Abstracts for Oral Presentations, Friday, Sept 28th

Oral Session: Neuroinflammation and Immunity

9:45-10:05 – Malú G. Tansey (Emory)

From the Gut to the Brain: Intestinal Inflammation as a Driver of Parkinsonian Neuropathology

Gastrointestinal (GI) problems are common features of PD, however, and they frequently manifest years before the development of motor symptoms. This has led to the theory that PD pathology could initiate in the intestine before advancing to the central nervous system (CNS). Given the abundant evidence supporting a role for inflammation in neurodegenerative disease, we investigated whether intestinal inflammation could mediate the progression from digestive dysfunction to CNS neuropathology in PD.

In a large-scale human study, we confirmed that the majority of PD patients experienced GI problems, and we identified elevated levels of specific soluble inflammatory mediators in stool from PD patients compared to controls. We determined that this inflammatory profile did not emerge as a result of advanced age or disease duration, suggesting that GI inflammation is involved in earlier stages of PD. Evaluation of colonic biopsies from PD patients affirmed these findings, revealing evidence of substantially increased immune cell infiltration, proinflammatory activity, and oxidative stress in gut tissue from PD patients compared to controls.

We then utilized mouse models to evaluate the impact that colonic inflammation could exert on neuron health and function in the brain. We discovered that the induction of damage and inflammation in the intestine was sufficient to perturb the functionality of dopaminergic neurons on its own, reducing levels of tyrosine hydroxylase and modulating dopamine transporter expression. We also found that colitis rendered mice more susceptible to the effects of the neurotoxic agent MPTP. The severity of certain neurological changes correlated with the severity of colitis in our model, further substantiating the relationship between GI inflammatory activity and central neuropathology. Our findings confirm that intestinal inflammation is present in PD and that such inflammation can induce dopaminergic neuropathology, lending support for the gut-to-brain theory of PD pathogenesis.

10:05-10:10 – Janna Jernigan (Emory)

Exploring transcriptional regulators of homeostatic and disease-associated microglial transcriptomic signatures

Background: Microglia are the resident innate immune cells of the central nervous system that transcriptionally transform from homeostatic to disease-associated-microglial (DAM) profiles in aging and neurodegeneration. Using network analysis of microglial transcriptomes, we recently identified distinct pro-inflammatory and anti-inflammatory gene co-expression modules within DAM in Alzheimer's disease (AD) models. Defining key transcriptional regulators of homeostatic, pro- and anti-inflammatory DAM microglial modules can be patho-physiologically and therapeutically important.

Methods: Transcriptional factors regulating homeostatic, pro- and anti-inflammatory DAM modules were predicted using canonical pathway analysis. Of these, top TFs for each module most highly expressed in microglia were silenced using small interfering RNA (siRNA) under resting and pro-inflammatory conditions. Using quantitative RT-PCR, gene expression of TFs, homeostatic and DAM genes were measured (n=3/condition).

Results: Among distinct predicted TFs for each microglial module IRF1, LXRβ and CEBPα were selected for PCR studies. We observed highly efficient silencing of all TFs (>70%) in primary microglia at 48h. Under resting conditions, CEBPα silencing upregulated pro-inflammatory DAM gene IL-1β and suppressed anti-inflammatory DAM genes (ApoE, Kcnj2) without altering homeostatic Tmem119. LXRβ silencing induced Ptgs2 mRNA and suppressed pro- (IL1β), anti-inflammatory DAM (ApoE, Kcnj2, Timp2) and Tmem119 expression. IRF1 silencing suppressed IL-1β, ApoE, Kcnj2, nceh1, Timp2 and Tmem119. Silencing IRF1 or LXRβ inhibited LPS-induced IL1β and Ptgs2 upregulation and augmented LPS-induced suppression of Apoe and Kcnj2. Silencing CEBPα prevented LPS-induced pro-inflammatory DAM (IL1β, Kcna3 and Ptgs2) upregulation and enhanced LPS-induced suppression of Apoe.

Conclusion: Our results suggest complex upstream regulation of homeostatic and DAM genes by TFs IRF-1, LXRβ and CEBPα. CEBPα also appears to play opposing roles in regulating IL1β expression under resting and activated microglial states. Global transcriptomic profiling of microglia under in-vivo relevant conditions will further clarify the role of these TFs in regulating microglial activation in health and disease.

10:10-10:20 – Aubrey Schonhoff (UAB)

Activated border-associated macrophages mediate peripheral cell infiltration in an AAV2 α-syn model of Parkinson disease

Parkinson disease (PD) is characterized by progressive loss of dopamine producing neurons in the substantia nigra pars compacta (SNpc) and widespread intracellular inclusions of the protein alpha-synuclein (α-syn). Evidence highlights the role of the immune system in progression of PD. In both human patients and rodent models, α-syn pathology is accompanied by microglial activation, T cell infiltration, hyper-reactive monocytes, and increased cytokine and chemokine release. However, the triggers responsible for initiating this immune response remain poorly understood. Additionally, many previous studies have not separated microglia from border-associated macrophages (BAMs, including perivascular, meningeal, and choroid plexus macrophages) and infiltrating cells during analysis, complicating the study of innate immune mechanisms. To determine the role of BAMs in models of PD, we utilized an adeno-associated virus (AAV) that overexpresses full-length human α-syn in neurons of the SNpc. We injected this into transgenic mice in which the first exon of CX3CR1 is replaced with GFP. Using flow cytometry and immunohistochemistry, we examined tissue resident CX3CR1+ cells (microglia and BAMs) for activation markers and proliferation. We found that α-syn led to an increase in the number of BAMs and in their expression of MHCII. To determine the functional role of BAMs, we administered clodronate-filled liposomes into the lateral ventricles to specifically deplete the BAMs without affecting other cell populations, such as microglia. Depletion of BAMs had an anti-inflammatory effect, as it significantly decreased infiltration of peripheral monocytes and T cells. Additionally, BAM depletion prevented upregulation of MHCII by microglia. These results demonstrate the importance of BAMs in the initiation of neuroinflammation and the recruitment of peripheral immune cells subsets in an α-syn PD model.

