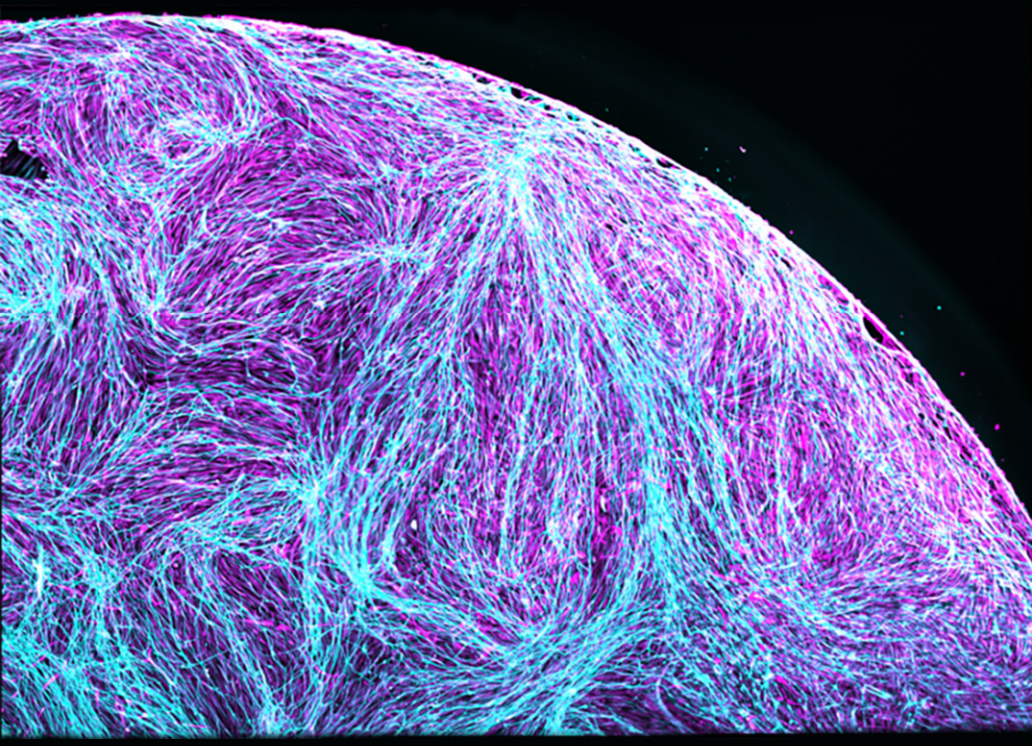
Abstracts of papers presented
at the 2020 *virtual* meeting on

NEURODEGENERATIVE DISEASES: BIOLOGY & THERAPEUTICS

December 2–December 4, 2020



Cold Spring Harbor Laboratory
MEETINGS & COURSES PROGRAM

Abstracts of papers presented
at the 2020 *virtual* meeting on

NEURODEGENERATIVE DISEASES: BIOLOGY & THERAPEUTICS

December 2–December 4, 2020

Arranged by

Aaron Gitler, *Stanford University*
Richard Ransohoff, *Third Rock Ventures*
Scott Small, *Columbia University*
Li-Huei Tsai, *Massachusetts Institute of Technology*

CSH Cold Spring Harbor Laboratory
MEETINGS & COURSES PROGRAM

Support for this meeting was provided in part by the **National Institute on Aging,** a branch of the **National Institutes of Health; Chan Zuckerberg Initiative (CZI), IRBO/International Brain Research Organization; Regeneron;** and **Stem Cell Technologies.**

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NEURODEGENERATIVE DISEASES: BIOLOGY & THERAPEUTICS
Virtual Meeting
Wednesday, December 2 – Friday, December 4, 2020

Wednesday	10:00 am–11:00 am	Keynote Speaker: Virginia Lee
Wednesday	11:00 am–1:00 pm	**1** Genetics, Genomics and Target Identification in Neurodegenerative Disease
Wednesday	2:00 pm–5:00 pm	**2** Neuroinflammation and Glial Biology of Neurodegeneration
Thursday	10:00 am–1:00 pm	**3** Therapeutic Initiatives in Neurodegenerative Disease
Thursday	2:00 pm–5:00 pm	**4** ApoE and Lipid Metabolism
Friday	10:00 am–12:45 pm	**5** Endolysosomal Dysfunction in Neurodegeneration
Friday	1:30 pm–3:00 pm	**Panel:** Science, Society and COVID-19
Friday	3:00 pm–6:00 pm	**6** New Technologies to Study Neurodegeneration
Throughout Meeting		*Virtual Poster Session*

Virtual Icebreaker, Wednesday, 5:30 pm

StemCell Technologies Workshop: Thursday, 5:30 pm (*p. T-1)*

Closing Social, Friday, 6:00 pm

All times shown are US EST: Time Zone Converter

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PROGRAM

WEDNESDAY, December 2—10:00 AM US EST

KEYNOTE SPEAKER

Virginia Lee
University of Pennsylvania School of Medicine

"Transmission of misfolded proteins in neurodegenerative diseases—A common mechanism of disease progression"

WEDNESDAY, December 2—11:00 AM US EST

SESSION 1 GENETICS, GENOMICS, AND TARGET IDENTIFICATION IN NEURODEGENERATIVE DISEASE

Chairperson: **Sally John,** Biogen, Cambridge, Massachusetts

Genomic investigation of the human brain transcriptome, CSF and plasma proteome and biomarkers of disease progression guides target validation in neurodegenerative diseases
Sally John.
Presenter affiliation: Biogen, Cambridge, Massachusetts. 1

Vacuolar tauopathy is associated with a hypomorph VCP mutation
Nabil F. Darwich, Jessica M. Phan, Boram Kim, EunRan Suh, John D. Papatriantafyllou, Lakshmi Changolkar, Sokratis G. Papageorgiou, Murray Grossman, Lauren Massimo, David J. Irwin, Corey T. McMillan, Ilya M. Nasrallah, Camilo Toro, Geoffrey K. Aguirre, Vivianna M. Van Deerlin, Edward B. Lee.
Presenter affiliation: University of Pennsylvania, Philadelphia, Pennsylvania. 2

Atlas of genetic regulatory effects on human microglia transcriptome
K.P. Lopes, G. Snijders, J. Humphrey, A. Allan, B. Schilder, R. A. Vialle, E. Navarro, F. Gigase, R. Kübler, M. Sneeboer, L. de Witte, T. Raj.
Presenter affiliation: Icahn School of Medicine, New York, New York. 3

WEDNESDAY, December 2—2:00 PM US EST

SESSION 2 NEUROINFLAMMATION AND GLIAL BIOLOGY OF NEURODEGENERATION

Chairperson: **Anne Schaefer,** Icahn School of Medicine at Mount Sinai, New York, New York

SESSION 3 THERAPEUTIC INITIATIVES IN NEURODEGENERATIVE DISEASE

Chairperson: **Chris Henderson,** Biogen, Cambridge, Massachusetts

THURSDAY, December 3—2:00 PM US EST

SESSION 4 ApoE AND LIPID METABOLISM

Chairperson: **Joachim Herz,** UT Southwestern, Dallas, Texas

SESSION 5 ENDOLYSOSOMAL DYSFUNCTION IN NEURODEGENERATION

Chairperson: **Henne Holstege,** Amsterdam UMC, the Netherlands

FRIDAY, December 4—1:30 PM US EST

PANEL SESSION: Science, Society, and COVID-19

Panelists

Sandra Encalada, The Scripps Research Institute
Walter Koroshetz, NINDS, National Institutes of Health
Anna Nordvig, Columbia University
Aaron Gitler, Stanford University School of Medicine

FRIDAY, December 4—3:00 PM US EST

SESSION 6 NEW TECHNOLOGIES TO STUDY NEURODEGENERATION

Chairperson: **Martin Kampmann,** University of California, San Francisco

VIRTUAL POSTER SESSION

Terminal complement activation drives neuropathology in Parkinson's disease through intracellular NLRP3 signaling
Eduardo A. Albornoz, Richard Gordon, Nathan Lin, John Lee, Anumantha G. Kanthasamy, Trent M. Woodruff.
Presenter affiliation: The University of Queensland, Brisbane, Australia.
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Cognitive deficits reported in the M323K mouse, a TDP-43 ALS model
Zeinab Ali, Remya Raghavan-Nair, Gareth Banks, Pietro Fratta, Abraham Acevedo Arozena, Elizabeth Fisher, Silvia Corrochano, Tom Cunningham.
Presenter affiliation: MRC Harwell, Oxford, United Kingdom.
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Uncovering the pathological role of astrocytes in SMA
R L. Allison, G Khayrullina, B G. Burnett, A D. Ebert.
Presenter affiliation: Medical College of Wisconsin, Milwaukee, Wisconsin.
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Disturbed BDNF / TrkB signaling in cortico-striatal networks of Parkinson's disease models
Thomas Andreska, Stefanie Rauskolb, Nina Schukraft, Patrick Lüningschrör, Robert Blum, Markus Sauer, Philip Tovote, Chi W. Ip, Michael Sendtner.
Presenter affiliation: University Hospital Wuerzburg, Wuerzburg, Germany.
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Increased transgene expression in rat retinal ganglion cells by using human mini-promoters compatible with rAAV
Victor G. Araujo, Mariana S. Dias, Thaís Gonçalo, Taliane Vasconcelos, Gabriel N. dos Santos, William W. Hauswirth, Rafael Linden, Hilda Petrs-Silva.
Presenter affiliation: Laboratório de Neurogênese, Rio de Janeiro, Brazil.
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Aberrant ganglioside metabolism in glaucomatous neurodegeneration and prospect for its modulation
Jennifer Arcuri, Sean Meehan, Sanjoy K. Bhattacharya.
Presenter affiliation: Bascom Palmer Eye Institute, Miller School of Medicine at University of Miami, Miami, Florida.
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Neuropeptide deficiency induces reactive astrocytes, upregulates immune processes, and induces AD-like hippocampal dysfunction
Brent Asrican, Josh Wooten, Yadong Li, Luis Quintanilla, Feiran Zhang, Connor Wander, Hechen Bao, Chia-Yu Yeh, Yanjia Luo, Reid Olsen, Szu-Aun Lim, Jessica Hu, Peng Jin, Juan Song.
Presenter affiliation: University of North Carolina, Chapel Hill, North Carolina.
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Development of the first selective chemical probe for the pleiotropic kinase CK2, an emerging target in neurodegenerative disease
Carrow I. Wells, David H. Drewry, Julie E. Pickett, Amelie Tjaden, Andreas Krämer, Stefan Knapp, Laszlo Gyenis, David Litchfield, Alison D. Axtman.
Presenter affiliation: University of North Carolina at Chapel Hill, Chapel Hill, North Carolina.
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Potential inefficiencies in the generation of RNA-seq data from an Alzheimer's disease-like mutation zebrafish model
Lachlan Baer, Morgan Newman, Nhi Hin, Stephen Pederson, Michael Lardelli.
Presenter affiliation: The University of Adelaide, Adelaide, Australia.
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Targeting RNA-binding protein MSUT2 for the treatment of tauopathies including Alzheimer's disease
Jeremy D. Baker, Rikki L. Uhrich, Brian C. Kraemer.
Presenter affiliation: University of Washington, Seattle, Washington; Veterans Affairs Puget Sound Health Care System, Seattle, Washington.
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Elucidating role of local secondary structure in modulating amyloid aggregation of intrinsically disordered proteins
Sofia Bali, Lukasz Joachimiak.
Presenter affiliation: UT Southwestern Medical Center, Dallas, Texas.
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Proteomic analyses of induced microglia uncover pathways relevant to Alzheimer's disease
Atoshi Banerjee, Juli Petereit, Jingchun Chen.
Presenter affiliation: University of Nevada, Las Vegas, Las Vegas, Nevada.
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The MEF2 network is a key regulator of cognitive function and confers resilience to neurodegeneration
Scarlett J. Barker, Ravikiran M. Raju, Jun Wang, Jose Davila-Velderrain, Fatima Gunter-Rahman, Fatema Abdurrob, Noah E. Milman, Karim Abdelaal, Manolis Kellis, Li-Huei Tsai.
Presenter affiliation: The Picower Institute for Learning and Memory, Cambridge, Massachusetts.
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A GNeo-GCase conjugate designed to target neurodegenerative Gaucher disease
Phillip L. Bartels, Lara E. Dozier, Gentry N. Patrick, Jeffrey D. Esko, Yitzhak Tor.
Presenter affiliation: UC San Diego, La Jolla, California.
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Brain transcriptome analyses of zebrafish early-onset familial Alzheimer's disease genetic models reveal effects-in-common on iron homeostasis, energy metabolism and protein synthesis in young adult brains
Karissa Barthelson, Morgan Newman, Nhi Hin, Yang Dong, Stephen M. Pederson, Michael Lardelli.
Presenter affiliation: Alzheimer's Disease Genetics Laboratory, The University of Adelaide, Adelaide, Australia.
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Characterizing the proteome of tau aggregation at its initiation
Sushobhna Batra, Marc I. Diamond.
Presenter affiliation: UT Southwestern Medical Center, Dallas, Texas; Graduate School of Biomedical Sciences, Dallas, Texas.
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Glymphatic function persists after partial *aqp4* mRNA knockdown
Roberta Battistella, Linda Decker, Hani Alsafadi, Berit Powers, Darcy Wagner, Frank Rigo, Iben Lundgaard.
Presenter affiliation: Lund University, Lund, Sweden; WCMM Wallenberg Centre for Molecular Medicine, Lund, Sweden.
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Synergistic toxicity between tau and amyloid drives neuronal dysfunction and neurodegeneration in transgenic *C. elegans*
Sarah J. Benbow, Timothy J. Strovas, Martin Darvas, Aleen Saxton, Brian C. Kraemer.
Presenter affiliation: University of Washington, Seattle, Washington; VAPSHCS, Seattle, Washington.
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FUS overexpression is linked to altered histone post-translational modifications in an amyotrophic lateral sclerosis yeast model
Seth A. Bennett, Mila Mirzakandova, George Angelakakis, Elizaveta Sun, Huda Yousuf, Arlet Olivera, Samantha N. Cobos, Mariana P. Torrente.
Presenter affiliation: The City University of New York Graduate Center, New York, New York; Brooklyn College, Brooklyn, New York.
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A model of traumatic brain injury using human iPSC-derived cortical brain organoids
Joshua Berlind, Jesse Lai, Gabriella Fricklas, Naomi Sta. Maria, Violeta Yu, Russell Jacobs, Justin Ichida.
Presenter affiliation: University of Southern California, Los Angeles, California.
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Pro-cognitive and morphological effects of specific GABA A receptor subunit modulation in a model of chronic stress
Ashley M. Bernardo, Philippe Lee, Michael Marcotte, Daniel E. Knutson, Dishary Sharmin, Md Y. Mian, James M. Cook, Etienne Sibille, Thomas D. Prevot.
Presenter affiliation: Centre for Addiction and Mental Health, Toronto, Canada.
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Biological sex modifies the impact of heat shock protein 70 in α-synucleinopathies
Tarun N. Bhatia, Patrick G. Needham, Kristin M. Miner, Elizabeth A. Eckhoff, Rachel N. Clark, Anuj S. Jamenis, Nevil Abraham, Xiaoming Hu, Jun Chen, Peter Wipf, Kelvin C. Luk, Jeffrey L. Brodsky, Rehana K. Leak.
Presenter affiliation: Duquesne University, Pittsburgh, Pennsylvania.
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Impaired cholesterol metabolism in glaucoma
Sanjoy K. Bhattacharya, Jennifer Arcuri, Noel Ziebarth, Vittorio Porciatti.
Presenter affiliation: University of Miami, Miami, Florida.
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Design and development of proteolysis targeting chimera against cytoplasmic TDP-43 in neurodegeneration
Krishna Bhavsar, Sharad Gupta.
Presenter affiliation: Indian Institute of Technology, Gandhinagar, Gandhinagar, India.
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Investigating the role of mitochondrial signaling in the maintenance of neuronal function and differentiation in the context of Parkinson's disease
Maria Bilen, Mohamed A. Iqbal, Jinwei Chen, Bensun C. Fong, Smitha Paul, Ruth S. Slack.
Presenter affiliation: University of Ottawa, Ottawa, Canada. 78

Linking molecular biomarkers and behavior in rodents and patients with Alzheimer's disease
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Presenter affiliation: The Norwegian University of Science of Technology, Trondheim, Norway. 79

Expanding the boundaries of human neurodegenerative disease models through the intersection of engineering, stem cell biology, and single-cell transcriptomics
Joel W. Blanchard, Li-Huei Tsai.
Presenter affiliation: Picower Institute for Learning and Memory, MIT, Cambridge, Massachusetts; Icahn School of Medicine at Mt. Sinai, New York, New York. 80

Unbiased epigenetic and phosphoproteomic profiling of *APOE4* NPCs and astrocytes suggests novel biology and new potential therapeutics
Julia M. Bonner, Karen Christianson, Joel Blanchard, James Mullahoo, Priyanaka Narayan, Sebastian Vaca, Deborah Dele-Oni, Michael Bula, Andrew Reiter, Tak Ko, Jennie Z. Young, Fatema Abdurrob, Agnese Graziosi, Noelle Leary, Malvina Papanastasiou, Steven A. Carr, Li-Huei Tsai. View Poster
Presenter affiliation: Massachusetts Institute of Technology, Cambridge, Massachusetts. 81

BMP5/7 protect dopaminergic neurons against α-synuclein induced neurotoxicity in a mouse model of Parkinson's disease
Zagorka Vitic, Hasem Safory, Vukasin M. Jovanovic, Simone Engelender, Claude Brodski. View Poster
Presenter affiliation: Ben-Gurion University, Beer Sheva, Israel. 82

The nuclear pore complex and nucleocytoplasmic transport—Structural and functional implications in Alzheimer's disease
Danielle L. Brokaw, Miriam Goras, Missy Tran, Jennifer Nolz, Elaine Delvaux, Diego Mastroeni, Paul Coleman. View Poster
Presenter affiliation: Arizona State University, Tempe, Arizona. 83

The endotoxin hypothesis of neurodegeneration—Roles of microglial phagocytosis
Guy C. Brown.
Presenter affiliation: University of Cambridge, Cambridge, United Kingdom. 84

Metabolomic associations with APOE-e4 in an ethnically diverse aging population
Vrinda Kalia, Meghan L. Bucher, Badri Vardarajan, Dean Jones, Richard Mayeux, Gary W. Miller. View Poster
Presenter affiliation: Mailman School of Public Health Columbia University, New York, New York. 85

Reduced function of C9orf72, leads to presynaptic defects and neuromuscular dysfunction in zebrafish View Poster
Zoe Butti, Jean Giacomotto, Kessen Patten.
Presenter affiliation: INRS - Centre Armand Frappier, Laval, Canada. 86

Manipulating PrP glycan structure to understand toxic signaling pathways driving prion-induced neurodegeneration
Julia A. Callender, Daniel B. McClatchy, Alejandro M. Sevillano, Timothy Kurt, Katrin Soldau, Jin Wang, Donald Pizzo, Annee Nguyen, May Madi Han, Helen Khuu, Henry Powell, Nigel Calcutt, John R. Yates, Christina J. Sigurdson. View Poster
Presenter affiliation: University of California San Diego, La Jolla, California. 87

The relationship between cognitive scores and detection of specific odours in the elderly
Gemma Camara, Majed Alotaibi, Melissa Lessard-Beaudoin, Kate Busch, Rona K. Graham.
Presenter affiliation: Bishop University, Sherbrooke, Canada; Research Centre on Aging CIUSSS de l'Estrie - CHUS, Sherbrooke, Canada; University of Sherbrooke, Sherbrooke, Canada. View Poster 88

mTOR-dependent translation amplifies microglia priming in ageing
Lily Keane, Ignazio Antignano, Sean-Patrick Richiers, Anaelle A. Dumas, Nina Offermann, Maria E. Bernis, Jenny Russ, Frederike Graelmann, Patrick N. McCormick, Julia Esser, Dario Tejera, Yvonne Biederbick, Annett Halle, Paolo Salomoni, Michael T. Heneka, Melania Capasso.
Presenter affiliation: German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany. 89

Nuclear receptor deletion in myeloid cells mitigates disease pathology in a mouse model of Alzheimer's disease
Brad T. Casali, Andy P. Tsai, Kathryn P. MacPherson, Erin G. Reed-Geaghan, Gary E. Landreth.
Presenter affiliation: Case Western Reserve University, Cleveland, Ohio; Stark Neuroscience Research Institute, Indianapolis, Indiana; Northeast Ohio Medical University, Rootstown, Ohio.
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Sex-specific differences in hippocampal blood flow and cerebrovascular reactivity in aged dahl salt-sensitive rats treated with an angiotensin converting enzyme (ACE) inhibitor lisinopril
Defne S. Cezayirli, Samuel O. Ajamu, Rachel C. Fenner, Jennifer Shearon, Yulia Grigorova, Mustapha Bouhrara, Edward G. Lakatta, Richard G. Spencer, Kenneth W. Fishbein, Olga V. Fedorova.
Presenter affiliation: National Institutes of Health, Baltimore, Maryland.
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Effect of Il-10 and its antagonism in mouse models of neurodegenerative diseases
Emily Koller, Samuel Cockey, Elsa Gonzalez De La Cruz, Mary Weinrich, Karen McFarland, David Borchelt, Todd Golde, Jada Lewis, Benoit Giasson, Paramita Chakrabarty.
Presenter affiliation: University of Florida, Gainesville, Florida; McKnight Brain Institute, Gainesville, Florida.
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Imaging the invaders in multiple sclerosis—PET imaging of CNS-infiltrating peripheral myeloid cells
Aisling M. Chaney, Haley Cropper, Edward Wilson, Poorva Jain, Michelle Swarovski, Marc Stevens, Hannes Vogel, Katrin Andreasson, Michelle James.
Presenter affiliation: Stanford University, Stanford, California.
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Significance of IL-1β in regulating monocytes/macrophages infiltrating the brain after tail amputation in zebrafish
Xiang-Ke Chen, Alvin Chun-Hang Ma, Raymond Chuen-Chung Chang.
Presenter affiliation: The University of Hong Kong, Pokfulam, China.
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An endogenous retrovirus drives the accumulation of TDP-43 proteinopathy and inter-cellular propagation of neurodegeneration in a *Drosophila* TDP-43 model
Yung-Heng Chang, Josh Dubnau.
Presenter affiliation: Stony Brook School of Medicine, New York, New York.
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Seed-competent tau monomer appears early in disease and its activity is independent of phosphorylation
Dailu Chen, Hilda Mirbaha, Vishruth Mullapudi, Lukasz Joachimiak, Marc Diamond.
Presenter affiliation: UT Southwestern Medical Center, Dallas, Texas.
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Investigation of epigenetic machinery genes in brain tissue of Alzheimer's disease
Tathyane C. Faria, Héctor L. Maldonado, Dalileia A. Santana, Carolina O. Gigek, Gustavo Turecki, Naguib Mechawar, Bernardo Lemos, Marilia A. Smith, Elizabeth S. Chen.
Presenter affiliation: Universidade Federal de Sao Paulo, Sao Paulo, Brazil; Harvard University, Cambridge, Massachusetts.
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Enhancing mitochondria integrity to restore neuronal function in Parkinson's disease
Jingwei Chen, Maria Bilen, Mohamed A. Iqbal, Smitha Paul, Ruth S. Slack.
Presenter affiliation: University of Ottawa, Ottawa, Canada.
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Probing and targeting motor neuron subtype differential vulnerability in ALS via miR-17~92
Jun-An Chen.
Presenter affiliation: Academia Sinica, Taipei, Taiwan.
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To develop ALS biosensors by tuning the low-complexity domain of TDP-43
Wen-Chen Wang, Yu-Shian Huang, Yu-Ping Meng, Ying-Chou Chen.
Presenter affiliation: National Yang-Ming University, Taipei, Taiwan.
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TDP-43 interacts with amyloid-β, inhibits fibrillization, and worsens Alzheimer's disease pathology
Yun-Ru Chen.
Presenter affiliation: Academia Sinica, Taipei, Taiwan.
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Prion infection is associated with reduced Rab7 activation and impaired vesicular trafficking
Pearl Cherry, Su Y. Shim, Govinda Sharma, Sabine Gilch, Li Lu.
Presenter affiliation: University of Calgary, Calgray, Canada.
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Studying brain tumor invasion with cerebral organoids from human pluripotent stem cells
Vorapin Chinchalongporn, Anjali Balakrishnan, Lakshmy Vasan, Oleksandr Prokopchuk, Dawn Zinyk, Rehnuma Islam, Cindi M. Morshead, Jennifer A. Chan, Carol Schuurmans.
Presenter affiliation: Sunnybrook Research Institute, Toronto, Canada.
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Microglial depletion reduces adeno-associated virus mediated tau propagation from the entorhinal cortex to the dentate granular cells in human APP^{NL-G-F} knock-in mice while increasing amyloid burden
Kevin A. Clayton, Jean-Christophe Delpech, Shawn Herron, Naotoshi Iwahara, Seiko Ikezu, Tsuneya Ikezu.
Presenter affiliation: Boston University, Boston, Massachusetts.
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Alzheimer's disease—Histone post-translational modification among amyloid-beta isoforms
Samantha N. Cobos, Muna Hugais, Jailene Paredes, Genevieve Foran, Mariana P. Torrente.
Presenter affiliation: The Graduate Center of the City University of New York, New York, New York; Brooklyn College, Brooklyn, New York.
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Sex- and region-dependent microglial morphology response to Alzheimer's disease progression
Gloria Colombo, Alessandro Venturino, Rouven Schulz, Ryan J. Cubero, Lida Kanari, Kathryn Hess, Sandra Siegert.
Presenter affiliation: IST Austria, Klosterneuburg, Austria.
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Single-cell epigenomic analyses implicate candidate causal variants at inherited risk loci for Alzheimer's and Parkinson's diseases
M. Ryan Corces, Anna Shcherbina, Soumya Kundu, Michael J. Gloudemans, Laure Fresard, Jeffrey M. Granja, Bryan H. Louie, Tiffany Eulalio, Shadi Shams, Sevgi T. Bagdatli, Maxwell R. Mumbach, Boxiang Liu, Kathleen S. Montine, William J. Greenleaf, Anshul Kundaje, Stephen B. Montgomery, Howard Y. Chang, Thomas J. Montine.
Presenter affiliation: Gladstone Institutes, San Francsico, California; Stanford University School of Medicine, Stanford, California.
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Small molecule Shc blockers ameliorate A-beta toxicity and ApoE4-dependent mitochondrial deficits and insulin resistance
Gino A. Cortopassi, Alexey A. Tomilov.
Presenter affiliation: Buto Corp, Davis, California.
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Multi-tissue proteomic signatures of genetically-defined Alzheimer disease cases—A window into precision medicine
Yun Ju Sung, Chengran Yang, Bruno Benitez, Oscar Harari, Carlos Cruchaga.
Presenter affiliation: Washington University School of Medicine, St. Louis, Missouri.
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Humanised and physiological mouse models of ALS
Thomas J. Cunningham, Remya R. Nair, David Thompson, Georgia Price, Charlotte Tibbit, Ross McLeod, Zeinab Ali, Samanta Gasco, Rosie Bunton-Stasyshyn, Anny Devoy, Francesca De Giorgio, Alasdair Allan, Gemma Codner, Lydia Teboul, Pietro Fratta, Abraham Acevedo-Arozena, Adrian M. Isaacs, Elizabeth M C. Fisher.
Presenter affiliation: MRC Harwell Institute, Oxfordshire, United Kingdom.
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Mitochondrial dysfunction in the brain alters the homeostasis of a newly identified population of mitochondria-derived extracellular vesicles
Pasquale D'Acunzo, Rocío Pérez-González, Yohan Kim, Melissa J. Alldred, Stephen D. Ginsberg, Chris N. Goulbourne, Efrat Levy.
Presenter affiliation: Nathan S. Kline Institute for Psychiatric Research, Orangeburg, New York; New York University School of Medicine, New York, New York.
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A Pur-based peptide of rational design binds and alters G-quadruplex secondary structure present in the expanded RNA repeat of *C9orf72* ALS/FTD
Margaret J. Wortman, Andrea M. Clark, Frank A. Lattanzio, Earl W. Godfrey, Steven M. Pascal, Edward M. Johnson, Dianne C. Daniel.
Presenter affiliation: Eastern Virginia Medical School, Norfolk, Virginia. 112

