ISMND NextGen 2023

PROGRAM & ABSTRACTS

Molecular and Cellular Mechanisms of Neurodegenerative Diseases

August 23-24, 2023

8:30 AM – 1:30 PM (EDT)

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MOLECULAR NEURODEGENERATION

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The International Society for Molecular Neurodegeneration (ISMND)

ISMND is a nonprofit organization with the goal to create a ***multidisciplinary global platform*** for scientists, physicians, trainees, and the public from different scientific disciplines to interact and collaborate. We are the ***only*** society specifically focusing on neurodegenerative brain and eye diseases.

We want to connect investigators, share scientific discoveries, and cultivate an ***inclusive and diverse*** place, while helping with ***training the next generation of scientists***.

Upcoming Webinars

September 21, 2023
Junmin Peng, Ph.D
St. Jude Children's Research Hospital, USA
The power of proteomics in neurodegenerative diseases

October 19, 2023
Mark Cookson, Ph.D
National Institutes of Health, USA
TBD

November 2023 (TBD)
Rosa Rademakers, Ph.D
VIB-UAntwerp, Belgium
Genetics and pathobiology of FTD (tentative title)

December 12, 2023
Shane Liddelow, Ph.D
NYU Langone Health, USA

Meet the Team

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The inaugural ISMND NextGen 2023 meeting is a virtual event for trainees and early-career investigators to shine and share their latest research on neurodegenerative diseases

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- *Hui Zheng*
  ISMND and Baylor College of Medicine, USA

ISMND NextGen 2023 Conference Program

DAY 1: AUGUST 23, 2023 (Eastern Daylight Time/EDT) 8:30 AM – 1:30 PM

8:30 - 8:40	**WELCOME REMARKS** *Henrietta Nielsen*, President of ISMND
8:40 - 9:40	**SESSION 1**: ***Molecular and Cellular Mechanisms Implicated in Neurodegeneration*.** Chairs: *Katarina Dittlau,* University of Copenhagen, Denmark *Lindsey Goodman,* Baylor College of Medicine, USA
8:40 – 8:47	**S1.1 Chronic lysosomal dysfunction induced differential changes in circRNAs levels in an Alzheimer's disease mouse model** *Skarleth Cardenas Romero*, Harvard Medical School, USA
8:47 – 8:54	**S1.2 Defective lysosomal acidification contributes to TNF-TNFR1 mediated neuronal death** *Chih Hung Lo***,** Nanyang Technological University Singapore, Singapore
8:54 – 9:01	**S1.3 Lipophorin Receptors Genetically Modulate Neurodegeneration Caused by Psn Knockdown in the Aging Drosophila Brain** *Chen Zhang,* Harvard Medical School, USA
9:01 – 9:08	**S1.4 Resistant and vulnerable motor neurons show unique temporal gene regulation in SOD1G93A ALS** *Irene Mei*, Stockholm University, Sweden
9:08 – 9:15	**S1.5 Glial Tau is required for lipid droplet formation and protection against ROS** *Lindsey Goodman*, Baylor College of Medicine, USA
9:15 – 9:22	**S1.6 Derailed protein turnover in the aging mammalian brain** *Nalini Rao*, Northwestern University, USA
9:22 – 9:40	**Q&A SESSION**
9:40 – 10:00	**BREAK**
10:00 – 11:05	**SESSION 2: *Applications of human cell models in neurodegeneration research*.** Chairs: *Amanada McQuade,* University of California-San Francisco, USA *Hayk Davtyan,* University of California-Irvine, USA
10:00 – 10:07	**S2.1 Long Noncoding RNA MEG3 Modulates Neuronal Necroptosis in Alzheimer's disease** *Sriram Balusu*, KU Leuven, Belgium
10:07 – 10:14	**S2.2 Reproducible and controllable human iPSC-derived cortical tissue models to investigate Alzheimer's disease** *Julien Klimmt*, LMU Munich, Germany
10:14 – 10:21	**S2.3 Optic nerve head astrocyte response to biomechanical strain using a 3D hydrogel system** *Ana Nicolle Strat*, SUNY Upstate Medical University, USA
10:21 – 10:28	**S2.4 Development of a human iPSC-based FTD model showing advanced**

	Tauopathy phenotypes *Angelika Dannert*, LMU Munich, Germany
10:28 – 10:35	**S2.5 IPSC-Microglia Transplantation Prevents Pathology in CSF1R FIRE/FIRE Chimeric Mouse Model of ALSP** *Jean Paul Chadarevian*, University of California-Irvine, USA
10:35 – 10:42	**S2.6 Transdifferentiation: a novel tool for disease modeling and mechanistic investigation in Alzheimer's disease** *Ching-Chieh (Ian) Chou*, Stanford University, USA
10:42 – 10:49	**S2.7 Uncovering novel regulators of an interferon-responsive microglial state** *Amanda McQuade*, University of California-San Francisco, USA
10:49 – 11:05	**Q&A SESSION**
11:05 – 11:20	**BREAK**
11:20 – 12:20	**KEYNOTE PRESENTATION** **Genomic approaches to study Alzheimer's disease** *David Gate*, Northwestern University, USA Chair: *Robert Vassar*, Northwestern University, USA
12:20 – 1:20	**PANEL DISCUSSION: *Disease Models - Road to Translation*** Panel Host: *Hui Zheng,* Baylor College of Medicine, USA Panelists: *Bruce Lamb,* Indiana University, USA *Julia TCW,* Boston University, USA *Jeroen Hoozemans,* Amsterdam UMC, The Netherlands *Kristine Freude,* University of Copenhagen, Denmark *Sriram Balusu,* KU Leuven, Belgium
1:20 – 1:30	**CLOSING REMARKS** *Lucy Job*, ISMND and Molecular Neurodegeneration, USA

Recorded poster presentations and commercial advertisements are available on-demand throughout the meeting, until October 24, 2023.

DAY 2: AUGUST 24, 2023 (Eastern Daylight Time/EDT) 8:30 AM – 1:30 PM

8:30 - 8:40	**GOOD MORNING ADDRESS** *Diane Bovenkamp,* BrightFocus Foundation, USA
8:40 - 9:40	**SESSION 3: *Sex, Vascular, Peripheral, and Systematic Factors*** Chairs: *Lesley Golden,* University of Kentucky, USA *Julia TCW*, Boston University, USA
8:40 – 8:47	**S3.1 Single-nucleus dissection of human brain vasculature and Transcriptomic analysis in Alzheimer's Disease** *Na Sun*, Massachusetts Institute of Technology, USA
8:47 – 8:54	**S3.2 Influence of Androgens in a Mouse Model of Multi-Etiology Dementia** *Charly Abi Ghanem,* Albany Medical College, USA
8:54 – 09:01	**S3.3 Mid-life APOE4 to APOE2 'Switching' Alters the Cerebral Transcriptome and Decreases AD Neuropathology** *Lesley Golden,* University of Kentucky, USA
9:01 – 9:08	**S3.4 Gut microbiome regulates astrocyte reaction to amyloidosis through microglial dependent and independent mechanisms** *Sidhanth Chandra*, Northwestern University, USA
9:08 – 9:15	**S3.5 Meningeal lymphatic drainage regulates oligodendrocytes survival and brain myelination** *Sofia Pereira das Neves*, Mayo Clinic Florida, USA
9:15 – 9:22	**S3.6 NMNAT2 supports vesicular glycolysis via NAD homeostasis to fuel fast axonal transport** *Sen Yang*, Indiana University, USA
9:22 – 9:40	**Q&A SESSION**
9:40 – 10:00	**BREAK**
10:00 – 11:00	**SESSION 4: *Searching for new therapeutic approaches to target neurodegenerative diseases*** Chairs: *Luke Dabin* (Indiana University, USA) *Daniel Twohig* (Lund University, Sweden)
10:00 – 10:07	**S4.1 Acidic nanoparticles restore lysosomal function and rescue α-syn Induced neuronal cell death in Parkinson's disease** *Jialiu Zeng*, Nanyang Technological University, Singapore
10:07 – 10:14	**S4.2 Does blocking arginase/polyamine pathway limit acute glaucomatous retina ganglion cell death?** *Syed Zaidi*, Augusta University, USA
10:14 – 10:21	**S4.3 Amelioration of Tau and ApoE4-linked glial lipid accumulation and neurodegeneration with an LXR agonist** *Alexandra Litvinchuk*, Washington University in St Louis, USA
10:21 – 10:29	**S4.4 Increased senescence is associated with α-synucleinopathy in the TgA53T mouse model and senolytic treatment delays disease onset** *Indrani Poddar,* University of Minnesota, USA
10:29 – 10:36	**S4.5 Alzheimer's disease associated isoforms of human CD33 distinctively modulate microglial cell responses in 5XFAD mice**

	Ghazaleh Eskandari-Sedighi, University of California-Irvine, USA
10:36 – 10:43	**S4.6 Assessing microglial states in the context of amyloid using targeted single cell profiling** *Luke Dabin*, Indiana University, USA
10:43 – 11:00	**Q&A SESSION**
11:00 – 11:20	**BREAK**
11:20 – 12:20	**KEYNOTE PRESENTATION** **Identification and validation of biomarkers for different dementias using antibody-based proteomics** *Charlotte Teunissen*, Amsterdam UMC, The Netherlands Chair: *Henrietta Nielsen,* Stockholm University, Sweden
12:20 – 1:20	**PANEL DISCUSSION**: ***Amyloid-targeted therapy for Alzheimer's disease - Are we there yet?*** Panel Host: *Guojun Bu*, Molecular Neurodegeneration, USA Panelists: *Robert Vassar,* Northwestern University, USA *Agneta Nordberg,* Karolinska Institutet, Sweden *Samuel Gandy,* Mount Sinai, USA *Charlotte Teunissen,* Amsterdam UMC, The Netherl*ands* *Hussein Yassine,* University of Southern California, USA *Daryl Rhys Jones,* Eisai, USA
1:20 – 1:30	**AWARD* CEREMONY & CLOSING REMARKS** *Guojun Bu,* ISMND and Molecular Neurodegeneration, USA *Henrietta Nielsen*, ISMND, USA and Stockholm University, Sweden *The winner of the *ISMND 2024 PBL Assay Science Travel Fellowship* will be announced in ISMND Science Webinar in September

Recorded poster presentations and commercial advertisements are available on-demand throughout the meeting, until October 24, 2023.

ORAL PRESENTATION ABSTRACTS

Oral Presentation Session Number - S1.1

Chronic lysosomal dysfunction induced differential changes in circRNAs levels in an Alzheimer's disease mouse model

Skarleth Cardenas Romero[1]*, Abdallah Eteleeb[2], Oscar Harari[2], Bruno A. Benitez[3]

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Background: Circular RNAs (circRNAs) modulate microRNA (miRNAs) levels by sequestration. MiRNAs regulate the transcripts, including APP, BACE1, and ADAM10, interfering in the amyloidogenic pathways. CircRNAs modulate key endolysosomal transcripts. Chronic lysosomal dysfunction (CLD) is linked to Alzheimer's Disease (AD). However, the role circRNAs downstream of CLD and AD is unclear. Here, we tested whether CLD affects the amyloidogenic pathway in 5xFAD mice through circRNAs. **Methods:** Cortical circRNA expression was obtained of 5xFAD, PPT1+/-, 5xFAD:PPT1+/- (P5X), Naglu+/-, 5xFAD:Naglu+/- (N5X) mice. TruSeq Stranded total RNA was extracted with Ribo-Zero to deplete rRNA and generate stranded-RNA-seq. In silico microRNA, binging was done using circAtlas 2.0 browser. The insoluble Aβ-40 and Aβ-42 were quantified by ELISA in the hippocampal fraction. The Aβ plaque load was quantified in coronal brain sections using immunohistochemistry. **Results:** The P5X and N5X mice exhibit increased amyloid plaque load, hippocampal Aβ-40 and Aβ-42 levels, and reduced lifespan compared to 5xFAD mice. Both linear and circRNAs of 4933406I18Rik, Zfp609, Zfp532, and Nnt changed between PPT1+/- and P5X compared to 5xFAD. The levels of circAdam10, circPan3, circMbtd1, circ4930402H24Rik, circSt6gal2, and circCdc14b were reduced, while circZranb1 and circMyo9a were increased in P5X and N5X compared with 5xFAD. CircNlgn1 levels changed only downstream of PPT1+/-. In silico analyses show amyloidogenic-related microRNAs exhibit circRNAs binding sites. MiR-361-3p has binding sites for circZfp609 and circADAM10, and miR-298-3p can bind circNlgn1. Both miR-361-3p and miR-298-3p trap and inhibit BACE1 function and Aβ production, interfering in the amyloidogenic pathway. CircPan3 regulates the autophagy-related miR-421. Overexpression of circPan3 suppresses autophagy through a miR-421/Pink1 pathway. **Conclusion:** AD mouse models have a positive feedback loop between CLD and circRNAs.

Oral Presentation Session Number - S1.2

Defective lysosomal acidification contributes to TNF-TNFR1 mediated neuronal death

Chih Hung Lo[1]*, Gavin Wen Zhao Loi[1], Eka Norfaishanty Saipuljumri[1], Lance M O'Connor[2], Richard Reynolds[3], Anna M Barron[1], Jialiu Zeng[1]

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Background: Tumor necrosis factor (TNF) stimulated TNF receptor 1 (TNFR1) signaling contributes to Alzheimer's disease (AD) pathogenesis by inducing autophagic dysfunction and neuronal death. Autophagy is a homeostatic mechanism involved in the disposal of damaged organelles and toxic protein aggregates with the final degradation of these cellular contents in sufficiently acidified lysosomes. In this study, we aim to investigate the role of lysosomal acidification dysfunction in mediating TNFR1 induced autophagic impairment and neuronal death. **Methods**: We elucidated the presence of TNFR1 activation and lysosomal acidification defects in post-mortem human AD brains and in APP knock-in (APP-KI/KI) mouse brains

using neuropathological approach. We then probed for pathogenic mechanisms of cellular dysfunctions associated with TNFR1 induced lysosomal acidification dysfunction using gene profilers in SH-SY5Y neuronal cells. Finally, we applied lysosome-targeting acidic nanoparticles (AcNPs) to restore lysosomal acidification and associated cellular functions in TNF treated SH-SY5Y cells and in the APP-KI/KI mice. **Results**: We reveal that TNFR1 activation correlates with downregulation of lysosomal vacuolar (H+)-ATPase required to maintain lysosomal acidification in both human AD brains and APP-KI/KI mouse brains. We then show that TNFR1 activation results in a downregulation of genes associated with lysosomal, autophagic, and mitochondrial functions and an upregulation of genes in cell death pathways in TNF treated SH-SY5Y neuronal cells. Importantly, we demonstrate that AcNPs restore lysosomal acidification, autophagic activity, and mitochondrial function, as well as rescues neuronal death in both TNF treated SH-SY5Y cells and APP-KI/KI mice. **Conclusion**: This opens avenues for new therapeutic directions to target lysosomal dysfunction, in addition to the existing efforts in developing receptor-specific inhibitors that target TNFR1 signaling.