10:20-10:40 – John Fryer (Mayo)

Translational profiling of microglia reveals shared signatures between aging, amyloid, and tau pathology

Alzheimer's disease (AD) is an age-associated neurodegenerative disease characterized pathologically by amyloidosis, tauopathy, and activation of microglia, the resident innate immune cells of the brain. We found that translational profiling of microglia (RiboTag) avoided cellular contamination and activation bias when directly compared to traditional cell sorting methods. We used this RiboTag approach to profile microglia in mouse models of amyloidosis, tauopathy, or aging alone. We found a striking overlap not only between amyloid and tau models, but also with aging alone. ApoE was among the top hits from amyloid, tau, and aging, and was upregulated to such as extent as to be in the 99% of all transcripts based on total abundance. Even in basal conditions, ApoE is abundantly (though not specifically) expressed by microglia and ranks in the top 95% of all transcripts based on absolute abundance. In addition to Cst7, Lgals3, Itgax, and Spp1, both Ccl3 and Ccl4 were among the most upregulated transcripts across all conditions, but these molecules have not been previously identified in these settings due to biases from traditional sorting methods. Pathway analysis of shared changes among amyloid, tau, and aging revealed that apoE was a critical driver of the top most enriched network that funneled into activation of CCL3 and CCL4, chemokines known to be involved in chemotaxis. This study has broad implications for microglial transcriptomic approaches and provides new insights into microglial pathways associated with different pathological aspects of aging and AD.

10:40-10:45 – Ngozi Nwabueze (Emory)

Age-dependent and age-independent microglial transcriptomic changes in the 5xFAD model of Alzheimer's Disease

Background: Microglia play disease-modifying roles in neurodegenerative diseases including Alzheimer's disease (AD) whereby homeostatic microglia progressively adopt disease-associated microglia (DAM) phenotypes. We aimed to determine the impact of aging on AD-associated microglial changes and of AD pathology on aging-related microglial changes.

Methods: Using a 770-gene panel (Nanostring), we performed transcriptomic profiling of flow-cytometrically sorted CD11b+CD45low microglia from adult (6 and 12 mo) wild-type (WT) and 5xFAD mice. Gene expression data were normalized to housekeeping genes (nSolver software). Differential expression (DEX) analyses were performed to identify 5xFAD-specific microglial transcriptomic changes and contrast early and advanced aging groups. DEX gene lists were also compared with DAM and homeostatic profiles.

Results: In 6 mo old mice, 34 gene transcripts showed DEX in 5xFAD microglia (17 upregulated, 17 downregulated) of which 27 met two-fold criteria (11 upregulated including Bdnf, Fgl2, Abcc3; 16 downregulated including Epsti1, Kdm3b, Rala). 60 genes showed DEX in 12 mo old 5xFAD mice (31 upregulated, 29 downregulated) of which two-fold DEX was observed for 48 genes (22 upregulated including Cst7, Apoe, Rps21, Clec7a; 26 downregulated including Bin1, Lrg1, Ctsf, Irak1). Despite robust amyloid beta (Aβ) deposition in both age groups, minimal overlap between early and late-stage differentially-expressed genes was observed. Upregulation of DAM genes (Cst7, Apoe, Clec7a, Ly9) and downregulation of homeostatic genes (Bin1, Ctsf, Jun) were seen in advanced-aged 5xFAD mice. Age-dependent changes unrelated to pathology (Sall1, Itgb5, Csf3r, C1qb, Itgam, Cd72) as well as those upregulated (Cd33, P2ry12, Tnf, Mpeg1, Supt7l, Igf1) or downregulated (Marco, Tet1, Bdnf, Rpl29, Msn, Xiap) specifically in aging 5xFAD mice were identified.

Conclusions: Despite established Aβ pathology, age appears to strongly impact microglial gene expression patterns whereby canonical DAM genes are upregulated in highly-aged mice. Our findings highlight key age-dependent and age-independent differences in microglial transcriptomic profiles in mouse models of AD pathology.

10:45-11:05 – Ashley Harms (UAB)

T cells are required for alpha-synuclein-induced myeloid activation and demyelination in a mouse model of multiple system atrophy

Multiple system atrophy (MSA) is a progressive neurodegenerative disorder characterized by abnormal accumulation of the protein alpha-synuclein (α-syn) in oligodendrocytes and is accompanied by inflammation, demyelination, and neuron loss in the brain. While little is known about the mechanisms of neurodegeneration in MSA specifically, recent work has highlighted a key role for the immune system in the pathophysiology of synuclein-related diseases including Parkinson disease. Using post mortem MSA patient brain, we show the presence of HLA-DR reactive microglia and T lymphocytes (CD4, CD8+) in the diseased caudate/putamen. To model MSA in mice in vivo, we utilized a new viral vector that selectively overexpresses α-syn in oligodendrocytes (Olig001-SYN) with >95% trophism in the dorsal striatum, more closely modeling many features of human MSA including inflammation, demyelination, and neurodegeneration. Using this model, we have found robust inflammatory responses including up regulation of MHCII+ expression on resident microglia and infiltration of pro-inflammatory monocytes into the CNS. Additionally, in response to α-syn we observed robust infiltration of CD4+ and CD8+ T cell populations specifically in the area of the lesion and an increase in antigen experienced CD44+, CD4+ T cells in the CNS draining cervical lymph nodes. Genetic deletion of TCRβ or CD4+ T cells robustly attenuated α-syn-induced inflammation and subsequent demyelination in vivo. These results demonstrate that T cell priming and infiltration into the CNS is a key mechanism of disease pathogenesis in MSA, and suggests that therapeutics targeting T cells may be neuroprotective in human and rodent models of MSA. Funding provided by NIH/NINDS 1R01NS107316-01 Harms, PI

11:05-11:25 – Elizabeth Kline (Emory)

Mechanistic investigation of Parkinson's disease risk factors: a common genetic variation in antigen presentation capacity and pesticide exposure

Antigen presentation via major histocompatibility complex class II (MHCII) controls immune response to specific targets. In Parkinson's disease (PD), substantia nigra (SN) dopamine neurons are burdened with α-synuclein aggregates. Cells expressing MHCII have been identified within post-mortem SN of PD patients, often near dopamine (DA) neurons and α-synuclein (asyn) aggregates. rs3129882, a common single nucleotide polymorphism in MHCII loci is associated with PD risk. Our group found that PD (high-risk, GG) patients exhibit a 300-fold greater inducible increase in MHCII mRNA compared to PD (AA). Thus, in PD, antigen presentation may contribute to neuroinflammation.