Modeling hereditary cerebral amyloid angiopathy with cerebral organoids
Elena Daoutsali, Stavros Stamatakis, Barry Pepers, Willeke van Roon-Mom.
Presenter affiliation: Leiden University Medical Center, Leiden, Netherlands.
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Optic nerve alterations in a rat glaucoma model of subacute ocular hypertension
Mariana Dias, Rafael Lani, Vinicius Ribas, Rafael Linden, Hilda Petrs-Silva.
Presenter affiliation: Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.
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Plasma markers of biological age suggest accelerated proteomic aging in Alzheimer's disease and ALS
Maria E. Diaz-Ortiz, Alice S. Chen-Plotkin.
Presenter affiliation: Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania; School of Engineering and Applied Sciences, University of Pennsylvania, Philadelphia, Pennsylvania.
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Gaining insights into the molecular mechanisms underlying X-linked Dystonia-Parkinsonism (XDP) with female carrier iPSC-derived striatal organoids
Laura D'Ignazio, Tomoyo Sawada, Kynon J. Benjamin, Ricardo Jacomini, Bareera Qamar, Kenneth Diffenderfer, William Hendriks, Cristopher Bragg, Apua Paquola, Jennifer A. Erwin.
Presenter affiliation: Lieber Institute for Brain Development, Baltimore, Maryland; Johns Hopkins School of Medicine, Baltimore, Maryland.
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CASY-1/Calsyntenin protects against axon degeneration caused by loss of mitochondria
Chen Ding, Agnes Roczniak-Ferguson, Youjun Wu, Lila Lyons, Shawn Ferguson, Marc Hammarlund.
Presenter affiliation: Yale University School of Medicine, New Haven, Connecticut.
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Transcriptome analyses of 7-day-old zebrafish larvae possessing a familial Alzheimer's disease-like mutation in *psen1* indicate effects on oxidative phosphorylation, ECM and mcm functions, and iron homeostasis
Yang Dong, Morgan Newman, Stephen M. Pederson, Nhi Hin, Michael Lardelli.
Presenter affiliation: Alzheimer's Disease Genetics Laboratory, Adelaide, Australia.
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Trans-synaptic transmission of mutant huntingtin aggregates is mediated by Draper-dependent phagocytosis in *Drosophila* brains
Kirby M. Donnelly, Aprem D. Zaya, Gabrielle E. Pisano, Margaret M. Panning Pearce.
Presenter affiliation: University of the Sciences, Philadelphia, Pennsylvania.
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Genome-wide detection of expression quantitative trait loci suggests regulation of T cell genes by tau variants in Alzheimer's disease patients
Dallin Dressman, Vilas Menon, Yiyi Ma, Maria Cimpean, Thomas Buttrick, Philip de Jager, David Bennett, Elizabeth M. Bradshaw, Badri Vardarajan, Wassim Elyaman.
Presenter affiliation: Columbia University Irving Medical Center, New York, New York.
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Identifying key regulatory mechanisms of TDP-43 nuclear export using a permeabilized cell assay
Lauren Duan, Petr Kalab, Jeffrey D. Rothstein, Lindsey R. Hayes.
Presenter affiliation: Johns Hopkins University, Baltimore, Maryland.
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AAV-mediated genome editing ameliorates amyloid-associated pathologies in familial Alzheimer's disease mouse models
Yangyang Duan, Tao Ye, Zhe Qu, Yuewen Chen, Abigail Miranda, Kit Cheung, Xiaopu Zhou, Yu Chen, Amy K.Y. Fu, Viviana Gradinaru, Nancy Ip.
Presenter affiliation: The Hong Kong University of Science and Technology, Kowloon, Hong Kong; Hong Kong Center for Neurodegenerative Diseases, Hong Kong.
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Lysosome-mediated structural and functional changes in neuronal nucleus in pathophysiology of Alzheimer's disease—A potential treatable target
Md Imamul Islam, Pandian Nagakannan, Tetania Shcholok, Fabio Contu, Sabina Mai, Benedict C. Albensi, Marc R. Del Bigio, Il-Seon Park, Eftekhar Eftekharpour.
Presenter affiliation: University of Manitoba, Winnipeg, Canada.
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Sequencing the previously unsequencable using amplification-free targeted enrichment powered by the CRSIPR-Cas9 system
Jenny M. Ekhom, Yu-Chih Tsai, Ian McLaughlin, Ting Hon, Janet Ziegle.
Presenter affiliation: Pacific Biosciences, Menlo Park, California.
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The pathological aggregation and functionality of *in vitro* modifications within TDP-43
Josephine C. Esposto, Sanela Martic.
Presenter affiliation: Trent University, Peterborough, Canada.
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Presenter affiliation: University of Sassari, Sassari, Italy.
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Presenter affiliation: University of Rhode Island, Kingston, Rhode Island.
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Presenter affiliation: FUJIFILM Cellular Dynamics, Inc., Madison, Wisconsin.
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Presenter affiliation: Washington University, St Louis, Missouri.
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Presenter affiliation: University of Sao Paulo, Sao Paulo, Brazil.
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Presenter affiliation: Washington University School of Medicine, St Louis, Missouri.
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Presenter affiliation: Pennsylvania State College of Medicine, Hershey, Pennsylvania; Pennsylvania State University, University Park, Pennsylvania.
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Presenter affiliation: The New York Stem Cell Foundation Research Institute, New York, New York.
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Presenter affiliation: UC Irvine, Irvine, California.
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Presenter affiliation: Institute of Molecular Genetics "Luigi Luca Cavalli Sforza", Pavia, Italy; FIRC Institute of Molecular Oncology, Milan, Italy.
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Presenter affiliation: Tel-Aviv University, Tel Aviv, Israel; Tel-Aviv University, Tel Aviv, Israel.
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Presenter affiliation: Indian Institute of Technology Gandhinagar, Gandhinagar, India.
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Presenter affiliation: The University of Texas Health Science Center at Houston, Houston, Texas; Universidad de Malaga, Malaga, Spain.
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Presenter affiliation: UC Davis School of Veterinary Medicine, Davis, California.
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Presenter affiliation: University of Malaga/IBIMA/CIBERNED, Málaga, Spain.
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Presenter affiliation: Nathan Kline Institute, Orangeburg, New York; NYU School of Medicine, New York, New York.
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Presenter affiliation: Alzheimer's Disease Genetics Laboratory, Adelaide, Australia.
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Presenter affiliation: University of Wuerzburg, Wuerzburg, Germany.
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Presenter affiliation: The Biodesign Institute, Tempe, Arizona.
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Presenter affiliation: Harvard University, Cambridge, Massachusetts; Vrije Universiteit Amsterdam, Amsterdam, Netherlands; Broad Institute of MIT and Harvard, Cambridge, Massachusetts.
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Presenter affiliation: Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, India.
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Presenter affiliation: Ruder Boskovic Institute, Zagreb, Croatia.
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Presenter affiliation: CIUSSS-NIM, Montréal, Canada; Université de Montréal, Montréal, Canada.
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Presenter affiliation: David Geffen School of Medicine at UCLA, Los Angeles, California.
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Presenter affiliation: Van Andel Institute, Grand Rapids, Michigan.
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Presenter affiliation: University of Toledo College of Medicine and Life Sciences, Toledo, Ohio.
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Alan M. Henrique, Merari de Fátima R. Ferrari.
Presenter affiliation: Institute of Biosciences, University of Sao Paulo, Sao Paulo, Brazil.
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Presenter affiliation: Indian Institute of Technology Gandhinagar, Gandhinagar, India.
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Presenter affiliation: University of Western Ontario, London, Canada; Robarts Research Institute, London, Canada.
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Presenter affiliation: German Center for Neurodegenerative Diseases, Bonn, Germany.
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Presenter affiliation: University of Pittsburgh, Pittsburgh, Pennsylvania.
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Presenter affiliation: University of California, Riverside, Riverside, California.
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Presenter affiliation: University of Southern California, Los Angeles, California.
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Presenter affiliation: University of Ottawa, Ottawa, Canada; University of Ottawa, Ottawa, Canada.
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Presenter affiliation: Washington University School of Medicine, St. Louis, Missouri.
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Presenter affiliation: Aarhus University, Aarhus C, Denmark.
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Presenter affiliation: University Hospital Wuerzburg, Wuerzburg, Germany.
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Presenter affiliation: Cornell University, Ithaca, New York.
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Presenter affiliation: Sanford Burnham Prebys Medical Discovery Institute, La Jolla, California.
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Presenter affiliation: Wayne State University, Detroit, Michigan.
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Presenter affiliation: Centre for Brain Research, Bangalore, India.
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Presenter affiliation: Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts.
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Presenter affiliation: Texas Woman's University, Denton, Texas.
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Presenter affiliation: University of Florida, Gainesville, Florida.
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Presenter affiliation: LUMC, Leiden, the Netherlands.
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Presenter affiliation: University of Massachusetts Medical School, Worcester, Massachusetts.
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Presenter affiliation: Royal Veterinary College, London, United Kingdom.
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Presenter affiliation: Massachusetts General Hospital, Boston, Massachusetts.
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Presenter affiliation: Harvard University, Cambridge, Massachusetts.
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Presenter affiliation: Temasek Life Sciences Laboratory, Singapore; National University of Singapore, Singapore.
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Presenter affiliation: BITS Pilani Hyderabad Campus, Hyderabad, India.
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Presenter affiliation: CWRU, Cleveland, Ohio.
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Presenter affiliation: Alector Inc., South San Francisco, California.
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Presenter affiliation: Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts.
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Presenter affiliation: West Virginia University School of Medicine, Morgantown, West Virginia.
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Presenter affiliation: Disarm Therapeutics, Cambridge, Massachusetts.
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Presenter affiliation: The State University of New York at Buffalo, Buffalo, New York.
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Presenter affiliation: Leiden University Medical Center, Leiden, Netherlands.
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Presenter affiliation: Sanofi, Framingham, Massachusetts.
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Presenter affiliation: Brigham and Women's Hospital, Boston, Massachusetts.
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Presenter affiliation: The Hong Kong University of Science and Technology, Hong Kong, China; Hong Kong Center for Neurodegenerative Diseases, Hong Kong, China.
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Presenter affiliation: University of Colorado Boulder, Boulder, Colorado; University of Colorado Anschutz Medical Campus, Aurora, Colorado.
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Presenter affiliation: Universitat de Barcelona, Barcelona, Spain; CIBERNED, Barcelona, Spain.
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Alejandro Lillo, Jaume Lillo, Iu Raïch, Gemma Navarro, Rafael Franco.
Presenter affiliation: University of Barcelona, School of Pharmacy, Barcelona, Spain.
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Presenter affiliation: Harvard University, Cambridge, Massachusetts; Broad Institute of MIT and Harvard, Cambridge, Massachusetts.
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Presenter affiliation: University of Southern California, Los Angeles, California.
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Presenter affiliation: IBMC - Instituto de Biologia Molecular e Celular/i3S - Instituto de Investigação e Inovação e Saúde, Porto, Portugal.
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Presenter affiliation: German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany.
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Presenter affiliation: Barrow Neurological Institute, Phoenix, Arizona.
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Presenter affiliation: Institute of Clinical Neurobiology, Wuerzburg, Germany.
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Presenter affiliation: University of South Florida, Tampa, Florida; University of Kentucky, Lexington, Kentucky.
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Presenter affiliation: Gladstone Institutes, San Francisco, California.
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Joana Magalhaes, Jessica Eira, Nídia Macedo, Sung-Tsang Hsieh, Márcia Liz.
Presenter affiliation: Instituto de Biologia Celular e Molecular and Instituto de Investigação e Inovação em Saúde, Porto, Portugal.
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Presenter affiliation: Washington University in St Louis, St Louis, Missouri.
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Presenter affiliation: University of California at San Diego, La Jolla, California.
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Presenter affiliation: Research Centre on Aging CIUSSS de l'Estrie – CHUS, Quebec, Canada; Université de Sherbrooke, Quebec, Canada.
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Presenter affiliation: 2N Pharma, Aalborg, Denmark.
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Presenter affiliation: University of Illinois College of Medicine Rockford, Rockford, Illinois; University of Illinois at Chicago, Chicago, Illinois.
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Presenter affiliation: Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, Massachusetts.
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GENOMIC INVESTIGATION OF THE HUMAN BRAIN TRANSCRIPTOME, CSF AND PLASMA PROTEOME AND BIOMARKERS OF DISEASE PROGRESSION GUIDES TARGET VALIDATION IN NEURODEGENERATIVE DISEASES

Sally John

Biogen, Cambridge, MA

It is now well established that drugs against targets supported by high confidence human genetic data are more likely to be approved as medicines across a broad range of disease areas. There has been considerable success in identifying both common and rare variants that influence disease susceptibility for neurodegenerative disease. Quantitative data that links DNA variants to disease relevant intermediate phenotypes is often needed to enable the translation to a therapeutic hypothesis.

However, to date, the majority of such studies on protein and gene expression have focused on non-brain tissues and peripheral circulating proteins. To address this issue, we have focused on the brain transcriptome, CSF proteins and longitudinal clinical phenotypes from cohort studies and clinical trial data as a more relevant source of intermediate phenotypes. We generated a large database of expression quantitative trait loci (eQTLs) specific to brain tissue and show that we identify more colocalized eQTLs with neurodegenerative disease than we observe when using blood eQTLs. We further demonstrate that performing GWAS on the CSF protein levels and clinical or radiological changes in disease progression can establish confidence that therapeutic modulation of the gene will influence disease progression and changes in relevant biomarkers.

These studies highlight the importance of access to disease relevant phenotypes and biomarkers to enable genetics research within the neurodegenerative disease and facilitate target validation and early drug discovery and development.

VACUOLAR TAUOPATHY IS ASSOCIATED WITH A HYPOMORPH VCP MUTATION

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Rare genetic causes of human disease have the potential to reveal mechanistic insights into sporadic disease. Identifying novel genetic forms of tauopathy may help our understanding of the pathophysiologic mechanisms that lead to neurofibrillary degeneration. We have identified two unrelated kindred with an autosomal dominant inheritance pattern of frontotemporal dementia associiated with a novel p.Asp395Gly VCP mutation. Neuropathologic and radiologic studies indicated that frontotemporal atrophy coincided with the accumulation of tau in the form of neurofibrillary tangles. Bioinformatic and recombinant protein biochemistry studies revealed p.Asp395Gly to be a hypomorph mutation, in contrast with hypermorphic VCP mutations which have been associated with Multisystem Proteinopathy, a disease with diverse clinical phenotypes that sometimes results in a TDP-43 proteinopathy. Mechanistically, recombinant VCP appeared to exhibit ATP- and polyubiquitin-dependent disaggregase activity against pathologic tau. Moreover, mutant VCP expression in cells and mice was associated with enhanced tau accumulation. Thus, vacuolar tauopathy appears to be a novel autosomal dominant tauopathy associated with a hypomorph VCP mutation which impairs disaggregase activity. Different mutations of the same gene (VCP) can lead to different underlying neuropathologies (tau versus TDP-43) but the same clinical presentation (FTD), a remarkable instance of allelic heterogeneity. These results suggest that VCP may be a novel target for the treatment of tauopathies.

ATLAS OF GENETIC REGULATORY EFFECTS ON HUMAN MICROGLIA TRANSCRIPTOME

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Genetic studies have strongly implicated microglial dysfunction in multiple neurodegenerative diseases. However, investigating genetically-driven changes in gene expression in microglia has been limited by lack of access to these cells from the number of subjects required to perform well-powered genomic analysis. Here we describe the transcriptome analysis of 255 primary human CD11b+ microglia samples isolated at autopsy from multiple brain regions of 114 human subjects with brain disorders. We performed systematic analyses to investigate various aspects of microglial heterogeneities including brain region, age, sex, and disease. By intersecting transcriptomics and genetics, we performed expression and splicing QTL (sQTL) analyses and by combining microglia from four different regions using a multivariate meta-analysis. We observed widespread transcriptome variation associated with microglia from different regions, suggesting that these genes may play important role in diversified responses to pathological stimuli of microglia at different locations. We also observed 2,174 genes (FDR<0.01) whose expression is associated with chronological age. We identified 3,611 eQTLs and 4,614 sQTLs (false sign rate < 0.05), of which 50% (1,791) show region-specific effects. We used full-length long-read Iso-seq (PacBio) to validate the disease-specific sQTLs altering novel microglia-specific isoforms (CD33, MS4A6A). We find that ~25-30% of AD and PD disease heritability is mediated by the cis-genetic component of microglial gene expression. We identified over 300 eQTLs that colocalize with a known risk locus for neurodegenerative or neuropsychiatric disease, nearly half of which are not found in prefrontal cortex or in peripheral monocytes. We prioritized 7 and 13 putative causal genes for AD and PD, respectively, many of which are novel genes (ITGAX, USP6NL, TSPOAP1, P2RY12, FCGR2C, and FGF20). Fine-mapping of these colocalized loci with CNS chromatin accessibility (ATAC-seq) and histone modification (H3K27ac) data nominates candidate causal variants that are within microglia-specific enhancers and are likely to modify disease susceptibility by regulating gene expression and/or splicing in microglia. In summary, we have built the most comprehensive catalog to date of genetic effects on the microglia transcriptome and propose molecular mechanisms of action of candidate functional variants in several neurological and neuropsychiatric diseases.

SINGLE CELL WHOLE GENOME SEQUENCING REVEALS INCREASED SOMATIC MUTATIONS IN ALZHEIMER'S DISEASE NEURONS

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Background: Alzheimer's disease (AD) is characterized pathologically by neuronal loss and the deposition of misfolded proteins β-amyloid and tau. Current pathogenesis paradigms based on β-amyloid and tau have led to important mechanistic and diagnostic advances in AD, but resulting therapeutic strategies have not shown clinical success. It is therefore important to examine pathogenesis from a broader lens.
Recent studies have found that neurons each harbor somatic single nucleotide variants (sSNV) in their genomes. The number of neuronal somatic mutations increases with age, at a rate of ~25 sSNV per year, a phenomenon known as genosenium. In AD, DNA damage is increased, leading to the question of whether these processes increase the somatic mutation burden.

Method: To test the role of somatic mutations in AD, we performed single cell whole genome sequencing on neurons isolated from postmortem brain from AD and age-matched controls. We performed mutational signature analysis of the nucleotide changes to assess for mutagenic patterns.

Result: We found significantly increased sSNV in AD, both in prefrontal cortex and hippocampus, with >800 additional somatic mutations per AD neuron, with a distinct mutational pattern. In contrast to normal aging, where clock-related Signature A accumulates, AD neurons show an increase in Signature C, which contains distinct nucleotide changes including C>A variants. Analysis of mutation genomic sites and transcription strand bias reveals increased sSNV in transcribed DNA strands, indicating a mechanistic role for transcription in sSNV generation. These somatic mutations stand to produce multiple deleterious effects on the neuron, including gene inactivation and the generation of neoantigens that may stimulate immune attack.

Conclusion: We found increased somatic mutations in AD, with multiple mutagenic causes that illuminate upstream components of disease pathogenesis including DNA oxidation and transcription-coupled DNA repair. Furthermore, somatic mutation levels appear to produce a toxic cellular state, positioning neurons for dysfunction and death. Somatic mutation accumulation is a novel process in neurodegeneration, through which we can dissect the cascade of events in disease pathogenesis.

A WHOLE-KINOME KNOCKOUT SCREEN IN POST-MITOTIC NEURONS TO ELUCIDATE THE KINASE NETWORKS UNDERLYING ER STRESS-INDUCED NEURODEGENERATION

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Endoplasmic reticulum (ER) stress is a common feature of neurodegenerative disorders that are characterized by the accumulation of misfolded proteins, including amyotrophic lateral sclerosis (ALS), Alzheimer's disease, and Parkinson's disease. If left unchecked, ER stress can ultimately trigger widespread neuronal death. We have previously shown that motor neurons, the primary cell type affected in ALS, are more sensitive to ER stress-inducing agents than other neuronal subtypes in the spinal cord, and that the expression of ALS-causing mutations further heightens their vulnerability. By performing pharmacological screens in mouse and human stem cell-derived motor neurons, we found that compounds targeting the mitogen-activated protein kinase kinase kinase kinase (MAP4K) family prevent ER stress-induced neurodegeneration. Knocking out MAP4Ks individually provided little neuroprotection, but in combination had additive protective effects, indicating that MAP4Ks act redundantly in the context of ER stress. Furthermore, the combinatorial knockout of MAP4Ks did not fully recapitulate the neuroprotective effects of pharmacological MAP4K inhibitors, suggesting that these compounds may have additional kinase targets that boost their efficacy. To identify these targets, we have developed a CRISPR-based whole-kinome knockout screen in post-mitotic motor neurons. The screen identified additional kinases beyond the MAP signaling pathway that contribute to ER stress-mediated motor neuron degeneration.