Oral Presentation Session Number - S1.3

Lipophorin Receptors Genetically Modulate Neurodegeneration Caused by Psn Knockdown in the Aging Drosophila Brain

Chen Zhang[1]*, Jongkyun Kang[1], Yuhao Wang[2], Jian Feng[3], Bonnie Berger[2], Norbert Perrimon[4], Jie Shen[1]

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Mutations in the Presenilin (PSEN) genes are the most common cause of early-onset familial Alzheimer's disease (FAD). Studies in cell-free biochemical systems, cell culture, and knockin mice showed that PSEN mutations are loss-of-function mutations, impairing g-secretase activity. Mouse genetic analysis highlighted the importance of Presenilin (PS) in learning and memory, synaptic plasticity and neurotransmitter release, and neuronal survival, and Drosophila studies further demonstrated an evolutionarily conserved role of PS in neuronal survival during aging. However, the molecular mechanisms by which PS protects neurons during aging remain unclear. To identify genetic modifiers that modulate PS-dependent neuronal survival, we developed a new Drosophila Psn model that exhibits age-dependent neurodegeneration and increase of apoptosis. Following a bioinformatic analysis, we tested the top ranked 25 candidate genes by selective knockdown (KD) of each gene expression in adult neurons using two independent RNAi lines. Interestingly, among the 9 genes that enhanced neurodegeneration caused by Psn KD, 4 of them, lpr2, lpr1, arr, and mgl, encode proteins that belong to the low-density lipoprotein receptor family, which is involved in lipid transport and metabolism. Specifically, neuron-specific KD of lipophorin receptors (LpR1 or LpR2) results in neurodegeneration and worsens Psn KD phenotypes. Furthermore, heterozygotic deletions of lpr1 and lpr2 or homozygotic deletions of lpr1 or lpr2 also lead to age-dependent neurodegeneration and further exacerbate neurodegeneration in Psn KD flies. These findings show that proteins involved in lipid transport and metabolism, such as LpRs, modulate Psn-dependent neuronal survival and are critically important for neuronal integrity in the aging brain.

Oral Presentation Session Number - S1.4

Resistant and vulnerable motor neurons show unique temporal gene regulation in SOD1G93A ALS

Irene Mei[1]*, Susanne Nichterwitz[1], Melanie Leboeuf[1], Jik Nijssen[2], Christoph Schweingruber[3], Eva Hedlund[3]

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Background: ALS is characterised by the degeneration of somatic motor neurons (MNs), that control voluntary muscle, while visceral motor neurons remain preserved. In this study we aim to investigate the longitudinal transcriptional dynamics of resistant and vulnerable MN populations in response to the SOD1G93A ALS-causative mutation. **Methods**: We have conducted RNAseq of resilient somatic oculomotor and trochlear (CN3/4) motor neurons, as well as visceral vagus nerve (CN10) motor neurons and compared to vulnerable hypoglossal (CN12) and spinal motor neurons (sMNs) isolated at presymptomatic (P56) and symptomatic (P112) stages. As sMNs displayed the largest number of Differential expressed genes (DEGs) with disease, we used three generations of pathway enrichment methods (over-representation, per-gene and network enrichment analysis) to analyze these. We have also performed a classification analysis using random forest on a published single cell RNAseq dataset with the SOD1E1OOG mutation in order to validate the DEGs in SOD1G93A. **Results**: Differential gene expression analysis showed that each neuron group and disease stage show a distinct gene regulation with only a minority of genes being regulated across ages and cell types. Results of the pathway analysis revealed deregulated pathways (shared also in other SOD1 mutation models) related to neuropeptide signaling, PERK-mediated unfolded protein response, ER stress and metabolic processes. **Conclusion**: In conclusion, our analysis shows that each motor neuron subpopulation responds uniquely to the SOD1G93A mutation, and that different disease stages give rise to distinct transcriptional responses. Classification analysis suggests that a subset of the DEGs we found dysregulated in SOD1G93A sMNs are general markers of SOD1-ALS. Cellular stress pathways were consistently enriched in sMNs across SOD1 mutations and data sets and could explain the unique and specific vulnerability of sMNs.

Oral Presentation Session Number - S1.5

Glial Tau is required for lipid droplet formation and protection against ROS

Lindsey D Goodman[1]*, Isha Ralhan[2], Oguz Kanca[1], Matthew J. Moulton[1], Maria S. Ioannou[2], Hugo J. Bellen[1]

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Oxidative stress, caused by the aberrant accumulation of reactive oxygen species (ROS), is a common feature in many neurodegenerative diseases, including Alzheimer's disease and tauopathies. The brain has many natural mechanisms to protect neurons against elevated ROS, including the transfer of peroxidated lipids from the neurons to glia by apolipoproteins like APOE1–3. Within the glia, these lipids are sequestered into lipid droplets (LD) and degraded by β-oxidation. If this process is inhibited, cells get overrun with peroxidated lipids and toxicity ensues1–3. Whether this impacts the neurons or the glia depends on the point at which the process is inhibited4. Here, we found that the overexpression of human Tau can disrupt glial LD formation in flies and rat neuron:astrocyte co-cultures. In aged flies, the glia degenerate in response to neuronal ROS, consistent with them becoming overrun with peroxidated lipids. Intriguingly, disruptions to glial LD formation were not specific to Tau overexpression as loss of endogenous Tau in flies and astrocytes also disrupted LD formation. This was similarly associated with progressive glial loss in response to neuronal ROS. Consistent with glial Tau being critical for maintaining brain health, novel Tau loss-of-function (LOF) mutant flies were found to have motor defects and reduced lifespans. Tau mutant phenotypes were robustly recapitulated in cell-type specific studies using RNAi or genetic manipulations where Tau was specifically depleted or rescued within the glia, glial Tau was found to be important during aging to maintain health. Overall, this work highlights a novel mechanism through which endogenous Tau is neuroprotective against ROS. Further, overexpressing Tau causes similar

effects suggesting that disruptions to glial LD formation contribute to disease mechanisms at multiple stages in disease progression.

Oral Presentation Session Number - S1.6

Derailed protein turnover in the aging mammalian brain

Nalini R Rao*, Nalini R. Rao, Arun Upadhyay, Jeffrey N. Savas

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Background: Efficient protein turnover is essential for cellular homeostasis and organ function. Loss of proteostasis is a hallmark of aging, which culminates as a severe reduction in protein turnover rates. Importantly, age is also the greatest known risk factor for most neurodegenerative diseases. Investigating protein turnover dynamics during aging may elucidate subcellular compartments with greater vulnerability to age-related disease impairments and losses in functionality. **Methods and Results:** In this study, we established a pulse-stepwise continuous metabolic labeling paradigm in mice and used quantitative proteomic analyses to uncover age-related fluctuations in protein turnover. We discovered the cortical proteome exhibits sex-specific, highly dynamic turnover during aging that is associated with distinct cellular compartments. We then investigated if protein groups exhibiting dissimilar turnover trends was due to protein misfolding. Analyses of insoluble proteins revealed select age-dependent protein turnover fluctuations. To further probe if the hampered turnover of the insoluble protein pool was a result of ubiquitin proteasome system (UPS) dysfunction, we biochemically isolated proteasomes. This revealed fluctuations in proteasome activity that correlated with the turnover of the catalytically active proteasome subunits. Our findings uncover proteome-wide changes in turnover during aging and revealed that protein turnover in the cortex is a nonlinear process that undergoes sex-specific and cellular compartment related fluctuations that may be caused by reduced proteasome fidelity. **Conclusion:** Taken together, our study provides a proteome-wide atlas of protein turnover across the aging continuum and highlights a link between the turnover of individual proteasome subunits and the age-associated decline in proteosome activity.

Oral Presentation Session Number - S2.1

Long Noncoding RNA MEG3 Modulates Neuronal Necroptosis in Alzheimer's disease

Sriram Balusu[1]*, Nicola Thrupp[2], An Snellinx[2], Katleen Craessaerts[2], Lutgarde Serneels[2], Dries T'Syen[2], Iordana Chrysidou[2], Amaia M. Arranz[3], Annerieke Sierksma[2], Joel Simrén[4], Thomas K. Karikari[4], Henrik Zetterberg[5], WeiTing Chen[2], Dietmar Rudolf Thal[6], Evgenia Salta[7], Mark Fiers[2], Bart De Strooper[2]

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Neuronal cell loss is a defining feature of Alzheimer's disease (AD), but it remains unclear how neurons die and how this relates to other defining characteristics of the disease. Here we demonstrate that human neurons xenografted in mouse brain exposed to

amyloid pathology develop sarkosyl-insoluble tau filaments, positive Gallyas silver staining, release phosphorylated tau (p-tau181 and p-tau231) into the blood, and display considerable neuronal cell loss, providing a model for the induction of full Tau pathology by simple exposure to amyloid pathology in AD. The alterations are specific to human neurons and contrast with the mild effects exhibited in the circumventing host mouse neurons or in transplanted mouse neurons. A core transcriptional program in the human neurons is characterized by strong upregulation of MEG3, a neuron-specific long noncoding RNA. MEG3 is also upregulated in neurons from AD patients in situ. MEG3 expression alone is sufficient to induce necroptosis in human neurons in vitro. Inhibiting necroptosis using orally administered small molecule receptor-interacting protein (RIP) kinase -1 and -3 inhibitors or RIPK3 knockout rescues neuronal cell loss in this novel AD model. Thus, xenografted human neurons, in contrast to mouse neurons, are uniquely sensitive to amyloid pathology, recapitulate neuropathological features of AD, and ultimately die by necroptosis.

Oral Presentation Session Number - S2.2

Reproducible and controllable human iPSC-derived cortical tissue models to investigate Alzheimer's disease

Julien Klimmt[1]*, Carolina Cardoso Gonçalves[2], Severin Filser[2], Stephan Mueller[3], Jochen Herms[3], Stefan Lichtenthaler[3], Christian Haass[3], Dominik Paquet[2]

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Background: Human iPSC-based Alzheimer's Disease (AD) models have great potential for mechanistic and translational studies, as they enable investigation of pathomechanisms in human brain cells. However, current iPSC-AD models show low reproducibility and cell type diversity, lack physiological cell-cell interactions, or show only earliest disease phenotypes. Therefore, we aim to develop more reproducible and controllable iPSC-AD models that combine multiple brain cell types and enable analysis of pathomechanisms in a human, 3D cortical tissue-like environment. **Methods:** We optimized protocols to differentiate iPSCs into cortical neurons, astrocytes, and microglia. We combined these differentiated cells into 3D co-cultures and characterized them using stainings, mass spectrometry and functional assays. Using CRISPR/Cas9, we introduced AD-causing mutations to study AD pathogenesis in an isogenic system. **Results:** By 3D co-culturing all cell types we established modular, human cortical tissue models that display dense networks of neuritic processes that are stable for >6 months without formation of a necrotic core. Added microglia migrate into and tile the cultures, surveil the environment and react to tissue damage. We further confirmed maturation of the cultures, e.g., by formation of synapses and deposition of a brain-like extracellular matrix. In AD cultures, we observed disease phenotypes such as increased Aβ secretion, age-dependent accumulation of extracellular and insoluble Aβ, and increased phospho-tau levels. Upon addition of exogenous Aβ42, we found aggregation into plaque-like structures with surrounding axonal dystrophies.

Conclusion

We developed reproducible and controllable, human cortical tissue models to study cell states, crosstalk, and functionality in health and disease. Currently, we expand the model by including additional cell types such as oligodendrocytes and test approaches to elicit endogenous formation of plaque pathology.

Oral Presentation Session Number - S2.3

Optic nerve head astrocyte response to biomechanical strain using a 3D hydrogel system

Ana Nicolle Strat[1]*, Ana N Strat[1], Alex Kirschner[1], Suhani Patel[1], Ayushi Singh[1], Michael P. Geiss[1], Samuel Herberg[1], Preethi S. Ganapathy[1]

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Purpose: Glaucoma is characterized by progressive loss of retinal ganglion cells (RGCs) within the optic nerve head (ONH). Elevated intraocular pressure (IOP) in glaucoma can induce ONH biomechanical strain. Local astrocytes respond by upregulating reactivity markers (i.e., GFAP, vimentin) and remodeling F-actin to provide neurotrophic support to RGCs. However, with chronically high IOP, astrocytes become gliotic and alter the extracellular matrix (ECM). Using our established 3D hydrogel system which allows for cell-to-cell and cell-ECM analysis, we investigated mouse optic nerve head astrocyte (MONHA) response to IOP-associated pathological compression. **Methods:** Cell purity was confirmed in primary MONHAs (N=3). MONHA-hydrogels were engineered by mixing 2.5x10^6 cells/ml with photoactive ECM components (i.e., collagen I, hyaluronic acid) and crosslinked with 0.025% Riboflavin and blue light (10.3mW/cm2) for 5 min. Hydrogels were cultured for 2 weeks prior to 0%, 3%, and 10% compressive strains for 24h. Cell viability and hydrogel stiffness were measured using MTS assays and rheometry. MONHA nuclear and F-actin morphology was analyzed; vimentin/GFAP, and ECM collagen IV and fibronectin levels were quantified. Collagen I fiber remodeling was assessed by reflection mode microscopy. **Results:** 24h-compression induced MONHA cytoskeletal response with cell viability and hydrogel stiffness unchanged. Increased strain was associated with reduced astrocyte process length, complexity, and remodeling of nuclei and F-actin centrally versus peripherally. Strained MONHAs upregulated levels of GFAP and vimentin (~1.4-fold, $p<0.005$), and altered ECM proteins. **Conclusion:** IOP-associated compression alters MONHA nuclear and cytoskeletal morphology, and ECM, akin to in vivo. Our system allows for detailed examination of MONHA response to mechanical strain in glaucoma. Ongoing RNA sequencing analysis will determine differentially expressed genes/pathways in response to compression.