To investigate effects of increased MHCII expression, we are evaluating CD4+ T helper subsets in the peripheral blood mononuclear cell population in healthy controls and PD patients (AA, GG, AG). We hypothesize that increased antigen presentation capacity via MHCII will skew the CD4+ subsets of GG individuals towards inflammatory Th1 phenotypes.

In order to better understand the interaction between MHCII and an asyn burden in the SN, we stereotaxically injected rAAV2/5-human WT asyn to the SN of mice lacking MHCII in peripheral myeloid cells. In a "double-hit", gene-by-environment interaction model, we also dosed these mice with the pesticide cypermethrin. Epidemiological studies report a connection between pesticide exposure and the incidence of PD. In addition to direct effects on DA neurons, cypermethrin exposure may affect immune cell function and has been show to increase odds for PD in GG individuals. By flow cytometry, we are characterizing peripheral immune cells as well as cells from deep cervical lymph nodes, the meningeal lymphatics' draining lymph nodes, in our "double-hit" mice. Additionally, we are immunohistochemically examining asyn expression in the SN and markers of brain immune cell activation. Mice with peripheral myeloid MHCII deletion display a different inflammatory response and dopaminergic dysfunction induced by human asyn expression and cypermethrin treatment.

Oral Session: Therapeutics I

1:00-1:10 – Jae-Kyung (Jamise) Lee (UGA)

Natural killer cells efficiently clear alpha-synuclein aggregates; the potential neuroprotective role in PD

The pathological hallmarks of Parkinson's disease (PD) are the presence of Lewy bodies (LBs), which are composed of intracellular fibrillar inclusions of alpha-synuclein ($\alpha-$syn). In addition to the pathologic effect of intracellular α-syn aggregates, the levels of extracellular α-syn species are increased in the serum and cerebrospinal fluid in PD, they exert the toxic effects and modulating immune responses both in the CNS and periphery. The number of circulating natural killer (NK) cells increased in PD number and their activities related to disease severity. Nevertheless, the role of NK cells in the context of PD has hardly been explored. Here, we first demonstrated that extracellular α-syn fibrils attenuated NK cell cytotoxicity with the dose-dependent manner by reducing the level of perforin. The extracellular α-syn also attenuated production of interferon (IFN)-γ, a major proinflammatory cytokine produced by NK cells. Remarkably, NK cells efficiently uptake and degrade extracellular α-syn species via the endosomal/lysosomal pathway. Our study has demonstrated a novel function of NK cell in clearing extracellular α-syn aggregates while extracellular α-syn aggregates not activated but inhibited NK cell effector functions. Our results suggest that NK cells may be the potential candidates of cell-based therapeutic target with their capability in clearance of extracellular α-syn aggregates without an aberrant activation.

1:10-1:15 – Kirsten Porter-Stransky (Emory)

Noradrenergic transmission at alpha1-adrenergic receptors in the ventral periaqueductal gray modulates wakefulness

Parkinson's disease (PD) causes multiple debilitating, non-motor symptoms in addition to the iconic motor impairments. Sleep disorders, especially excessive sleepiness, is one of the most commonly reported non-motor symptoms that significantly reduces the quality of life of PD patients. While degeneration of the dopaminergic substantia nigra pars compacta is considered the hallmark neuropathology of PD, an underappreciated facet of the disease is the catastrophic loss of locus coeruleus (LC) neurons. Previous research has revealed that an understudied population of dopamine (DA) neurons in the ventral periaqueductal gray (vPAG) are involved in wakefulness; yet, unlike substantia nigra DA neurons, vPAG^{DA} remain relatively intact in PD. While vPAG^{DA} neurons may be spared in PD, they are likely dysfunctional due to lack of noradrenergic input. Using behavioral pharmacology, Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), electrophysiology, and immunoelectron microscopy, the present study tested the hypothesis that noradrenergic transmission from the LC modulates wake-promoting vPAG^{DA} neurons. We found that DREADD-induced activation of LC projections to the vPAG or vPAG^{DA} neurons promoted wakefulness. Similarly, agonist stimulation of vPAG α1-adrenergic receptors (α1ARs) increased latency to fall asleep, while α1AR blockade had the opposite effect. α1AR stimulation drove vPAG^{DA} activity in a glutamate-dependent, action potential-independent manner. Compared to other dopaminergic brain regions, α1ARs were enriched on astrocytes in the vPAG, and mimicking α1AR transmission specifically in vPAG astrocytes via Gq-DREADDs was sufficient to increase arousal. Together, these results support an arousal circuit whereby noradrenergic transmission at astrocytic α1ARs activates wake-promoting vPAG^{DA} neurons via glutamate transmission. This circuit may be a potential therapeutic target for treating excessive sleepiness in PD.

1:15-1:25 – Hunter Futch (UF)

Immunotherapeutic Targeting of Corticotropin Releasing Hormone in Alzheimer's Disease

Background: Epidemiologic and biomarker studies have posited an association between the experience of chronic psychologic stress and increased incidence of Alzheimer's Disease (AD). Corticotropin-releasing hormone (CRH) and cortisol are two of the main mediators of the response to psychologic stress in humans, and both CRH and cortisol have been found to exacerbate amyloid ß deposition and tau phosphorylation in AD relevant transgenic mouse models. In addition, several independent studies have now shown that increased cortisol levels are associated with a more rapid rate of decline in AD patients. Therefore, substantial epidemiologic, biomarker, and preclinical data support the potential of CRH and cortisol as therapeutic targets for AD. Unfortunately, CRH receptor (CRHR1) antagonists have failed in clinical trials for psychiatric disorders, and can potentially increase amyloid ß production through ß-arrestin mediated pathways. In order to circumvent suboptimal receptor-based approaches for targeting this axis, we aimed to develop a novel strategy for directly targeting CRH using a high affinity antibody. We have successfully generated and purified a high affinity (K_d<1.0E-12) monoclonal Anti CRH antibody for use as a potential therapeutic. Studies investigating Anti-CRH as a therapeutic in both mutant APP and tau transgenic mice. In wildtype mice, this antibody is able to suppress the corticosterone response to acute restraint stress by greater than 85%, and has a half-life of one week *in vivo*. In all, we have generated a high affinity monoclonal CRH antibody that is able to reduce the glucocorticoid response to acute restraint stress by greater than 80% while also maintaining basal glucocorticoid levels in mice. These studies have broad implication for stress-related disorders in addition to AD, and may herald a new class of central nervous system therapies that target neuropeptides using high affinity antibodies.