SINGLE-CELL DISSECTION OF APOE4 EFFECTS ON ALZHEIMER'S DISEASE

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Apolipoprotein E4 (APOE4) is the strongest known genetic risk variant for sporadic Alzheimer's disease (AD), however, we lack a comprehensive understanding of cell-type-specific APOE4 effects in the human brain in the presence and absence of AD pathology. Here, we investigate the cell-type-specific transcriptomic signature of APOE4 by 10x single-cell-RNA sequencing of the human prefrontal cortex. We analyze ~ 165,000 cells from a sex-balanced cohort of 32 individuals, comprising APOE3 and E4 carriers (12 E3/E3, 12 E3/E4, and 8 E4/E4). We confirm that APOE expression is highest in human microglia and astrocytes and find cell-type-specific signatures of APOE-associated receptors, lipid transporters, and apolipoproteins. Together, these observations suggest that cell-type-specific APOE effects in non-APOE-expressing cell types, including excitatory and inhibitory neuronal subtypes, oligodendrocytes, and oligodendrocyte precursor cells, are mediated by a diverse set of specific ligand-receptor interactions. This suggests a cell-type specific response to secreted APOE from microglia and astrocytes. Importantly, we find that a subset of these receptors and ligands show cell-type-specific changes in expression with AD pathology and that these alterations are modified by APOE genotype. Next, we ask to what extent APOE genotype mediates differential responses to AD pathology. To this end, we curate a set of 16 biological processes, which have previously been associated with AD. In line with previous findings, we observe that these pathways show cell-type-specific activity. We identify multiple processes that are perturbed in AD pathology, exclusively in the context of APOE4. These include glucose metabolism, perturbed in all excitatory neuronal subtypes and in PV-Basket-, RELN+-, and VIP- interneurons, insulin signaling, perturbed in layer 2/3-, 4/5-, and 5/6-excitatory neurons, and inflammatory signaling, perturbed in oligodendrocytes, among other biological process perturbations. In addition, we find pathways that are uniquely altered in APOE3 cells, as well as pathways perturbed in AD pathology in both APOE genotypes. These results suggest that APOE genotype modifies the cell-type-specific response to AD pathology. Our data indicate that this may occur as a function of cell-type- specific receptor-ligand profiles and baseline pathway specificity. Collectively, our study provides a systematic comparison between the transcriptomic landscape of APOE3 and E4 carriers, considering their interaction with AD pathology at single cell resolution, and pinpoints genes and molecular pathways whose collective function may contribute to the multicellular effect of APOE on AD risk.

NEGATIVE FEEDBACK CONTROL OF NEURONAL ACTIVITY BY MICROGLIA

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Differentiated neurons exist as highly specialized neuronal subtypes with distinct activation states and functions. Neuronal fitness is surveyed by microglia, the innate immune cells in the brain, which eliminate non-functional cells and/or synapses. Given the multitude of neuronal states, the notion of functionality is likely to differ between different neurons and hence requires microglia adaption to distinct neuronal states. We found that microglia adapt to the neighboring neurons and that this adaptation relies on epigenetic mechanisms. Our findings show that microglia brain region-specific transcriptional and functional adaptation enables co-regulation of microglia and neurons by common triggers including specific neuromodulators. Accordingly, changes in phenotypes of either neurons or microglia can prompt a neuron-microglia mismatch. We will discuss how neuronal specification is communicated to neighboring microglia and how the mismatch between neurons and microglia can prompt microglia activation and/or neurotoxicity. Our data further show that regulation of neuronal circuits is not the exclusive prerogative of neurons but is controlled by microglia. We found that microglia can sense changes in local neuronal activity and identified a neuronal activity-induced, microglia-mediated feedback mechanism that suppresses local neuronal responses. Our findings suggest that this novel microglia-driven negative feedback mechanism plays an important role in protecting the brain from excessive neuronal activity and controls animal behaviors in health and diseases.

MICROGLIAL HANDLING OF NEUROTOXIC PROTEIN AGGREGATES

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The accumulation of neurotoxic protein aggregates along with neuronal loss are key pathological hallmarks of Alzheimer's disease and other neurodegenerative diseases. The brain has been considered as an immune-privileged organ, however, increasing evidence from translational, genetic, and pathological studies suggests that activation of microglia and their capacity to degrade and remove these protein aggregates represent an important mechanism by which innate immune pathways contributes to either brain protection or disease progression.

Microglia are activated by binding of aggregated proteins to pattern recognition receptors subsequently leading to two major immunological reactions: Immune activation and release of inflammatory mediators, but also phagocytic debris clearance. Data will be shown that point to a NLRP3-dependent phagocytic block of phagocytosis in microglia that underwent proliferation. Proliferating microglia showed upregulation of DDX3X, a co-activator of the NLRP3 inflammasome. Inhibition of DDX3X completely blocked NLRP3 inflammasome dependent generation and secretion of interleukin-1 beta and restored phagocytic clearance of beta-amyloid aggregates, thereby representing a novel therapeutic target.

SINGLE-CELL TRANSCRIPTOMICS AND EPIGENOMICS REVEAL DISEASE-SPECIFIC STATES OF OLIGODENDROGLIA IN MULTIPLE SCLEROSIS

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Oligodendrocytes are glial cells that mediate myelination of neurons, a process that allows efficient electrical impulse transmission in the central nervous system (CNS). An autoimmune response against myelin triggers demyelination in multiple sclerosis (MS). Oligodendrocyte precursor cells (OPCs) can initially differentiate and promote remyelination in MS, but this process eventually fails in progressive MS. In order to clearly define transcriptional of oligodendrocyte lineage cells in multiple sclerosis, we performed single-cell RNA-Seq in the experimental autoimmune encephalomyelitis (EAE) mouse model of MS and in different kind of CNS lesions from MS patients. We identified disease-specific of oligodendrocytes and OPC populations in EAE, and altered heterogeneity of the oligodendrocyte lineage in MS patients. One of the populations expressed genes involved in antigen processing and presentation and immunoprotection, and presented immunomodulatory properties. We also performed single-cell ATAC-Seq in oligodendroglia from the EAE mouse model of MS and observed transitions in chromatin accessibility correlating with the single-cell transcriptomics data. Thus, our single cell transcriptomics and epigenomics analysis unveiled a transcriptional overhaul during chronic inflammatory demyelination in multiple sclerosis.

METABOLIC REPROGRAMMING OF MYELOID CELLS REVERSES COGNITIVE DECLINE IN AGING

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Aging is characterized by the development of persistent pro-inflammatory responses that promote diseases like atherosclerosis, metabolic syndrome, cancer, and frailty. The aging brain is vulnerable to inflammation, as demonstrated by the high prevalence of age-associated cognitive decline and Alzheimer's dementia. Systemically, circulating pro-inflammatory factors can promote cognitive decline and in brain, microglia lose the ability to maintain immune homeostasis and clear misfolded proteins that are associated with neurodegeneration. However, the underlying mechanisms that initiate and sustain maladaptive inflammation with aging are not well defined.

Here we show that in aging mice, myeloid cell bioenergetics are suppressed in response to increased signaling by the lipid messenger prostaglandin E2 (PGE_2), a major modulator of inflammation. In aging macrophages and microglia, PGE_2 signaling through its EP2 receptor promotes the sequestration of glucose into glycogen, reducing glucose flux and mitochondrial respiration. This energy deficient state shifts myeloid polarization state and immune responses towards a maladaptive pro-inflammatory phenotype and is further aggravated by dependence of aged myeloid cells on glucose as a principal fuel source. In aged mice, inhibition of myeloid EP2 signaling restores youthful energy metabolism in peripheral macrophages and microglia, rejuvenates systemic and brain inflammatory states, and prevents loss of hippocampal synaptic plasticity and spatial memory. Moreover, blockade of peripheral myeloid EP2 signaling is sufficient to restore cognition in aged mice. Our study suggests that cognitive aging is not a static or irrevocable condition but can be reversed by reprogramming myeloid glucose metabolism and restoring youthful immune function.

MECHANISMS MEDIATING *CSF1R*-RELATED LEUKOENCEPHALOPATHY

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Adult onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) is a dementia resulting from dominantly-inherited CSF1R inactivating mutations. Similar to ALSP patients, Csf1r+/- mice exhibit behavioral and histopathologic alterations associated with microgliosis and demyelination. Csf1r is mainly expressed in microglia, but also in cortical layer V neurons that are gradually lost in Csf1r+/- mice. Microglial but not neural Csf1r heterozygosity was sufficient to cause the behavioral deficits, pathologies and elevation of Csf2 expression previously described in the Csf1r+/- mice. These data confirm that ALSP is a primary microgliopathy and support therapeutic approaches that aim to replace mutant with wild type microglia or to limit microglial activation. For the latter approach, prompted by observations that CSF2 expression is elevated both in presymptomatic ALSP mice and in the brains of ALSP patients, we have tested the therapeutic potential of targeting Csf2. Csf2 heterozygosity rescued most behavioral deficits and histopathological changes in Csf1r+/- mice by preventing cerebral microgliosis and attenuating transcriptomic changes in microglia. Monoallelic deletion of Csf2 also reduced oxidative stress and the expression of Cst7, a marker of demyelinating microglia. Elevated expression of several CSF-2 downstream target genes in the brains of ALSP patients suggested that similar mechanisms are functional in man. Csf1r heterozygosity in microglia triggered the expansion astrocytes and oligodendrocytes which are of the primary sources of CSF-2. These data establish an important role for CSF-2 in ALSP.
The expression of CSF3 was also elevated both in ALSP mice and in the brains of ALSP patients. Csf3 heterozygosity in ALSP mice failed to attenuate the behavioral phenotypes, callosal demyelination and cortical neurodegeneration but improved motor coordination and reduced of cerebellar microgliosis. These data suggest that in ALSP, CSF-2 and CSF-3 contribute to the expansion and activation of different populations of microglia that mediate forebrain demyelination and dysregulation of cerebellar homeostasis, respectively and inform future therapeutic and experimental approaches.

REACTIVE OR TRANSGENIC INCREASE IN MICROGLIAL TYROBP REVEALS A TREM2-INDEPENDENT TYROBP-APOE LINK IN WILD-TYPE AND ALZHEIMER'S-RELATED MICE

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Microglial TYROBP (also known as DAP12) has been identified by computational transcriptomics as a network hub and driver in late-onset sporadic Alzheimer's disease (AD) and as an important regulator of the microglial environmental sensing function. TYROBP is the transmembrane adaptor of AD-related receptors TREM2 and CR3, but importantly, TYROBP interacts with many other receptors, and little is known about its roles in microglial action and/or in the pathogenesis of AD. Herein, using dual RNA *in situ* hybridization and immunohistochemistry, we demonstrate that endogenous *Tyrobp* transcription is increased specifically in recruited microglia in the brains of wild-type and AD-related mouse models. To determine whether chronically elevated TYROBP might modify microglial phenotype and/or progression of AD pathogenesis, we generated a novel transgenic mouse overexpressing TYROBP in microglia. TYROBP-overexpressing mice were crossed with either *APP/PSEN1* or $MAPT^{P301S}$ mice, resulting in a decrease of the amyloid burden in the former and an increase of TAU phosphorylation in the latter. Apolipoprotein E (*Apoe*) transcription was upregulated in $MAPT^{P301S}$ mice overexpressing TYROBP and transcription of genes previously associated with *Apoe*, including *Axl*, *Ccl2*, *Tgfβ* and *Il6*, was altered in both *APP/PSEN1* and $MAPT^{P301S}$ mice overexpressing TYROBP. Lastly, *Tyrobp* and *Apoe* mRNAs were clearly increased in *Trem2*-null mice in microglia recruited around a cortical stab injury or amyloid-β (Aβ) deposits. Conversely, microglial *Apoe* transcription was dramatically diminished when *Tyrobp* was absent. Our results provide compelling evidence that TYROBP-APOE signaling in the microglial sensome does not require TREM2. We propose that activation of a TREM2-independent TYROBP-APOE signaling could be an early or even initiating step in the transformation of microglia from the homeostatic phenotype to the Disease-Associated Microglia (DAM) phenotype.

PHYSIOLOGICAL FUNCTION OF TMEM106B, A GENE ASSOCIATED WITH MULTIPLE BRAIN DISORDERS

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TMEM106B, encoding a lysosome membrane protein, has been recently associated with brain aging, hypomyelinating leukodystrophy (HLD) and multiple neurodegenerative diseases, such as frontotemporal lobar degeneration (FTLD) and limbic-predominant age-related TDP-43 encephalopathy (LATE). However, the exact function of TMEM106B on lysosome membrane and its role in the brain disorders remain to be elucidated. Using CRISPR/Cas9 technology, we generated TMEM106B deficient mice and found that TMEM106B deficiency leads to accumulation of lysosome vacuoles at the distal end of the axon initial segment in motor neurons and the development of FTLD-related pathology during aging. In addition, TMEM106B ablation results in myelination defects with a significant reduction of protein levels of proteolipid protein (PLP), the main membrane proteins found in the myelin sheath, by affecting lysosome exocytosis and PLP trafficking. Moreover, we found that the HLD associated D252N mutation abolished lysosome enlargement and lysosome acidification induced by wild-type TMEM106B overexpression. Instead, it stimulates lysosome clustering near the nucleus as seen in TMEM106B-deficient cells. Ablation of TMEM106B and the FTLD protein progranulin (PGRN) in mice results in exacerbated lysosomal abnormalities, severe neuronal loss and glial activation and enhanced manifestation of FTLD phenotypes. These results provide novel insights into the role of TMEM106B in the lysosome, in brain aging, and in HLD and FTLD pathogenesis.

OPPORTUNITIES AND RESOURCES TO HELP EARLY CAREER SCIENTISTS NAVIGATE THE NIH: THE NUTS AND BOLTS OF NIH PEER REVIEW

Laurent Taupenot

Center for Scientific Review, National Institutes of Health, Bethesda, MD

NIH, as the largest funder of biomedical research in the world, directly supports a large proportion of the science and scientists. Understanding the NIH peer review process can improve one’s chance of getting funded. The majority of peer review takes place through the NIH Center for Scientific Review (CSR). CSR conducts the review of: 90% of R01s, 85% of Fellowships, and 95% of SBIR applications. Outreach efforts by CSR Scientific Review Officers help investigators and reviewers understand important aspects of the grant submission and review process. With the goal to educate potential NIH grant applicants, particularly early career scientists, key areas of the presentation will include the basics of peer review, review criteria, what reviewers look for, the review timeline, who at NIH to talk to about your application, how to find the right study section. Outreach efforts at NIH help to minimize the influence of grantsmanship and differential knowledge of NIH priorities, policies, and practices, to ensure that review outcomes reflect the strength of applicants' ideas and capabilities as scientists.

MAKING SENSE OF ANTISENSE DRUGS FOR NEUROLOGICAL DISEASES

C. Frank Bennett

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Antisense oligonucleotides (ASOs) are synthetic, chemical modified nucleic acid analogs designed to bind to RNA by Watson-Crick base paring and upon binding, modulate the function of the targeted RNA. There are a variety of mechanisms by which ASOs can modulate RNA function dependent on the chemical design of the ASO, the type of RNA and where on the RNA the ASO is designed to bind. These include promoting the degradation of the target RNA and modifying intermediate metabolism such as splicing or polyadenylation. Both protein coding, as well as non-coding RNAs, can be targets of ASO based drugs, significantly broadening therapeutic targets for drug discovery compared to small molecules and protein based therapeutics. The approval of nusinersen (Spinraza™) as a treatment for spinal muscular atrophy (SMA) validates the utility of antisense drugs for the treatment of motor neuron diseases. The key learnings from the development of SMA will be summarized as they apply to treatment of other neurodegenerative diseases. Finally the application of antisense technology as potential therapy for other neurological diseases and neurodevelopmental disorders will be discussed.

THE ROLE OF INTERLEUKIN-33 SIGNALING IN ALZHEIMER'S DISEASE

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The identification of AD risk genes that are associated with the innate immune system underscores the contribution of innate immunity to AD pathogenesis. Hence, understanding the roles of innate immunity in AD can lead to new therapeutic strategies for the disease. Interleukin 33 (IL-33), a member of the IL-1 family, was originally identified as an alarmin to maintain tissue homeostasis by activating its cognate receptor ST2 on target cells. Our recent work has shown that increased plasma levels of the IL-33 decoy receptor sST2 is associated with AD progression. Restoration of IL-33/ST2 signaling by replenishing IL-33 in an AD transgenic mouse model rescues hippocampal synaptic dysfunctions and contextual memory deficits. Importantly, we have demonstrated that the beneficial actions of IL-33 are mediated in part by regulating the Aβ clearance of microglia. Furthermore, single-cell RNA sequencing analysis has revealed that in AD transgenic mice, IL-33 induces a subpopulation of microglia (IL-33RM) that exhibit a distinctive transcriptome profile with an enhanced expression of MHC-II genes. Epigenetic studies by ATACseq and ChIPseq analysis have further revealed that PU.1 transcriptional control is essential for regulating the transcriptomic state transition of microglia upon IL-33 treatment. In humans, we have identified a non-coding genetic variant of ST2 which is associated with decreased plasma levels of sST2, and intriguingly, AD patients carrying this protective variant exhibit a slower progression of the disease pathology. Our work thus highlights the role of dysregulated IL-33/ST2 signaling in the pathogenesis of AD, providing new insights into AD drug discovery and development.

FROM GENETIC TO SPORADIC: MANAGING RISK IN NEUROSCIENCE DRUG DEVELOPMENT

Chris Henderson

Biogen, Cambridge, MA

Drug development for neurodegenerative disease is a high priority for society but has historically had a low success rate. Finding ways in which programs can be de-risked before they engage the daunting costs in time, money and patient engagement represented by a large Phase 3 clinical study is therefore a pre-requisite for long-term viability of CNS-focused pipelines. Many approaches to this are currently being considered, with no single solution that fits all therapeutic challenges. It is clearly important to base each program on strong (preferably human) data that validate a given target as disease-modifying. But it is also vital to structure programs so that each successive experiment – nonclinical or clinical – provides a cost-efficient means to measure the action of the drug on the target and the disease mechanism, allowing a crisp decision whether to pursue or terminate. This presentation will focus on some examples of how use of biomarkers and novel endpoints can support early decision-making. It will do so in the context of a strategy involving progression from higher- to lower-confidence targets in progressively broader subsets of patients with a single disease.

One main area of focus will be amyotrophic lateral sclerosis (ALS), a devastating disease for which current treatments confer very moderate benefit. I will trace how an initial focus on genetic forms of the disease linked to mutations in SOD1 and C9orf72, combined with the use of target engagement biomarkers and others for disease progression (e.g. neurofilament) can not only address the urgent needs of this subset of patients but also provide a basis for taking on the far more frequent (90% of all cases) sporadic forms of ALS. Our collaboration with Ionis Pharmaceuticals (see talk by Frank Bennett) and many academic clinicians and researchers has been essential in moving forward this strategy. Now, as we launch programs in sporadic ALS, the degree of target confidence is necessarily one degree lower, but emerging human genetics and clinical data can considerably reinforce the prior validation of some targets (see talk by Sally John). We hope that this, combined with many learnings from the trials in patients with genetic ALS, will help us develop clinically meaningful therapies for people and their families living with sporadic ALS.

PILOT STUDIES OF SUPPRESSION OF *SOD1* AND *C9ORF72* GENES IN ALS PATIENTS

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Most mutations that cause familial ALS are transmitted as dominant traits. The cascade of pathological processes evoked by these mutations are complex and only partially understood. Nonetheless, several lines of evidence argue that the course of the disease is attenuated by suppressing expression of the offending mutant alleles. In collaboration with the laboratories of Drs. Chris Mueller and Jonathan Watts (also at UMMS), we have pursued ALS gene silencing with two strategies: AAV-mediated delivery of a microRNA targeting the *SOD1* gene, and anti-sense oligonucleotides that target the *C9orf72* gene. In both instances, extensive preclinical studies in mice and non-human primates documented feasibility and safety. Using intrathecal administration of AAVrh10 to deliver a microRNA that targets the *SOD1* gene, we have treated two patients with *SOD1* gene mutations. In the first case, the clinical course and pathological findings suggested some limited benefit, despite an inflammatory reaction to the virus (not seen in the monkey studies). In the second case, a concurrent course of immunosuppression appeared to suppress the adverse immunoreactivity. Again using IT administration, we have treated a single patient with our ASO that targets the two isoforms of *C9orf72* harboring the mutation. After multiple doses, this intervention has been well tolerated and associated with a reduction in CSF levels of the polyGP dipeptide and potential clinical benefit. Details of these studies will be presented. Our pilot findings are aligned with other reports from human trials that gene suppression therapy is promising in dominantly inherited neurodegenerative disorders.

PR001 GENE THERAPY INCREASED GCASE ACTIVITY AND AMELIORATED GBA1-ASSOCIATED DISEASE PHENOTYPES

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Mutations in the GBA1 gene are believed to be the most common etiology of lysosomal storage diseases. GBA1 mutations cause Gaucher disease (GD), an autosomal recessive inherited disorder, with severe mutations leading to neurological manifestations (neuronopathic Gaucher disease, or nGD). Additionally, GBA1 mutations are the most common known genetic cause of Parkinson's disease (PD), with a prevalence of 7-10% of the patient population. Deficiency in the GBA1 encoded enzyme glucocerebrosidase (GCase), a key lysosomal enzyme required for the normal metabolism of glycolipids, leads to the accumulation of glycolipid substrates and lysosomal dysfunction that ultimately results in neuroinflammation and neurodegeneration. With our gene therapy product, PR001, we aim to increase GCase activity in nGD and PD patients with GBA1 mutations (PD-GBA) in order to ameliorate the lysosomal dysfunction and slow or stop disease progression. We evaluated PR001 in in vivo and in vitro studies. We used two established mouse models of GCase deficiency that display phenotypic characteristics consistent with nGD and PD-GBA. Intracerebroventricular (ICV) injection of PR001 increased GCase activity, decreased glycolipid substrate accumulation, improved motor abnormalities, and reduced neuropathological changes. These therapeutic benefits were persistent, with lasting effects observed at 6 months post-treatment. Select safety endpoints from these studies, as well as toxicology studies in nonhuman primates, demonstrated that PR001 was well-tolerated at all doses tested. Supportive in vitro studies showed that PR001 increased GCase activity and decreased α-synuclein levels in a cell line and primary rodent neurons. Overall, these findings support the clinical development of PR001 for nGD and PD-GBA. We have recently initiated Phase 1/2 clinical trials to investigate safety, tolerability, as well as biomarker and behavioral measures of efficacy in nGD and PD GBA patients.

A BRAIN PENETRANT PROGRANULIN BIOTHERAPEUTIC RESCUES LYSOSOMAL AND INFLAMMATORY BIOMARKERS IN GRN KNOCKOUT MOUSE BRAIN

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Introduction: Heterozygous loss of function (LOF) mutations in the GRN gene culminate in frontotemporal dementia (FTD), a neurodegenerative disorder associated with lysosomal dysfunction, inflammation, and neuronal loss. GRN encodes progranulin (PGRN), a soluble protein that is abundantly found within lysosomes of microglia where growing evidence suggests it may regulate lysosomal function and inflammatory responses. Accordingly, PGRN LOF *in vitro* and *in vivo* models are associated with lysosomal defects, hyperinflammatory responses, and decreased neuronal viability. Because GRN-FTD patients exhibit reduced levels of PGRN in biofluids and tissues, including brain, a protein replacement therapy capable of crossing the blood brain barrier (BBB) efficiently provides a potentially powerful approach to slow or prevent disease progression.

Method: Here we describe a novel therapeutic for increasing brain concentrations of human PGRN, termed Protein Transport Vehicle (PTV):PGRN. PTV:PGRN consists of recombinant human PGRN fused to a modified Fc domain that has been engineered to bind to the apical domain of the human transferrin receptor (huTfR), promoting receptor-mediated transcytosis across the BBB and into the brain parenchyma.