Oral Presentation Session Number - S2.4

Development of a human iPSC-based FTD model showing advanced Tauopathy phenotypes

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Background: Malfunction of the protein Tau is a hallmark of neurodegenerative Tauopathies such as Alzheimer's disease and Frontotemporal Dementia (FTD). In the healthy human brain, Tau expression and splicing are highly regulated and dys-regulation of the ratio between 3-repeat (3R) and 4R splice isoforms leads to FTD. Despite its importance in physiology and disease, current Tauopathy models largely do not recapitulate adult human Tau isoform expression at a 3R to 4R ratio of 1:1. In particular, human induced pluripotent stem cell (iPSC)-derived neurons express only the fetal 3R isoform and little or no 4R Tau. **Methods:** We developed a novel iPSC-based cortical neuron model that by default expresses 3R and 4R Tau in the 1:1 ratio found in adult human neurons using a multi-step CRISPR/Cas9 genome editing strategy that alters endogenous Tau isoform expression from the genomic MAPT locus. To create a disease model, we further included disease-causing Tau mutations. **Results:** 4R Tau expression in these cortical neurons was required to elicit strong and robust formation of late-stage Tau pathology in the presence of synergistic Tau mutations. In particular, these neurons endogenously accumulated seeding-competent, misfolded, fibrillar Tau in tangle-like structures inside the somata of affected neurons. Exclusive expression of mutant 4R Tau in the absence of 3R Tau disproportionately intensified pathology, resulting in abundant Tau misfolding and aggregation. **Conclusion:** Our human iPSC-derived neuronal model, which recapitulates the adult human 3R/4R Tau ratio and develops endogenous late-stage Tau pathology in the presence of

synergistic Tau mutations, will provide a valuable experimental platform to study mechanistic disease processes and complement drug screening efforts.

Oral Presentation Session Number - S2.5

IPSC-Microglia Transplantation Prevents Pathology in CSF1R FIRE/FIRE Chimeric Mouse Model of ALSP

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Background: Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) is a rare leukodystrophy caused by mutations in coding and non-coding regions of colony-stimulating factor-1 receptor (CSF1R). CSF1R is implicated in neuronal survival and imperative for microglia homeostasis. As such, disruptions in CSF1R signaling in ALSP result in fewer microglia accompanied by progressive brain atrophy, parenchymal calcification, axonal swelling, white matter lesions, and in rare cases cerebral microbleeds. In even rarer cases, homozygous CSF1R mutations result in no microglia with extensive calcification and premature death. **Methods:** To recapitulate microglia loss and explore human microglia replacement strategies, we generated a xenotolerant chimeric mouse model harboring a homozygous deletion of CSF1R fms-intronic regulatory element(hFIRE) that lacks murine microglia. Surprisingly, despite lacking microglia, we found hFIRE mice exhibit normal brain gross anatomy with little to no pathology observed at 2 months of age. Therefore, mice were transplanted with microglia precursors or vehicle at 2 months. After 6.5 months, mice were sacrificed and whole brain and plasma were collected for transcriptomic analysis, immunohistochemistry (IHC), and biochemical assays. **Results:** hFIRE mice progressively develop axonal swellings, calcification, astrogliosis, and irregular myelination indicative of ALSP. Transplantation with human microglia precursors prevents these pathologies and returns synaptic and glial markers to WT levels. Ongoing studies transplanting ALSP patient-corrected microglia also reveal microglia transplantation can further rescue pathology when transplanted into older mice. **Conclusion:** Taken together, these results indicate the humanized-FIRE mouse model recapitulates the diverse pathologies of ALSP and suggest microglia replacement with CSF1R-WT microglia as a possible therapeutic strategy to treat ALSP.

Oral Presentation Session Number - S2.6

Transdifferentiation: a novel tool for disease modeling and mechanistic investigation in Alzheimer's disease

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Background: Most Alzheimer's disease (AD) cases manifest symptoms after age 65, suggesting advanced age as the prominent risk factor. A tool for modeling aged human neurons, the cell type mostly impacted by AD, is still lacking. This study aims to develop a new cell model that overcomes rejuvenation caused by stem cell reprogramming approach for dissecting mechanisms underlying aging and AD and exploring therapeutic strategies.

Methods: We leverage powerful direct reprogramming paradigm to transdifferentiate human adult fibroblasts into functional neurons. Fibroblasts are derived from healthy controls differing in age and patients with genetic and sporadic forms of AD. We use quantitative proteomics, high-throughput imaging and biochemical analysis of cell phenotypes, supported by histological validation in mouse and human brain tissue. We integrate CRISPR genome engineering and drug discovery techniques to provide pharmacological strategies for AD. **Results**: Transdifferentiated neurons (tNeurons) exhibit cortical glutamatergic neuron identity. tNeurons show age-related changes to DNA repair and histone modifications, and AD-related amyloid-β and hyper-phosphorylated tau deposits. Proteomics and trajectory analysis reveal neuronal proteome markers associated with AD risk and unexpectedly link lysosomal quality control (LQC) pathway to AD. We molecularly define lysosomal repair deficits and the associated inflammatory responses in AD tNeurons and accumulations of LQC markers containing lysosomal proteins, proteostasis factors and amyloid-β inclusions in AD mouse and patient brain tissue. Treatment of our newly discovered drug that targets lysosomal v-ATPases ameliorates these AD neuropathologies. **Conclusion**: This study characterizes the next generation of neuronal model for late-onset AD. We demonstrate that tNeurons are a tractable and predictive model for disease mechanism exploration and therapeutics development.

Oral Presentation Session Number - S2.7

Uncovering novel regulators of an interferon-responsive microglial state

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Background: Human genetic studies and eQTL analyses implicate microglia as critical players in Alzheimer's disease (AD). In response, the field has focused on defining microglial transcriptional states across many models of neurodegeneration. Integrating these studies, we find several microglial activation states commonly enriched in AD. These include Disease Associated Microglia, Antigen-Presenting Microglia, and Interferon-Responsive Microglia. To truly make use of this data, we now must move towards understanding the functional consequence of these microglial activation states in disease. **Methods**: To determine regulators of the interferon-responsive microglial activation state, we performed a functional genomic screen using CRISPR interference. To avoid bottlenecking of CRISPR guide RNAs and accommodate the large numbers of cells needed for screening, we implemented a novel transcription factor driven approach to differentiate iPSC-derived microglia. After differentiation, microglia harboring individual gene knockdowns were primed with type I interferon and screened on expression of IFIT1, a key marker of the interferon-responsive microglial state. **Results**: These experiments have uncovered both canonical regulators of interferon signaling as well as novel regulators of the interferon-responsive microglial state such as phosphoinositide signaling. Furthermore, several known AD risk genes were shown to inhibit microglial entry into this state, suggesting a potential mechanism whereby these nodes may influence disease risk. **Conclusion**: Previous studies have uncovered significant heterogeneity of microglial states including the discovery of several disease associated states. However, it remains unclear whether these states represent beneficial, disease-fighting functions or detrimental, disease-promoting functions. Gaining the ability to selectively control entry into specific microglial states will allow novel investigations into how these specific microglial states interact with and guide disease trajectories.

Oral Presentation Session Number - S3.1

Single-nucleus dissection of human brain vasculature and transcriptomic analysis in Alzheimer's Disease

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Background: Cerebrovascular dysregulation is a hallmark of Alzheimer's Disease (AD), but the changes that occur in specific cell-types have not been fully characterized. **Methods:** we perform the first single-cell characterization of the human cerebrovasculature using both ex vivo fresh tissue experimental enrichment and post mortem in silico sorting of human cortical tissue samples in 6 brain regions from 220 individuals with AD and 208 age-matched control individuals. **Results**: We capture ~27k cerebrovascular nuclei across 11 subtypes, including endothelial cells, mural cells, and three distinct subtypes of perivascular fibroblasts along the vasculature. We uncover human-specific expression patterns along the arteriovenous axis and determine previously uncharacterized cell type-specific markers in endothelial and mural cells. We find three subtypes of fibroblasts with specific gene expression patterns. We identify 2,676 differentially expressed genes in AD, including downregulation of PDGFRB in pericytes, and of ABCB1 and ATP10A in endothelial cells, and validate the downregulation of SLC6A1 and upregulation of APOD, INSR, and COL4A1 in post-mortem AD brain tissues. We find the vasculature-glial-neuronal co-expressed gene modules, suggesting coordinated neurovascular unit dysregulation in AD. Integration with AD genetics reveals 125 AD-differentially expressed genes directly linked to AD-associated genetic variants. Lastly, we show that APOE4-genotype associated differences are significantly enriched among AD-associated genes in capillary and venule endothelial cells, and subsets of pericytes and fibroblasts. **Conclusion:** Our study provides a comprehensive resource molecular atlas of the human cerebrovasculature to guide future biological and therapeutic studies.

Oral Presentation Session Number - S3.2

Influence of Androgens in a Mouse Model of Multi-Etiology Dementia

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Low testosterone (T) levels in men, such as those caused by age-related decline and androgen depleting therapy, are a risk factor for Alzheimer's disease (AD). Men with AD have significantly lower T levels. Decreased circulating androgens are associated with declining cognitive performance and with increased levels of soluble Aβ. Androgen supplementation to hypogonadal men results in improved memory performance. Up to 80% of AD patients suffer from vascular contributions to cognitive impairment and dementia (VCID) resulting in multi-etiology dementia. VCID is slightly more prevalent in men however the effects of androgens on multi-etiology dementia are unknown. Here we investigate the importance of androgens by comparing intact and gonadectomized (GDX) males in a mouse model multi-etiology dementia using chronic cerebral hypoperfusion (unilateral carotid artery occlusion) in the AppNL-F knock-in Alzheimer's disease mouse model that do not overexpress amyloid precursor protein but instead expresses human App mutations under the endogenous mouse promoter. Cognitive impairment differences between groups were investigated using several behavioral tests. We investigated locomotor activity, anxiety like behavior, object recognition and spatial memory. We will further assess the underlying pathology in the brain that could mediate cognitive deficits (i.e. amyloid pathology, white matter damage and neuroinflammation). Results will provide valuable insight into the effects of androgens on pathology underlying multi-etiology dementia especially that in the US almost 40% of men aged over 45years suffer from hypogonadism.

Oral Presentation Session Number - S3.3

Mid-life APOE4 to APOE2 'Switching' Alters the Cerebral Transcriptome and Decreases AD Neuropathology

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OBJECTIVES: Compared to the 'neutral' E3, the E4 allele of Apolipoprotein E (APOE) confers up to a 15-fold increase in Alzheimer's Disease (AD) risk. Conversely, the neuroprotective E2 allele decreases AD risk by a similar degree. Here, we aimed to assess the therapeutic potential of allelic 'switching' by investigating the physiological changes associated with an inducible, in vivo APOE4 to APOE2 transition in a novel transgenic mouse model. **METHODS**: The APOE "switch mouse" (APOE4s2) uses the Cre-loxP system to allow for inducible APOE allele switching from E4 to E2. These mice express a floxed human APOE4 coding region followed by the human APOE2 coding region. Allelic discrimination (RT-PCR) and mass spec-based proteomic analyses were employed to validate the E4 to E2 transition. Single-cell RNAseq was used to measure physiological changes following the E4 to E2 allele switch. Behavioral measures and neuropathological analyses were applied to assess the effects of the allelic switch on AD pathology. **RESULTS**: mRNA and protein analyses confirm that APOE4s2 mice synthesize full-length human APOE4 pre-switch, and that tamoxifen induces an efficient recombination and expression of human APOE2 in target tissues. Single-cell RNAseq reveals that global, genetic replacement of APOE4 with APOE2 results in distinct alterations to glial cell transcriptomes affecting pathways involved with metabolism, inflammation, and amyloid beta. As scRNAseq implicated astrocytes as the most affected cell type post-switch, we next explored the effects of an astrocyte-selective (Aldh1l1-Cre) E4 to E2 transition. This astrocyte-specific E4 to E2 'switching' significantly decreases amyloid-associated astro- and microgliosis and improves cognition when compared to controls. **CONCLUSIONS**: Together, these data suggest that a successful transition from E4 to E2 has broad impact on the cerebral transcriptome and that an astrocyte-specific E4 to E2 'switch' improves AD associated pathologies.

Oral Presentation Session Number - S3.4

Gut microbiome regulates astrocyte reaction to amyloidosis through microglial dependent and independent mechanisms

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Background: Previous studies show that antibiotic-mediated (abx) alteration of the gut microbiome (GMB) results in a reduction of amyloid beta (Aβ) plaques and proinflammatory microglial phenotype in male APPPS1-21 mice. However, the effect of GMB perturbation on astrocyte phenotypes and microglial-astrocyte communication in the context of amyloidosis has not been examined. **Methods**: To study whether the GMB modulates astrocyte phenotype in the context of amyloidosis, APPPS1-21 mice were treated with abx, fecal matter transplants (FMT), or housed in germ-free (GF) or conventional environments leading to GMB perturbation. GFAP+ astrocytes, plaque-associated astrocytes (PAA), PAA morphological parameters, and astrocyte complement component C3 levels were quantified using a combination of immunohistochemistry, immunoblotting, widefield microscopy, and confocal microscopy. **Results**: We find that postnatal treatment of male APPPS1-21 mice with abx or GF housing reduces GFAP+ reactive astrocytosis and PAAs, suggesting that the GMB plays a role in

regulating reactive astrocyte induction and recruitment to Aβ plaques. Additionally, PAAs in abx-treated and GF housed male APPPS1-21 mice exhibit an altered morphology with increased number and length of processes and reduced astrocytic complement C3, consistent with a homeostatic phenotype. Abx-induced astrocyte phenotypes are reversed when abx-treated mice are subject to FMT from untreated APPPS1-21 male donor mice. Finally, using CSF1R inhibitors to deplete microglia, we determined that abx-mediated reduction in GFAP+ astrocytosis, PAAs, astrocytic C3 expression are independent of microglia. However, abx-induced astrocyte morphological alterations are dependent on the presence of microglia. **Conclusions**: We conclude that the GMB plays an important role in controlling reactive astrocyte phenotypes through both microglial independent and dependent mechanisms.