1:25-1:30 – Abbi Hernandez (UF)

Utilizing the Ketogenic Diet to Restore Metabolic and Neurobiological Health in Aged Rats

With the cognitive health span exceeding the average life expectancy, and 10% of Americans over 65 diagnosed with Alzheimer's Disease (AD), it's imperative that therapeutic strategies for combating cognitive dysfunction are developed and empirically tested. Unfortunately, despite such high prevalence, there are currently no treatment options to cure or prevent the development of AD and other types of cognitive aging. Critically, dysfunction in glucose metabolism is a hallmark of normal aging, and Type-II Diabetes (or insulin insensitivity) is comorbid with AD in >85% of diagnosed cases. Furthermore, declines in metabolic capacity are first detected within brain regions that show the earliest vulnerability to both aging and AD pathology, including the hippocampus (HPC) and prefrontal cortex (PFC). The current study therefore investigated the ability of a high-fat ketogenic diet (KD) to normalize metabolic and neurobiological function in young and aged rodents within the HPC and PFC. While consuming a KD, fat-derived ketone bodies replace glucose as the primary fuel source for the central nervous system and other organs. Following 12 weeks of dietary intervention, transporters for glucose were downregulated within the PFC and HPC, while transporters for ketone bodies were upregulated, indicating rats were utilizing ketosis, not glycolysis, for cellular metabolism. Age-related HPC decreases in vesicular glutamate transporter, which is a marker for excitatory synapses, were ameliorated in aged KD-fed rats. Interestingly, expression for the vesicular GABA transporter, a marker for inhibitory synapses, was increased in the PFC and HPC of both age groups. While aged rats required significantly more time to enter ketosis, both KD-fed age groups were more resilient against metabolic challenge, such as fasting, than age-matched control-fed rats. Collectively, these data suggest KDs have the potential to alleviate symptoms of both AD and cognitive aging.

1:30-1:35 – Leah Truckenbrod (UF)

Ketogenic diet as a potential metabolic strategy for improving cognitive and peripheral health in aged subjects

Age-related cognitive impairments are a hallmark of typical aging that are exacerbated in neurodegenerative diseases, such as Alzheimer's Disease (AD). One physiological consequence of aging is a decrease in cerebral glucose metabolic capacity, which has been linked to cognitive decline. In contrast to glucose, the brain's ability to utilize ketone bodies derived from fat for energy metabolism does not appear to decline with age. The current study therefore tested the ability of a high fat, low carbohydrate ketogenic diet (KD), which switches the body's main fuel source to ketone bodies, to improve cognitive outcomes in young and aged rats. Young (4 m.o.) and aged (24 m.o.) rats were placed on a KD or nutrient- and calorie-matched control diet and tested on a variety of behavioral tasks known to be dependent on brain regions often affected by advancing age, including the prefrontal cortex and medial temporal lobe. Anxiety-like behaviors, examined by willingness to enter an unenclosed arm of the maze, were reduced in KD-fed rats. This may be related to upregulation of the vesicular GABA transporter in the hippocampus and prefrontal cortex (see Hernandez et al.). Additionally, the ability of rats to simultaneously perform a bi-conditional object-place paired association and working memory task was significantly improved in KD-fed rats. These data suggest that a KD may improve cognitive multi-tasking, which is particularly vulnerable in old age and the early stages of dementia. Lastly, aged rats on the KD had significantly improved peripheral health, as indicated by decreased body fat without commensurate loss of lean tissue or motor skills. Future studies should investigate at what point the ketogenic diet should be implemented to optimize improvements in cognitive function, as well as possible pharmacological interventions to induce ketosis without restricting dietary carbohydrate intake.

1:35-1:45 – Marshall Goodwin (UF)

Functionalized Intrabodies as novel therapeutics for Alzheimer's Disease and other Tauopathies

Background: Several neurodegenerative diseases known as tauopathies are characterized by the intracellular accumulation of misfolded tau, including Alzheimer's Disease and Frontotemporal dementia with parkinsonism-17. Although there are currently no cures for these debilitating diseases, novel antibody-based therapies targeting disease specific proteins such as the microtubule associated protein tau are currently under development. However, these therapies may be limited by the ability of extracellular antibodies to enter the cytoplasm and engage intracellular proteins. This potentially limiting factor may be avoided by utilizing intracellular antibody technology to target misfolded proteins directly within the cytoplasm. The intracellular expression of antibody fragments known as intrabodies has been shown to alter the folding, interaction, localization, and degradation of cytoplasmic proteins. Intrabodies specific for intracellular proteins such as huntingtin have shown efficacy in animal models, yet there has been little effort to target intrabodies against pathological tau. Our lab has generated several tau-specific intrabodies which were found to reduce tau pathology both in vitro and in two transgenic mouse models. We hypothesize that this reduction may be improved by fusing intrabodies to functional domains which target tau for increased proteasomal or lysosomal degradation. **Methods**: Molecular cloning techniques were used to fuse the most effective phospho-tau specific CP13 intrabody to protein domains which target bound tau for either proteasomal or lysosomal degradation. These new intrabody fusion proteins were tested in both seeded and non-seeded HEK cell models of tau aggregation. **Results**: Our preliminary data shows that expression of a lysosomal targeted intrabody greatly reduced the formation of insoluble tau aggregates as compared to a proteasomal targeted or intrabody alone. We are currently testing these constructs in *ex vivo* brain slice cultures and mouse models of tauopathy.