Result: To initially test whether PTV:PGRN could rescue Grn KO phenotypes *in vitro*, PTV:PGRN was applied extracellularly. We found that Grn KO cell phenotypes were fully rescued with PTV:PGRN, including lysosomal proteolysis and levels of the endolysosomal phospholipid bis(monoacylglycero) phosphate (BMP) that is involved in lysosomal lipid catabolism. We next systemically administered PTV:PGRN to huTfR knock-in ($TfR^{mu/hu}$) mice and saw a significant increase in brain PGRN levels relative to non-huTfR binding Fc:PGRN fusion control, highlighting the ability of huTfR binding in increase brain uptake of PGRN. To ultimately test whether this increase in brain PGRN could impact disease relevant endpoints, Grn KO x $TfR^{mu/hu}$ mice were treated acutely and chronically with PTV:PGRN. We found that at low doses in acute and chronic treatment paradigms, PTV:PGRN fully corrected disease associated lysosomal lipid changes, including BMP deficiency, as well as inflammatory markers in the brains of Grn KO x $TfR^{mu/hu}$ mice.

Conclusion: Our data suggest that PTV:PGRN may represent a viable therapeutic strategy for the treatment of GRN-FTD and potentially other neurological disorders associated with PGRN deficiency.

MALE SEX CHROMOSOMES ATTENUATE CYTOKINE RESPONSE TO INFLAMMATION IN AGING BRAIN

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Alzheimer's Disease (AD) exhibits sex differences, with two-fold higher prevalence for women than for men. Overall AD pathology is also increased in female AD patients compared to male patients. As the most predominant risk allele in late onset sporadic AD, apoliprotein E4 exhibits sex-biased effects with female *APOE4* carriers exhibiting significantly higher risk than male carriers. Interestingly, clinical studies have shown that *APOE4* carriers with low grade inflammation demonstrate higher risk of AD. Moreover, studies have shown that microglia expressing *APOE4* demonstrate heightened pro-inflammatory response to lipopolysaccharide (LPS) neurotoxic stimulus compared to all other *APOE* isoforms. Our previous studies showed sex-specific microglial response to tau pathology, further supporting the notion that sex-specific neuroinflammatory response could underlie the increased female susceptibility to AD. To begin to test this hypothesis and dissect underlying mechanisms, we took advantage of the Four Core Genotype mouse model, which allows for dissociating the effects of sex chromosome from those of sex hormones. Using lipopolysaccharide to model acute inflammatory response, our aged female mice exhibited the highest pro-inflammatory response to LPS, while mice with XY sex chromosomes and either testes or ovaries exhibit significantly attenuated cytokine response compared to mice with XX sex chromosomes. Specifically, the XY-ovaries genotype demonstrated the most attenuated cytokine induction in response to LPS. We next examined inflammation in response to cuprizone-induced demyelination in Four Core Genotype mice. Consistent with the responses to LPS, aged female mice exhibited the highest pro-inflammatory response to demyelination while mice with XY chromosome, especially on an ovarian background, exhibited the lowest. Ongoing investigation will dissect the mechanisms underlying sex differences in innate immune response, and the sex difference in *APOE4*-induced alternations, which could lay foundation for developing patient-specific therapeutic strategies for AD.

A GENE ONLY IN MALES CONTRIBUTES TO MALE BIAS IN EXPERIMENTAL PARKINSON'S DISEASE

Joohyung Lee[1], Paulo Pinares-Garcia[1], Hannah Loke[1], Seungmin Ham[1], Eric Vilain[2], Vincent Harley[1]

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Parkinson's disease (PD) is a debilitating neurodegenerative disorder caused by the loss of midbrain dopamine (DA) neurons. While the cause of DA cell loss in PD is unknown, male sex is a strong risk factor. Aside from the protective actions of sex hormones in females, emerging evidence suggests that sex-chromosome genes contribute to the male bias in PD. We previously showed that the Y-chromosome gene, SRY, directly regulates adult brain function in males independent of gonadal hormone influence. SRY protein colocalizes with DA neurons in the male substantia nigra, where it regulates DA biosynthesis and voluntary movement.

Given the presence and function of SRY in male DA neurons, we hypothesised that dysregulation of SRY may contribute to male susceptibility to PD. To test our hypothesis, human cell culture and toxin-induced rat models of PD were combined with repeated Sry antisense oligonucleotide (ASO) infusions. Motor behaviours, histological and molecular analyses were undertaken.

Here we demonstrate that nigral SRY expression is highly and persistently up-regulated in animal and human cell culture models of PD. Lowering nigral SRY expression with ASOs in male rats diminished motor deficits and nigral DA cell loss in toxin-induced rat models of PD. The protective effect of the SRY ASOs was associated with male-specific attenuation of DNA damage, mitochondrial degradation, and neuroinflammation in the toxin- induced rat models of PD.

Moreover, reducing nigral SRY expression diminished or removed the male bias in nigrostriatal degeneration, mitochondrial degradation, DNA damage, and neuroinflammation in the 6-OHDA rat model of PD, suggesting that SRY directly contributes to the sex differences in PD. These findings demonstrate that SRY directs a previously unrecognized male-specific mechanism of DA cell death and suggests that suppressing nigral Sry synthesis represents a sex-specific strategy to slow or prevent DA cell loss in PD.

Additionally, new data addressing the mechanism of ASO induced neuroprotection by SRY will be discussed.

A RATIONAL THERAPEUTIC APPROACH TO THE PREVENTION OF LATE-ONSET AD

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With a prevalence of as high as 40% in some populations, ApoE4 genotype is the most important risk factor for late-onset AD. ApoE4 differs from the most frequent ApoE3 allele by a single amino acid that changes the isoelectric point of ApoE4 to the pH present in early endosomes. Endolysosomal dysfunction is a hallmark of AD and the result of numerous mutations in genes that function in endolysosomal trafficking. We have shown that the shift in the isoelectric point of ApoE4 leads to the delayed trafficking of ApoE4 containing early endosomes. On the basis of the biophysical properties of ApoE4 and the established inverse relationship protein solubility in aequeous solutions around their isoelectric point, we rationalized that lowering of early endosomal pH should relieve the ApoE4 trafficking defect and restore normal neuronal physiology. This can be achieved by inhibition of the early endosomal sodiumhydrogen exchanger NHE6. We have tested this approach in cultured neurons, as well as in vivo, using pharmacological NHE6 inhibition and conditional genetic NHE6 inactivation. Inhibition or genetic inactivation of NHE6 not only restores normal vesicular trafficking, but also prevents plaque formation in a humanized APP/ApoE4 mouse model. NHE inhibitors have been used in clinical practice for over 50 year, suggesting that this approach is safe and adaptable to the prevention of late-onset AD in humans.

NEURONAL APOE DRIVES SELECTIVE NEURODEGENERATION IN ALZHEIMER'S DISEASE

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Selective neurodegeneration is a critical causal factor in Alzheimer's disease (AD); however, the mechanisms that lead some neurons to perish while others remain resilient are unknown. We sought potential drivers of this selective vulnerability¬ using single-nucleus RNA sequencing and discovered that apoE expression level is a substantial driver of neuronal variability. Strikingly, neuronal expression of apoE—which has a strong genetic linkage to AD—correlated strongly, on a cell-by-cell basis, with cellular stress and immune response pathways in neurons in the brains of wildtype mice, human apoE knock-in mice, and humans—both with and without AD. Elimination or over-expression of neuronal apoE revealed a causal relationship between apoE expression, immune response pathway expression, tau pathology, and neurodegeneration. Functional reduction of immune response pathways rescued AD-related tau pathology in wildtype primary neurons, in human apoE4-expressing primary neurons, and in wildtype mouse hippocampi expressing pathological human tau. Together these findings suggest a mechanism linking neuronal expression of apoE to neuronal immune response and, subsequently, to tau pathology and selective neurodegeneration, providing potential new targets to combat AD.

APOLIPOPROTEIN E: EVIDENCE FOR ROLES IN MODULATING AMYLOID-β (Aβ) PATHOLOGY AND TAU MEDIATED NEURODEGENERATION

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The Apolipoprotein E (APOE) gene is the strongest genetic risk factor for Alzheimer disease (AD). ApoE is a 299 amino acid protein expressed at highest levels in the liver but also at high levels in the central nervous system. In humans, there are 3 common ApoE alleles, E2, E3, and E4. ApoE4 is associated with increased risk and ApoE2 decreased risk for AD relative to ApoE3. Evidence will be presented that demonstrates that ApoE has a very strong effect on modulating Aβ pathology in an isoform-specific fashion in both humans and animal models. This effect is modulated by lipidation of ApoE-containing lipoproteins in the CNS as well as via ApoE receptors. In addition to ApoE's effect on Aβ, there is also now strong evidence that ApoE influences tau pathology and tau-mediated neurodegeneration. This effect appears to require microglia. The different cell types that produce ApoE in the brain in the context of these effects will be discussed.

APOE4 DISRUPTS INTRACELLULAR LIPID HOMEOSTASIS

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The *E4* allele of apolipoprotein E (*APOE*) has been firmly established as a genetic risk factor for many diseases including cardiovascular diseases and Alzheimer's disease (AD), yet its mechanism of action remains poorly understood. APOE is best known to function as a lipid transport protein. The dysregulation of lipids has recently emerged as a key feature of several neurodegenerative diseases including AD. However, it is unclear how *APOE4* perturbs the intracellular lipid state.

During the meeting, we will report details on how APOE4 disrupts the cellular lipidome in human iPSC-derived astrocytes. We combine lipidomics and unbiased genome-wide screens with functional and genetic characterization to demonstrate that APOE4 induces widespread changes in lipid homeostasis. Additionally, we identify genetic and chemical modulators of these lipid disruptions which restore cellular lipidome in *APOE4* expressing cells to its basal state.

Given the central role of lipid metabolism in cell physiology, our study illuminates key molecular abnormalities that may underlie the disease risk linked to the *APOE4* genotype. Importantly, our study suggests that targeted manipulations to lipid metabolism could alleviate the consequences of the *APOE4* allele.

TAU AGGREGATE SEEDS ENTER THE CELL BY DIRECT TRANSLOCATION ACROSS THE PLASMA MEMBRANE.

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Propagation of tau aggregation (termed “seeding”) between brain cells may underlie progression of neurodegenerative tauopathies. The molecular basis of this process has been studied extensively by our laboratory and others using simple cell systems, especially “biosensor” cells that express the tau repeat domain (RD) containing a single disease-associated mutation (P301S) fused to complementary fluorescent proteins. Exposure of biosensor cells to exogenous tau seeds triggers intracellular aggregation that can be detected by fluorescence resonance energy transfer (FRET). Despite over a decade of study, the fundamental mechanism by which a tau seed enters the cytoplasm is unknown. We previously determined that tau aggregates bind heparan sulfate proteoglycans (HSPG) on the cell surface. This triggers tau uptake into the cell via macropinocytosis, and pharmacologic or genetic interventions that block tau binding to HSPG prevent uptake and intracellular seeding. Based on this data, we concluded that uptake via macropinocytosis underlies intracellular tau seeding. However, this model fails to explain the relative inefficiency of seeding into cells upon tau exposure, whereby only a small subset exhibits intracellular aggregation. We used a whole genome CRISPR screen to explore mechanisms of tau uptake. This led us to determine that genetic and chemical inhibitors of vesicle acidification block this process. Yet they paradoxically increase seeding. Indeed, halting endocytosis entirely by exposing cells to tau at 4°C prior to returning them to culture at 37°C increases seeding several fold. Furthermore, after loading the endolysosomal system with tau aggregates, we have found an immediate drop in seeding within the cytosol when tau is removed from the extracellular space. This is most consistent with seed entry primarily by direct crossing of the plasma membrane. Finally, we have tested for tau uptake into giant plasma membrane vesicles—artificial membrane-derived structures produced by exposure of cells to specialized buffer—which lack the capacity for endocytosis. Tau aggregates readily translocate into these vesicles *in vitro* in an HSPG-dependent manner, indicating that, like other cell penetrating peptides, even large tau assemblies can directly traverse the plasma membrane. Our results suggest two modes of entry into the cell for tau seeds. While the majority are taken up by macropinocytosis, these species do not trigger intracellular aggregation. By contrast, seeds that mediate intracellular aggregation most likely enter the cytoplasm via direct plasma membrane translocation. This work raises important biochemical and biophysical questions about how a charged macromolecule such as a tau assembly can directly translocate across a lipid bilayer. It suggests a unique biological mechanism to traffic large molecules from the outside to the inside of a cell.

MICROGLIAL BIN1 FAVORS THE SPREADING OF TAU VIA EXTRACELLULAR VESICLES

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Objectives: BIN1 represents the most statistically significant susceptibility locus associated to Late Onset Alzheimer's Disease, after ApoE. Nevertheless, the mechanisms underlying the contribution of BIN1 to AD pathogenesis are still not understood. Given the link between BIN1, Tau and extracellular vesicles, we investigated whether BIN1 could affect Tau spreading via exosomes secretion.

Methods: First, we biochemically characterized extracellular vesicles purified from cerebrospinal fluid of AD-affected individuals. The effects of BIN1 expression in modulating the release of Tau via extracellular vesicles in vitro was analyzed biochemically in 293T cells and in primary microglia. Subsequently, the effects of BIN1 expression in modulating Tau spreading in vivo was investigated by immune-histochemistry in mice expressing Tau P301S (PS19), and then after crossing PS19 with BIN1 microglial knock-out mice.

Results: We observed that BIN1-associated Tau-containing extracellular vesicles purified from cerebrospinal fluid of AD-affected individuals are seeding-competent. We demonstrated that BIN1 over-expression promotes the release of Tau via extracellular vesicles in vitro as well as exacerbation of Tau pathology in vivo. Genetic deletion of Bin1 from microglia resulted in reduction of Tau secretion via extracellular vesicles in vitro, and in decrease of Tau spreading in vivo in male, but not female, mice. Deletion of Bin1 in microglia of male mice resulted in significant reduction in the expression of heat-shock proteins, previously implicated in Tau proteostasis.

Conclusions: These observations suggest that BIN1 could contribute to the progression of AD-related Tau pathology by altering Tau clearance and subsequent Tau-enriched extracellular vesicles secretion by microglia.

ALZHEIMER'S DISEASE BRAIN-DERIVED EXTRACELLULAR VESICLES SPREAD TAU PATHOLOGY IN INTERNEURONS

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Extracellular vesicles (EVs) are highly transmissible and play critical roles in the propagation of tau pathology, although the underlying mechanism remains elusive. Here, for the first time, we comprehensively characterized the physicochemical structure and pathogenic function of human brain-derived EVs isolated from Alzheimer's disease (AD), prodromal AD, and non-demented control (CTRL) cases. AD EVs were significantly enriched in epitope-specific tau oligomers in comparison to prodromal AD or CTRL EVs as determined by dot-blot and atomic force microscopy. AD EVs were more efficiently internalized by murine cortical neurons, as well as more efficient in transferring and misfolding tau, than prodromal AD and CTRL EVs in vitro. Strikingly, the inoculation of AD or prodromal AD EVs containing only 300 pg of tau into the outer molecular layer of the dentate gyrus of 18 months-old C57BL/6 mice resulted in the accumulation of abnormally phosphorylated tau throughout the hippocampus by 4.5 months, whereas inoculation of an equal amount of tau from CTRL EVs, isolated tau oligomers, or fibrils from the same AD donor showed little tau pathology. Furthermore, AD EVs induced misfolding of endogenous tau in both oligomeric and sarkosyl-insoluble forms in the hippocampal region. Unexpectedly, phosphorylated tau was primarily accumulated in GAD67 GABAergic interneurons and, to a lesser extent, glutamate receptor 2/3-positive excitatory mossy cells, showing preferential EV-mediated GABAergic interneuronal tau propagation. Whole-cell patch clamp recordings of CA1 pyramidal cells showed significant reduction in the amplitude of spontaneous inhibitory post-synaptic currents. This was accompanied by reductions in c-fos+ glutamic acid decarboxylase 67+ neurons and glutamic acid decarboxylase 67+ neuronal puncta surrounding pyramidal neurons in the CA1 region, confirming reduced GABAergic transmission in this region. Our study posits a novel mechanism for the spread of tau in hippocampal GABAergic interneurons via brain-derived EVs and their subsequent neuronal dysfunction.

RAISING CYCLIC GMP ACTIVATES 26S PROTEASOMES, UBIQUITINATION, AND PROTEIN DEGRADATION AND HAS THERAPEUTIC EFFECTS IN ZEBRAFISH AND MOUSE MODELS OF NEURODEGENERATIVE DISEASES

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It is widely believed that protein degradation by the Ubiquitin Proteasome System (UPS) is regulated solely at the level of ubiquitination. However, it is now clear that proteasomal activities are also tightly regulated and influence rates of protein degradation. We had previously shown that drugs, hormones, and physiological stimuli (e.g. fasting, exercise) that raise cAMP enhance 26S proteasome activity, the phosphorylation of subunit Rpn6 by Protein Kinase A, and the degradation of short-lived cell proteins, including the breakdown of misfolded disease-associated proteins. We have now investigated whether agents that raise cGMP may regulate protein degradation similarly. Treating various cell lines with inhibitors of phosphodiesterase 5 (e.g., sildenafil) or stimulators of soluble guanylyl cyclase (e.g. riociguat) rapidly increased multiple proteasome activities by activating protein kinase G (PKG). PKG stimulated purified 26S proteasomes by phosphorylating a different 26S component than is modified by Protein Kinase A. In cells and cell extracts, raising cGMP also enhanced within minutes ubiquitin conjugation to cell proteins. Raising cGMP, like raising cAMP, stimulated the degradation of short-lived cell proteins, but unlike cAMP, cGMP also increased proteasomal degradation of long-lived proteins (the bulk of cell proteins) without affecting lysosomal proteolysis. We also tested if raising cGMP, like cAMP, can promote the degradation of mutant proteins that cause neurodegenerative diseases. Treating zebrafish larva models of tauopathies or Huntington's disease with a PDE5 inhibitor reduced the levels of the mutant tau and poly Q-expanded huntingtin, the associated neuronal death, and the resulting morphological abnormalities. Thus, the activation of proteasomes, ubiquitination, and protein degradation by cGMP can have therapeutic benefits against proteotoxic diseases.
To determine if agents that raise cGMP may help combat the progression of other neurological diseases, we studied a mouse model of Charcot Marie Tooth 1B, a peripheral neuropathy caused by the expression of a mutant myelin protein zero (S63del). In the sciatic nerve of the S63del mouse, 26S proteasome activities are reduced, ubiquitinated proteins accumulate and the Unfolded Protein Response is activated. Treatment of these mice with sildenafil restored proteasome function and protein homeostasis and reduced the levels of polyubiquitinated proteins. Moreover, treatment for 14 days restored myelin thickness in the peripheral nerves, decreased amyelinated axons, and increased nerve conduction velocity. Thus, cGMP is an important regulator of proteasome activity and intracellular protein homeostasis, and pharmacological agents that raise cGMP have the potential to combat diverse neurodegenerative and other proteotoxic diseases.

CRISPR MEDIATED KNOCKOUT OF STATHMIN-2 IN MICE LEADS TO MOTOR NEUROPATHY

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Amyotrophic lateral sclerosis (ALS) is a fatal, neurodegenerative disorder in which the average lifespan is 2-5 years after diagnosis. Approximately 90% of patients are termed sporadic, lacking a family history of disease or known genetic risks. As the result of the genetic heterogeneity and complexity of ALS, progression in biomarker discovery and therapeutic development have been slow and costly. Interestingly, 98% of all ALS patients exhibit toxic cytoplasmic TDP43 aggregates, generating both a loss-of-function (LOF) and gain-of-toxicity. Modeling TDP43 LOF in cultured human motor neurons results in a dysfunctional spliceosome and dozens of misregulated transcripts such as STMN2, a microtubule regulator important for neuronal development and repair. Significant loss of full STMN2 and increased levels of the misspliced form have been confirmed in the brain and spinal cord of ALS patients featuring TDP43 pathology. Thus far it remains unresolved to what extent TDP43 pathology directly contributes to neurodegeneration or if it is an event that happens in conjunction with degenerative processes. Furthermore, the role of STMN2 in vivo and how its loss contributes to neuropathy as seen in patients has never been investigated. Therefore, we used CRISPR Cas9 guides directed towards Stmn2 to create a Stmn2 LOF murine model. Loss of Stmn2 transcript and protein was confirmed within the brain and lumbar spinal cords of mice. In comparison to controls, preliminary work has shown loss of Stmn2 led to substantial impairments in strength and coordination as determined by rotarod and hanging wire. Furthermore, loss of Stmn2 led to significant loss of innervation within the hind limb muscles, increased presence of fractured neuromuscular junctions, and significant alterations within the musculoskeletal system. Together, these results show Stmn2 plays a significant role in neuropathy as seen in ALS patients and suggest restoring STMN2 could provide a potential avenue to control and stunt the progressive deterioration of the motor neuropathy in patients.

GENETIC VARIANTS IN THE *SORL1* TRAFFICKING GENE AND THEIR ASSOCIATION WITH ALZHEIMER'S DISEASE

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Background: Extremely rare protein truncating or missense variants in the *SORL1* gene are associated with an increased risk and possibly a causative effect on developing Alzheimer's Disease (AD). Here we aimed to **(1)** investigate the association between the predicted variant pathogenicity, variant frequency and increased AD risk. **(2)** To investigate: the relationship between the predicted *SORL1* variant pathogenicity and the age at AD onset (a.a.o.).
Method: We obtained sequencing data from 12,675 AD cases and 8,693 controls by combined sequencing data from the ADES consortium with data from the ADSP consortium. We selected variants in the *SORL1* gene with allele frequencies <1% in the sample: nonsynonymous variants were annotated using the REVEL algorithm, with predicted damaging effect scores 1 - 100 (low – high damagingness). Loss of function (LOF) variants were selected using LOFTEE, and damagingness was predicted using the CADD algorithm; a CADD score >30 is considered pathogenic. Independent variant allele frequencies (VAF) were derived from the GNOMAD database. For a subset of the cases the age at onset (a.a.o.) was available.
Result: We identified 470 rare nonsynonymous missense or LOF *SORL1* variants. *SORL1* variants with varying predicted damagingness scores and variant allele frequencies occur across the coding sequence of the entire *SORL1* gene. Missense variants with REVEL>75 and MAF <0.001% associated with estimated 6.6-fold increased risk of developing AD. We identified 47 unique LOF variants with CADD score >30, carried by 57 AD patients and one 75-year old control, and together, these associated with 36.0-fold increased risk of AD (95%CI 5.2 – 239.4; $p = 2.7x10^{-12}$). AD patients who carried a LOF variant had a median a.a.o. of 63 years, and carriers of nonsynonymous variants with REVEL>75 had a median a.a.o. of 70 years. We observed the highest fraction of LOF variants in patients with a.a.o <65 (0.8%), and the highest fraction of variants with REVEL 75-100 was observed in patients with a.a.o. 65-70 years (1.3%). Of the AD patients with a.a.o. <65, 2.8% carried at least one predicted damaging variant compared to respectively 2.2%, 1.6% and 1.1% of the patients with a.a.o. between 65-70, 70-80 and 80+. All patients and controls carried a similar fraction of variants with REVEL 1-25.
Conclusion: LOF variants in the *SORL1* gene occurred almost exclusively in AD cases: LOF variants associated with a >36-fold increased risk of AD with relatively early age at onset. These results open the discussion of whether carriers of LOF *SORL1*-variant and their (presymptomatic) family-members should be clinically counseled. For non-synonymous missense variants, pathogenicity screens that distinguish between pathogenic and non-pathogenic variants are warranted. The profound effects of LOF and damaging nonsynonymous variants on AD risk makes the *SORL1* protein product a promising target for the design of selective treatment strategies for individuals affected with *SORL1*-associated AD.