Oral Presentation Session Number - S3.5

Meningeal lymphatic drainage regulates oligodendrocytes survival and brain myelination

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Background: The meningeal lymphatic vessels are constantly draining cerebrospinal fluid content to the cervical lymph nodes, and functional defects in this previously unappreciated brain lymphatic cleansing pathway have been linked to poor brain function. However, the impact of impaired meningeal lymphatic drainage on specific classes of brain molecules, namely on lipids is still unknown. The main purpose of this study is to explore if and how impaired meningeal lymphatic function can alter lipid composition and myelination in the brain. **Methods:** Animals were injected with an adeno-associated virus (AAV) 9 encoding domains 1–3 of vascular endothelial growth factor receptor-3 (VEGFR3) coupled to an Ig domain, which traps circulating vascular endothelial growth factor (VEGF)-C and D, and leads to initial lymphatic vessel loss in the meninges. Control mice were injected with an AAV9 expressing the innocuous domains 4–7 of VEGFR3-Ig. Additionally, the cuprizone model was used to investigate the role of meningeal lymphatic loss during remyelination. The oligodendrocyte cell density was evaluated in mice with intact or ablated meningeal lymphatic vasculature at different time points post AAV9 injection and after different regimens of cuprizone. **Results:** Decreased VEGF-C/D signaling resulted in reduced numbers of mature oligodendrocytes and in the coverage of the myelin protein MBP. These results were recapitulated in a genetic model of lymphatic endothelial cell loss. Importantly, the demyelination observed was dependent on a proper brain immune surveillance, as models of impaired innate and adaptive immune function did not present decreased number of mature oligodendrocytes. We additionally observed that animals with impaired meningeal lymphatic function show a delay in the spontaneous remyelination process that occurs after cuprizone withdrawal. **Conclusion:** Decreased meningeal lymphatic drainage is associated with a marked loss of mature oligodendrocytes, brain demyelination, and with delayed remyelination.

Oral Presentation Session Number - S3.6

NMNAT2 supports vesicular glycolysis via NAD homeostasis to fuel fast axonal transport

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Background: Bioenergetic maladaptations and axonopathy are often found in the early stages of neurodegeneration. Nicotinamide adenine dinucleotide (NAD), an essential cofactor for energy metabolism, is mainly synthesized by Nicotinamide mononucleotide adenylyl transferase 2 (NMNAT2) in CNS neurons. NMNAT2 mRNA levels are reduced in the brains of Alzheimer's, Parkinson's, and Huntington's disease. Here we addressed whether NMNAT2 is required for axonal health of cortical glutamatergic neurons, whose long-projecting axons are often vulnerable in neurodegenerative conditions. We also tested if NMNAT2 maintains axonal health by ensuring axonal ATP levels for axonal transport, critical for axonal function. **Methods**: We generated mouse and cultured neuron models to determine the impact of NMNAT2 loss from cortical glutamatergic neurons on axonal transport, energetic metabolism, and morphological integrity. In addition, we determined if exogenous NAD supplementation or inhibiting a NAD hydrolase, sterile alpha and TIR motif-containing protein 1 (SARM1), prevented axonal deficits caused by NMNAT2 loss. This study used a combination of techniques, including genetics, molecular biology, immunohistochemistry, biochemistry, fluorescent time-lapse imaging, live imaging with optical sensors, and anti-sense oligos. **Results**: We provide in vivo evidence that NMNAT2 in glutamatergic neurons is required for axonal survival. Using in vivo and in vitro studies, we demonstrate that NMNAT2 maintains the NAD-redox potential to provide "on-board" ATP via glycolysis to vesicular cargos in distal axons. Exogenous NAD+ supplementation to NMNAT2 KO neurons restores glycolysis and resumes fast axonal transport. Finally, we demonstrate both in vitro and in vivo that reducing the activity of SARM1, an NAD degradation enzyme, can reduce axonal transport deficits and suppress axon degeneration in NMNAT2 KO neurons. **Conclusion**: NMNAT2 ensures axonal health by maintaining NAD redox potential in distal axons to ensure efficient vesicular glycolysis required for fast axonal transport.

Oral Presentation Session Number - S4.1

Acidic nanoparticles restore lysosomal function and rescue α-syn-induced neuronal cell death in Parkinson's disease

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Background: Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder worldwide. PD pathogenesis has been associated with the abnormal build-up of alpha-synuclein (α-syn) in Lewy bodies, resulting in functional decline of dopaminergic neurons. Recent work in models of PD induced by α-syn overexpression and preformed fibrils (PFFs) has shown that the endolysosomal pathway is compromised, although the exact pathogenic mechanism remains unclear. Herein, we aim to investigate this mechanism through utilizing novel acidic nanoparticles (acNPs) which can specifically localize to lysosome and induce lysosomal acidification. **Methods:** We overexpressed A30P α-syn in SH-SY5Y neuronal cells and compared the changes in lysosomal pH, autophagic function, mitochondrial function and α-syn secretion, to untreated control cells and cells with both A30P α-syn and acNPs. In a A30P transgenic mouse model, we probed for lysosomal V-ATPase subunits content via immunoblotting and immunostaining. We also studied lysosomal acidification in a mouse model with intrastriatal injection of α-syn PFFs. **Results:** A30P α-syn overexpression induced lysosomal pH elevation, lysosomal enzyme dysfunction, impaired autophagic

and mitochondrial function. Re-acidification of the impaired lysosomes using acNPs ameliorates defects in autophagic and mitochondrial function and reduced the release of α-syn, reducing α-syn induced cell death. Transcriptomics analysis show that there is a downregulation of pathways regulating lysosomal function in A30P α-syn overexpression models, and acNPs treatment restored this downregulation. In the α-syn PPFs-induced PD model, re-acidification of impaired lysosomes with acNPs reduced exosome-mediated α-syn spreading. **Conclusion:** acNPs show great potential as a valuable investigative tool for studying the intricacies of lysosomal acidification mechanisms. Moreover, their use as a potential therapeutic strategy holds promise for addressing PD.

Oral Presentation Session Number - S4.2

Does blocking arginase/polyamine pathway limit acute glaucomatous retina ganglion cell death?

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Background: We have shown that global arginase 2 (A2) deletion limits retinal ganglion cell (RGC) death after optic nerve crush (ONC). We examined the mechanism of this protective effect and the role of A2's downstream target, the polyamine-generating enzyme ornithine decarboxylase1 (ODC1), in the injury. **Methods**: We subjected C57BL/6J (WT) mice to ONC and treated them with ODC1 inhibitor, difluoromethylornithine (DFMO, 1%), in drinking water. We assessed levels of A2/ODC1 products using liquid-chromatography-mass-spectrometry (LC-MS). To study the role of polyamines in excitotoxic RGC injury, we injected WT and A2KO mice intravitreally with 20nM NMDA (N-Methyl-D-Aspartate). We measured visual function by pattern ERG and OptoMotry. We overexpressed A2 in primary RGC cultures, treated them with L-glutamate and DFMO (24h), and studied mitochondrial dysfunction using Seahorse analyzer. **Results**: LC-MS showed increased arginine, ornithine, and polyamine putrescine levels in WT retinas 7d post-ONC ($p<0.05$, n=6). DFMO treatment significantly increased survival of RBPMS-positive RGC ($p=0.006$, n=8) and improved contrast sensitivity ($p=0.02$, n=8) and pattern ERG amplitude ($p=0.01$, n=8) in WT ONC retinas compared to control at 14d post-ONC. Additionally, NMDA treatment of WT mice decreased RGC density by 43% at 7d post-injury. This loss was blocked in A2KO mice ($p=0.04$, n=8). Western blot showed increased expression of the cysteine protease calpain2 and its downstream substrates (PARP1, RIP3) and increased phosphorylation of p38/MAPK and DRP1 (mitochondrial dysfunction indicator) in WT retinas at 6h-post NMDA. A2 deletion inhibited this effect ($p<0.05$, n=6). Overexpression of A2 in RGC culture and L-glutamate treatment induced polyamine production and decreased mitochondrial respiration, whereas DFMO treatment blocked polyamine-mediated mitochondrial dysfunction. **Conclusion**: Blocking polyamine production by inhibiting ODC1 offers a therapeutic strategy to limit RGC death.

Oral Presentation Session Number - S4.3

Amelioration of Tau and ApoE4-linked glial lipid accumulation and neurodegeneration with an LXR agonist

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Background: Apolipoprotein E4 plays an essential role in brain`s lipid homeostasis; the presence of the

apoE4 allele is the strongest genetic risk factor for developing AD compared to most common apoE3 allele. Recent studies in iPSC microglia and astrocytes demonstrate that apoE modulates cholesterol dysfunction and inflammatory responses in an isoform-specific fashion. However, it is not known if lipid accumulation in glia mediates toxic effects in vivo in the context of tauopathy. **Methods:** In this work we used the combination unbiased lipidomics with immunostainings to dissect the role of apoE isoforms in glial lipid metabolism of P301S tau transgenic mice with a targeted replacement of murine apoE with human ApoE4, ApoE3, or ApoE KO. We further treated the P301S/ApoE4 mice with an LXR agonist GW3965 to test if promoting cholesterol efflux would reduce tau pathology and neurodegeneration. **Results:** By performing unbiased lipidomics, we demonstrate that P301S/ApoE KI mice accumulate significant amounts of specific cholesteryl esters in an ApoE-isoform and Tau-dependent manner. We further show that microglia from aged 9.5 months old P301S/ApoE4 mice accumulate significant amounts of neutral BODIPY-positive lipids within lysosomes. We next demonstrate that the oral administration of the LXR agonist for 3 months significantly reduces AT8 p-tau levels, protects from neurodegeneration, and improves nesting behavior in 9.5 months old P301S/ApoE4 mice. Using bulk RNAseq and immunostaining, we show that LXR agonist diet induces changes in cholesterol biosynthesis and metabolism that result in a significant reduction of neuroinflammatory responses. Using unbiased lipidomics we then demonstrate that P301S/ApoE4 mice on LXR diet exhibit significantly lower levels of cholesterol esters. **Conclusions:** Together, we show that ApoE4 promotes lipid accumulation in glia in the setting of tauopathy and that reducing the accumulation of these lipids by LXR activation is neuroprotective.

Oral Presentation Session Number - S4.4

Increased senescence is associated with α-synucleinopathy in the TgA53T mouse model and senolytic treatment delays disease onset

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Emerging evidence indicates that cellular senescence is a pathological factor in aging and neurodegenerative diseases, including Parkinson's Disease (PD). Because α-synuclein(aS)-pathology and aS-dependent neurodegeneration are mechanistically linked to pathogenesis of PD, we examined the pathological relationship between a-synucleinopathy and cellular senescence. To study the in vivo relevance, we used a transgenic mouse model of a-synucleinopathy (TgA53T), where rapid and reliable onset of disease was induced by intramuscular inoculation with human αS PFF. Analysis of TgA53T mice show that a-synucleinopathy is associated with increased levels of senescence markers including signs of DNA damage response (DDR; γH2Ax, HMGB1), p16INK4a, p21Cip1, and SASP factors. Cellular localization of senescence markers using Immunohistochemistry and RNAscope analysis show that multiple cell types exhibit increased p16INK4a and/or p21Cip1, including neurons with aS aggregates. Significantly, expression of A53T mutant human aS seem to induce DDR in neurons in absence of overt aS pathology or other signs of senescence. To determine the pathologic significance of senescence, the mice were treated with senolytic cocktail [Dasatinib (12 mg/kg) and Quercetin (50 mg/kg) (D+Q)] or vehicle (DMSO) once weekly orally, starting 21 days post αS PFF inoculation. Analysis of motor behavior (rotarod and open field) show the D+Q treatment attenuated preclinical motor abnormalities in TgA53T mice. More important, D+Q treatment significantly delayed the onset of a-synucleinopathy. While we are currently analyzing the D+Q treated mice for neuropathology and levels of senescence markers, our initial studies indicate that D+Q treatment reduces aS pathology and reduces senescence markers in TgA53T mice. Analysis of transcriptional changes via Nanostring nCounter analysis show that a-synucleinopathy is associated with increases in senescence-associated

pathways, including increased neural inflammation and D+Q treatment significantly attenuates these changes. Our data show that cellular senescence is induced by α-synucleinopathy and targeting senescent cells using senolytics may provide neuroprotection from α-synucleinopathy.

Oral Presentation Session Number - S4.5

Alzheimer's disease associated isoforms of human CD33 distinctively modulate microglial cell responses in 5XFAD mice

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Background: Neuroinflammation and microglia are key pathological players of Alzheimer's disease (AD), with genetic susceptibility factors skewing microglial cell function to influence AD risk. CD33 is an immunomodulatory receptor associated with AD susceptibility through a single nucleotide polymorphism that modulates mRNA splicing, skewing protein expression from a long protein isoform (CD33M) to a short isoform (CD33m). Understanding how human CD33 isoforms differentially impact microglial cell function in vivo has been challenging due to functional divergence of CD33 between mice and humans. **Methods**: To address this challenge we generated transgenic mice expressing either of the human CD33 isoforms crossed with the 5XFAD mouse model of amyloidosis and applied a combination of techniques including immunofluorescent microscopy, biochemistry, transcriptomics and proteomics analysis to decipher the function of each human CD33 protein isoform in the context of amyloid pathogenesis. **Results**: We find that human CD33 isoforms have opposing effects on the response of microglia to amyloid-β (Aβ) deposition. Mice expressing CD33M have increased Aβ levels, more diffuse plaques, fewer disease-associated microglia, and more dystrophic neurites compared to 5XFAD mice. Conversely, CD33m promotes plaque compaction and minimizes neuritic plaque pathology, highlighting an AD protective role for this isoform. Protective phenotypes driven by CD33m are detected at an earlier timepoint compared to the more aggressive pathology in CD33M mice, suggesting that CD33m has a more prominent impact on microglia cell function at earlier stages of disease progression. In addition to divergent roles in modulating phagocytosis, scRNAseq and proteomics analyses demonstrate that CD33m+ microglia upregulate nestin, an intermediate filament involved in cell migration, at plaque contact sites. **Conclusion**: Overall, our work provides new functional insights into how CD33 modulates microglia.