Oral Session: Genetics/Modeling/Systems Biology I

1:45-1:55 – Jada Lewis (UF)

Exacerbation of tau pathology by pre-existing Abeta pathology in novel trigenic transgenic mice

One of the gaps in our understanding of the mechanisms of Alzheimer's disease (AD) is how amyloid triggers tauopathy. One hypothesis we have pursued is that the deposition of Abeta induces tau misfolding by directly or indirectly diminishing function of the proteostasis network. Previously, we demonstrated that deposition of Abeta in the brains of older (>12 months) APPswe/PS1dE9 mice (line 85; hereafter "APP") changes the solubility of over 100 cytosolic proteins. This "secondary" proteome pathology could be indicative of reduced proteostatic function in this model. We have now asked whether inducing mutant human tau[P301L] in the context of preexisting amyloid pathology the brains of older APP mice would accelerate "secondary" tau pathology. We crossed the APP mice with mice that express 4R0N human tau[P301L] under the transcriptional control of a doxycycline (Dox)-regulated promoter (rTg4510). In both cohorts, we suppressed tau expression with Dox until APP-containing mice developed high amyloid burden (14 months) before withdrawing Dox to induce tau expression for the subsequent 6 to 9 months. If diminished proteostatic function contributes to tau misfolding, then expressing human tau[P301L] in a setting of high amyloid burden should accelerate or exacerbate tau pathology. We found that the APP/rTg4510 mice (n=5) developed striking tau pathology with misfolded tau forming pre-tangle and tangle-like inclusions when tau was induced for 6 months in the context of amyloid pathology, but none of the rTg4510 transgenic mice lacking the APP transgene (n=9) showed tauopathy at the same age. When we extended tau expression through 8-9 months post-induction, the tau pathology in rTg4510 mice (n=3) was similar to that of the older APP/rTg4510 mice in which tau expression had only been induced for 6 months. These data provide additional support for the ability of amyloid to influence the development of tauopathy and this new model system could be useful in uncovering the mechanisms by which Abeta pathology exacerbates tau pathology.

1:55-2:00 – Yu Yamazaki (Mayo)

Vascular specific expression of apoE4 induces endothelial dysfunctions and cognitive deficits

Background: The ε4 allele of the apolipoprotein E (*APOE*) gene is the strongest genetic risk factor for late-onset Alzheimer's disease (AD) among its three polymorphic alleles (ε2, ε3 and ε4). In addition to inhibiting amyloid-β clearance and promoting its aggregation and deposition, substantial evidence suggests that apoE4 disrupts cerebrovascular homeostasis, contributing to neuronal dysfunction. Because multiple cell types express apoE, elucidating the cell type-specific function of apoE isoforms is critical for understanding how apoE4 contributes to AD risk and how this pathway can be therapeutically targeted.

Methods: We generated novel mouse models conditionally express human apoE3 or apoE4 in vascular mural cells (VMCs, referring to a population of perivascular cells including pericytes and smooth muscle cells). Upon breeding to the murine *Apoe*-KO background, effects of apoE isoforms on vascular function and behaviors were determined. *In vitro* blood-brain barrier (BBB) model, composed of endothelial cells, pericytes, and astrocytes, was used to assess effects of apoE isoforms expressed in VMCs on endothelial cell phenotypes and barrier integrity.

Results: Conditional expression of apoE4, but not apoE3, in VMCs in the background of murine *Apoe*-KO mice led to increased anxiety-like phenotype, impaired learning, and reduced arteriole blood flow compared to their respective littermate controls. Both apoE3 and apoE4 in VMCs rescued aortic atherosclerotic phenotype in *Apoe*-KO mice, suggesting that the differential effects of apoE isoforms are driven primarily by apoE expressed in the brain vasculature rather than periphery. In the setting of reconstituted BBB model, pericytes isolated from apoE4-targeted replacement (apoE4-TR) mice showed reduced ability to stimulate extracellular matrix protein induction and endothelial barrier formation compared to those from apoE3-TR mice.

Conclusions: Our findings demonstrate a pathogenic role of apoE4 in VMCs in impairing vascular homeostasis and cognitive functions, further supporting a gain-of-toxic role of apoE4 in the pathogenesis of AD.

2:00-2:05 – Michael Pace (UF)

Changes in proteome solubility indicate widespread proteostatic disruption in mouse models of neurodegenerative disease

The deposition of pathologic misfolded proteins in neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, frontotemporal dementia and amyotrophic lateral sclerosis is hypothesized to burden protein homeostatic (proteostatic) machinery, potentially leading to insufficient capacity to maintain the proteome. This hypothesis has been supported by previous work in our laboratory, as evidenced by the perturbation of cytosolic protein solubility in response to amyloid plaques in a mouse model of Alzheimer's amyloidosis. In the current study, we demonstrate changes in proteome solubility are a common pathology to mouse models of neurodegenerative disease. Pathological accumulations of misfolded tau, α-synuclein and mutant superoxide dismutase 1 in CNS tissues of transgenic mice was associated with changes in the solubility of hundreds of CNS proteins in each model. We observed that changes in proteome solubility were progressive and, using the rTg4510 model of inducible tau pathology, demonstrated that these changes were dependent upon sustained expression of the primary pathologic protein. In all of the models examined, changes in proteome solubility were robust, easily detected, and provided a sensitive indicator of proteostatic disruption. Interestingly, a subset of the proteins that display a shift towards insolubility were common between these different models, suggesting that a specific subset of the proteome is vulnerable to proteostatic disruption. Overall, our data suggest that neurodegenerative proteinopathies modeled in mice impose a burden on the proteostatic network that diminishes the ability of neural cells to prevent aberrant conformational changes that alter the solubility of hundreds of abundant cellular proteins.

2:05-2:10 – Erica Modeste (Emory)

A Proteomic Analysis of Sex-Specific Differences Linked to Alzheimer's Disease Risk

Alzheimer's disease (AD) is an age-dependent neurodegenerative disease that is increasing in prevalence worldwide. Despite the growing burden of AD, current treatments have limited effects on the progression of the disease. In addition to age, there are many factors that increase an individual's risk to develop AD. For example, women are disproportionally affected by AD compared to men. Although clinical explanations for this difference have been postulated, research at the molecular and biochemical level remains sparse. To bridge this gap in knowledge we used an analytical approach to analyze proteomics and transcriptomics of post-mortem human brain tissues from three different groups defined as controls, AD, and asymptomatic carriers of amyloid pathology (AsymAD) across two brain regions (inferior temporal cortex and dorsolateral prefrontal cortex) and three independent cohorts (Mayo, Banner/SunHealth, and Mt. Sinai). Weighted Correlation Network Analysis (WGCNA) revealed proteomic subnetworks, represented by modules, that indicated sets of proteins differentially expressed between sexes. We further examined whether these modules or other subnetworks correlated to AD pathology. Sex-based differences in the human brain proteome were also benchmarked against proteomic networks generated from male and female mouse models of AD (5xFAD). We identified sex differences in brain protein co-expression subnetworks involving mitochondrial and transcriptional pathways or ontologies which may cause or stem from brain sex-specific differences in AD risk. Our findings begin to resolve sex-driven protein co-expression modules preserved across mouse and human brain tissue and inform a deeper mechanistic understanding of the biological underpinning of female risk for AD.