MUTANT PRION PROTEIN AGGREGATES TARGET THE AXONAL CYTOSKELETON AND TRAFFICKING MACHINERY TO DISRUPT MITOCHONDRIAL FUNCTION

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A ubiquitous hallmark of neurodegeneration is the accumulation of misfolded protein aggregates inside swellings along axons. These axonopathies also contain accumulations of abnormal amounts of cytoskeletal filaments, microtubule-associated molecular motor proteins, vesicles, and mitochondria, but how they form as well as how they disrupt neuronal viability, remains unknown. Previous work showed that intra-axonal aggregates impair or block the proper transport of cargoes from the soma to the synapse including of mitochondria. Moreover, neuronal mitochondrial dysfunction, including increased mitochondrial fragmentation is often observed in neurons with intracellular pathologies. The subcellular mechanisms by which axonal aggregates impair transport and mitochondrial dynamics remain undefined. Using an in cellulo neuronal system expressing a mutant prion protein (PrP^{PG14}) that causes prion diseases in humans, we uncovered an endo-lysosomal pathway that accounts for the formation of PrP^{PG14} aggregates uniquely within endo-lysosomal compartments, which cause axonal swellings in axons and disrupt selectively the axonal cytoskeleton and impair the transport and dynamics of mitochondria. Super-resolution and electron microscopy studies as well as dynamic live super-resolution imaging show that intra-axonal PrP^{PG14} aggregates disrupt selectively a subset of post-translationally modified microtubules, and result in the impairment of the anterograde transport of mitochondria as they try to make their way through the aggregate swellings sites. While some succeed getting through, many mitochondria accumulate at these sites and we show that the sequestration of kinesin-1 away from these organelles at those sites disrupts the association between kinesin-1 and cargo adaptors as these complexes try to more through swellings. We furthermore show that sequestered mitochondria undergo significantly more fragmentation, and this is dependent on enhanced mitochondria-Rab7-positive endosome contacts at aggregate sites where fragmentation is promoted by the Rab7 GTPase activity and contacts with mitochondria. Our findings point toward mutant prion aggregate sites as local ‘sinks’, whereby disruptions in the microtubule cytoskeleton promote the sequestration of molecular motors, mitochondria and endosomes, enhancing organelle-organelle contacts, and thus selectively disrupting axonal function. This study points to intra-axonal aggregates and the cytoskeleton as potential targets for the development of strategies to modulate neuronal dysfunction in the prionopathies.

A NEW SORL1-DEFICIENT ANIMAL MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most common form of dementia and no available treatment exist to cure the disease. The rare familial form of AD is caused by variants in the genes *APP, PSEN1* and *PSEN2*, but also the more common sporadic form of AD show a strong genetic component that increase risk for development of the disease. One of the most frequently affected genes is *SORL1*, encoding a sorting receptor involved in trafficking of multiple cargoes between the cell surface and golgi and endosomal compartments. Many of the identified genetic variants in *SORL1* has not been functionally characterized, and the majority is therefore considered as variants of unknown significance. However, a group of variants that leads to premature truncation of the *SORL1* translation product is almost exclusively identified among AD patients, and therefore considered causal of the disease.

Here, we present a large animal model of *SORL1* deficiency. Göttingen minipigs carrying only a single copy of a functional *SORL1* allele have increased Amyloid β-peptide in their CSF, and changes in their brain structures and metabolism that phenocopies the situation for patients with AD. These data confirm that compromised *SORL1* expression leads to AD pathologies, and suggest the *SORL1-KO* minipigs as a novel AD model that could be used to study longitudinal aspects of AD development and for testing drug and treatment strategies against AD.

THE ROLES OF NDR1/2 KINASES IN NEURONAL AUTOPHAGY

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In neurons constitutive autophagy plays a vital role in ensuring a constant turnover of organelles and proteins for proper neuronal function. A failure to carry out autophagy in brain cells results in neurodegeneration. NDR kinases are serine/threonine kinases that are evolutionarily conserved from yeast to mammals, implicated in membrane trafficking, cell polarisation, neuronal differentiation and autophagy. Two highly similar isoforms, NDR1 and NDR2, are found in mammals. The roles of NDR1/2 in mammalian neurons *in vivo* are unknown. Using a conditional knockout mouse model, we deleted both NDR isoforms in excitatory neurons in the cortex and hippocampus. We found that dual loss of *NDR1/2* causes excessive membrane protrusions and Atg9 mislocalisation from Golgi to peripheral compartments in neurons as early as postnatal day 20. In later ages, we observed progressive accumulation of p62-positive aggregates and ubiquitinated proteins and a reduction in the number of LC3-positive autophagosomes, consistent with impaired autophagy. These alterations result in degeneration of upper cortical layers and the hippocampal neuropil in parallel with astrocytic and microglial activation, hallmarks of neurodegeneration. Individual *NDR1* or *NDR2* knock-out mice are unaffected, indicating that there is compensation between the two isoforms. Tamoxifen-induced deletion of *NDR1* and *NDR2* in adult mice results in a similar phenotype showing that NDR1/2 kinases are required for the maintenance of efficient autophagy and neuronal homeostasis. In order to determine the molecular pathways downstream of NDR1/2 we carried out a quantitative mass spectrometry analysis of *NDR1/2* knock-out mice; the results revealed major alterations in endocytosis proteins and confirmed previously-reported NDR1 substrates implicated in membrane trafficking (Ultanir et. al., Neuron, 2012). To test the role of NDR1/2 in membrane trafficking, we infected primary neurons with NDR1/2 shRNAs. We found that transferrin uptake is significantly reduced and the surface levels of transferrin receptor as well as Atg9 are increased, confirming defects in membrane recycling. Retrograde Atg9 trafficking in axons is also significantly impaired. We conclude that NDR1/2 are required for membrane and Atg9 recycling as well as efficient autophagy and when altered these processes lead to neurodegeneration *in vivo*.

TAU AND OTHER PROTEINS FOUND IN ALZHEIMER'S DISEASE SPINAL FLUID ARE LINKED TO RETROMER-MEDIATED ENDOSOMAL TRAFFIC IN MICE AND HUMANS

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Endosomal trafficking has emerged as a defective biological pathway in Alzheimer's disease (AD), and the pathway is a source of cerebrospinal fluid (CSF) protein accumulation. Nevertheless, the identity of the CSF proteins that accumulate in the setting of defects in AD's endosomal trafficking pathway remains unknown. Here, we performed a CSF proteomic screen in mice with a neuronal-selective knockout of the core of the retromer complex VPS35, a master conductor of endosomal traffic which has been implicated in AD. We then validated three of the most relevant proteomic findings: The n-terminal of the transmembrane proteins APLP1 and CHL1, and the mid-domain of tau, which is known to be unconventionally secreted and elevated in AD. In patients with AD dementia, the concentration of n-terminus APLP1 and CHL1 in the CSF correlated with tau and phosphorylated tau. Similar results were observed in healthy controls, where both proteins correlated with tau and phosphorylated tau and were elevated in approximately 70% of patients in the prodromal stages of AD. Collectively, the mouse-to-human studies suggest that retromer-dependent endosomal trafficking can regulate tau, APLP1 and CHL1 CSF concentration, informing on how AD's trafficking pathway might contribute to disease spread, and how to identify its trafficking impairments in vivo.

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DISRUPTED ENDOLYSOSOMAL ACIDIFICATION AS A DRIVING PATHOLOGICAL STRESS IN EARLY ONSET FAMILIAL ALZHEIMER'S DISEASE

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To prevent or delay Alzheimer's disease (AD), we must understand the initial molecular changes/stresses that ultimately lead to cognitive changes and neurodegeneration. Rare mutations, mostly in the gene *PSEN1*, cause early onset familial AD (EOfAD). However, we cannot study the brains of living people in molecular detail to determine what is happening before they develop AD. To do this we must use animal models. There is disagreement about the validity of current animal models of AD. The designs and evaluation of current transgenic models are based on the idea that the mutations causing AD do so by altering production of the Amyloidβ peptide. However, an explanation more consistent with the mutation data is that all the mutations reduce endolysosomal acidification, with consequences for iron homeostasis and cellular energy production. Our approach to understanding the effects of EOfAD mutations is to replicate, as closely as possible, the genetic states of human EOfAD mutation carriers – i.e. we examine single EOfAD-like mutations in single copies of single endogenous genes. We do this using the zebrafish to exploit its advantages in advanced " 'omics" analyses. Our analyses of young adult zebrafish brains support that EOfAD-like mutations disrupt endolysosomal acidification, iron homeostasis, and energy production. Indeed, disrupted energy production is observed across a variety of EOfAD mutations in different genes and so represents an "EOfAD signature" disturbance. Our work suggests that EOfAD and Sanfilippo syndrome childhood dementia may share a common pathological mechanism.

DEPLETION OF NEURONAL ESCRT-0 ACCELERATES SYNAPTIC DEGENERATION IN PRION DISEASE

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Endolysosomal trafficking defects and synaptic dysfunction are central to the progression of prion and other neurodegenerative diseases. Prions traffic through the multivesicular body for degradation in lysosomes or release in exosomes, yet how endolysosomal prion transport impacts neuronal proteostasis is unclear. Here we found a massive reduction in ESCRT-0 (Hrs and STAM1) protein levels in prion-infected mice. To determine how an impaired ESCRT pathway impacts prion conversion and toxicity in vivo, we genetically deleted Hrs from astrocytes, microglia, and neurons and inoculated mice with prions. We found that Hrs depletion from neurons markedly decreased survival time despite unaltered prion levels. In the prion-infection brain, pre- and post-synaptic structural and protein alterations occurred prematurely with Hrs-depletion, including expanded post-synaptic densities and increased phosphorylated AMPA receptor levels. Collectively these data show that severely diminished Hrs accelerates synaptic pathology and reduces survival time in prion disease, suggesting that a reduction in ESCRT-0 may contribute to disease progression as a driver of altered synaptic homeostasis.

EMERGING IMAGING TECHNOLOGIES TO STUDY CELL ARCHITECTURE, DYNAMICS AND FUNCTION

Jennifer Lippincott-Schwartz

Janelia Research Campus, HHMI, Ashburn, VA

Powerful new ways to image the internal structures and complex dynamics of cells are revolutionizing cell biology and bio-medical research. In this talk, I will focus on how emerging fluorescent technologies are increasing spatio-temporal resolution dramatically, permitting simultaneous multispectral imaging of multiple cellular components. In addition, results will be discussed from whole cell milling using Focused Ion Beam Electron Microscopy (FIB-SEM), which reconstructs the entire cell volume at 4 voxel resolution. Using these tools, it is now possible to begin constructing an “organelle interactome”, describing the interrelationships of different cellular organelles as they carry out critical functions. The same tools are also revealing new properties of organelles and their trafficking pathways, and how disruptions of their normal functions due to genetic mutations may contribute to important neurodegenerative diseases.

CRISPR-BASED FUNCTIONAL GENOMICS IN IPSC-DERIVED MODELS OF NEURODEGENERATIVE DISEASE

Martin Kampmann

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Human genes associated with brain-related diseases are being discovered at an accelerating pace. A major challenge is the identification of the mechanisms through which these genes act, and of potential therapeutic strategies. To elucidate such mechanisms in human cells, we established a CRISPR-based platform for genetic screening in human iPSC-derived neurons, astrocytes, microglia and 3D neuron-glia assembloids. Complex libraries of sgRNAs enable us to conduct genome-wide or focused loss-of-function and gain-of-function screens. Such screens uncover molecular players for phenotypes based on survival, stress resistance, cellular functions and dysfunction, and cell states captured by single-cell RNA-Seq. To uncover disease mechanisms and therapeutic targets, we are conducting genetic modifier screens for disease-relevant cellular phenotypes in patient-derived neurons and glia with familial mutations and isogenic controls.

iPSCs-BASED MODELLINGS OF ALS AND KII ALS AND PARKINSONISM-DEMENTIA COMPLEX (KII ALS/PDC)

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Recently, we identified a new drug candidate for ALS, ropinirole hydrochloride (ROPI) by iPSC technology and drug-repositioning strategy (Nat Med 2018). ROPI was shown to suppress ALS-associated phenotypes in a dish for about 70% of sporadic ALS patients. In December 2018 by our group started a clinical trial testing ropinirole hydrochloride in ALS patients was started. This is a phase I/IIa randomized, double-blind, placebo-controlled, open-label continuation clinical trial, named ROPALS trial (UMIN000034954) (Trends Pharmacol Sci 2020, Regen Ther 2019). The primary aim is to assess the safety and tolerability of ropinirole hydrochloride in patients with ALS. Secondary aims include evaluations of effectiveness. A total of 29 patients have been recruited; 21 of these patients are enrolled in the 24-week double-blind phase. ALSFRS-R score was 40 +/- 3, with a mean reduction of -3 points. Finally, 15 patients were assigned to the active drug and 5 patients to the placebo. Of these, 18 patients completed the double-blind phase in March 2020 and started the open-label phase. Eight patients have completed the whole trial schedule in July 2020. In parallel with this, we have established iPSCs from all the participants of ROPALS trial. Subsequent to their rapid motor neurons differentiation by using transcription factors, we could make stratifications of ALS patients among ROPALS trial's participants using neurite retraction phenotypes and other biomarkers such as TDP-43 aggregation, stress granule formation, mitochondrial dysfunction, and increase of lipid peroxidation, in response to ROPI.

As an ALS-related disease, we are focusing on ALS and Parkinsonism-dementia complex (ALS/PDC). ALS/PDC is a unique endemic neurodegenerative disease, with high-incidence foci in Kii Peninsula, Japan, Guam island, U.S., and Western New Guinea, Indonesia. To gather new insights into the pathological mechanisms underlying ALS/PDC in Kii peninsula (Kii ALS/PSC), we performed transcriptome analyses of patient brains, and revealed that expression levels of genes associated with heat shock proteins, DNA binding/damage, and senescence were significantly altered in patients with Kii ALS/PDC compared with healthy individuals. Additionally, pathway and network analyses indicated that the molecular mechanism underlying Kii ALS/PDC may be associated with oxidative phosphorylation of mitochondria, ribosomes, and the synaptic vesicle cycle; in particular, upstream regulators of these mechanisms may be found in synapses and during synaptic trafficking. As a result, determining the relationship between stress-responsive proteins, synaptic dysfunction, and the pathogenesis of Kii ALS/PDC may provide new understanding of this mysterious disease. Additionally, we succeeded to develop the iPSC model of Kii ALS/PDC for the first time and found some new findings of the pahomechanism.

THE iPSC NEURODEGENERATIVE DISEASE INITIATIVE (iNDI)

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Over the past few decades, breakthroughs in genomics, CRISPR/Cas9, and iPSCs have provided an unprecedented opportunity to understand the basic biology of Alzheimer's disease and related dementias (ADRD). However, practical application of these advances by the ADRD research community has, to a large degree, been hampered by a lack of foundational cellular disease models and datasets. Here we describe a large-scale iPSC genome engineering and phenotyping effort for ADRD, the iPSC Neurodegenerative Disease Initiative (iNDI). iNDI is a two-phase collaborative project between the NIH, non-profits, and industry to generate a large series of disease-relevant iPSC lines and related foundational datasets for the ADRD research community. In Phase 1, iNDI will create >500 genetically-engineered isogenic iPSC lines, including those harboring ADRD mutations, gene knockouts, and endogenous tags. All lines will be deeply characterized through a panel of genomic and phenotypic quality control assays, accompanied by the release and sharing of all associated datasets. Following their validation, lines will be distributed to the research community on a rolling basis, with the first lines becoming available in 2021. In Phase 2, through the use of high-throughput robotic platforms, iNDI will differentiate mutation-harboring iPSC lines into CNS-relevant cell types such as neurons and microglia. We will use a series of phenotypic assays, including transcriptomics, proteomics, imaging, and functional genomics to characterize the effect of ADRD mutations on downstream molecular pathways. We anticipate that these resulting tools and datasets will enable discovery of fundamental disease mechanisms, thereby unlocking new therapeutic targets and reducing the burden of these diseases on affected patients, their families, and society.

GENOME-WIDE CRISPR SCREENS FOR TDP-43 AND FUS AGGREGATION STATUS REVEALS NOVEL PROTEIN QUALITY CONTROL REGULATORS AND THERAPEUTIC TARGETS

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TDP-43 and FUS are RNA binding proteins that form cytoplasmic aggregates in patients with ALS and other neurodegenerative diseases. Multiple lines of evidence point to a causal role between the formation of these aggregates and disease onset, highlighting the urgent need to find modifying therapeutic avenues for this process. We performed a genome wide pooled CRISPR knockout screen, in a human cell line, using the aggregation status of each one of these proteins as our screened phenotype. We utilized a FACS based reporter system that enabled us to separate cells based on the aggregation status of an exogenously expressed TDP-43 or FUS. Our screens revealed known and novel protein quality control (PQC) components including stress response genes, chaperones, protein degradation and genes that have not been previously associated with PQC. We are using several approaches to validate the effect of our modifier genes using additional experimental systems. These includes yeast toxicity models, which revealed high level of protection for our top, previously uncharacterized, gene hit. Patient iPSC models, in-vitro aggregation assays and fly models. As part of our FUS screen, we identified a protein that when knocked down resulted in less FUS aggregation. The same protein was previously shown contribute to axonal degeneration. Using a fly model, we show that knockdown results also in increased axonal regeneration making it an intriguing drug target. Altogether, our results provide new routes for further investigation of protein quality control and aggregation modifying pathways.

A GENOME-WIDE SCREEN FOR SUPPRESSORS OF MOTOR DYSFUNCTION IN A TDP-43 MODEL OF ALS

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Background: Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease. ALS manifests as focal motor weakness which inexorably worsens, causing muscle wasting and paralysis. Available pharmacological treatments are largely ineffective, prolonging life an average of 6 months. One established hallmark of ALS is the abnormal cytosolic aggregation of TDP-43, a protein normally localized to the nucleus, and implicated in diverse functions related to RNA metabolism and gene expression. Several laboratories have used TDP-43 overexpression systems to trigger cytosolic aggregation and screen for genetic modifiers that either ameliorate or exacerbate cellular toxicity. These screens identified some promising avenues to the underlying biology, but are limited to non-neuronal systems or to using readouts that reflect effects on developmental biology. Here, we report a genome-wide screen in *Drosophila* to identify genes whose knock-down ameliorates the toxicity of TDP-43. Importantly, our screen is conducted in motor neurons and with a behavioral readout on an age-dependent locomotion defect that we propose is a more faithful cognate to human disease.
Methods: We used the *Drosophila* UAS-Gal4 binary expression system to simultaneously drive a human TDP-43 transgene in a subset of fly motor neurons and a panel of short hairpin RNAs (shRNAs), each of which targets one from among thousands of genes in an unbiased fashion. We quantified the ability of the animals to climb the side of a vial. Wild-type flies climb quickly, while flies expressing TDP-43 in motor neurons climb slowly. Our screen identifies shRNA lines that ameliorate the defects from TDP-43 expression in motor neurons.
Results: We have screened an RNA interference library and find that approximately 1% of shRNAs tested robustly suppress TDP-43 toxicity in a replicable manner. Here we report our full screen pipeline, data from the behavior screen in introgressed animals and validation in a separate glial expression system. One key finding from our screen is that several of our suppressors have previously been found to ameliorate the neurotoxic effects of C9orf72 hexanucleotide repeat expansion. We also identified several gene loci not previously implicated in ALS.
Conclusions: Our screening system proves to be robust and consistent, and identifies both genes with previously established links to ALS biology, and ones that have not been implicated yet. With this platform, we are poised to provide novel insight into the underpinnings of the initiation and progression of the disease.

C9ORF72 DIPEPTIDE REPEAT PROTEINS INHIBIT UPF1-MEDIATED RNA DECAY VIA TRANSLATIONAL REPRESSION

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Expansion of an intronic $(GGGGCC)_n$ repeat region within the *C9orf72* gene is a main cause of familial amyotrophic lateral sclerosis and frontotemporal dementia (c9ALS/FTD). A hallmark of c9ALS/FTD is the accumulation of misprocessed RNAs, which are often targets of cellular RNA surveillance. Here, we show that RNA decay mechanisms involving upstream frameshift 1 (UPF1), including nonsense-mediated decay (NMD), are inhibited in c9ALS/FTD brains and in cultured cells expressing either of two arginine-rich dipeptide repeats (R-DPRs), poly(GR) and poly(PR). Mechanistically, although R-DPRs cause the recruitment of UPF1 to stress granules, stress granule formation is independent of NMD inhibition. Instead, NMD inhibition is primarily a result from global translational repression caused by R-DPRs. Overexpression of UPF1, but none of its NMD-deficient mutants, enhanced the survival of neurons treated by R-DPRs, suggesting that R-DPRs cause neurotoxicity in part by inhibiting cellular RNA surveillance.

C9ORF72-GENERATED POLY(GR) DIRECTLY CONTRIBUTES TO TDP-43 PATHOLOGY IN C9-ALS/FTD

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One of the defining pathological hallmarks associated with ALS/FTD is the aggregation of TAR DNA-binding protein (TDP-43). Thus far, the underlying factors that trigger or initiate TDP-43 aggregation remain elusive. Several lines of evidence suggest that TDP-43 pathology is downstream of the genetic burden that causes ALS/FTD. The discovery of the G_4C_2 repeat expansion in *C9orf72* as the leading genetic cause of ALS/FTD, and shortly thereafter, the discovery of the non-canonical translation of the repeat expansion and production of toxic dipeptide-repeat proteins (DPRs), paved the way to elucidate the potential mechanisms that drive TDP-43 pathology. However, a mechanistic understanding of how DPRs interact with TDP-43 remains unclear. Given that the vast majority of the DPR proteome comprises of RNA-binding proteins with low-complexity domains, we hypothesized that arginine-rich DPRs (R-DPRs) might directly interact with TDP-43, accelerating its fibrillization and exacerbating TDP-43-mediated neuropathy. In this study, we provide the first evidence for a direct effect of poly(GR) on TDP-43 aggregation, both *in vitro* and in C9-mice. Poly(GR) accelerated and enhanced TDP-43 aggregation, causing the formation of large, dense aggregates. Intriguingly, by utilizing a previously discovered disaggregase, the nuclear-import receptor TNPO1, we demonstrate that TNPO1 can sequester poly(GR) away from interacting with TDP-43, buffering against the detrimental effects of poly(GR) on TDP-43 aggregation. Finally, we also established that the injection of RNA-targeting antisense oligonucleotides (ASOs) in C9-mice not only reduces DPRs protein levels, but also diminishes TDP-43 pathology and mitigates neurodegeneration. Overall, our work provides mechanistic insights on the detrimental and direct roles R-DPRs play in worsening TDP-43 pathology, and presents promising therapeutic strategies to combat those deleterious outcomes.

TDP-43 AND HSP70 PHASE SEPARATE INTO INTRANUCLEAR LIQUID SPHERICAL ANNULI

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The RNA binding protein TDP-43 naturally phase separates within cell nuclei and forms aggregates in multiple age-related neurodegenerative diseases including amyotrophic lateral sclerosis (ALS) and frontal temporal dementia (FTD). We show that RNA-binding deficient TDP-43 (produced by ALS/FTD causing mutations or post-translational acetylation in either of its two RNA recognition motifs) drives TDP-43 de-mixing into symmetrical, intranuclear liquid spherical shells. The major components of these shells' cores are identified to be Hsp70 family chaperones, whose ATPase activity is required to maintain liquidity of TDP-43. Thus, we identify that acetylation, and Hsp70 chaperone activity can regulate TDP-43 phase separation.

NUCLEAR EXPORT AND TRANSLATION OF CIRCULAR REPEAT-CONTAINING INTRONIC RNA IN *C9ORF72*-ALS/FTD

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C9ORF72 hexanucleotide GGGGCC repeat expansion is the most common cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Repeat-containing RNA mediates toxicity through nuclear granules and dipeptide repeat (DPR) proteins produced by repeat-associated non-AUG translation. However, it remains unclear how the intron-localized repeats are exported and translated in the cytoplasm. We used single molecule imaging approach to examine the molecular identity and spatiotemporal dynamics of the repeat RNA. We demonstrate that the spliced intron with G-rich repeats is stabilized in a circular form due to defective lariat debranching. The spliced circular intron, instead of pre-mRNA, serves as the translation template. The NXF1-NXT1 pathway plays an important role in the nuclear export of the circular intron and modulates toxic DPR production. This study reveals an uncharacterized disease-causing RNA species mediated by repeat expansion and demonstrates the importance of RNA spatial localization to understand disease etiology.

DETERMINANTS OF TAU AGGREGATION IN HUMAN NEURONS

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Tauopathies, including Alzheimer's Disease, are a leading and growing cause of death worldwide. There is currently no treatment to slow or stop the progression of these diseases. Tauopathies are characterized by the intracellular deposition of aggregates of the protein tau. We lack, however, a comprehensive understanding of the cellular factors that drive the initiation of this process in human neurons. We recently completed a genome-wide CRISPRi screen for factors that control tau oligomerization in iPSC-derived human neurons expressing the Frontotemporal Lobe Dementia (FTD)-associated tau V337M mutation. We have identified pathways and factors responsible for both increases and decreases in both tau levels and tau oligomer levels. These include autophagy, components of the ubiquitin-proteasome system, and factors responsible for mitochondrial function. These results reveal the underlying mechanisms that control tau aggregation in human neurons and targeting these factors could lead to the development of new therapeutics for tauopathies.