Oral Presentation Session Number - S4.6

Assessing microglial states in the context of amyloid using targeted single cell profiling

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Background: Historically microglial state has been described as either "activated" (M1) or "homeostatic" (M2) microglia. With the advent of single-cell RNA sequencing technology, unique subtypes of microglia such as disease-associated microglia, Alzheimer's Disease 1 and 2 (AD1, AD2) microglia, and lipid-droplet accumulating microglia have been identified and profiled. Researchers now question whether such microglial subtypes are unique and differentiated forms of microglia, or whether microglia exist in a dynamic state capable of shifting between transcriptional phenotypes in response to their environment. **Methods**: Microglia

are poorly represented in single-nucleus studies of brain compared to neurons and astrocytes, and whole-transcriptome data is sparse, meaning that many genes go undetected from cell to cell. To resolve this, we optimized a single-cell dissociation protocol that depletes neurons and enriches for microglia. We also designed a novel, targeted single-cell RNA-sequencing (scRNAseq) panel to profile 598 genes associated with different microglial subtypes/states at dramatically increased depth and sensitivity. **Results**: Using this unique targeted scRNAseq approach, we profiled microglia from three different models of amyloidosis (5XFAD, NLGF, APPPS1) at approximately equal levels of amyloid burden. We then compared signatures across different states of microglia between amyloid and control mice, and assessed overlap between the models. This well-powered study (163,370 cells) identifies disease associated microglia in 5XFAD and NLGF mice, as well as complex shifts in microglial states across all three comparisons. **Conclusion**: Targeted scRNAseq is a cost-effective and highly sensitive method of identifying changes in microglial phenotypes across disease models, and for validating published markers of microglial states.

POSTER PRESENTATION ABSTRACTS

Poster #01

Repetitive traumatic brain injuries induce Alzheimer's disease-related protein pathogenesis via microtubule disruption

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Background: Alzheimer's disease (AD) is the major cause for dementia, however, there is no cure for such devastating disease. Tau accumulation is the hallmark of AD, and its burden and spreading correlates well with AD patients' cognitive decline. Traumatic brain injury (TBI) has been reported as a risk factor for AD, however, the exact association between TBI and AD, and the underlying mechanisms are absent. In this study, we aim to extensively examine the association between TBI and AD-related pathogenesis and neurodegeneration. **Methods:** We developed a repetitive traumatic brain injury (rTBI) mouse model, combined with our existing AD transgenic mouse models and our recently developed seeding mouse models, we extensively examined the effects of rTBI on AD-related protein pathogenesis, including tau, A□, TDP43 and α-Synuclein, as well as associated tissue degeneration. **Results:** We reported that rTBI disrupted axonal microtubules, induced tau pathogenesis and caused neurodegeneration in wild-type (WT) mice, as well as in APP transgenic mice harboring abundant A□ plaques. In addition to tau, Aβ and TDP-43 pathologies were also increased in rTBI-treated mice. Notably, rTBI could also facilitate neuronal pathological tau spreading in mice with either pre-existing tau pathology or those in which tau pathology was induced through brain injection of human-derived tau seeds. In addition, rTBI also promoted glial tau pathology and transmission. Normalizing microtubules with a microtubule-stabilizing molecule reduced TBI-induced tau and TDP-43 pathogenesis, and the associated neurodegeneration. **Conclusion:** Our study suggests rTBI not only trigger initial pathological tau seed formation, but also facilitate subsequent transmission of tau pathology and neurodegeneration. And microtubule alterations play a primary role in TBI-mediated pathological changes. Furthermore, our study provides a therapeutic strategy for treatment of TBI and related neurodegenerative diseases.

Poster #02

Congo Red-Derived Carbon Dots as Dual Inhibitor of Tau and Amyloid Aggregation in Alzheimer's Disease

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Alzheimer's Disease (AD) is the leading form of senile dementia, affecting ~6 million Americans and having a national economic impact of $321 billion, numbers expected to double by 2050. The major pathological hallmarks of AD include Amyloid Beta (Aβ) plaques and Tau neurofibrillary tangles (NFT). The first goal of this research was to develop a novel form of carbon dots (CD) using Congo red, a known aggregation inhibitor, and citric acid. Then we characterized Congo red-derived carbon dots (CRCDs) using multiple chemical and biophysical methods. Finally, we aimed to utilize CRCDs to inhibit tau and Aβ aggregation in vitro. Thioflavin T (ThT) aggregation assays were utilized and verified by atomic force microscopy (AFM) which can visualize these aggregates. All three CRCDs inhibited both tau and Aβ aggregation, suggesting the use as a dual inhibitor. CRCD1 demonstrated IC50 values of 0.2 ± 0.1 mg/mL for tau and 2 ± 2 mg/mL for Aβ aggregation, whereas CRCD2 (0.5 ± 0.3 mg/mL for tau and 3.3 ± 0.7 mg/mL for Ab) and CRCD3 (3 ± 1

mg/mL for tau and 6 ± 2 mg/mL for Ab) showed slightly higher IC50 values for their inhibition of aggregation. This data suggested CRCD1 as the best dual inhibitor, and CRCD3 being least efficient. However, experiments in zebrafish testing blood brain barrier (BBB) penetration suggested that only CRCD3 could efficiently cross the blood brain barrier. Overall, the data suggests investing future research into CRCDs for AD treatment in the future.

Poster #03

Dementia Training for Primary Health Care (PHC) Workers in Ethiopia

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Background: Most cases of dementia are seen at primary health care centers by primary health care (PHC) workers. There is a big gap in knowledge of dementia, among PHC workers' leading to underdiagnosis or misdiagnosis of dementia with primary psychiatric disorders. Because of this, there will be a shortfall in providing evidence-based and patent centered care for people living with dementia. Implementing competency-based training would bring a change to the shortfall. **Objective:** To develop competency-based dementia training material and implement it in Primary health care workers. And evaluate the outcome of the training value in terms of knowledge, practice, and referrals. **Methods:** a total of 55 primary healthcare workers consisting of MSc, and BSc psychiatry nurses will be involved in the training from 3 geographic pilot sites in Addis Ababa. Training will be implemented at Amanuel Mental Specialized Hospital for a total of 22 hrs. A rating scale “ADKS” and “CODS” will be used to collect data on the knowledge soon after completing the training. An online survey will be used to assess practice in the 6th and 9th months after completion of the training. Referral rates and case identification rates will be collected from the health management information system (HMIS) at the 3rd, 6th, 9th, and 12th months from the 3 health bureaus of the city. Data will be analyzed using SPSS V.25 with a model of Paired sample T-test for Knowledge, and ANOVA for the practice and referrals. **Expected result:** Development of training materials will be started soon after a grant is awarded. The total budget for this pilot will be $25,000. The expected result of the pilot would be improved knowledge, and practice of dementia among primary healthcare workers, and increased diagnosis and referral.

Poster #04

The role of synaptic mitochondrial dysfunction in dopamine oxidation in Parkinson’s disease

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The preferential degeneration of dopaminergic neurons in the substantia nigra is a hallmark of Parkinson’s disease (PD), but the molecular mechanisms underlying this selective vulnerability remains unknown. Excessive dopamine oxidation has been observed across many genetic and idiopathic forms of PD, and may represent a common pathological phenotype. In addition, the identification of mutations in a variety of PD genes has highlighted the importance of mitochondrial function for the disease and with this a potential pathological cascade of mitochondrial oxidant stress eventually resulting in elevated dopamine oxidation. Given that impaired mitochondrial function leads to loss of ATP, and that packaging of dopamine into synaptic vesicles by vesicular monoamine transporter (VMAT2) is ATP-dependent, I hypothesize that this may result in excess dopamine in the cytosol and, consequently, excess dopamine oxidation into neurotoxic quinones. Mutations in DJ-1, a key regulator of mitochondrial

function in dopaminergic neurons, have been associated with familial and sporadic forms of PD. Therefore, I utilized CRISPR-edited DJ-1 knockout human induced pluripotent stem cells differentiated into dopaminergic neurons as a model system. By verifying the depolarized state of mitochondria accompanied by decreased ATP level in mutant neurons, I showed a correlation between mitochondrial alterations and resulting cytosolic energy content. In addition, I observed reduced VMAT2 activity together with revealed abnormalities in vesicle dynamics, suggesting irregularities in vesicle formation and vesicular dopamine uptake in PD neurons, that may explain measured excess of oxidized dopamine. Summarized, these findings highlight a potential role for ATP and dopamine metabolism in dopaminergic neuronal vulnerability.

Poster #05

A deep sequencing investigation of mitochondrial DNA damage in cholinergic neurons of the Pedunculopontine Nucleus

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In Parkinson's disease (PD) patient brains, higher levels of somatic mitochondrial DNA (mtDNA) damage, manifesting as large-scale deletions, are frequently seen. Such mtDNA damage associates with neuronal loss, particularly the nigral dopaminergic neurons. However, cholinergic neuronal loss also occurs, particularly within a brainstem structure termed the Pedunculopontine Nucleus (PPN). We previously showed that, in contrast to nigral dopaminergic neurons, which show reduced mtDNA copy number (mtCN) in the presence of mtDNA deletions, PPN cholinergic neurons attempt to maintain the pool of wild-type mtDNA mtCN by increasing mtCN. Here we will isolate single cholinergic neurons from post-mortem PPN tissue of aged controls versus PD patients followed by ultra-deep sequencing. At a single-cell resolution, we will consider the location of mtDNA deletions, their size, the nature of deletion breakpoints and their heteroplasmy level. Our study aims to understand how mtDNA deletions are generated and explain the different compensatory responses by mtDNA in nigral (reduced mtCN) versus PPN cholinergic (increased mtCN) neurons to the accumulation of mtDNA deletions. Finally, we will use single cells qRT-PCR to determine expression levels of critical nuclear genes involved in mitochondrial biogenesis, mitophagy and mtDNA maintenance. Preliminary results support earlier observations of higher levels of large deletions in PD compared to controls. The deletions range between 5-140bp (small) and >1,400bp (large). Analysis of large deletion location 'hotspots' is consistent with generation due to errors during replication. We find no significant difference in the levels of point mutations between cases and controls. The low concentration of small deletions suggests that oxidative damage is not a significant driver of excess mtDNA damage affecting PPN cholinergic neurons in PD patients.

Poster #06

Heterozygous loss of the Alzheimer's risk factor iRhom2 leads to a partial reduction of the amyloid pathology in mice

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Background: Neuroinflammation, induced by Aβ deposition, plays a key role in Alzheimer's Disease (AD) pathogenesis. Subsequently, microglia start to

release pro-inflammatory cytokines, such as TNFα, cleaved by the protease ADAM17. Immature ADAM17 is localized to the membrane of the ER. Inactive rhomboid 2 (iRhom2, RHBDF2) is an essential upstream regulator of ADAM17 and necessary for its trafficking and maturation in microglia. iRhom2 has recently been identified as an epigenetic risk factor for AD. A genetic inactivation of iRhom2 may prevent the activation of ADAM17 in microglia and thus, the release of pro-inflammatory cytokines. **Methods:** We investigated the effect of iRhom2 deficiency in APP/PS1 mice. We analysed number and size of plaques, number and activation pattern of microglia and the area of plaque associated dystrophic neurites, as well as the amount of Aβ in brain homogenates. **Results**: The histological results indicate a beneficial effect on AD pathology caused by the heterozygous knockout of iRhom2 (iR2(+/-);APP/PS1). This effect was characterized by reduced number of diffuse and dense core amyloid plaques, determined by Abeta stainings. Additionally, the number of Iba1+ microglia cells was reduced, indicating an attenuated neuroinflammation. The area of the dystrophic neurites was significand reduced especially in the male iR2+/-;APP/PS1 within the hippocampus compared to the females and the other genotype groups. The decreased protein levels of insoluble Abeta40 and especially of the toxic Abeta42 correlated with the estimated number of congophilic plaques within the iR2(+/-);APP/PS1 genotype group and thus strengthen our histological data. **Conclusions:** Taken together, the partial loss of iRhom2 due to a heterozygous knockout of the Rhbdf2 gene harbours potential neuroprotective functions in an AD mouse model. Our findings provide an encouraging approach for testing iRhom2 as a potential AD drug target.

Poster #07 **[Also in Oral Presentation]**

Mid-life APOE4 to APOE2 'Switching' Alters the Cerebral Transcriptome and Decreases AD Neuropathology

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OBJECTIVES: Compared to the 'neutral' E3, the E4 allele of Apolipoprotein E (APOE) confers up to a 15-fold increase in Alzheimer's Disease (AD) risk. Conversely, the neuroprotective E2 allele decreases AD risk by a similar degree. Here, we aimed to assess the therapeutic potential of allelic 'switching' by investigating the physiological changes associated with an inducible, in vivo APOE4 to APOE2 transition in a novel transgenic mouse model. **METHODS:** The APOE "switch mouse" (APOE4s2) uses the Cre-loxP system to allow for inducible APOE allele switching from E4 to E2. These mice express a floxed human APOE4 coding region followed by the human APOE2 coding region. Allelic discrimination (RT-PCR) and mass spec-based proteomic analyses were employed to validate the E4 to E2 transition. Single-cell RNAseq was used to measure physiological changes following the E4 to E2 allele switch. Behavioral measures and neuropathological analyses were applied to assess the effects of the allelic switch on AD pathology. **RESULTS:** mRNA and protein analyses confirm that APOE4s2 mice synthesize full-length human APOE4 pre-switch, and that tamoxifen induces an efficient recombination and expression of human APOE2 in target tissues. Single-cell RNAseq reveals that global, genetic replacement of APOE4 with APOE2 results in distinct alterations to glial cell transcriptomes affecting pathways involved with metabolism, inflammation, and amyloid beta. As scRNAseq implicated astrocytes as the most affected cell type post-switch, we next explored the effects of an astrocyte-selective (Aldh1l1-Cre) E4 to E2 transition. This astrocyte-specific E4 to E2 'switching' significantly decreases amyloid-associated astro- and micro-gliosis and improves cognition when compared to controls. **CONCLUSIONS:** Together, these data suggest that a successful transition from E4 to E2 has broad impact on the cerebral transcriptome and that an astrocyte-specific E4 to E2 'switch' improves AD associated pathologies.