2:10-2:15 – Emily Koller (UF)

Exploring the role of Apolipoprotein E in tauopathy

The ε4 allele of apolipoprotein E (APOE4) is the strongest genetic risk factor for Alzheimer's disease (AD) whereas APOE2 is protective. Although there is a large body of evidence demonstrating how APOE isoforms alter amyloid β clearance and thus modify AD pathology, the effect of APOE isoforms on tau pathology is unclear. Using bitransgenic mice, a recent study showed that APOE4 leads to increased neurodegeneration in PS19 tau transgenic mice. However, there is a large gap in our understanding of the underlying mechanisms: for example, whether this is a brain-specific effect of APOE, whether there are isoform-specific alterations in tau tangles and further if this effect can be recapitulated in other tauopathy models. To understand the synergy between tau and APOE, we overexpressed human APOE4 or APOE2 in neonatal rTg4510 mice using adeno-associated viral (AAV) delivery into the brain. Using AAVs to create somatic brain transgenesis models allows us a convenient and rapid platform to evaluate the effects of a target gene limited to the CNS. We investigated phosphorylated tau, conformationally altered tau and tangle burden in 4 month and 6 month old rTg4510 mice expressing APOE2 or APOE4. We observed that levels of phosphorylated tau as well as NFT burden (Gallyas) remained indistinguishable in the presence of either APOE protein compared to control cohorts., Interestingly, there were differential effects of APOE expression on synaptic proteins, such as PSD95 was altered in APOE4 expressing mice whereas spinophilin did not show any change. Our study demonstrates that APOE exerts isoform-dependent effects in rTg4510 mice. Future studies are warranted to fully characterize the pathogenesis of AD type pathologies in the context of APOE isoforms, especially its effect on tau spreading and resulting memory impairment.

2:15-2:20 – Yuliya Voskobiynyk (UAB)

The Alzheimer's disease risk gene BIN1 regulates network hyperexcitability

Alzheimer's Disease (AD) affects about five million Americans, who receive only a very modest benefit from current treatment options. Multiple treatment trials have failed in the past, raising interest in identifying new targets to treat AD. GWAS studies have identified bridging integrator 1 (*BIN1*) as one of the leading genetic risk factors in AD. Neurons express unique BIN1 isoforms, and a growing body of evidence indicates loss of neuronal BIN1 in AD. However, the function of neuronal BIN1 remains unclear and its contribution to AD is critical to investigate. We generated brain-specific BIN1 knockout (KO) mice and discovered that the loss of BIN1 in the brain leads to network hyperexcitability, with increased seizure susceptibility. Network hyperexcitability is observed in AD: patients with mild cognitive impairment or dementia due to AD have epileptiform activity. Such aberrant activity is recapitulated in multiple rodent models of AD. Multiple lines of evidence suggest that increased network hyperexcitability results from inhibitory neuron dysfunction in AD patients. Network synchrony is tightly regulated by the activity of inhibitory GABAergic interneurons that coordinate synchronous excitatory neuron firing required for proper brain oscillatory activity. Therefore, interneuron impairment may play an important role in AD pathogenesis. To investigate the mechanisms by which BIN1 loss brain-specifically induces network hyperexcitability, we generated mice lacking BIN1 in inhibitory and excitatory neurons. Strikingly, we found that loss of BIN1 in inhibitory neurons increased seizure susceptibility, phenocopying BIN1 loss in the whole brain, while BIN1 loss in excitatory neurons decreased seizure susceptibility. Mice lacking BIN1 in inhibitory neurons had age-dependent behavioral deficits and decreased survival. In addition, initial electrophysiological studies provide evidence that loss of neuronal BIN1 decreases neuronal activity. These data generate fundamental insights about the mechanistic role BIN1 plays in AD to provide promising therapeutic strategies for targeting inhibitory neuron dysfunction and network hyperexcitability in AD.

2:20-2:25 – Deepak Chhangani (UF)

A Massive RNAi screen in *Drosophila* reveals potential targets for TDP-43 proteinopathies

TAR DNA-binding protein 43 (TDP-43) is associated with neurodegenerative conditions including Amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), Frontotemporal dementia (FTLD) and Chronic traumatic encephalopathy (CTE). This highly-conserved DNA/RNA binding protein is primarily distributed in nuclei. However, it translocates to the cytoplasm and gets hyperphosphorylated and ubiquitinated, which may subsequently impart protein aggregation. Despite considerable efforts to investigate the physiological role of TDP-43, we still have a very limited understanding of the molecular and cellular mechanisms of underlying TDP-43 proteinopathies. A key challenge, therefore, is to identify critical proteins and pathways mediating TDP-43 neurotoxicity. To shed light on this issue, we searched for genetic modifiers of neurotoxicity in transgenic flies expressing human TDP-43^{M337V}, a prevalent mutation in TDP-43 proteinopathies. These flies display a reliable eye phenotype, thus, we crossed them with a library of 6,261 RNAi strains obtained from the Vienna Drosophila Resource Center. We identified 300 modifiers of mutant TDP-43 toxicity in a primary screen. After eliminating genes that have dominant effect on eye phenotype, we validated 80 robust suppressors, 60 enhancers and 25 lethals. Furthermore, we found that many suppressors are linked to transcription, mRNA elongation, splicing and nucleocytoplasmic shuttling. In addition, we also found many modifiers linked to other functions such as neurogenesis, syntaxin binding, mitochondrial transport, ubiquitin activity, phosphatidylinositol signaling, and protein quality control. In summary, this loss-of-function screen has led to the identification of several genes and molecular pathways not previously known to be associated with TDP-43 pathologies.

2:25-2:45 – Rebecca Brennan (Mayo)

Mitochondrial genomic variation, and the interaction with nuclear DNA variants in PSP

Progressive supranuclear palsy (PSP) is a primary tauopathy which develops after 60 years of age and is characterised by abnormal tau aggregates in cortical and striatal neuronal regions, causing parkinsonian-like movement and cognitive defects. PSP is a rare, sporadic, and complex neurodegenerative disorder whereby genetics and environmental factors contribute to its onset. Variations in tau-coding gene, *MAPT,* are consistently the major genetic risk factors associated with PSP however they do not account for complete disease pathogenesis. Mitochondrial DNA (mtDNA) variation has been associated with age-related disease and similar parkinsonian disorders; however no study has investigated the role of mtDNA variation in PSP. Utilising Agena Bioscience iPlex technology, we have genotyped 39 mtDNA SNPs to define mtDNA haplogroups and assess association with PSP risk. Additionally, nuclear DNA (nDNA) variants reported to be associated with PSP from published genome-wide association studies were genotyped to further investigate the interplay between mtDNA and nDNA variations. Employing an age-matched case-control study of n>1000 controls, and n>1000 PSP cases, all from European descent, this is the largest and most comprehensive study of mtDNA variation associated with PSP to date, and is the first study to investigate nuclear-mitochondrial interactions with disease pathogenesis.