MICROBIAL METABOLITES AND NEUROINFLAMMATION IN PARKINSON'S DISEASE

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Parkinson's disease (PD) affects 1% of the population over the age of 65 and is the second most common neurodegenerative disease after Alzheimer's. One of the contributing factors to neurodegeneration in PD is chronic neuroinflammation, driven primarily by aberrant microglial activation. Although PD is predominantly classified as a brain disorder, neurological manifestations of the disease are preceded by gastrointestinal distress in over 80% of patients. This suggests that the billions of microbial organisms that inhabit our gut, collectively termed the gut microbiota, may be implicated in disease pathogenesis. One class of bacterial metabolites that may be mediating gut-brain interactions in PD are short chain fatty acids (SCFAs), metabolic products formed by the breakdown of dietary fiber by gut bacteria. SCFAs have anti-inflammatory properties in a variety of disease contexts, however the effects of SCFAs on microglia in PD is poorly understood. Through the use of custom fiber-based diets (i.e. prebiotics) we hope to increase endogenous production of SCFAs by the gut microbiome and investigate the effects on microglial-driven neuroinflammation in a mouse model of PD.

TRANS HEMISPHERIC CIRCUIT DYSFUNCTION IN ALZHEIMER'S DISEASE MICE

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In mammals, including humans and mice, callosal projection neurons pass through corpus callosum to form the largest commissural white matter bundle in the brain and are thought to be necessary for the integration of the information between left and right cerebral hemispheres. While brain wide imaging studies have revealed that the corpus callosum and white matter tract are affected in humans with Alzheimer's disease (AD), the structural and functional aspect of specific transhemispheric circuit that is affected in AD remains poorly understood. Here, we use mouse models of AD to show that homotopic callosal circuit in the primary visual cortex (V1) is severely affected in AD mice with a significant loss of spines in trans-hemispheric neurons and altered theta gamma coupling between left and right V1. Homotopic trans-hemispheric neurons are primarily excitatory non-GABAergic cells and provide monosynaptic input to excitatory neurons as well as parvalbumin expressing interneurons. Chemo-genetic inhibition of trans-hemispheric neurons impact cross frequency coupling in V1, and repeated resetting of gamma phase by sensory evoked entrainment alleviates spine loss in trans-hemispheric neurons and improves cross frequency coupling in AD mice. Furthermore, performance in a visual novelty discrimination task that is severely impaired in AD mice and mediated by trans hemispheric neurons can be improved by repeated gamma in AD mice. Together, our data reveal a novel trans-hemispheric circuit and cross frequency coupling dysfunction between brain hemispheres in AD.

GENETIC AND POLYGENIC RISK SCORE ANALYSIS FOR ALZHEIMER'S DISEASE IN THE HONG KONG CHINESE POPULATION

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Dozens of Alzheimer's disease (AD)-associated loci have been identified in the European-descent populations, but their effects have not been thoroughly investigated in the Hong Kong Chinese. By conducting array genotyping in Hong Kong Chinese AD cohort, we successfully replicated the AD-association of several reported variants. Meanwhile, we identified several individuals harboring *TREM2* H157Y in our cohorts. Using the replicated variants, we successfully developed a polygenic risk score model that is indicative of the individual AD risk and cognitive performance. Moreover, we showed that the *TREM2* H157Y might influence the amyloid-beta 42/40 ratio and levels of specific immune-associated proteins in plasma. Further studies are warranted to investigate the effects of these genetic risk factors in AD.

REDUCING THE SULFATION OF HEPARAN SULFATE CHAINS PROLONGS SURVIVAL AND DECREASES AMYLOID DEPOSITION IN PRION-INFECTED MICE

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Heparan sulfate (HS) glycosaminoglycans are major constituents of the brain extracellular matrix, neural cell glycocalyx, and vascular basement membrane. HS is associated with amyloid-β (Aβ) plaques and tau tangles in Alzheimer's disease, and with prion plaques in Creutzfeldt-Jakob disease. The interaction of HS with Aβ and PrP proteins is modulated by the length and the sulfation of the HS chains. We previously found that prion infection of mice with shortened HS chains (*Ext1*$^{+/-}$) led to a dramatically prolonged survival and a redistribution of prions from parenchymal plaques to blood vessels, suggesting that impairing the HS-prion interaction may increase prion clearance into the cerebrospinal fluid (CSF). Here we investigated how decreasing HS sulfation impacts the replication of prions in vivo. *Ndst1*$^{f/f}$*SynCre*+ mice, which express reduced neuronal HS sulfation due to the conditional deletion of *Ndst1* in neurons, displayed 50% longer survival compared to *Ndst1*$^{f/f}$*SynCre*- littermates when infected with prions. *Ndst1*$^{f/f}$*SynCre*+ brains showed 60% reduced prion levels and a redistribution of prion parenchymal plaques to vasculature and periventricular areas. Prion plaques in *Ndst1*$^{f/f}$*SynCre*+ brains were also smaller and appeared fragmented, which was associated with an increased aggregate solubility. However, the electrophoretic mobility, glycoprofile, and aggregate stability of the prions in the *Ndst1*$^{f/f}$*SynCre*+ and *Ndst1*$^{f/f}$*SynCre*- brains were indistinguishable. Collectively, our results suggest that HS chains trap prions in the brain extracellular matrix, promoting assembly into insoluble fibrillar aggregates and parenchymal plaques and potentially preventing their clearance into the CSF. Our study provides a new target for the rational design of neuroprotective therapies based on manipulating HS sulfation to disrupt the interaction of HS with misfolded prions.

TERMINAL COMPLEMENT ACTIVATION DRIVES NEUROPATHOLOGY IN PARKINSON'S DISEASE THROUGH INTRACELLULAR NLRP3 SIGNALING

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The activation of the innate immune system in the CNS is a key trigger of dopaminergic neuronal loss in Parkinson's disease (PD). Innate immune activation in the CNS is modulated by inflammasomes and the complement system. We recently demonstrated that microglial NLRP3 inflammasome inhibition prevents α-synuclein pathology and dopaminergic neurodegeneration in mice. In the current study, we investigated whether complement signaling also underpins the activation of microglial NLPR3 inflammasomes in PD. We first demonstrated the complement pathway is upregulated in experimental PD mice models. We next evaluated the contribution of key complement effectors, C3aR, C5aR1, and C9 in the 6-OHDA PD model using knockout mice, demonstrating that C5aR1-deficient mice showed the greatest neuroprotection. C5aR1 was expressed on activated microglia in experimental PD models and in human postmortem PD brains. In vitro studies using primary microglia stimulated with fibrillar α-synuclein to induce inflammasome activation, lead to mature IL-1β secretion, which was markedly reduced in microglia isolated from C5aR1-deficient mice. Pharmacological inhibition of C5aR1 using two distinct antagonists was similarly able to diminish α-synuclein-mediated inflammasome activation. Furthermore, C5aR1 was also required for full activation of microglial inflammasomes mediated by other NLPR3 activators such as ATP. In support of these in vitro findings, we demonstrated that pharmacological inhibition of C5aR1 with PMX205 mitigates inflammasome activation, motor deficits, and nigrostriatal dopaminergic degeneration in the 6-OHDA and the PFF synuclein mouse models of PD. Finally, we demonstrated that fibrillar α-synuclein could activate complement directly in human plasma, and C5a was upregulated in post-mortem patient CSF and serum, providing support for this pathway in human PD pathology. Taken together, our results suggest that C5aR1 is an essential co-factor in microglial NLRP3 inflammasome activation. Therapeutic targeting of C5aR1 could, therefore, be a viable strategy to reduce microglial-mediated neuroinflammation, to slow disease progression in PD and other neurodegenerative disorders.

COGNITIVE DEFICITS REPORTED IN THE M323K MOUSE, A TDP-43 ALS MODEL

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Rationale: Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disease which is characterised by progressive muscle weakness and subsequent motor defects, due to degenerative changes of upper and lower motor neurons in the brain and spinal cord. Traditionally, ALS was believed to spare cognitive functions. However, ~50% of ALS patients develop cognitive and behavioural impairments, whilst ~15% of patients develop frontotemporal dementia (FTD). ALS and FTD are now considered parts of the same spectrum disorder.
TDP-43 is a RNA-binding protein which is implicated in both ALS and FTD pathology. This protein is normally localised in the nucleus and is involved in a number of RNA metabolism processes, including splicing. Cytoplasmic aggregates of TDP-43 protein are found in >90% of ALS cases and in 45% of FTD cases. Currently, there is no physiological TDP-43 model that exhibits both the motor and cognitive defects seen in ALS-FTD. The M323K mouse contains a point mutation in the TDP-43 gene (Tardbp). Homozygous mice have already been shown to display a mid-to-late life onset neurodegenerative phenotype, mainly motor symptoms (Fratta et al, 2018). However, cognitive testing on these mice has remained limited.
Methodology: A longitudinal study, consisting of a comprehensive phenotyping pipeline, was conducted on a large cohort of M323K female mice (*n=9* wild-types and *n=9* homozygous). This study focused on identifying progressive phenotypic changes in homozygous mice; heterozygous mice were omitted since they show minimal neurodegenerative changes. A range of motor, cognitive and metabolic tests were conducted at an early (3-months) and late (1-year) time point.
Results: Preliminary data show that homozygous mice display cognitive deficits from 3-months. General well-being tests, like marble burying and nesting, revealed impairments in normal rodent behaviours; indicative of non-specific hippocampal dysfunction. Further in-depth cognitive tests, spontaneous alternation and fear conditioning, revealed learning and memory problems in the homozygous mice at 1-year.
Conclusion: The M323K mouse is an excellent physiological model of ALS-FTD. Its ability to display motor and cognitive phenotypes, which mimic clinical observations, make it a unique and invaluable tool to further understand ALS-FTD TDP-43 pathophysiology.

UNCOVERING THE PATHOLOGICAL ROLE OF ASTROCYTES IN SMA

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Spinal muscular atrophy (SMA) is the most common genetic cause of infant mortality and is caused by mutations of the survival motor neuron 1 gene (SMN1) resulting in reduced expression of the SMN protein. Motor neuron loss is the primary phenotypic outcome in SMA patients though it is unclear why motor neurons are particularly impacted. SMA motor neurons show intrinsic deficits in splicing and electrophysiological function, but these defects alone are not sufficient to induce overt motor neuron loss. Data from us and others indicate that there are significant non-cell autonomous astrocyte and microglia influences on motor neuron health and survival, raising the possibility that glia directly contribute to the vulnerability of neurons in SMA.

We have previously found that SMA astrocytes undergo morphological and functional changes very early in disease and are capable of inducing motor neuron pathology. We found that SMA astrocytes have reduced growth factor release and abnormal microRNA production. Additionally, others have shown that SMA microglia exhibit activation later in the disease process and are involved in synapse engulfment. However, the mechanisms of glia-mediated neuron dysfunction and the temporal contributions of astrocyte-microglia crosstalk to SMA pathology have not been elucidated.

We hypothesize that early SMA astrocyte malfunction induces motor neuron pathology and simultaneously activates microglia. In support of this, we found that induced pluripotent stem cell (iPSC)-derived astrocytes exhibit aberrant expression of the transcription factor GATA6, increased NFκB nuclear localization, and increased complement factor C3 release. Moreover, we found that SMA iPSC-derived astrocyte conditioned medium (ACM) is sufficient to induce motor neuron death. Interestingly, lentiviral knockdown of GATA6 in SMA iPSC-derived astrocytes abrogated ACM-mediated motor neuron toxicity. Separately, we found that SMA iPSC-derived microglia adopted a reactive morphology, increased phagocytosis, and increased expression of complement C1q compared to control microglia. Exposure of SMA iPSC-derived microglia to SMA ACM significantly increased their phagocytic activity compared to untreated microglia, consistent with a role of astrocytes in exacerbating microglial activation in SMA.

These data indicate the significant role of astrocyte secreted factors in contributing to disease phenotypes in SMA. Gaining a better understanding of the non-cell autonomous processes involved in SMA will help elucidate the mechanisms of motor neuron malfunction and can help identify targets for therapeutic intervention.

DISTURBED BDNF / TrkB SIGNALING IN CORTICO-STRIATAL NETWORKS OF PARKINSON'S DISEASE MODELS

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Impaired motor performance is a central symptom in Parkinson's disease (PD). This is caused by dysfunction of the motor loop between cortex and basal ganglia structures. BDNF expression in layer II/III and V cortico-striatal projections plays an essential role in motor learning by modulating synaptic plasticity on cortico-striatal synapses. Depletion of BDNF from cortical neurons causes significant reduction in striatal BDNF levels, along with defects in motor learning and performance. Thus, disturbed BDNF / TrkB signaling on cortico-striatal synapses is thought to contribute to motor symptoms in PD. Using 6-OHDA rats, we found that nigro-striatal dopamine modulates MSN target cell sensitivity for BDNF by control of TrkB cell surface expression. TrkB forms intracellular clusters in DRD1 expressing direct pathway striatal medium spiny neurons (dMSNs) in dopamine depleted rats. These clusters are closely associated with SORCS2 which functions in cell surface expression and the lysosomal recycling pathway. Analyses of FACS enriched direct pathway striatal medium spiny neurons (dMSNs) confirmed that DRD1 activation promotes TrkB cell surface expression and increases target cell sensitivity for BDNF. In contrast, activation of DRD2 in indirect pathway MSNs reduces TrkB cell surface expression and sensitivity for BDNF. This could have impact for a better functional understanding and the optimization of therapeutic strategies such as deep brain stimulation that is thought to alter circuit function and synaptic activity at DRD1 and DRD2 receptor expressing medium spiny neurons.

INCREASED TRANSGENE EXPRESSION IN RAT RETINAL GANGLION CELLS BY USING HUMAN MINI-PROMOTERS COMPATIBLE WITH RAAV

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Introduction: As retinal gene therapy field matures, it became crucial to improve experimental protocols to facilitate its translation to human trials. Some clinical trials use ubiquitous promoters such as CBA/CMV, which promote high expression level but can cause side effects since they are not cell specific. To limit such effects, specific promoters have successfully been used to target a variety of retinal cell types. However, between the promoters designated for retinal ganglion cells (RGCs), there are some restrictions: too large, not specific, and not strong in promoting expression. Thus, an important step for gene therapies under development for RGC-related disorders is the availability of small promoters, capable of promoting specific gene expression at suitable levels of expression for the therapy.
Aim: In this work, we assess the capacity of transgene expression in RGCs of six mini-promoters (MiniPs) designed by bioinformatics tools
Methods: MiniPs sequences were packaged in rAAV vectors driving the direct reporter green fluorescent protein (GFP). Adult Lister Hooded rats (CEUA #083/17) had intravitreal injection of rAAV vectors and their retinas were immunolabeled 4 weeks post-injection and analyzed by confocal microscopy. To quantify the number of GFP-expressing RGCs, we used the RCG marker, Brn3a. Moreover, Image J software was used to determine GFP intensity in individual GFP-expressing Brn3a+ cells. Statistics were assessed using one-way analysis of variance with Dunnett's post hoc test in GraphPad Prism 7.00 software. $p < 0.05$.
Results: Our results demonstrate that different retinal cell types can be efficiently target using the rAAV-MiniPs, especially RGCs. Indeed, three MiniPs, Ple25 2kb (36.43%±5.49; n=5); Ple25 0.75kb (37.45%±5.79; n=5); and Ple53 (43.36%±3.52; n=5), have potentially increased the number of RGCs expressing the transgene compared to ubiquitous promoter CBA/CMV (11.25%±3.88; n=5). Furthermore, quantification of GFP fluorescence intensity in individual Brn3a-positive RGCs showed that MiniPs Ple53 drove strong GFP expression in RGCs with significantly higher intensity levels at 1.31-fold increase.
Conclusion: Thus, this study identified a functional set of human mini-promoters suitable for AAV-mediated ocular gene delivery applicable in basic and preclinical research, making them potential tools for gene therapies where preferential transgene expression in RGCs is desired.

ABERRANT GANGLIOSIDE METABOLISM IN GLAUCOMATOUS NEURODEGENERATION AND PROSPECT FOR ITS MODULATION

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Glaucoma represent a group of progressive blinding diseases characterized by slow degeneration of retinal ganglion cell (RGC) axons. Advent of power full omics tools within last decade has made a comprehensive quantitative evaluation of almost all members of different lipid classes. Our evaluation of human cadaveric control and glaucomatous optic nerve (ON) demonstrated accumulation of GM3 and simultaneous decreased levels of GM1 gangliosides. This is consistent with our prior lipidomics analyses. The bioinformatic analysis of available genomic datasets suggested impaired levels of several interconversion enzymes in the pathways leading to ganglioside synthesis as well as within the different ganglioside conversion branched pathways. Our proteomic analysis is consistent with findings from analyses of genomic data.
We next utilized a mouse model (DBA/2J) of glaucoma. A genomic large dataset of DBA/2J clinically well characterized with respect to elevated intraocular pressure, the major risk factor in glaucoma, ON structure and electrophysiological function (non-invasive longitudinal follow up) is available that correlates with genomic mRNA analyses of the ON. Our analyses is consistent with findings in human optic nerve of elevated ASAH1 in glucosylsphingosine pathway as well as HexA and SLC33A1 enzymes in the ganglioside interconversion pathway. These findings set us to evaluate prospect of modulation of these enzymes during induced regeneration of RGC axons after injuries. This is consistent with findings of reverse level of ganglioside, glucosylsphingosine and other sphingolipids in regenerative ON compared to glaucomatous ON.

NEUROPEPTIDE DEFICIENCY INDUCES REACTIVE ASTROCYTES, UPREGULATES IMMUNE PROCESSES, AND INDUCES AD-LIKE HIPPOCAMPAL DYSFUNCTION.

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The dentate gyrus (DG) of the hippocampus has many important functions, involving learning, memory, and mood regulation, and also contains a specialized local niche that supports the production of adult-born neurons. Neurodegenerative conditions such as Alzheimer's disease (AD) or aging are typified by altered immune states, and are known to have concurrent reductions in the production of newborn cells. How activity states of the local niche cells interact at the circuit level to ensure continuous neurogenesis and healthy hippocampal function remains unknown. Here, we report that an endogenous neuropeptide, cholecystokinin (CCK), released from dentate CCK interneurons, modulates DG niche cells and robustly controls NSCs through astrocyte-mediated glutamatergic gliotransmission. These DG-CCK interneurons however, begin displaying degenerative signs in an animal model of AD, exhibiting dystrophic neurites by 4.5 months of age. Furthermore, viral-mediated injections of shRNA against CCK into the DGs of wild-type animals cause several AD-like phenotypes, including: induction of reactive astrocytes, upregulation of genes involved in immune processes, reductions in stem-cell proliferation, and behavioral impairments in hippocampal-dependent memory tasks. To determine relevance to human disease, hippocampal lysates from human AD patients were donated and found to exhibit reduced levels of endogenous CCK peptide as compared to age-matched healthy individuals. Our findings provide novel circuit-based information on how the tone of CCK signaling in the DG may be a contributing factor in the loss of cognitive function in neurodegenerative disease through recruitment of inflammatory signaling, activation of immune processes, and the loss of newborn neuron production.

DEVELOPMENT OF THE FIRST SELECTIVE CHEMICAL PROBE FOR THE PLEIOTROPIC KINASE CK2, AN EMERGING TARGET IN NEURODEGENERATIVE DISEASE.

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Protein kinase CK2 (casein kinase 2) has received a plethora of attention in oncology due to evidence that it is overexpressed in many cancer subtypes as well as its reported participation in cell survival pathways. CK2 is also highly expressed in the mammalian brain and has many validated substrates that are critical in neuronal or glial homeostasis and signaling processes across synapses. These roles have indicated that CK2 is a key regulator of neuronal function and may represent a novel target to treat neurodegenerative diseases. Building upon a wealth of published knowledge surrounding the pyrazolopyrimidine scaffold, we designed a small library around the most selective small molecule CK2 inhibitors reported. Through extensive evaluation of this library we identified SGC-CK2-1 as a potent, selective, and cell-active CK2 chemical probe. Remarkably, despite years of research pointing to CK2 as a key driver in cancer, our chemical probe did not elicit an antiproliferative phenotype in >90% of more than 140 cell lines tested. While many publications have attempted to characterize CK2 function, CK2 biology is complex and a high-quality chemical tool like SGC-CK2-1 will aid in connecting CK2 functions to phenotypes. Development of SGC-CK2-1 will enable exploration of the putative role of CK2 in neurodegenerative diseases for the first time.

POTENTIAL INEFFICIENCIES IN THE GENERATION OF RNA-SEQ DATA FROM AN ALZHEIMER'S DISEASE-LIKE MUTATION ZEBRAFISH MODEL

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Ribonucleic acid sequencing (RNA-seq) has emerged as a ubiquitous and valuable tool for understanding the molecular basis of neurodegenerative diseases. As our ability to generate gene expression data has progressed, so too has our understanding of a number of associated technical biases. Techniques have evolved to remove these undesired effects, however, there are still frequent observations of complex bias from unknown origins. Technical variance in RNA-seq can be attributed to the library preparation and sequencing procedures that involve a series of complex chemical reactions. The flexibility of RNA-seq has also allowed for the development of many different protocols depending on the desired outcome. As such, the data resulting from an RNA-seq experiment contains properties dependent on the library preparation and sequencing strategy chosen. Comparison of two datasets generated from two families of zebrafish harbouring the same familial Alzheimer's disease-like mutation showed stark differences in gene expression patterns. The variability between the datasets is too substantial to be explained by biological variability, and therefore must be impacted by sizeable technical artefacts. As the datasets were generated by different sequencing providers, it is likely that differing library preparation protocols are the source of such observations. Further evidence upon examination of additional familial Alzheimer's disease mutation models has provided reason to believe that particular sample characteristics can amplify these effects or even cause additional undescribed artefacts. For example, oxidation of RNA molecules has been shown to occur in the early stages of neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. The consequences of RNA oxidation are relatively unexplored, especially in the context of RNA-seq studies of neurodegeneration. Our work highlights a number of aspects of RNA-seq that need to be carefully considered when designing an RNA-seq experiment. We also consider previously undescribed sources of RNA-seq technical bias relevant to neurodegeneration.

TARGETING RNA-BINDING PROTEIN MSUT2 FOR THE TREATMENT OF TAUOPATHIES INCLUDING ALZHEIMER'S DISEASE.

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Abnormal neuronal deposition of the protein tau is a hallmark of Alzheimer’s disease and a host of disorders termed tauopathies. Our lab has previously identified and validated the RNA-binding protein MSUT2 as a determinant of toxic tau accumulation. We have shown that overexpression of MSUT2 in a mouse model of tauopathy exacerbates tau pathology leading to behavioral deficits, while MSUT2 KO in these animals prevents tau-mediated neurodegeneration. Although the precise mechanism of MSUT2 induced neurotoxicity is largely unknown, we have demonstrated that the RNA-binding domain is required for toxic tau accumulation. This RNA-binding domain, consisting of five zinc finger domains, primarily interacts with poly(A) and has a role in overall poly(A) tail length of mRNA transcripts. Because we have shown this region to be critical to MSUT2-induced tau toxicity, we hypothesize that inhibiting the MSUT2 ZF:poly(A) RNA interaction will prevent toxic tau accumulation. Here we present our in vitro screening paradigm for targeting the MSUT2:RNA interaction including Alpha Screen, Fluorescence Polarization, and TR-FRET modalities, a cell model of target engagement, as well as results from our latest drug discovery initiative.