Poster #08

Examining Noradrenergic Changes in the AppNL-G-F Mouse Model of Alzheimer's Disease

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Background: Alzheimer's disease (AD)-associated neuronal death has been hypothesized to include degeneration of the norepinephrine (NE) producing locus coeruleus (LC) neurons as an initial region of loss responsible for propagating death to efferent projection areas. However, the mechanisms explaining this early and preferential dysfunction of the noradrenergic system remain unclear. **Methods:** we compared littermate control six-month-old male and female C57BL/6 wild type mice to the AppNL-G-F knock-in model of AD (n=6). Immunostaining for tyrosine hydroxylase, as the rate-limiting enzyme for norepinephrine synthesis, was used to quantify cell loss in the LC. As a relevant efferent output of the LC, we quantified norepinephrine levels in the hippocampus and compared this, via western blot, to levels of the catabolic enzymes, monoamine oxidase A (MAO-A), catechol-O_x0002_methyltransferase (COMT), and monoamine oxidase B (MAO-B). Western blot analysis was also used to assess overall presynaptic and post-synaptic compartment integrity through quantifying levels of synaptophysin and PSD95, respectively. **Results**: As a possible consequence of the NE deficiency, we quantified a decrease in hippocampal protein levels of beta-2 adrenergic receptors (β2-AR) in male and female AppNL-G-F mice compared to wild-type controls. There were no differences in synaptophysin or PSD95 in AppNL-G-F compared to wild type mice in either sex suggesting no overall synaptic loss in the hippocampus. **Conclusion**: Our findings revealed a specific modulation of β2-AR and MAO-A levels in the AppNL-G-F mice that is not associated with robust cell death or synaptic loss. These data suggest a potential selective disruption in adrenergic signaling. Moreover, this change in NE neuron function may identify an early phenotype change during disease that precedes or contributes to eventual neuron and synaptic loss.

Poster #09

G protein-coupled receptor kinase 2 modulates tau pathogenesis in human neurons

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Background: Accumulation of the tau protein in neurofibrillary tangles (NFTs) is a pathological hallmark of Alzheimer's Disease (AD) brains. Kinases heavily contribute to the pathological phosphorylation and aggregation of tau in AD. Previously, we showed that the G protein-coupled receptor (GPCR) kinase 2 (GRK2) is abundantly expressed in neurons, positively correlated with soluble tau levels, and associated with NFTs in the human AD brain. **Methods**: We tested a putative direct role for GRK2 on tau phosphorylation and aggregation using in vitro CRISPR HEK293 modified lines, mass spectrometry, optogenetic tools, and patient-derived neuronal cultures. **Results**: Genetic deletion of Grk2 induces global changes in the tau phosphoproteomic profile, while GRK2 overexpression increases tau phosphorylation (pTau). Using a novel optogenetic system to induce tau aggregation (optoTAU) we show that optoTAU aggregation, and specifically soluble tau, is reduced in Grk2-deficient cells. We also show that GRK2 directly interacts with tau and pathogenic species in vitro. Moreover, we find that GRK2 modulates pTau in a kinase activity-independent manner. Specifically, ERK-mediated phosphorylation of GRK2 species at residue 670 is protective against pTau formation and is this found downregulated in AD brains. Interestingly, pharmacological inhibition of GRK2 in human directly converted induced neurons (iNs) increases both ERK activation and GRK2-s670 levels, while decreasing pTau and reactive-oxygen stress in

AD-derived iNs. Lastly, Grk2 genetic deletion enhances tau ubiquitination and interaction with HSP90, which further increases tau degradation, protecting the cells from aggresome formation upon proteasome inhibition. **Conclusions**: These studies causally implicate GRK2 as a multifactorial modulator of tau pathology through changes in phosphorylation, aggregation, and degradation of tau and support further investigation of therapeutic interventions against GRKs in AD.

Poster #10

RLN3 Immunoreactivity and In Situ Hybridization Reveal the Human Nucleus Incertus: A Step towards Understanding its Function and Pathological Implications in Dementia

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Background: The nucleus incertus (NI), was originally described by Streeter in 1903 as a midline region in the floor of the fourth ventricle of the human brainstem with an 'unknown' function. A century later, the NI has been shown to play a key role in memory formation in mice through direct inhibition of the hippocampus (Hip). The anatomy of the NI and its projections have been mapped in vertebrates, but not in humans. In these novel studies, we mapped the human NI using the neuropeptide relaxin-3 (RLN3). In vertebrates, RLN3 is produced mostly in the NI. **Methods:** Serial free-floating sections of formalin-fixed postmortem tissue were prepared from the pons of one elderly female with Alzheimer's disease (AD) and two elderly control females, without a history of dementia. Then, we analyzed the Hip of one elderly control female and the testis of one elderly control male. We mapped the distribution of microtubule-associated protein-2 (MAP2) and RLN3+ neurons in the pons and in the Hip, using immunohistochemistry. Specificity of RLN3 antibody was tested by dot-blot (negative control) and pre-adsorption with native RLN3 peptide, in testis (positive control). Also, RNAscope in situ hybridization for RLN3 mRNA was tested in the pons and Hip. Finally, we used a phosphorylated-tau antibody (AT8) to determine if AD NI neurons were reactive for phosphorylated tau. **Results:** MAP2-immunoreactivity (IR) revealed the neuronal distribution throughout the pons. RLN3-IR and mRNA were detected in neurons in the dorsal, anterior-medial region, revealing the anatomy of the human NI. Also, RLN3-IR was found in pyramidal cells of the Hip. Finally, AT8-IR was detected in the NI of the AD case co-localizing with RLN3+ neurons. **Conclusion:** RLN3 is synthesized in neurons of the human NI and aspects of its anatomy are shared across species, including the macaque. Accumulation of phosphorylated-tau in the NI and RLN3-IR in the Hip, suggests its possible involvement in the pathology of AD.

Poster #11

Age related neuropathology in a novel mouse model of Adult-onset leukoencephalopathy (ALSP)

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Background: Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) is a rare human disease that is caused by mutations in CSF1R, a gene that is critical for the differentiation, proliferation, and survival of microglia. ALSP patients typically develop dementia, motor impairments, and seizures during their 30s or 40s. Upon autopsy, patients' brains exhibit decreased numbers of microglia, white matter atrophy, astrocytosis, axonal spheroids, enlarged ventricles, and calcification. Two recent reports have further described rare cases of homozygous CSF1R mutations that lead to perinatal lethality, a complete absence of microglia, and an acceleration of ALSP pathologies. Unfortunately, there is currently no effective treatment for this devastating disease. **Method:** To better understand the role of microglia in the development and progression of ALSP we utilized the 'FIRE' mouse model that harbors a homozygous deletion in a microglial-specific enhancer within the CSF1R gene. **Result:** Surprisingly, at 2 months of age, despite absence of microglia, FIRE mice exhibit normal brain gross anatomy, minimal white matter alterations, and little to no pathology. However, by 10-months of age FIRE mice exhibit many of the pathologies and symptoms observed in ALSP patients, including axonal spheroids, calcification, demyelination, seizures, and behavioral deficits. Importantly, postnatal transplantation of wildtype murine microglia prevents nearly all of these pathologies, demonstrating the importance of decreased microglial numbers on the development of ALSP. Additional ongoing examinations of 2- and 10-month-old FIRE mice are exploring the sex-related impact of microglia absence on synaptic density and utilizing MRI imaging to examine white matter integrity and calcification. **Conclusion:** Taken together, these data suggest that aged FIRE mice may provide a novel and informative, albeit rapidly progressing, model of human ALSP.

Poster #13

Pegylated Arginase 1 Protects Against Indirect Traumatic Optic Neuropathy

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Among the causes of blindness, traumatic optic neuropathy (TON) has a low incidence (0.5 to 5%) worldwide. However, in combat situations TON is much more prevalent. TON is an acute event that frequently triggers partial or complete loss of vision. TON can occur by either direct or indirect means. The direct form occurs when an object directly damages the optic nerve. Indirect TON is more common and occurs when concussive or tensile forces are transferred through the skull and twist the optic nerve. Current options for treatment, corticosteroids and optic canal decompression, offer limited effectiveness. Though the pathophysiology of this trauma has not been fully elucidated, indirect TON has been associated with axonal degeneration and a reduced population of retinal ganglion cells (RGC). Thus, clinical studies are focused on limiting RGC death and promoting optic nerve regeneration. In a prior study, we showed that intraperitoneal injections (IP) of a stable form of arginase 1 (Peg-Arg1) in mice subjected to retinal ischemia-reperfusion protected against neurovascular retinal injury by increasing neural growth factors and blocking inflammation. We have now produced indirect TON by sonication of the orbital bone in 9-week old wild-type mice treated IP with Peg-Arg1 at (25mg/kg) or vehicle every 3 days for 7 days and 14 days. Sonication-induced TON (SI-TON) increased

expression of macrophages colony stimulant factor (MCSF), glial fibrillary acidic protein (GFAP) and significantly decreases the number of RGCs. SI-TON mice treated with Peg-Arg1 showed significantly reduced death of RGCs, promoted expression of brain derived neurotrophic factor (BDNF), blocked macrophages infiltration and glial cell activation. These data suggest Peg-Arg1 as a novel therapy for indirect TON.

Poster #14

Investigating the Role of Protein Disulphide Isomerase in DNA Damage and Repair in Amyotrophic Lateral Sclerosis

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Background: The DNA damage response is crucial for maintaining genomic integrity and is increasingly linked to neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). Our previous studies have demonstrated that protein disulphide isomerase (PDI), a unique chaperone with oxidoreductase activity, protects against multiple ALS-associated pathologies. However, the protective role of PDI in DNA damage remains unexplored. In this study, we aimed to investigate the protective effects of PDI in DNA repair. **Methods:** Using neuroblastoma cell lines (Neuro-2A), we determined PDI expression levels through overexpression and siRNA knockdown. DNA damage was induced using etoposide, hydrogen peroxide, and mutant TDP-43. Fluorescence microscopy and software analysis were employed to assess DNA damage foci. **Results:** Our findings revealed that overexpression of PDI effectively inhibited DNA damage induced by etoposide, H2O2, and ALS mutant TDP-43. Knockdown experiments further demonstrated that PDI plays a protective role against DNA damage in neuroblastoma cells following etoposide or H2O2 treatment. Additionally, we observed the translocation of PDI to the nucleus upon etoposide-induced DNA damage, suggesting a direct involvement in DNA repair mechanisms. Investigating ALS risk-causing mutants, PDI-D292N and PDI-R300H, as well as a PDI-Quad mutant lacking redox active cystine sites, revealed that while PDI-R300H exhibited partial protective activity, PDI-D292N and the Quad mutant did not confer protection against DNA damage. **Conclusions:** Hence these results indicate that the redox activity of PDI is protective against DNA damage, but this is perturbed in ALS. By defining these mechanisms further in future studies, these results can lead to design future therapeutics for ALS based on PDI that can prevent DNA damage.

Poster #16

Imaging glial activation in virally-suppressed individuals with HIV

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Background: Emerging data suggests that chronic neuroinflammation may be associated with cognitive impairment in people living with HIV, despite viral suppression. Microglia, the brain's resident immune cells, play an important role in the neuroimmune response. [11C]DPA-713 positron emission tomography (DPA-PET) may be used to map the availability of translocator protein 18 KDa (TSPO), a marker of glial (microglia, astrocyte) activation, and this radiotracer has some advantages over other second-generation radiotracers for imaging TSPO. Here we present data from the largest DPA-PET study to date in a population of virally-suppressed people with HIV (VS-PWH) and HIV-uninfected individuals. We hypothesized higher regional TSPO levels in the VS-PWH. **Methods:** Twenty-four VS-PWH and 18 demographically-similar HIV-uninfected individuals completed one DPA-PET scan. Emission scans lasted

90 minutes post slow intravenous push of [11C]DPA-713. Each participant also completed a structural brain MRI for segmentation and selection of regional volumes of interest. Regional [11C]DPA-713 total distribution volume (VT) values were estimated using Logan graphical analysis with metabolite-corrected arterial input function. Regional VT values were compared between groups using a linear mixed model with repeated measures in SAS (Version 9.4, SAS Institute Inc., Cary, NC). **Results:** Higher [11C]DPA-713 VT values were found in VS-PWH compared to HIV-uninfected individuals ($P<0.05$). The magnitude of the group difference in [11C]DPA-713 VT was similar across all regions. **Conclusion:** These DPA-PET data support a persistent glial activation in the brains of VS-PWH, which warrants further longitudinal investigation to evaluate the temporal course of high TSPO in VS-PWH and its relationship to cognition cross-sectionally and over time.

Poster #17

Study of reelin's effects on a chemical model of Parkinson's disease induced by rotenone.

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Background: Parkinson's disease (PD) is one of the neurodegenerative diseases that has become highly prevalent in the last few years and a burden for health systems worldwide. Patients present α-synuclein aggregates and selective degeneration of the Substantia Nigra. Even when the PD etiology is unknown, it has been proposed that genetic and environmental causes, especially chronic exposure to the pesticide rotenone, are relevant. Rotenone blocks the mitochondrial electron transport chain and recapitulates the PD's main phenotype. On the other hand, reelin is a secreted glycoprotein crucial for the nervous system. It is associated with neuronal survival in adults, synaptic plasticity and memory. It has been reported that reelin decreases α-synuclein preformed fibrils in cell lines, and it is neuroprotective in Alzheimer's disease. Considering all these, we hypothesize that reelin has a neuroprotective effect on a PD model induced by rotenone. **Methods**: Two neuron-like cell lines were used, the mouse dopaminergic MN9D and the human SH-SY5Y, both expressing the Reelin receptor ApoER2. Cells were treated with 100nM of rotenone in co-incubation with reelin or mock for 24 and 48 hours. Then, MTT assays to measure cell survival were performed, along with western blot and immunofluorescence analysis. As rotenone impairs the autophagic flux, we measured the endo-lysosomal system. **Results:** rotenone reduces the cell viability in MN9D at 24 and 48 hours. It also induces aggregation of α-synuclein. Conversely, reelin treatment induces metabolic activity in SH-SY5Y, increases the neurite length in the vehicle condition and protects from the neurite retraction induced by rotenone in MN9D. Overall, Reelin improves endo-lysosomal functioning. **Conclusions:** Reelin has a neuroprotective effect on a PD model induced by rotenone, improving cell survival and the function of the endo-lysosomal system. It remains to characterize the molecular mechanisms by which Reelin exerts its role.

