family protein, followed by induction of mitophagy through treatment with the mitochondrial uncoupling agent FCCP. Confocal image-based analysis was then used to assess the involvement of each Rab family protein in depolarization-induced mitophagy to identify novel mediators of mitophagic dysfunction in PD. Results indicated positive and negative regulators of mitophagy within the family of Rab proteins, and future studies will investigate the role of identified targets in the development of mitochondrial dysfunction in models of LRRK2 PD.
Tobilola Akingbade	NOVEL BIOMARKER DISCOVERY FOR LIMBIC PREDOMINANT AGE RELATED TDP-43 ENCEPHALOPATHY DIAGNOSIS	NOVEL BIOMARKER DISCOVERY FOR LIMBIC PREDOMINANT AGE RELATED TDP-43 ENCEPHALOPATHY DIAGNOSIS Tobilola Akingbade,1 Ling Wu,2,4 Shih-Hsiu J. Wang,3,4 and Bin Xu1,2,4 1Department of Pharmaceutical Sciences, College of Health and Sciences, 2Biomanufacturing Research Institute and Technology Enterprise (BRITE), North Carolina Central University, Durham, NC 27707. 3Department of Pathology and Neurology, Duke University Medical Center, 4Affiliated Member, Duke-UNC Alzheimer's Disease Research Center, Durham, NC 27710. Abstract: Limbic-Predominant Age-Related TDP-43 Encephalopathy (LATE), featured by a TDP-43 proteinopathy signature, is a recently characterized type of dementia. Autopsy findings indicate that LATE can worsen cognitive impairment both independently and in combination with other neurodegenerative diseases, particularly in individuals aged 80 and above. LATE is often misdiagnosed because it shares similar memory-related symptoms with those of Alzheimer's disease (AD) and patients with pure LATE are typically classified as Cognitively Normal (CN), with only mild cognitive complaints until death. Currently, there are no robust fluid biomarkers to distinguish LATE from CN or AD or LATE with other comorbidities. However, numerous studies have demonstrated the direct relationship between LATE and TDP-43 proteinopathy and between LATE and tau proteinopathy. This study investigates site-specific phosphorylated tau (p-Tau) antibodies/epitopes as potential LATE-specific biomarkers in brain tissues. From western blotting analysis and ELISA quantification of postmortem temporal cortex, we identified four site-specific p-tau antibodies that showed statistically significant increase of p-Tau levels in AD samples compared to those in LATE samples. While no p-Tau epitopes effectively distinguished LATE from CN or AD from LATE with AD comorbidity, we identified four p-Tau epitopes which can differentiate stage 3 LATE from CN. One p-Tau epitope distinguished stage 1 from stage 3 LATE. These findings highlight the site-specific p-Tau antibodies as potential new LATE biomarkers and emphasize the need for further research to develop and improve diagnosis for LATE. (Sponsored BY: NIH grants R01AG067607, R03AG085058, and NC Biotechnology Center Translational Research Grant 2023-TRG-001)

Tuyana Malankhanova	Role of microtubules in the mediation of LRRK2 activity	Microtubule dysfunction is associated with several neurodegenerative disorders including Parkinson's disease (PD). Recent reports suggest microtubules may stabilize the kinase-active conformation of the LRRK2 protein kinase. LRRK2 activity is linked to PD risk, possibly through the phosphorylation of Rab protein substrates that can control vesicle and lysosome dynamics. Our studies in macrophages that have high levels of endogenous LRRK2, Rab substrates, and dynamic microtubules, genetic and complementary pharmacological approaches reveal the underlying mechanisms of microtubule and LRRK2 interactions. Preliminary data suggests stable LRRK2 alignment along microtubules does not occur under physiological conditions and instead requires the over-expression of LRRK2 fused to a bulky N-terminal tag in combination with type II inhibitors. However, fast-scanning proximity ligation proteomics highlights prominent LRRK2 kinase-independent interactions with tubulins, which is not influenced by microtubule polymerization. Forced recruitment of LRRK2 to the outer surface of microtubules using molecular trapping system does not affect LRRK2 kinase activity, whereas forced recruitment to the lysosome in macrophages strongly upregulates LRRK2 activity. Acute microtubule destabilization abolishes Rab positioning on the membranes and rescues the lysosomal stress-induced increases in LRRK2 activity and phosphorylated Rab10 levels. Restoration of GTP levels under microtubule destabilization conditions allows for normal LRRK2 mediated Rab phosphorylation. Together, these results suggest that microtubule polymerization does not have a direct effect on the mediation of LRRK2 activity, and that stable binding to microtubules may be a consequence of over-expression and bulky N-terminal LRRK2 tags.