ELUCIDATING ROLE OF LOCAL SECONDARY STRUCTURE IN MODULATING AMYLOID AGGREGATION OF INTRINSICALLY DISORDERED PROTEINS

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Amyloid fibrils are highly stable cross-β sheet protein assemblies that are a hallmark of neurodegenerative diseases. Fibril formation is driven by short sequences called amyloid motifs, which compromise the core of the fibril and are sufficient to drive assembly. Structure-based approaches predicting amyloid motifs suggest their ubiquitous presence in proteins. Still, only a relatively small fraction of proteins adopt cross-β structures in cells. In globular proteins, which fold into stable tertiary structures, the amyloid motifs are either buried or interacting in local secondary structures that prevent self-assembly. However, a subset of the human proteome encodes intrinsically disordered proteins (IDPs), which must balance between the exposure of functionally important binding epitopes and amyloid motifs. Yet, there is little known on how aggregation is regulated in IDPs in the absence of a stable tertiary structure. In the intrinsically disordered microtubule-associated protein tau (MAPT), several disease-associated mutations localize near its amyloidogenic sequences, such as 306VQIVYK311. Through cross-linking mass spectrometry, peptide systems, and in-silico modeling we have shown how these mutations are disrupting local structure in tau. These disruptions, therefore, increase the exposure of amyloid motifs and drive aggregation. I have developed a computational pipeline to identify novel local secondary structure elements that encode amyloid motifs in both globular proteins and IDPs. I experimentally validated their aggregation behavior and used molecular dynamics to identify key interactions that stabilize compact conformations. This strategy could reveal previously unidentified regulatory elements in IDPs that can prevent aggregation in protein misfolding diseases that lead to neurodegeneration.

PROTEOMIC ANALYSES OF INDUCED MICROGLIA UNCOVER PATHWAYS RELEVANT TO ALZHEIMER'S DISEASE

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Microglia are the resident immune cells of the central nervous system and important for brain homeostasis. Microglial activation is highly dynamic and can be triggered by pathogen-associated molecular patterns (PAMPs) and/or endogenous damage-associated molecular patterns (DAMPs). Microglial activation mediates neuroinflammation and neurodegeneration which are common features of many neurodegenerative diseases such as Alzheimer's disease (AD). Understanding the microglial proteome will help to define the specialized functions of microglia in maintaining brain homeostasis or disease progression. In this study, we utilized data-independent acquisition mass spectrometry, a high-throughput label-free quantitative proteomics approach to determine the global proteomic signatures from an in vitro induced microglia-like cells (iMGLCs) derived from peripheral monocytes of three individuals. For comparison, in vitro induced macrophages (iMACs) were also generated from the same individual's monocytes. Principal component analysis showed that iMGLCs were distinct from iMACs; 795 differentially expressed proteins were statistical significant (p-value < 0.01). Among the top, upregulated proteins in iMGLCs were IL-34, CLEC5A, HDDC2, TRAF3IP3 and LAMC1 whereas F9, IGFBP2, HMGCR, ALB and RNF214 were the top downregulated proteins. To gain further biological insights into the microglial proteome, we conduced gene ontology enrichment and pathway analyses via iPathwayGuide. Highly upregulated biological processes in iMGLCs were extracellular matrix organization, amyloid fibril formation, and lipid metabolism. iMGLCs' upregulated pathways compared with iMACS included PPAR signaling, complement and coagulation cascades, and AD. Interestingly, a total of 31 AD-associated proteins were differentially expressed in iMGLCs suggesting the importance of the microglial regulation in the AD progression. Overall, our study provides a comprehensive proteomic assessment for human microglia representing important biological processes and diseases-relevant pathways. Our data highlights the potential of moocyte-derived human microglia for targeting and manipulating microglia specific responses in many neurodegenerative diseases, including AD.

THE MEF2 NETWORK IS A KEY REGULATOR OF COGNITIVE FUNCTION AND CONFERS RESILIENCE TO NEURODEGENERATION

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Recent increases in human longevity have been accompanied by a rise in the incidence of dementia, raising an important question about how to preserve cognitive functioning in an aging population. Interestingly, a small percentage of individuals with pathological hallmarks of neurodegenerative disease are able to maintain normal cognition. The molecular mechanisms that govern this neuro-protected state remain unknown, but individuals that exhibit cognitive resilience (CgR) represent a unique source of insight into potential therapies that could preserve brain function in the face of aging and neurodegenerative disease. In both humans and animal models, living in an enriched, cognitively stimulating environment is the most effective known inducer of CgR. To gain insight into potential modulators of this phenomenon, we began by studying the molecular changes that arise from environmental enrichment in mice. Global chromatin and transcriptomic profiling in cortical neurons led to the identification of Mef2a/c, a group of transcription factors (TFs) induced by neuronal activity. Conditional knockdown of Mef2a/c prior to enrichment blocked the cognitive enhancement typically afforded by an enriched environment. In order to assess the importance of Mef2 activity in regulating CgR in humans, we next turn to repositories of clinical and brain transcriptomic data, where we find that levels of MEF2C are positively associated with cognitive ability, and that MEF2 target genes are significantly overrepresented among the genes that are most predictive of cognition. Through single-nucleus RNA-sequencing of cortical tissue from a cohort of resilient and non-resilient individuals, we pinpoint upregulation of MEF2C and its transcriptional network in resilient individuals to a subpopulation of cortical, excitatory neurons. Finally, to determine the causal impact of Mef2 on cognition in the context of neurodegeneration, we overexpress Mef2a/c in the PS19 mouse model of tauopathy. Remarkably, Mef2 overexpression alone is sufficient to improve cognition and reduce hyperexcitability in PS19 mice. Overall, our findings reveal a novel role for MEF2 TFs in promoting cognition and cognitive resilience, highlighting their potential as novel biomarkers or therapeutic targets for healthy aging and neurodegeneration.

A GNEO-GCASE CONJUGATE DESIGNED TO TARGET NEURODEGENERATIVE GAUCHER DISEASE

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Mutations in both copies of the GBA1 gene, which encodes a lysosomal hydrolase known as glucoslyceramidase (GCase), can result in the lysosomal storage disorder Gaucher disease (GD). Neurodegenerative symptoms occur in two forms of GD, and mutations in a single copy of the gene are further linked to Parkinson's Disease. Enzyme replacement therapy (ERT) using intravenous delivery can alleviate non-neuronal GD symptoms, but the blood-brain barrier presents an obstacle to treating neuronal symptoms in GD as well as Parkinson's Disease. This work aims to address the accessibility problem by conjugating recombinant GCase to Guanidinylated Neomycin (GNeo), a small molecule known to enhance cellular uptake and lysosomal delivery through a heparan sulfate-dependent pathway. Recent work has shown that intranasal delivery of GNeo conjugates of another lysosomal enzyme, α-L iduronidase, could enter and clear substrate throughout the brain of a mouse model, and we are undertaking a similar approach for GCase. At this point, recombinant GCase has been obtained using the ExpiCHO expression system, and fully active GNeo-GCase conjugates have been prepared. Uptake is being assessed first in human GD fibroblasts, using enzymatic activity in cell lysate to confirm cell entry. Further experiments will be performed with mixed neuronal cultures from a GD mouse, and we are also preparing a fluorophore-modified version of GNeo to visualize cell entry and localization. Finally, we will assess lysosomal storage and α-synuclein clearance in GD mouse models using the intranasal delivery system described above for iduronidase.

BRAIN TRANSCRIPTOME ANALYSES OF ZEBRAFISH EARLY-ONSET FAMILIAL ALZHEIMER'S DISEASE GENETIC MODELS REVEAL EFFECTS-IN-COMMON ON IRON HOMEOSTASIS, ENERGY METABOLISM AND PROTEIN SYNTHESIS IN YOUNG ADULT BRAINS.

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Alzheimer's disease (AD) develops silently over decades. To delay or prevent the onset of AD, we must understand the initial molecular stresses that drive the disease. However, we cannot easily investigate these changes in great molecular detail in humans as we cannot access pre-symptomatic brain material. Therefore, use of animal models is warranted.
The vast majority of AD cases arise sporadically with a late age of onset of > 65 years of age (LOAD). However, in rare cases, AD arises with an onset of < 65 years of age (EOAD). Some of these cases are familial (EOfAD) due to heterozygous mutations in either *PRESENILIN 1/2* (*PSEN1* and *PSEN2*), *AMYLOID β A4 PRECURSOR PROTEIN* (*APP*) or *SORTILIN-RELATED RECEPTOR 1* (*SORL1*). We have created a suite of endogenous gene (knock-in) models of these disease-causing mutations in zebrafish, and have performed RNA-seq analyses on whole brains from young adults to predict the cellular functions altered by such mutations when heterozygous (i.e. replicating, as closely as possible, the human disease genetic state). These cellular processes may give insight into the early pathological changes in brain which eventually lead to EOfAD. Analysis of single families of heterozygous mutants and their non-mutant siblings raised together in an identical environment (the same tank) reduces both genetic and environmental noise and improves statistical power to detect subtle effects due to the mutations.
We have identified that genes involved in oxidative phosphorylation, and encoding ribosomal subunits, appear altered in-commonly by different EOfAD-like mutations in different genes. We have also found evidence that iron homeostasis is altered in young zebrafish EOfAD-like mutation carrier brains, as shown by changes to expression of genes which have iron-responsive elements in the untranslated regions of their mRNAs. Analysis of publicly available RNA-seq data of brains from human allele-replacement *APOE* mice (a knock-in model of LOAD) revealed similar transcriptomic signatures similar to our zebrafish EOfAD models. This provides molecular support for the identity of EOfAD and LOAD as similar disease states.
Together, our transcriptomic analyses imply changes to iron homeostasis, energy metabolism and protein synthesis as early cellular disturbances driving development of EOfAD pathology.

Characterizing the Proteome of Tau Aggregation at its Initiation

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Tauopathies are characterized by aggregation of the tau protein. Propagation of tau prions, whereby aggregated tau taken up by a cell recruits endogenous tau monomer to a growing assembly is hypothesized to underlie disease progression. This phenomenon, termed “seeding,” is robust in cell and animal models. The molecular mechanisms that underlie this process, however, remain unknown. We seek to identify the cellular factors that govern intracellular seeding by tau assemblies at the initiation of this process. We are using ascorbate peroxidase (APEX2)-dependent proximity labeling to identify the aggregation-specific tau proteome. Repeat domains of wild type (WT), anti-aggregation dual proline (2P), and pro-aggregation prone P301S tau were tagged to the split fragments of APEX2 (sAPEX) and overexpressed in HEK 293T cells. These cells were transduced with recombinant full-length WT tau fibrils via cationic lipid complexes and seeding was allowed to occur for five hours post-treatment. Proteins biotinylated by the enzymatic activity of reconstituted sAPEX at this early stage of the aggregation process were identified using streptavidin pull downs followed by tandem mass tag mass spectrometry. A list of hits was established and we are determining the role of these proteins in the aggregation process using CRISPRi/a targeting the genes involved.

Glymphatic function persists after partial *aqp4* mRNA knockdown

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The glymphatic system is a brain waste clearing system which is impaired in many neurodegenerative diseases. It involves the influx of cerebrospinal fluid (CSF), mediated by the water channel aquaporin 4 (AQP4), into the brain parenchyma, where the CSF can exchange nutrients and waste substances with the interstitial fluid (ISF). Thus far, to study the effects of lack of AQP4 on glymphatic function, AQP4 knockout mouse lines have been used. However, they are characterized by a constitutive lack of AQP4 and do not enable studies where a spatially and temporally controlled removal of AQP4 is of interest. In this study, we assessed glymphatic function in wild type (WT) mice 4 weeks after they were injected unilaterally in the lateral ventricle with *aqp4* specific antisense oligonucleotides (ASOs). We observed a 50% reduction of *aqp4* mRNA in the knockdown model and a trend towards less glymphatic influx in the brain, while the levels of AQP4 protein and its polarization on astrocytic endfeet were not significantly changed. Our results suggest that a significant reduction in *aqp4* mRNA can be achieved using ASOs and that it lasts at least 4 weeks. However, further modifications of the experimental design are necessary to achieve impairment of glymphatic function.

SYNERGISTIC TOXICITY BETWEEN TAU AND AMYLOID DRIVES NEURONAL DYSFUNCTION AND NEURODEGENERATION IN TRANSGENIC *C. ELEGANS*

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Aggregates of Aβ peptide and the microtubule-associated protein tau are key molecular hallmarks of Alzheimer's disease (AD). However, the interaction between these two pathologies and the mechanisms underlying disease progression have remained unclear. Numerous failed clinical trials suggest the necessity for greater mechanistic understanding in order to refine strategies for therapeutic discovery and development. To this end, we have generated a transgenic *Caenorhabditis elegans* model expressing both human Aβ1-42 peptide and human tau protein pan-neuronally. We used behavioral, morphological and biochemical assays to assess pathological progression. We observed exacerbated behavioral dysfunction and age-dependent neurodegenerative changes in the Aβ;tau transgenic animals. Further, these changes occurred in the Aβ;tau transgenic animals at greater levels than worms harboring either the Aβ1-42 or tau transgene alone and interestingly without changes to the levels of tau expression, phosphorylation or aggregation. Functional changes were partially rescued with the introduction of a genetic suppressor of tau pathology. Taken together, the data herein support a synergistic role for both Aβ and tau in driving neuronal dysfunction seen in AD. Additionally, we believe that the utilization of the genetically tractable *C. elegans* model will provide a key resource for dissecting mechanisms driving AD molecular pathology.

FUS OVEREXPRESSION IS LINKED TO ALTERED HISTONE POST-TRANSLATIONAL MODIFICATIONS IN AN AMYOTROPHIC LATERAL SCLEROSIS YEAST MODEL

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Amyotrophic Lateral Sclerosis (ALS) is a debilitating and incurable disease. As our population ages, the incidence of ALS has increased and we still know little about what causes it. Further complicating matters, the overwhelming majority of diagnoses are sporadic, meaning there is no family history of the disease. Recent evidence points to the contributions from epigenetic mechanisms to ALS disease etiology and progression. Although the majority of ALS cases do not involve a specific gene mutation, over 40 genes have been associated with the disease, including FUS. Epigenetics is broadly defined as any change in gene expression where the underlying DNA sequence is unaltered. Chromatin, a complex primarily involving DNA and histone proteins, controls access to the DNA. Histones post-translational modifications (PTMs) help regulate transcription by controlling access to transcriptional machinery. We have previously categorized the histone PTM landscape of a FUS overexpression yeast model of ALS, finding that there is significantly decreased levels of histone H3 phosphorylation at serine residue 10 (H3S10ph) and H3 acetylation at lysine residues 14 (H3K14ac) and 56 (H3K56ac). We now show that inhibiting protein phosphatase 1/2A (PP1/2A) with Okadaic Acid (OKA) and histone deacetylases (HDACs) with Trichostatin A (TSA), which are responsible for the removal of the histone PTMs H3S10ph and H3K14/56ac, respectively, reduce the toxicity associated with FUS overexpression in yeast. Our results suggest that epigenetic mechanisms play an important role in ALS and are an attractive new target in the development of novel therapeutics and treatments for this devastating disease.

A MODEL OF TRAUMATIC BRAIN INJURY USING HUMAN IPSC-DERIVED CORTICAL BRAIN ORGANOIDS.

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Traumatic brain injury (TBI) is a significant burden on healthcare systems with nearly 70 million cases worldwide, and increasing evidence suggests that TBI constitutes a major environmental risk factor for dementia and other chronic neurodegenerative diseases. Pathologic hallmarks of TBI have been well characterized and include neuronal aggregates of misfolded, hyper-phosphorylated tau and TDP-43, axonal injury, astrogliosis, excitotoxicity, metabolic dysfunction and progressive neural degeneration. However, how acute mechanical injury contributes to long-term neurodegeneration and dementia is poorly understood, in part due to differences between current rodent and cellular TBI models with degenerative and biophysical properties of the human brain, respectively. As a result, available treatment options remain limited and are largely ineffective. To address these issues, we have developed a unique system of focused ultrasonic injury to mimic TBI *in vitro* using a 3-D cortical organoid culture system grown from human induced pluripotent stem cells (iPSCs) which retains key aspects of human cellular diversity and cytoarchitecture. Our results show that mechanically injured organoids recapitulate key hallmarks of *in vivo* TBI, including increased phosphorylated tau and TDP-43 aggregation, metabolic dysfunction, and neurodegeneration. A genome-wide CRISPR interference screen on injured organoids identified ion homeostasis as a major regulator of neuron survival following traumatic injury. Specifically, we find that injury induces activation of mechanosensitive ion channels (MSCs), and that pharmacologic inhibition of MSCs partially rescues neuronal survival and injury-induced phenotypes. Moreover, we find that iPSC-organoids derived from frontotemporal dementia patients with a V337M disease-causing mutation in *MAPT* produce higher levels of phosphorylated tau and TDP-43 upon mechanical injury compared to CRISPR-corrected isogenic controls. This suggests that the *MAPT* mutation sensitizes neurons to degenerative mechanisms in TBI. Together, these studies enhance the current understanding of TBI pathogenesis by establishing MSC activation as a potential therapeutic target and provides a platform to integrate environmental and genetic contributions to neurodegeneration while preserving human-specific biology.

PRO-COGNITIVE AND MORPHOLOGICAL EFFECTS OF SPECIFIC GABA A RECEPTOR SUBUNIT MODULATION IN A MODEL OF CHRONIC STRESS

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Chronic stress has an established connection to anxiety, depression and Alzheimer's disease, all of which are linked to reduced cognitive performance and neuronal atrophy. Somatostatin neurons and postsynaptic α5-GABAA receptor functions are related to mood and cognition and are reduced in chronically stressed rodents. Common GABAergic drugs, such as Benzodiazepines acting at α1,2,3,5-GABAA receptors cause many side effects (sedation, dependence, amnesia) mixed with mild anxiolytic effects, and limited cognitive benefits. First, this study aims to evidence beneficial properties of positive allosteric modulation (PAM) devoid of the α1-GABAA receptor activity and associated side-effects, for improvement of mood and cognitive deficits induced by unpredictable chronic mild stress (UCMS). GL-II-73 and GL-I-54 have preferential PAM selectivity at α5-GABAA receptors followed by selectivity for α3 and α2. Acute and chronic (N=48 per regimen; 50% female) administration of a GL-II-73/GL-I-54 racemic mixture (termed "GL-RM") improved cognitive performance of UCMS exposed mice (acute: $p=0.0022$; chronic: $p=0.0006$). Several anxiolytic and depressive phenotypes induced by UCMS were also affected by GL-RM. Second, this study aims to assess a chronic regimen on morphological features in brain regions involved in cognition. Golgi staining morphological analysis (N=12) of pyramidal neurons in the prefrontal cortex (PFC) and hippocampus CA1 found chronic GL-RM rescued spine density depletions caused by UCMS at apical and basal dendrites ($p<0.0001$ in PFC and CA1). Spine densities were correlated to cognitive performance and exhibited ameliorative benefits of GL-RM. Together, results support selective α-subunit targeting of GABAA receptors to overcome UCMS induced cognitive deficits and detriments in neuronal morphology. Stress is a significant risk factor for many disorders affecting cognition and both anxiety and depression are prodromal states of neurodegenerative disorders such as Alzheimer's disease. This study supports investigation of α5-GABAA receptors in other neurodegenerative disorders affecting cognition and considers GL-RM as a potential targeted therapeutic.

COI: Authors TDP, GL, JMC and ES are listed inventors on PCT application ##62805009 for GL-II-73 and GL-I-54. All other authors declare no conflicts of interest.

BIOLOGICAL SEX MODIFIES THE IMPACT OF HEAT SHOCK PROTEIN 70 IN α-SYNUCLEINOPATHIES

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Lewy body disorders are characterized by hallmark inclusions composed partly of aggregated α-synuclein. Misfolded, aggregated proteins can be refolded or degraded by the coordinated activities of chaperones such as heat shock protein 70 (Hsp70) and autophagic/proteasomal disposal systems. Men are at greater risk of Lewy body disorders than women, but the impact of biological sex on chaperones in Lewy body disorders is unclear. We examined chaperones in olfactory bulb (OB) and amygdala tissues acquired via Neurobiobank (UCLA and U Miami cohorts). In the UCLA cohort, women with Lewy body disorders showed higher Hsp70 expression in both brain regions than age-matched male counterparts (n=3/group). This effect was not observed in the Miami cohort (n=3-4/group), but a statistical interaction between sex and disease state was noted in Hsp70 expression in OB tissues from both cohorts. Next, we treated primary hippocampal mixed neuron/astrocyte cultures from male or female rat pups with α-synuclein fibrils and an inhibitor of Hsp70, VER155008. Male cultures were more vulnerable to cell death after VER155008 exposure than female cultures. Fibril-treated hippocampal cultures from both sexes responded to VER155008 with a ~2-fold increase in α-synuclein+ inclusions in the somata, but VER155008 led to an increase in the average size of all inclusions (somal & neuritic) in male cells only. Hsp70 is known to be released into the extracellular space, where it serves as an immunogenic signal and may also restrict the intercellular transmission and propagation of α-synucleinopathy. Thus, we treated primary hippocampal cultures with α-synuclein fibrils and low-endotoxin, exogenous Hsp70 (eHsp70). eHsp70 reduced α-synuclein+ inclusions in male, but not female cells. α-synuclein fibrils elicited mild cell loss, but only in male cells, and this toxicity was prevented by eHsp70. Finally, we delivered eHsp70 through the nostrils of 20-month-old male mice that were infused in the OB with α-synuclein fibrils. Daily eHsp70 delivery for 28d reduced inclusion counts and the latency to locate buried food. eHsp70 entered the OB, limbic system, brainstem, and spinal cord of male mice within 3h, but was cleared within 72h. Unexpectedly, female mice did not display uptake of eHsp70 from the nose into the brain at any time. These findings reveal sex differences in the function of Hsp70 and support its therapeutic potential in Lewy body disorders.

IMPAIRED CHOLESTEROL METABOLISM IN GLAUCOMA

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Glaucoma refers to a group of irreversible blinding diseases. Primary open angle glaucoma (POAG) is the most common form. The neurodegeneration of the optic nerve (ON), specifically the damage and/or impairment of retinal ganglion cell axon is the characteristic feature of these diseases. Progressive peripheral vision loss occurs without and pain or warning in glaucoma. We found aberrant accumulation of Zymosterol, an intermediate species in post-squalene cholesterol synthesis pathway in the aqueous humor (AH) as well as in the ON of POAG compared to control human donor eyes. The DBA/2 mouse is a model of glaucoma, characterized by a spontaneous elevation of intraocular pressure (IOP), a risk factor for POAG. They are heterogenous with respect to IOP elevation, ON damage and vision loss. In a cohort of DBA/2J AH Zymosterol accumulation occurred in those DBA/2J mice that presented elevated IOP for next 30 days but not those where IOP remained within normal range. Parallel finding of elevated Zymosterol was found in the cadaveric human optic nerves in POAG as noted above. Accumulation of Zymosterol was commensurate with elevated levels of Nsdhl, HSD17B7 and Sc4mol, the enzymes that catalyze preceding steps of Zymosterol conversion and reduced Ebp level respectively. The Ebp feeds on Zymosterol for next conversion step in the post-squalene pathway. The glaucomatous AH also is characterized by accumulation of 15-alpha-hydroxycholestane and aberrant level of TM7Sf2, an enzyme that is involved in conversion of this species in the pathway. Zymosterol accumulation AH is consistent with elevated acetate availability in the POAG AH.

Our mass spectrometric analysis also revealed increased total unsaturated sterol and decreased total unsaturated cholesterol in glaucoma. Cholesterol esters were significantly less unsaturated in glaucomatous optic nerves. Lower unsaturation corresponds with increased stiffness of the plasma membrane. Exogenous lipid additions to alter the lower unsaturation is consistent with lowering the stiffness (or elastic modulus) and improved inner retinal visual function as evidenced by electrophysiological pattern electroretinogram (PERG) amplitude. The cholesterol species distribution of regenerating optic nerve (after crush injury) is reverse of that observed in glaucoma. Finally, we found that visual function in HSD17B7, Nsdhl inhibitor treated DBA/2J eyes with or without Ebp level elevation is improved as measured by PERG amplitude and/or latency. In conclusion, glaucomatous eyes present cholesterol impairment and their reversal is associated with improved visual function.