Poster #18

Neuronal Tau Pathology Alters Human Microglial Morphology, Transcriptome, and Function

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Background: The V337M mutation in MAPT is associated with frontotemporal dementia and leads to aggregation of hyperphosphorylated tau in humans and transgenic mouse models. Previous

research has revealed conflicting roles for microglial in FTLD-Tau and other tauopathies, and many of these roles have only been studied in mouse models. **Methods:** CRISPR gene editing has been used to introduce the V337M mutation into a Tet-regulatable Neurogenin-2 expressing iPSC line that enables efficient differentiation into human glutamatergic neurons. To study the interactions between V337M neurons and human microglia, V337M NGN2-iPSCs and isogenic control NGN2-iPSCs were differentiated into neurons and cultured with wild-type hiPSC-derived microglia. To determine whether microglia grown with mutant tau neurons exhibit altered gene expression or visa versa, RNA sequencing was performed on cocultures, mutant and wildtype neurons grown alone, and microglia isolated from co-cultures. **Results:** Microglia grown with neurons homozygous for the V337M mutation develop altered morphology and enrichment for genes associated with proliferation and the cell cycle. Tau mutant neurons grown with microglia exhibit less β3-tubulin immunoreactivity than when grown alone, indicating decreased neuronal survival or diminished dendritic arborization. V337M mutant neurons also displayed enrichment for genes associated with neurotransmitter release and protein folding chaperones, but reduced expression of genes associated with translation. **Conclusions:** These results may indicate that impaired protein processing or increased synaptic activity in V337M neurons drives microglial proliferation in the early stages of tauopathy. Characterizing the interactions between tau-accumulating human neurons and microglia will help us to better understand the role of microglia in human tauopathies and establish a novel in vitro system for testing pharmacological approaches.

Poster #20

Excitation/Inhibition Imbalance in a Murine Model of Epileptogenesis Induced by Pentylenetetrazol and Its Impact on Temporal Fidelity in CA1 Hippocampal Neurons

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Epilepsy is a neurological disorder that can coexist with a wide range of other neurological conditions, including neurodegenerative diseases such as Alzheimer's, Down Syndrome, and Parkinson's. It is characterized by hyperexcitability and hypersynchrony of neural circuits, which have been associated with increased glutamatergic neurotransmission and decreased GABAergic neurotransmission. However, in many epilepsy models, it remains unclear whether the balance between excitation and inhibition (E/I) is disrupted and how it affects the excitability and input integration of principal neurons in the hippocampal circuit. In this study, we investigated the E/I balance in the PTZ induced chemical kindling model of epileptogenesis. Using electrophysiological techniques, we simultaneously examined glutamatergic and GABAergic neurotransmission in CA1 pyramidal neurons of the hippocampus in mice with PTZ induced epilepsy. We recorded evoked excitatory and inhibitory postsynaptic currents (EPSCs/IPSCs) at 40 mV and analyzed various parameters, including the amplitude ratio, paired pulse ratio (PPR), frequency of monosynaptic and disynaptic spontaneous currents, and spike probability, to assess the temporal integration of

neuronal inputs. Our results revealed that PTZ kindled mice exhibited a two-fold increase in the E/I amplitude ratio compared to the control group, primarily driven by a higher PPR of EPSCs. Additionally, the PTZ group showed higher frequency values of miniature postsynaptic currents (mEPSCs and mIPSCs) and an increased frequency of spontaneous excitatory postsynaptic currents (sEPSCs) compared to the control group. Moreover, PTZ treated animals displayed an enhanced spike probability, indicating that the hyperexcitability associated with epilepsy may extend the time window during which CA1 pyramidal cells can integrate multiple subthreshold inputs. Furthermore, in the presence of PTX, we observed higher input/output function compared to control groups, while the population spike threshold was lower in PTZ kindled mice. Taken together, our findings suggest that in PTZ induced epileptogenesis, there is a dysregulation of the E/I balance, which contributes to the upregulation of the input/output function of CA1 pyramidal neurons.

Poster #21

Endothelin-converting enzyme-2 (ECE-2) regulates endogenous synaptosomal and secreted β-amyloid in distinct brain regions

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Background: Levels of the Alzheimer's disease (AD) associated β-amyloid (Aβ) peptide are tightly regulated by proteases responsible for its production and degradation. Among known Aβ degrading enzymes, endothelin-converting enzymes (ECEs) have the unique trait of cleaving Aβ within the endosomal vesicles where it is produced and pharmacological inhibition of ECEs causes rapid accumulation of both intravesicular and secreted Aβ. Compared to ECE-1, ECE-2 expression in the brain is both spatially and neuronal cell-type restricted, with high expression in interneurons and enrichment in dentate gyrus, hypothalamus, midbrain, and cerebellum. Furthermore, impaired ECE-2 activity has been implicated as a risk factor for late-onset AD. Understanding the spatial relationship between ECE-2 activity and endogenous Aβ metabolism will provide insight into regional and cell-type-specific Aβ regulation. **Methods**: Using ECE-2 KO and wild-type mice, whole brain or microdissected regions were homogenized to prepare crude synaptosomal vesicles and separate them from the extracellular (ISF-enriched) fraction. ECE-2 protein expression in synaptosomes was confirmed by western blot and Aβ was measured by ELISA. **Results**: ECE-2 localizes to synapses and, globally, ECE-2 KO mice had significantly increased synaptosomal and secreted Aβ. Subcortical structures and cerebellum had the highest ECE-2 protein expression and, in ECE-2 KO mice, the largest increases in synaptosomal Aβ. Secreted Aβ was significantly increased in all brain regions except cortex, with hippocampus showing the largest change and overall Aβ levels. **Conclusion**: Our results demonstrate that ECE-2 regulates endogenous synaptosomal and secreted Aβ within brain regions known to be important for cognition and impacted early in AD pathogenesis. Future research will determine how ECE-2 regulation may relate to the physiological function of Aβ and whether changes in endogenous ECE-2 activity can alter the pathogenesis of AD.

Poster #22

Rescuing the Secretion and Lipidation Deficits of APOE4 Using HDL Mimetic Peptides in Primary Glial Cells

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Background: Alzheimer's disease (AD) is one of the most prevalent neurodegenerative diseases. Genome-wide association studies have identified apolipoprotein E4 (APOE4) as the greatest genetic

risk factor for developing sporadic AD; however, the exact underlying mechanism remains undeciphered. The human APOE, encoded by three alleles—ε2, ε3, ε4, is a key constituent of HDL-like particles in the brain. Lipidation of APOE is isoform dependent (ε2>ε3>ε4) and is crucial for HDL formation and its function including cholesterol homeostasis, synaptic growth. APOE4 exhibits altered physiological functions because of its decreased propensity to form HDL in the brain, which may be a potential mechanism driving APOE4 linked AD pathology. We hypothesize that reversing APOE4's lipidation deficit using HDL-mimetic peptides corrects its functions and ameliorates APOE4 linked AD pathology. **Methods:** Primary murine astrocytes derived from homozygous human APOE3 and APOE4 knock in (KI) mice were treated with peptides 4F and a modified 4F (X-4F) in the presence or absence of aggregated amyloid-β (Aβ). Secretion and lipidation of APOE isoforms were investigated using gel electrophoresis. **Results:** We confirmed the well-established lipidation deficit of APOE4 and further demonstrated that 4F and X-4F increase the secretion and lipidation of both APOE4 and APOE3. Importantly, 4F and X-4F improve lipidation of APOE4 to a greater extent than APOE3, reversing its lipidation deficit. In addition, 4F and X-4F mediated enhancement of APOE secretion and lipidation persists in the presence of Aβ as well as in APOE4 KI astrocytes overexpressing APP/PS1 that model endogenous Aβ production in vitro. **Conclusion:** This study provides additional evidence of HDL-mimetic peptides as potential APOE4 modulating agents. Future studies are warranted to assess the efficacy of HDL-mimetic peptides to restore the beneficial functions of HDL and APOE in the brain and mitigate the pathogenic processes in AD.

Poster #23

EMBER multidimensional spectral microscopy enables quantitative determination of disease- and cell-specific amyloid strains

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In neurodegenerative diseases, proteins fold into amyloid structures with distinct conformations (strains) that are characteristic of different diseases. However, there is a need to rapidly identify amyloid conformations in situ. Here, we use machine learning on the full information available in fluorescent excitation/emission spectra of amyloid-binding dyes to identify six distinct different conformational strains in vitro, as well as amyloid-β (Aβ) deposits in different transgenic mouse models. Our EMBER (excitation multiplexed bright emission recording) imaging method rapidly identifies conformational differences in Aβ and tau deposits from Down syndrome, sporadic and familial Alzheimer's disease human brain slices. EMBER has in situ identified distinct conformational strains of tau inclusions in astrocytes, oligodendrocytes, and neurons from Pick's disease. In future studies, EMBER should enable high-throughput measurements of the fidelity of strain transmission in cellular and animal neurodegenerative diseases models, time course of amyloid strain propagation, and identification of pathogenic versus benign strains.

Poster #24

Level of blood Tau and pTau : a non-invasive approach for the diagnosis of Alzheimer's Disease(AD)

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Background: Prominent expression of Tau and phosphorylated Tau in cerebrospinal fluid (CSF) is one of the well-known hallmarks of the Alzheimer's disease (AD). High levels of p-Tau can easily differentiate AD from other neurodegenerative disease. However, the serum levels of these proteins in AD patients are not well explored. This study provides an alternative non-invasive approach to diagnose the disease by evaluating the level of Tau and p-Tau181 in serum samples. **Methods:** Blood samples were collected from 39 AD patients, 37 MCI patients and 37 elderly individuals as controls. Quantification of tau and pTau in serum samples was done with the help of a label free real time Surface Plasmon Resonance technology, and further validated by Western Blot. Statistical analysis, including Receiver Operating Characteristic (ROC), was done for further affirmation. **Results:** The concentrations of serum Tau and p-Tau181 were significantly higher ($p<0.00001$) in AD (Tau; 47.49±9.00ng/µL, p-Tau181; 0.161±0.04 ng/µL) compared to MCI (Tau; 39.26±7.78 ng/µL, p-Tau181; 0.135±0.02 ng/µL) and were further compared to elderly controls (Tau; 34.92±6.58 ng/µL, p-Tau181; 0.122±0.01 ng/ µL). A significant ($p<0.0001$) downhill correlation was found between Tau as well as p-Tau181 levels with HMSE and MoCA score. **Conclusion:** This study for the first time reports the concentration of Tau and p-Tau181 in serum of AD and MCI patients. The cut-off values of Tau and p-Tau181 of AD and MCI patients with sensitivity and specificity reveal that serum level of these proteins can be used as a predictive marker for AD and MCI.

Poster #25

FOXO3A serum protein: A potent marker for early diagnosis of Alzheimer's disease

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FOXO3A serum protein: A potent marker for early diagnosis of Alzheimer's disease
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Background: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive decline. In any ageing society it's a major health concern and a public health challenge. Diagnosis of Alzheimer's disease (AD) is often difficult because of distinct and subjective clinical features, especially in the early stage. FOXO3a protein present in the cognitive centre of brain in inferior temporal region and parahippocampus. FOXO3a can be a potential novel target against AD. **Method:** Older subjects aged 60 Years and above comprised three groups such as AD, Mild Cognitive impairment (MCI) and Geriatric Control (GC) were recruited after diagnosis by clinical assessment, MRI, Tau PET and FDG PET. We have quantified serum FOXO3a by surface plasmon resonance (SPR) and compare with Tau PET between of AD, MCI patients and GC. **Result:** Serum FOXO3A was significantly lower in AD (1.42 ± 0.09 ng/µl) compare to MCI (1.61 ± 0.14 ng/µl) and GC (1.89 ± 0.07 ng/µl). ROC curve for FOXO3A protein level was plotted and showed a reasonably good sensitivity and specificity at the cut-off value for detecting cognitively impaired patients (AD and MCI) in compared to control older subjects. For Tau PET in inferior temporal region, the AUC for predicting AD from GC was 0.72 and AD from MCI was 0.71. **Conclusion:** Serum FOXO3A could significantly differentiate AD vs MCI, MCI vs GC and AD vs GC. It may serve as novel blood marker for early detection for AD and target for therapeutic intervention. **Key words** FOXO3A, Alzheimer's disease, SPR

Poster #26

Integrated Stress Response in alpha-synucleinopathy

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Abnormalities in α-synuclein (αS) is directly linked to the pathogenesis of Parkinson's disease (PD) and related disorders called α-synucleinopathies. We showed that α-synucleinopathy in αS transgenic mouse model (TgA53T) and humans causes chronic Integrated Stress Response (ISR). Treatment of TgA53T with salubrinal, an inhibitor eIF2α dephosphorylation, can significantly delay onset of αS pathology and motor deficits. Thus, we further studied the mechanistic relationships between of eIF2α phosphortulation and α-synucleinopathy. First, we examined if the loss of eIF2α phosphorylation by the Protein kinase R-like ER Kinase (PERK) in neurons exacerbates α-synucleinopathy. We show that conditional deletion of PERK in neurons of TgA53T accelerates the onset of α-synucleinopathy. Further, loss of PERK was associated with increased severity of the disease, including the levels of phosphor-Ser129S (pS129S). The results show the pathologic importance of PERK-eIF2α pathway in α-synucleinopathy. Because Salubrinal inhibits both the protein phosphatase 1 regulatory subunit 15A (PPP1R15A, Gadd34) and PPP1R15B (CReP), we tested the pathological importance of each of these phosphatases independently. While inhibition of Gadd34 is neuroprotective multiple models of neurodegeneration, inclusing motor neuron disease and multiple sclerosis, we show that neither the pharmacological inhibition of Gadd34 or genetic loss Gadd34 function attenuated α-synucleinopathy in TgA53T model. To test the role of CReP, we treated the TgA53T mouse model with Raphin1, a selective inhibitor of CReP. Treatment of TgA53T model with an Raphin1 significantly delays disease onset. The neuroprotective effects of Raphin1 treatment reduces pS129S in neuronal cells via activation of autophagy and attenuates αS preform fibril induced neurotoxicity. Our data show that PERK-eIF2α pathway is an important pathological axis for α-synucleinopathy.