Weiping Wang	Novel pharmacodynamic markers and assays in biofluids for LRRK2 kinase activity	Parkinson's disease (PD) is an age-related neurodegenerative disorder that profoundly impacts patients' quality of life and imposes a significant burden on healthcare systems. Mutations within the leucine-rich repeat kinase 2 (LRRK2) gene are among the most common genetic risk factors for both familial and sporadic PD. Pathogenic mutations in LRRK2 are known to increase LRRK2 kinase activity. This underscores the therapeutic potential of LRRK2 kinase inhibitors for PD, though pharmacodynamic biomarkers to track LRRK2 kinase activity, for example in response to candidate therapeutics, have not been sufficiently developed for use in routinely collected and banked biofluids. A specific, sensitive, and accessible platform for measuring LRRK2 kinase activity from banked biofluids may facilitate the success of LRRK2-targeting drugs in clinical trials through facilitating measures of target engagement on the patient level for the study duration, as well as assist patient selection for therapeutic intervention based on LRRK2 activity profiles. Herein we introduce an ultra-sensitive single-molecule array assay developed and validated by our laboratory to evaluate extracellular levels of LRRK2, Rab10, and phosphorylated Rab10 (pT73-Rab10) in small volumes of serum and cerebrospinal fluid (CSF). In mice, strains with PD-linked VPS-35 mutations and over-expression of SNCA were treated with different LRRK2 kinase inhibitors to track pharmacodynamic responses in serum related to the ratio of pT73-Rab10 to total Rab10. Rats were also treated with LRRK2 kinase inhibitors, with blood serum procured at different time-points post oral dosages of LRRK2-targeting drugs. Finally, pharmacodynamic responses in these markers have been evaluated in non-human primate biofluids treated acutely or chronically with LRRK2 kinase inhibitors. Preliminary results suggest that both serum and CSF concentrations of total LRRK2, and the ratio of pT73-Rab10 to total Rab10, are highly pharmacodynamic and rapidly diminish with LRRK2 inhibition. These studies will reveal the relationship between free drug levels and the fluid biomarkers to help establish a foundation for the successful integration of these markers into ongoing clinical trials for LRRK2-targeting therapeutics.
ZIJIAN ZHANG	Cell Type-Specific Dissection and Correction of Neural Circuit Changes during the Pathogenesis of Alzheimer's Disease	Alzheimer's disease (AD) is characterized by cognitive decline and progressive memory loss, driven by pathological changes affecting specific neuronal circuits. Synaptic degeneration, a key early feature of AD, disrupts neural connectivity, underscoring the need to map circuit alterations at high resolution to inform therapeutic strategies. However, traditional approaches such as electrophysiology and viral tracing with fluorescent imaging are limited in throughput. Recent advances in single-cell genomics, combined with transsynaptic rabies virus (RV) tracing, have enabled more comprehensive circuit analysis. To leverage these high-throughput methods, we have developed inducible barCoded Rabies Virus (inCodeRV), an innovative tool integrating single-cell transcriptomics with temporally controlled transsynaptic labeling. Based on monosynaptic RV tracing, inCodeRV incorporates a 20-nucleotide barcode and a molecular switch to enable precise, time-resolved mapping of neuronal networks. Upon injection, each starter cell is uniquely barcoded, and controlled activation of viral spread propagates these barcodes to presynaptic partners, allowing systematic circuit reconstruction via single-cell RNA sequencing (scRNA-seq). This method provides high-throughput, dynamic mapping of neuronal connectivity with unprecedented resolution. By applying inCodeRV to AD models, we aim to dissect progressive circuit disruptions and identify therapeutic targets to restore network integrity.