Oral Session: Genetics/Modeling/Systems Biology II

3:30-3:50 – Tom Kukar (Emory)

Lysosome dysfunction and inflammation are common pathogenic pathways in GRN knockout mice and human FTD-GRN

Mutations in the *GRN* gene, which encodes the progranulin (PGRN) protein, are one of the most common causes of Frontotemporal dementia (FTD) and lead to haploinsufficiency of PGRN. PGRN is composed of 7 repeating domains termed granulins (GRNs 1-7) that are joined by linker regions. We have recently found that PGRN is processed into ~6kDa GRNs within the lysosome, where we hypothesize they are critical for lysosome homeostasis (Holler et al, *eNeuro,* 2017 Aug 18;4(4)). However, the molecular mechanisms caused by loss of lysosomal PGRN/GRNs that lead to neurodegeneration are still unknown. To gain insight into the mechanisms of FTD pathogenesis, we performed an unbiased quantitative proteomic analysis of whole-brain tissue from wild-type (WT) and Progranulin knockout ($Grn^{-/-}$) mice at 3 and 19-months of age. We utilized a 10-plex Tandem Mass Tag (TMT) isobaric labeling mass spectrometry approach for peptide labeling and quantification that enabled a deep proteome analysis of mouse brain and detection of 8,695 proteins. Differential expression analysis of the brain proteome of 3-month old $Grn^{-/-}$ versus WT mice revealed 29 up and 26 down-regulated proteins, while 119 proteins were up and 20 proteins were down-regulated in 19-month old $Grn^{-/-}$ mice. Next, we performed weighted correlation network analysis (WGCNA) on the brain proteome of $Grn^{-/-}$ vs WT mice and identified 29 modules of highly co-expressed proteins. In particular, 3 modules were strongly correlated to Grn deficiency and increased with age and were enriched with lysosomal proteins (Ctsd, Fuca2, Tpp1) and inflammatory proteins (GFAP, CD44, S100a C1qa). Finally, we used WGCNA to compare our $Grn^{-/-}$ mouse proteome dataset to the transcriptome and proteome from human FTD-GRN brain (Chen-Plotkin AS et al, *Acta Neuropathol.*, 2010 Jan;119(1):111-22). We identified similar dysregulated networks of lysosomal and inflammatory proteins, suggesting that common networks of proteins are dysregulated in human and mouse brains with deficient levels of PGRN and GRNs. The commonality of these pathogenic pathways suggests new therapeutic targets in neurodegenerative diseases caused by decreased levels of PGRN/GRNs.

3:50-4:10 – Diego Rincon-Limas (UF)

A new and powerful therapeutic tool against multiple neurotoxic amyloids: proof of principle in flies

Despite tremendous advances in the field of neurodegenerative proteinopathies, no curative treatments are yet available for these disorders, including Alzheimer's disease, tauopathies, frontotemporal dementia, amyotrophic lateral sclerosis, and polyglutamine diseases. Interestingly, although these disorders have distinct clinical symptoms and pathologies, they share common mechanisms such as protein aggregation, oxidative damage, abnormal phosphorylation, transcriptional dysregulation, and ER stress to name a few. Therefore, based on these commonalities, it has been suggested that identification of targets acting at common downstream events may lead to novel therapeutic strategies that could result in more effective symptomatic therapies. Unfortunately, a common target that could robustly protect against these distinct disorders is still elusive.

To address this, my group recently identified a new gene in flies that elicits a dramatic protection against a broad spectrum of neurotoxic amyloids. This gene, referred to as RAF2 (RING Associated Factor 2), dramatically blocks the toxicity of Abeta42 when expressed in the *Drosophila* eye and brain neurons. This protection is also effective against the insults of tau, and even against the aggressive toxicity elicited by co-expression of Abeta42+tau. Strikingly, RAF2 also blocks the toxicity of other pathologically relevant amyloids. Preliminary data in Aβ42--expressing flies suggests that RAF2 mediates Aβ42 degradation, however we do not know if similar observations would apply to the other amyloidogenic proteins. Interestingly, RAF2 is predicted to have a significant degree of disorderness and thus it may have unique properties in terms of function, regulation and interactions. However, the physiological role of this protein is largely unknown. Thus, we are currently conducting a mutational analysis of RAF2 to address this critical gap and the results will be presented.

4:10-4:30 – Nick Cochran (HudsonAlpha)

Findings from Whole Genome Sequencing for over 1000 Early-Onset AD and FTD Cases

Through ongoing collaborations with UCSF, UCSB, UAB, and the University of Antioquia in Colombia, we are sequencing genomes from both families and singletons with early onset neurodegenerative diseases. While numbers continue to increase, to date we have sequenced 1339 new genomes at HudsonAlpha in these projects and are analyzing a total of 727 early onset AD cases, 520 FTD cases (many of which are also early onset), and 1348 matched controls (most of which were sequenced at HudsonAlpha; 190 total cases were sequenced at HLI and NYGC using the same technology). Interesting findings from these efforts include genetic explanations for atypical disease presentations (for example, mutations such as Tau R406W in AD cases with FTD features), enrichment of APOE4 alleles along with additional established risk factors identified in other case-control studies (ABCA7, SORL1, and TREM2), and early signals for novel genetic associations from initial analysis of individuals of European ancestry in TET2 and PDZRN3. Enrichment of rare variation in TET2 is present in both AD and FTD samples, and many of the rare variants observed are canonical loss-of-function mutations at a similar level of enrichment vs. loss-of-function mutations in population databases as the level of enrichment for LoF variants in SORL1 observed in other studies. PDZRN3 is enriched for rare variation in FTD cases. For both TET2 and PDZRN3, an important contributor to the rare variation signal (and almost all of the signal for PDZRN3) comes from private non-coding variation. Further analysis of these cohorts, along with eventual meta-analysis with other similar cohorts of early onset neurodegeneration cases, is likely to provide important insights into the genetic underpinnings of early onset neurodegeneration.