Poster #27

Exogenous alpha-synuclein fibrils cause postsynaptic deficits in Lewy body dementia

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Background: Alpha-synuclein (aS) is currently the primary pathological protein implicated in Parkinson's disease dementia (PDD) and dementia with Lewy bodies (DLB). Previously we have shown that A53T aS transgenic mice (TgA53T) have tau-dependent age-related cognitive deficits. Further, A53T expressing neurons have GSK3b-dependent tau mislocalization to dendritic spines leading to synaptic dysfunction. Dementia in PDD/DLB is associated with high neuritic aS pathology in CA2, likely from afferents of the entorhinal cortex (EC), without overt neurodegeneration. Thus, we hypothesize that in sporadic DLB, the neurites from EC neurons are releasing toxic aS, impacting hippocampal dendrites, leading to tau mislocalization and synaptic deficits that leads to cognitive deficits. **Methods:** To directly test our hypothesis, we evaluated sporadic PD hippocampal pathology and exposed mouse primary hippocampal neurons to aS preformed fibrils (PFF). The neurons were transfected to express DsRed and Tau-eGFP to monitor the neurites/spines and tau, respectively. **Results:** Analysis of hippocampal sections from sporadic PD cases with neuritic aS pathology exhibit tau mislocalization to somatodendritic areas compared to controls. Live cell imaging of mouse primary hippocampal neurons at 24 hours post-PFF treatment shows that even low doses (0.05-4.0ug/mL; 3.5-280nM), cause tau mislocalization. Tau mislocalization is independent of endogenous aS but requires GSK3b. aS PFF causes significant loss of spines, as well as markers synapsin and PSD95 by 14 days after PFF. Finally, to define the in vivo significance of our hypothesis, we show that WT mice injected with aS PFF to the CA1 region have mEPSC frequency and amplitude deficits 48 hours after injection, as well as a lack of LTP. **Conclusion:**

We conclude that in PDD/DLB, Lewy neuritic pathology may be releasing toxic aS species leading to tau mislocalization and synaptic deficits that lead to circuit dysfunction and cognitive deficits.

Poster #28

GSK3β: Serum based Detection of Alzheimer's Disease

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Background: The characteristic feature of Alzheimer's Disease (AD) is the atrophy of brain cell networks leading to memory impairment. Since there is presently no cure for Alzheimer's disease, it is critical to detect it at the earliest possible stage in order to fully understand its pathogenesis. The inflammatory molecules such as GSK3-β and p53's upregulation leads to taupathy and amyloid-beta deposition, the key hallmarks of AD. This study aimed to estimate GSK3-β and p53 expression levels as potential blood based biomarkers for early diagnosis of Alzheimer's Disease. **Methods**: Blood samples were collected from AD pateints, mild cognitive impairment (MCI) pateints and geriatric control (GC) subjects and the level of GSK3-β, p53 and their phosphorylated states were quantified utilizing Surface Plasmon Resonance (SPR) technology and validated using Immunoblotting in the serum and statistical analysis (ROC curves) was performed. The neurotoxic SH-SY5Y cell line was treated with antioxidant Emblica Officinalis (EO) for rescue effect. **Results**: In SPR data analysis, GSK3-β, P- GSK3-β (Y-216), P- GSK3-β (S-9), p53 and P-p53 (T-155) in serum were significantly upregulated ($p<0.001$) in AD and MCI patients, compared to GC and validated via immunoblotting. Percentage increase in AD in comparison to GC for GSK3-β, P-GSK3-β (Y-216), P- GSK3-β (S-9), p53 and P-p53 (T-155) was 44.44%, 84.71%, 38%, 59.42% and 43.91%. The best percentage increase (84.71%) was found in P- GSK3-β (Y-216) with sensitivity: 77.74% and specificity: 79.31%. The expression level of these proteins decreases in SH-SY5Y cells after the treatment of EO in a dose-related manner. **Conclusion:** Expression levels of GSK3-β, P- GSK3-β (Y-216), P- GSK3-β (S-9), p53 and P-p53 (T-155) can be a part of a panel of efficient blood based biomarkers for early diagnosis of AD and EO can suppress their level.

Poster #29

Identification of Molecular Mechanisms in Different Alzheimer's Disease Subtypes Using Unbiased Localized Proteomics in Human Post-mortem Tissues

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Background: Alzheimer's disease (AD) accounts for an estimated 60% to 80% of the total cases of dementia. The number of cases of AD is projected to increase as the worldwide population ages. Therapeutic strategies against AD have focused on the aggregation of amyloid-beta and tau, thought to be the main culprits of AD symptomatology. However, hundreds of proteins associated with amyloid plaques and tau that play mechanistic roles in AD are often overlooked and poorly understood. **Methods:** We characterized the protein signature of amyloid plaque pathology and adjacent non-plaque tissue in three subtypes of AD: sporadic early-onset AD (EOAD), late-onset AD (LOAD) and Down syndrome (DS) using our unbiased localized proteomics approach. We microdissected cortical plaques from post-mortem human brain tissues and identified enriched proteins and their associated signaling pathways. **Results:** We found a strong correlation in the proteome of amyloid plaques between the AD subtypes evaluated but a weaker

correlation when comparing neighboring non-plaque tissue, thus elucidating an amyloid plaques protein signature. We also identified differentially enriched proteins in each disease group, which may account for the differences in brain pathology and cognitive decline outcomes. Furthermore, functional enrichment analyses revealed GO terms and pathways involved in amyloid-beta binding and clearance regulation, endo/lysosomal pathways and regulation of immune response and inflammation. Strikingly, some pathways relevant in AD are differentially enriched in the different AD subtypes. **Conclusion:** Our study highlights that the altered signaling pathways associated with amyloid plaques regulate fundamental functions in the neuropathology of AD, which might be essential to explain enhanced vulnerability and resilience observed in the pathology and may be informative to develop potential therapeutic targets and biomarkers.

Poster #30

Synthesis, single crystal structure, and Amyloid Beta Aggregation Inhibition Activity of Novel Alpha Ketoamide Derivative

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Alzheimer's disease (AD) is a multifaceted, severe condition affecting a considerable number of individuals worldwide. There is an urgent necessity to discover effective treatments, and a breakthrough in this domain would be an outstanding accomplishment globally. Amyloid beta, a peptide resulting from the degradation of a larger protein named the amyloid precursor protein (APP), plays a key role in the disease process. The build-up of Aβ fibrils leads to the generation of Aβ plaques, which cause neuronal damage and the emergence of dementia symptoms. As such, managing Aβ fibril accumulation is crucial to neutralize its harmful effects by either halting Aβ aggregation or speeding up the transition of toxic Aβ oligomers to harmless mature Aβ fibrils. In our research, we targeted the synthesis of different ketoamides and examined their potential as Aβ aggregation modulators. We discovered that the compound N-benzyl-4-(4-chlorophenyl)-2-oxobutanamide functioned as an Aβ aggregation modulator through ThT assay testing. To delve deeper into the compound's structural and electronic attributes, we utilized single crystal X-ray diffraction to determine its 3D structure, later optimized via DFT computations using the B3LYP/6-311G(d,p) basis set. The interplay of practical and theoretical methodologies in this study offers a detailed exploration of the compound's structure and electronic characteristics. This research underscores the potential of α-ketoamides as Aβ fibril modulators, and endeavors to augment our comprehension of the principles governing their functionality. It also highlights the prospective utility of α-ketoamides as potential medicinal molecules in treating AD and similar neurodegenerative ailments.

Poster #31

Understanding the Mechanism of Choline Acetyltransferase Inhibition by Proton Pump Inhibitors

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Background: Proton pump inhibitors (PPIs) have revolutionised the management of stomach acid suppression in patients with gastro-oesophageal reflux disease. However, evidence suggests that Long-term PPI use may increase the risk of developing Alzheimer's disease (AD). In our previous report, we presented findings on the inhibitory effects of PPIs on choline acetyltransferase (ChAT), an enzyme involved in the biosynthesis of acetylcholine. **Method:** Here in this study we have

employed a series of computational tools, namely molecular docking and classical molecular dynamics to gain a mechanistic understanding of the molecular interactions between PPIs and the binding pocket of ChAT. Enabling the elucidation of protein-ligand complexes binding interactions, conformational stability, and dynamic evolution within a time frame of 200 nanoseconds. Further, the binding free energies for the complexes under investigation were calculated using Molecular Mechanics Poisson-Boltzmann Surface Area. **Result:** The findings indicate that the PPIs have comparable or greater binding affinity to the ChAT catalytic tunnel. Additionally, it was observed that the pyridine ring of the PPIs predominantly interacts with the catalytic residue His324. Moreover, the free energy landscape analysis showed that the folding process was linear, and the residue interaction network analysis provided insight into the roles of various amino acid residues in stabilization of the PPIs in the ChAT binding pocket. **Conclusion:** As a major factor for the onset of Alzheimer's disease is linked to cholinergic dysfunction, our previous and the present findings give clear insight into the PPI interaction with ChAT. The scaffold can be further simplified to develop novel ChAT ligands, which can also be used as ChAT tracer probes for the diagnosis of cholinergic dysfunction and to initiate timely therapeutic interventions to prevent or delay the progression of AD.

Poster #32

Molecular and cellular pathways of microvascular dysfunction in Alzheimer's Disease

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Neurovascular coupling describes the signalling pathways between neurons, glia and vascular cells that ensure regional increases in brain activity are matched by corresponding increases in local blood flow. Increasing evidence suggests that these pathways are dysfunctional in Alzheimer's disease (neurovascular uncoupling) and are a significant contributor to cognitive impairment and disease progression. Vasoactive molecules such as nitric oxide and potassium ions are key for neurovascular coupling, and emerging evidence suggests that their roles are disrupted in mouse models of Alzheimer's disease. Furthermore, the two characteristic pathological molecules in Alzheimer's disease, hyperphosphorylated tau and amyloid beta, have been shown to have deleterious effects on neurovascular coupling. Pericytes are contractile cells which regulate cerebral blood flow using finger-like projections that influence the diameter of brain capillaries in response to the neurovascular coupling signals. Looking beyond controversies in their classification, it is now well documented that pericyte dysfunction is a feature of Alzheimer's disease. Given that microvascular dysfunction (neurovascular uncoupling) in Alzheimer's disease leads to cognitive impairment and neurodegeneration, study of neurovascular uncoupling is important to discover potential future therapeutic targets. This poster will provide an overview of the pathophysiology of neurovascular uncoupling in AD and how these disturbances in cerebral blood flow and subsequent ischaemia may activate biochemical pathways that lead to the hallmark Aβ plaques and NFTs found in symptomatic AD.

Poster #33

Autophagy as a therapeutic target in Huntington's disease: insights from a fly model

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Huntington's disease (HD) is an autosomal dominant disorder caused by mutations in the Huntingtin gene (Htt). Mutations in the gene leading to expansion of poly-Q/CAG repeats in the coding region of the protein cause the mutant HTT protein to form toxic

aggregates which affect processes including synaptic transport and high ROS levels, eventually leading to the death of neurons. A protein homeostasis pathway, namely the autophagy pathway has the ability to degrade large protein aggregates. Harnessing this degradative ability of the autophagy pathway mainly via pharmacological agents, has been shown to mitigate the toxic effect of mutant proteins. However, one of the questions that is least explored is, whether autophagy induction is equally effective in different circuits (functionally and spatially) present in the brain. Using a genetic approach and Drosophila as our model system, we aimed to understand the contribution of the autophagy pathway in mitigating the toxicity caused by the mutant HTT protein. We found that overexpression of a core autophagy pathway gene "Atg8a" could rescue behavioral defects caused by the expression of mutant HTT protein containing 128 polyQ repeats (mHtt-Q128) in only a few targeted circuits among all. Interestingly, while Atg8a-mediated behavioral rescue was dependent on the autophagy pathway, complete degradation of mutant protein aggregates was not observed. Further, we found that the rescue in the targeted circuits is possibly an outcome of improved synaptic transmission to the downstream circuits. Thus, our study in a highly tractable genetic model system sheds light on the idea that autophagy modulation might not be helpful in mitigating toxicity of the mutant HTT protein among all the affected circuits in Huntington's disease.

Poster #34

Neuroprotective effect of Chromenopryazol-4-ones ameliorating Aβ25-35-induced damage in SH-SY5Y cell lines.

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BACKGROUND: Alzheimer's disease pathogenesis is believed to be driven by the production, deposition and clearance of the β-amyloid peptide (Aβ). BACE1 is responsible for the production of amyloid beta by cleaving APP at the minor Asp1 site, generating C99 and leading to Aβ generation. Due to the complex pathology of AD it is reasonable to think that strategies such as multi-target drugs can be a good alternative as an effective treatment. The current study aims to design marine based MTDLs targeting Aβ peptide and MAO-B. **METHODS:** Employing BREED algorithm and Ligand Designer module of Schrodinger suite, a total of 8 chromenopryazolone derivatives (ALZ 1-8) were designed, synthesized and evaluated for their anti-alzheimer's activity. In-vitro MTT assay (SH-SY5Y cell lines) assay in the presence of synthesized compounds ALZ 1-8showed high percentage of cell viability (> 100µg/mL) indicating less cytotoxicity. **RESULTS AND CONCLUSION:** Compounds ALZ3 and ALZ4 showed reduction in Aβ25-35 induced cytotoxicity in SH-SY5Y cells thus confirming the neuroprotection (increase in cell viability 80-98%). In DPPH assay compounds ALZ2, ALZ4, and ALZ7 exhibited significant antioxidant activity when compared with standard. The in-silico molecular docking and MMGB/SA studies against the respective targets provided crucial information about binding modes of ALZ 1-8.

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