4:30-4:50 – Edgardo Rodríguez-Lebrón (UF)

Novel insights into Matr3 function in the CNS

Mutations in the DNA/RNA binding nuclear matrix protein Matrin-3 (Matr3) are directly linked to familial ALS. Matr3 pathology is also observed in C9ORF72+ and sporadic ALS. To date, our understanding of Matr3's function in the CNS remains limited. Matr3 is one of the most enriched DNA/RNA binding proteins in the nuclear matrix proteome from where it can coordinate diverse gene expression programs including those controlled by Pit1 and p53. Thus, Matr3 sits at the interface of nuclear matrix dynamics and the regulation of gene expression, influencing the nuclear response to numerous signaling events. Relying on numerous molecular techniques and acute mouse spinal cord culture experiments we have discovered that Matr3 coalesces into de novo nuclear assemblies in response to nuclear stress. This novel Matr3 stress response is not caused by transcriptional inhibition and is unique, as no other ALS-related RNA binding protein tested responds in this fashion. Matr3 self-assembly is driven by liquid-liquid phase transition of a nuclear matrix-associated Matr3 fraction and results in the sequestration of other nuclear factors such as PTBP1. This response is reversible and driven by its N-terminal domain. Importantly, it is impaired by the disease-linked S85C mutation. The results highlight a role for Matr3 as an essential component of a nuclear matrix network that connects changes in nuclear architecture to gene expression programs and RNA processing events in neurons. These studies open up a new area in ALS research by implicating changes in nuclear matrix dynamics to motor neuron disease.

4:50-5:10 – Yingxue Ren (Mayo)

***TMEM106B* haplotypes have distinct gene expression patterns in aged brain**

Single nucleotide polymorphisms (SNPs) inherited as one of two common haplotypes at the *transmembrane protein 106B* (*TMEM106B*) locus are associated with the risk of multiple neurodegenerative diseases, including frontotemporal lobar degeneration with pathological inclusions of TDP-43. Among the associated variants, rs3173615 (encoding p.T185S) is the only coding variant; however, non-coding variants may also contribute to disease risk. It has been reported that the risk haplotype is associated with higher levels of TMEM106B and increased levels of TMEM106B cause cytotoxicity; however, the precise mechanism through which *TMEM106B* haplotypes contribute to neurodegeneration is unclear. We utilized RNA sequencing data derived from temporal cortex (TCX) and cerebellum (CER) from 312 North American Caucasian subjects neuropathologically diagnosed with Alzheimer's disease, progressive supranuclear palsy, pathological aging or normal controls to analyze transcriptome signatures associated with the risk (TT) and protective (SS) *TMEM106B* haplotypes. When comparing TT to SS carriers, we detected 593 differentially expressed genes in TCX and 7 in CER. Gene co-expression network analyses further showed that in both TCX and CER the SS haplotype was positively correlated with gene networks involved in synaptic transmission, whereas the TT haplotype was positively correlated with gene networks enriched for immune response. Gene expression patterns of 5 cell-type-specific markers revealed significantly reduced expression of the neuronal marker and relative increases in all other cell markers in TT as compared to SS carriers in TCX with a similar but non-significant trend in CER. Our study identified significant and partly conserved transcriptional differences across TCX and CER and striking changes in cell-type composition, especially in TCX. These findings illustrate the profound effect of *TMEM106B* haplotypes on brain health and highlight the importance to better understand TMEM106B's function and dysfunction in the context of neurodegenerative diseases.

5:10-5:30 – Jose Abisambra (UF)

The PERK inhibitor GSK2606414 reduces hyperphosphorylated tau and rescues neurological deficits in tau transgenic mice

Tauopathies are a group of more than twenty known neurodegenerative disorders that affect nearly eight million people in the United States. Currently, there is no cure for tauopathies, and there are temporary and limited benefits to current therapeutic strategies. The endoplasmic reticulum (ER) stress sensor PERK (protein kinase R-like ER kinase) participates in the pathogenesis and progression of tauopathies. However, the mechanism by which the PERK pathway causes neuronal dysfunction is still unknown. In this study, we treated rTg4510 tau transgenic mice with a potent PERK inhibitor, GSK2606414, at the onset of increasing pre-tangle tau pathology and cognitive impairment but before evident tangle pathology and brain atrophy. The treatment significantly reduced hyperphosphorylated tau species and led to improvement of neuronal function, as determined with a sensitive and innovative imaging technique called manganese-enhanced magnetic resonance imaging (MEMRI) with quantitative R1 mapping. We found that PERK inhibition mediated these improvements via a mechanism distinct from the canonical PERK pathway. Instead, we found PERK inhibition at this early time point rescues measures of oxidative damage as measured by protein nitrosylation. Our results highlight a novel mechanism by which this PERK inhibitor mediates tau pathogenesis and potentiates progression of early-stage pathology. Finally, although the complete mechanism is not yet elucidated, this study suggests that structural analogs of GSK2606414 are a viable therapeutic to ameliorate neuronal function in tauopathies.

5:30-5:50 – Zane Zeier (Miami)

Breaking RAD52: DNA damage repair in C9ORF72 ALS/FTD

The C9ORF72 hexanucleotide repeat expansion (HRE) encodes mutant RNAs and proteins that contribute to the onset of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Among five dipeptide repeat proteins (DPRs) produced by the HRE, proline-arginine (PR) disrupts nucleolar function and is neurotoxic. Here, we identified multivalent contact points necessary for the interaction of PR and nucleophosmin (NPM1), a multifunctional nucleolar protein involved in ribosomal RNA biogenesis and DNA damage repair. Moreover, we found the interaction of PR and NPM1 is independent of nucleolar localization. Next, we show that PR selectively impedes single strand annealing (SSA) and non-homologous end joining, two double strand DNA break repair pathways used by non-dividing cells including neurons. We further show that RAD52 - a protein that facilitates annealing of complementary DNA strands during SSA - is activated in patient derived iPSC motor neurons relative to control neurons or isogenic neurons in which the HRE was removed by genomic editing. This molecular phenotype was confirmed in pathological post-mortem brain tissue. Collectively, we have identified novel mechanisms whereby the HRE leads to genomic instability and contributes to neurotoxicity in C9ORF72 ALS/FTD.