DESIGN AND DEVELOPMENT OF PROTEOLYSIS TARGETING CHIMERA AGAINST CYTOPLASMIC TDP-43 IN NEURODEGENERATION

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Neurodegenerative disorders (ND) are increasingly recognized as the major cause of disability and deaths, currently affecting 47.5 million people globally with dementia in people aging 60 or above. One common feature linked to mostly all ND is the misfolding and aggregation of certain proteins, which generates the cascade of pathological events leading to failure to protein homeostasis in the physiological state. TAR DNA binding protein 43 (TDP-43), is one such protein responsible for Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal lobar degeneration (FTLD). Physiologically TDP-43 is a regulator of gene expression, RNA processing, etc. However, the mutation in TDP-43, leads to the excess deposition and formation of misfolded toxic protein aggregates in the cytoplasm of the cell, leading to disease. The aggregation-prone proteins are pharmacologically difficult to target because of their misfolded and disordered structure. To tackle this stumbling block, we contemplated the Targeted Protein Degradation approach and developed a peptide-based Proteolysis Targeting Chimera (PROTAC) against aggregated TDP-43 degradation. PROTAC is a heterobifunctional molecule, composed of two different binding domains connected by a linker. One is for engaging an E3 ubiquitin ligase and the other for the binding to targeted protein. E3 ligase recruitment leads to the ubiquitination of targeted protein, and recognition by the Ubiquitin proteasome system for the degradation. As a protein quality control system, the UPS plays a key role in the degradation of misfolded or aggregated proteins and maintaining protein homeostasis. We synthesized novel PROTAC with Fmoc-based solid-phase peptide synthesis. The purity and characterization of the molecule were done using RP-HPLC and MALDI mass spectrometer. The cell-penetrating ability of the PROTAC was checked in-vitro using the SH-SY5Y neuroblastoma cell line and was characterized via confocal microscopy. We believe that the synthesized novel PROTAC will regulate the aggregated cytosolic TDP-43 towards the degradation, which will be characterized in-vitro.

INVESTIGATING THE ROLE OF MITOCHONDRIAL SIGNALING IN THE MAINTENANCE OF NEURONAL FUNCTION AND DIFFERENTIATION IN THE CONTEXT OF PARKINSON'S DISEASE

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Parkinsons Disease (PD) is characterized by the degeneration of dopaminergic neurons in the Substantia Nigra leading to motor deficits. Overwhelming evidence now suggests that mitochondrial dysfunction is a key feature contributing to the pathology in PD. We have shown that mitochondrial dysfunction can alter the proliferation and differentiation of adult neural stem and progenitor cells, resulting in the dedifferentiation of newborn neurons. Using Opa1 KO as a model for mitochondrial dysfunction, we examined the mitochondrial signaling mechanisms underlying neuronal dedifferentiation. Bulk RNA sequencing was performed in differentiated neurons over a time course of 1-5 days. We have identified a decrease in the expression of genes involved in neuronal differentiation, including those that regulate neuronal excitability and synapse formation. Using bioinformatic tools, we identified the E2F and ATF4 transcription factors as potential regulators of gene expression changes following mitochondrial dysfunction. This evidence led us to hypothesize that mitochondrial dynamics signal in a retrograde manner to the nucleus to repress the expression of genes involved in neuronal differentiation, resulting in impaired neurological function. We will manipulate these key pathways in an effort to restore neuronal function in vitro and in vivo. Elucidating this pathway is key for understanding mechanisms underlying neurodegeneration, and will identify novel therapeutic targets by which to improve neurological function in PD.
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LINKING MOLECULAR BIOMARKERS AND BEHAVIOR IN RODENTS AND PATIENTS WITH ALZHEIMER'S DISEASE.

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Alzheimer's disease (AD) is a debilitating neurodegenerative disorder that causes severe deterioration of memory, cognition, behavior, and the ability to perform daily activities. In its earliest stages, AD is associated with marked neuronal loss and dysfunction localized largely to entorhinal cortex (EC). In particular, morphological and molecular features of entorhinal cells, especially in layer two, relate to increased susceptibility to AD pathology. At a molecular level, the disease is characterized by the accumulation of two proteins in fibrillar form; Amyloid-β forms fibrils that accumulate as extracellular plaques while tau fibrils form intracellular tangles. This project aims to address three current issues within the Alzheimer's research field.

First, the timing of interventions during the disease cascade. By longitudinally inhibiting EC layer two principal cells using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) and following the development of neuropathological hallmarks of the disease in anatomically connected regions, we aim to indicate whether these cells are the origin of the molecular disease cascade. We also aim to label tau after injection into EC layer two cells and follow the cell-to-cell transmission of tau in order to see whether its propagation is activity-dependent.

Second, the translational aspect of staging the AD molecular disease cascade between rodents and human AD patients has remained inadequate. This study will for the first time measure long-term in vivo changes in molecular AD biomarkers in rodents, which will be translated to human AD cerebrospinal fluid (CSF) data. We aim to longitudinally sample CSF using microdialysis of awake, behaving mice, in order to measure neuropathological markers during the disease cascade. Additionally, we aim to use microdialysis in order to longitudinally infuse drugs in rodents aimed at halting the development of neuropathological hallmarks of AD. Our preliminary microdialysis results suggest that the slower the sampling rate, the better the recovery of Amyloid-β and tau proteins from CSF, and the levels of proteins mirror changes observed along the AD disease cascade in patients.

Third, to link the neuropathological processes that initiate the disease with early cognitive symptoms. We aim to use a contextual memory paradigm and predict that animals with advanced AD will perform worse on this task compared to animals at pre-clinical or intermediate AD stages.

EXPANDING THE BOUNDARIES OF HUMAN NEURODEGENERATIVE DISEASE MODELS THROUGH THE INTERSECTION OF ENGINEERING, STEM CELL BIOLOGY, AND SINGLE-CELL TRANSCRIPTOMICS

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The human brain of many individuals is genetically susceptible to neurodegenerative diseases like Alzheimer's disease (AD) or poor recovery from traumatic injury and chemotherapy. However, pinpointing the genetic elements that promote this pathogenesis, establishing their underlying cellular and molecular mechanisms, and developing therapeutic strategies remain major challenges. These challenges are reflected in the growing patient population and the near absence of therapeutics for AD and related dementias. The intersection between engineering, stem cell biology, and single cell transcriptomics offers new approaches to understand the uniquely human biology that is fundamental to neurodegeneration and innovative technology that facilitates the discovery and development of new therapeutic and preventative strategies. We recently applied principles of engineering to reconstruct the human Blood-Brain Barrier in vitro (iBBB). Subsequently, employing the iBBB in conjunction with single-cell transcriptomic studies of post-mortem human brains revealed how the strongest risk factor (APOE4) for AD predisposes vascular amyloid pathology. By pinpointing the cell-type (pericytes), mechanism (increased soluble APOE), and regulatory pathway (calcineurin/NFAT) that APOE4 employs to increase the risk for cerebrovascular AD pathology, we uncovered FDA-approved drugs that can reverse the pathology in vivo. This work validates a promising new approach for investigating and targeting genetic and environmental risk factors that underlie neurodegeneration. We have since incorporated additional cell-types into the iBBB including microglia, neurons, and oligodendrocytes to generate human in vitro models of neuro-immune vascular system, myelination, and ultimately a multicellular integrated human brain on a chip (miBrain). We will present the application of these new model systems to understanding and therapeutically targeting pathologies associated with Alzheimer's disease.

UNBIASED EPIGENETIC AND PHOSPHOPROTEOMIC PROFILING OF *APOE4* NPCS AND ASTROCYTES SUGGESTS NOVEL BIOLOGY AND NEW POTENTIAL THERAPEUTICS

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Current approaches for treatment of AD focus on symptomatic therapy but do not target the underlying molecular causes of the disease. New approaches aimed at understanding the molecular mechanisms involved in this debilitating disease are required in order to improve therapeutic targeting. The E4 allele of apolipoprotein E (*APOE*) is a genetic risk factor for many diseases including Alzheimer's disease (AD), yet its mechanism of action remains poorly understood. Here, we present a resource to explore perturbations to biology arising from possession of the AD risk *APOE4* allele compared to the non-risk *APOE3* allele using both epigenetic and phosphoproteomic profiling. We define a series of *APOE4* "risk signature" profiles, from which we hypothesize novel biology. We further leverage a recently developed, novel data analysis pipeline to deeply interrogate phosophoproteomic changes associated with APOE4, detailing thousands of phosphosite changes and allowing prediction of putative kinase involvement. Finally, we developed a novel platform for unbiased drug discovery by profiling the epigenetic and phosphoproteomic effects of a library of drugs, using the Neuro-LINCS library and querying 119 unique compounds. From these data, the net effect of drug treatment on histone marks and key phosphosignaling marks can be used to predict which drugs may shift a given profile towards either an "AD-risk" state or "control" state. We validate key drug predictions in GCP profile, and P100 drug predictions against AD related defects in astrocytes. These new datasets provide a rich resource for understanding fundamental APOE biology and predicting novel therapeutic avenues.

BMP5/7 PROTECT DOPAMINERGIC NEURONS AGAINST A-SYNUCLEIN INDUCED NEUROTOXICITY IN A MOUSE MODEL OF PARKINSON'S DISEASE

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Neurotrophic factors, particularly GDNF and NTN, protect the degeneration of dopaminergic (DA) neurons in toxin-based Parkinson's disease (PD) animal models. This has led to clinical trials aiming to test their therapeutic potential. However, so far, clinical trials have not been able to meet their clinical endpoint. In contrast to toxin-induced DA neurodegeneration, GDNF does not protect substantia nigra (SN) neurons against α-synuclein-induced DA neurodegeneration in a rat PD model. Thus, raising the question as to whether neurotrophic factors can slow or halt α-synuclein-induced neurodegeneration.

Here, we report the neuroprotective effects of the neurotrophic factors bone morphogenetic proteins 5/7 (BMP5/7) and SMAD1, which mediates BMP receptor activation intracellularly. Moreover, we show that SMAD6, which inhibits BMP signal transduction, is upregulated in PD post-mortem brains.

Using an established viral-vector based mouse model in which the human α-synuclein, containing the A53T mutation, was expressed we found that striatal injection of viral vectors expressing BMP5/7 fully prevented the loss of DA neurons and striatal projections that was induced by α-synuclein overexpression. Associated with the prevention of DA neuronal loss, α-synuclein induced gliosis, as studied by activated microglia and astrocyte markers, was significantly reduced by BMP5/7 treatment. Moreover, BMP5/7 could significantly improve α-synuclein induce motor deficits as assessed by the cylinder and pole test.

To gain insights into the molecular mechanisms mediating the therapeutic effects of BMP5/7 we studied their potential to modulate α-synuclein accumulation in DA neurons. α-synuclein treated mice showed a significant increase of cells double-positive for TH and α-synuclein immunostaining and the p-S129 form of α-synuclein, an effect which was significantly reduced by BMP5/7 expression.

To further study the effects of BMPs on α-synuclein, we complemented our BMP treatment experiments with BMP pathway inactivation experiments. We conditionally deleted in embryonic mouse neural stem cells the *Smad1* gene by using a Nestin-Cre driver ($Smad1^{Nes}$). Thus, by deleting the *Smad1* gene, we significantly attenuated intracellular BMP signaling in neurons. In adult $Smad1^{Nes}$ animals, there was a significant increase in TH/α-synuclein positive neurons and proteinase K resistant aggregates in the SN, cortex, and olfactory bulb compared to controls, which was associated with a reduction in DA neurons.

To study the relevance of our findings in humans, we investigated the expression of the inhibitory SMAD6 protein in the SN of PD post-mortem brains. SMAD6 protein was significantly upregulated in the SN of PD patients compared to age-matched controls, providing evidence of a downregulation of the BMP/SMAD pathway in PD. Taken together, we suggest that BMP5/7 are promising disease-modifying drug candidates for PD.

THE NUCLEAR PORE COMPLEX AND NUCLEOCYTOPLASMIC TRANSPORT: STRUCTURAL AND FUNCTIONAL IMPLICATIONS IN ALZHEIMER'S DISEASE

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The nuclear pore complex (NPC) is a large structure composed of more than thirty types of subunits, or nucleoporins (NUPs), which mediate the selective transportation of cargo to and from the nucleus. All molecules larger than 40 kDa are unable to diffuse independently through the NPC and rely on an assortment of cargo-specific protein chaperones, termed transport factors, to efficiently transport the cargo between the nucleoplasm and cytoplasm. Our laboratory has previously shown that nuclear proteins are mislocalized into the cytoplasm in early stages of Alzheimer's disease (AD) progression, suggesting structural changes to the NPC and functional aberrations to nucleocytoplasmic transport in AD. Changes to the NPC have been described in neurodegenerative diseases, including amyotrophic lateral sclerosis, Huntington's disease, and AD. Specifically, in AD tau has been implicated in mislocalization of NUPs from the NPC to the cytoplasm in AD and functional deficits in nucleocytoplasmic transport. Here, we present results from an integrated bioinformatic analysis of NUPs and nucleocytoplasmic transport factor expression in four brain regions (hippocampus, superior frontal gyrus, entorhinal cortex, and postcentral gyrus), as well as from laser-capture microdissected neurons. Immunohistochemistry and Western Blotting were performed on human tissue samples to validate significant results. Our findings indicate expression changes to NUPs throughout the NPC and mislocalization of some NUPs in the cytoplasm in postmortem human tissue in AD, as well as differential expression of multiple transport factors in AD. Together, these results suggest an interactive impact of NPC structural disruption and aberrant nucleocytoplasmic transport in AD. Future work will focus on the mechanistic relationship between AD neuropathology and NPC abnormalities as well as implications of disrupted NPC-nucleocytoplasmic transport to downstream nuclear function in AD.

THE ENDOTOXIN HYPOTHESIS OF NEURODEGENERATION: ROLES OF MICROGLIAL PHAGOCYTOSIS

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The lipopolysaccharide (LPS) endotoxin enters the blood from gut, gums and infections. Elevated blood levels of endotoxin are associated with neurodegeneration, and at those levels, rapidly induce microglial activation in healthy volunteers. Parkinson's disease has: relatively low genetic inheritability, early gut dysfunction and elevated blood endotoxin levels. Endotoxin induces alpha-synuclein aggregation by direct binding, and induces microglial activation via TLR4.

In culture, we find that endotoxin induces microglia to phagocytose neurons and synapses, and the loss of neurons and synapses is prevented by blocking microglial phagocytosis in five different ways, for example, by inhibiting the engulfment receptor P2Y6. Endotoxin induces microglia to: i) release oxidants that stress live neurons to release UDP and expose phosphatidylserine, ii) release opsonins galactin-3, calreticulin and C1q, and iii) desialylate the microglial cell surface, activating TLR4 and complement receptor CR3. All of this enables microglial phagocytosis of live neurons and synapses.

In vivo, injection of endotoxin into striatum of rats induced uptake of neuronal nuclei into microglia, measured at 3 days, and neuronal loss measured at 7 days, both of which were prevented by blocking microglial phagocytosis at P2Y6 or other phagocytic receptors. Recently we found that chronic peripheral endotoxin induced loss of brain neurons, specifically in the substantia nigra, which was prevented in P2Y6 knockout mice. Thus, endotoxin might contribute to loss of neurons in Parkinson's disease, and be prevented by blocking microglial phagocytosis at P2Y6.

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METABOLOMIC ASSOCIATIONS WITH APOE-E4 IN AN ETHNICALLY DIVERSE AGING POPULATION

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A few studies have used untargeted metabolomics to study the relationship between APOE genotype and metabolism, however, not many have considered the interplay between ethnicity, APOE genotype and metabolism. We use an untargeted high-resolution metabolomic approach to profile the plasma of 119 cases and controls of Alzheimer's disease (AD) from the Washington Heights and Inwood Columbia Aging Project (WHICAP), an ethnically diverse aging cohort with more than 2000 participants. Our analysis included 39 individuals of Caucasian ancestry (12.8% APOE-e4 carriers), 40 of African American ancestry (42.5% APOE-e4 carriers) and 40 of Hispanic ancestry (25% APOE-e4 carriers). We determined the relationship between plasma metabolomic features and APOE genotype (APOE-e4 carriers v APOE-e4 non-carriers), controlling for sex and ethnicity, which are known factors associated with APOE genotype and metabolism. We found that in the 60 controls (20% APOE-e4 carriers), APOE genotype was associated with altered omega-6-fatty acid metabolism and de novo fatty acid metabolism, determined using the mummichog pathway analysis algorithm. Features annotated as hexadecanoic acid, eicosanoic acid, butanoic acid, phenyl pyruvate, and sorbitol were associated with APOE genotype and showed different levels of abundance in the three ethnic groups. In the 59 cases (33.9% APOE-e4 carriers), APOE genotype was associated with changes in amino sugars metabolism and beta-alanine metabolism. Features associated with APOE genotype and with different abundances in the three ethnic groups included glutamate, lactate, and a folate metabolite. These findings suggest different associations between APOE genotype and metabolism in different ethnicity, which could be a result of differences in diet and environmental exposures in the ethnic groups. We also see differences in association based on AD status, suggesting changes in plasma metabolism as a result of AD or its treatment. In the future, a targeted analysis for these metabolites of interest will be conducted in this cohort.

REDUCED FUNCTION OF C9ORF72, LEADS TO PRESYNAPTIC DEFECTS AND NEUROMUSCULAR DYSFUNCTION IN ZEBRAFISH

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A hexanucleotide repeat expansion within the C9orf72 gene is the most common genetic cause of amyotrophic lateral sclerosis (ALS) and fronto-temporal dementia (FTD). Reduced levels of C9orf72 mRNA and protein have been found in ALS/FTD patients, but the role of this protein in disease pathogenesis is still poorly understood. To investigate the role of C9orf72 loss-of-function in ALS, we used synthetic micro-RNAs to specifically target and silence the zebrafish C9orf72 gene (C9-miRNA) and generated a stable C9orf72 loss-of-function model in the zebrafish. Upon loss-of-function (LOF) of C9orf72, we observed that zebrafish C9-miRNA mutants displayed severe motor deficits starting at 6 days postfertilization (6 dpf) and a majority die premature as of 15 dpf. Additionally, TDP-43 was found to be mislocalized in C9-miRNA zebrafish. Analysis of the structure and function of the neuromuscular junctions (NMJs) of the C9-miRNA larvae, revealed a significant reduction in the number of presynaptic and postsynaptic structures and an impaired release of quantal synaptic vesicles at the NMJ. We also found a downregulation of SV2a upon C9orf72-LOF and a reduced rate of synaptic vesicle cycling. Furthermore, we observed a reduced number and size of Rab3a-postive synaptic puncta at NMJs. Among the few fishes that survived till adulthood, we also found that these fish exhibited motor defects, muscle atrophy and motor neuron loss. Altogether, this new zebrafish C9-miRNA LOF model replicates aspects of ALS and reveal a key function for C9orf72 in the control of presynaptic vesicle trafficking and release at the zebrafish larval NMJ. Our study demonstrates a novel role for C9orf72 in ALS/FTD pathogenesis, where it regulates synaptic vesicle release and neuromuscular functions.

MANIPULATING PRP GLYCAN STRUCTURE TO UNDERSTAND TOXIC SIGNALING PATHWAYS DRIVING PRION-INDUCED NEURODEGENERATION

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Prion proteins cause an infectious and rapidly progressive neurodegenerative disease characterized by prion aggregates, spongiform encephalopathy, dystrophic neurites, and neuronal death. These processes depend on the neuronal expression of prion protein (PrP^C), which exists on the outer leaflet of the cell membrane as a glycosylphosphatidylinositol (GPI)-anchored glycoprotein containing two variably occupied N-linked glycosylation sites on its carboxy terminus. Previous work has shown that glycan modifications may impact PrP aggregation and neuronal toxicity. To investigate the role of glycans in prion-induced neurotoxicity, we engineered a new knockin mouse model that expresses PrP with an additional glycan. This glycan is sensitive to PNGase F digestion, but not to endoglycosidase H digestion, indicating the presence of a complex N-linked glycan. This mouse spontaneously develops neurodegeneration characterized by spongiform encephalopathy in the CA3 region of the hippocampus and dystrophic neurites. In contrast to other murine models of prion disease, this neurodegenerative pathology develops in the absence of PrP aggregates or infectivity, as shown by several in vitro and in vivo methods. Therefore, this model provides the opportunity to investigate the neurotoxic role of PrP^C, uncoupled from its aggregation. We show that although the extra glycan does not affect PrP^C expression, stability, or turnover in cells, primary hippocampal neurons isolated from these mice display signs of excitotoxicity. Additionally, immunoprecipitation-mass spectrometry (IP-MS) studies reveal that introduction of this third glycan significantly alters the PrP interactome as compared to wild-type littermates. These studies hold relevance to both prion diseases and also other neurodegenerative diseases; PrP^C has been shown to act as a cell surface receptor for extracellular aggregated proteins such as amyloid-β in Alzheimer's disease and α-synuclein in Parkinson's disease. Therefore, understanding PrP^C-mediated neurotoxic signaling can aid in understanding not only prion diseases, but also the neurotoxic pathways instigated by other aggregated, misfolded proteins.

THE RELATIONSHIP BETWEEN COGNITIVE SCORES AND DETECTION OF SPECIFIC ODOURS IN THE ELDERLY.

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Olfactory dysfunction is a common symptom in many neurodegenerative disorders. It is regarded as an early sign of neurodegeneration and indeed as an early predictor of impending cognitive decline. Olfaction dysfunction may be caused by several factors other than ageing, such as but not limited to strokes, infections, head trauma, genetics and/or environmental causes. The goal of this study is to determine if the olfactory dysfunction observed in the elderly is due to a general loss of smell or lack of detection of specific odours, and if that correlates with cognitive scores. Participants for the ORCA study were recruited from the NuAge cohort of which 93 participants (50 females and 43 males, age range 80-94 yrs) agreed to participate. Our study identified the olfactory function and cognitive scores of seniors. The measure of olfaction was done using a scratch and sniff test (UPSIT). Cognitive scores were recorded using the t-MMSE. The results show that females performed better than males in odour identification when detecting specific odours. It also demonstrated that the seniors had severe difficulty identifying lemon, using a cut-off point of <20% . Furthermore, the participants had moderate difficulty identifying pizza, bubble gum, motor oil, banana, fruit punch, cheddar cheese, lime and rose by using a cut-off of <50%. There was a high success rate (>80% cut off) amongst, onion, chocolate, gingerbread, paint thinner, smoke and peanut butter. Detection of the odours pizza, cherry, soap and rose showed a significant difference between sexes. Furthermore, participants who had a lower cognition score (t-MMSE 18-20) had an average of 60% of total odours correctly identified. In contrast, those with t-MMSE scores of 21 - 23 (mid cognitive score) identified 63% correctly, and those with t-MMSE scores of 24 -26 (high cognition score) averaged 67%. Our data reveals that there is a loss of odour detection in seniors for specific scents. The data shows that there is a correlation between cognitive scores and the percentage of correctly identified scents. The relationship between the cognitive scores and scent identification suggests that impending cognitive decline may be due to the lack of detection of specific odours. Our study provides additional support for the testing of olfactory function in the elderly and suggests that loss of smell for particular scents may be a useful diagnostic tool.

mTOR-DEPENDENT TRANSLATION AMPLIFIES MICROGLIA PRIMING IN AGEING

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Microglia maintain homeostasis in the brain. However, with age, they become primed and respond more strongly to inflammatory stimuli. We show here that microglia from aged mice upregulated mammalian target of rapamycin (mTOR) complex 1 signaling regulating translation, as well as cytokine protein levels. Genetic ablation of mTOR signaling showed a dual, yet contrasting effect on microglia priming: it caused an NF-κB-dependent upregulation of priming genes at mRNA level; however, mice displayed reduced cytokine protein levels, diminished microglia activation and milder sickness behavior. The effect on translation was dependent on reduced phosphorylation of 4EBP1 and increased 4EBP1 expression, resulting in decreased binding of eIF4E to eIF4G. Similar changes were present in aged human microglia and in damage-associated microglia, indicating upregulation of mTOR-dependent translation is an essential step licensing microglia priming in ageing and neurodegeneration

NUCLEAR RECEPTOR DELETION IN MYELOID CELLS MITIGATES DISEASE PATHOLOGY IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a highly prevalent neurodegenerative disorder typified by extracellular deposition of β-amyloid (Aβ) plaques, neurofibrillary tangles, robust neuroinflammation, and progressive cognitive decline. Nuclear receptor agonist administration upregulates genes associated with Aβ clearance and phagocytosis and represses inflammatory gene expression to ameliorate these hallmarks in various AD mouse models. In microglia, the brain-resident myeloid cells, these agonists activate liver X receptor (LXR) and peroxisome proliferator-activated receptor (PPAR) target genes, which form obligate heterodimers with retinoid X receptor (RXR). While nuclear receptors play a prominent role in peripheral myeloid cells such as monocytes, how they contribute to modification of AD pathology is unknown. Furthermore, it is unclear whether myeloid-cell selective deletion of RXRs would exacerbate AD pathology, given the well-characterized beneficial roles of nuclear receptors in neurodegenerative diseases. We demonstrate the inducible deletion of the predominant isoform of RXR, RXRα, in either peripheral monocytes or microglia ameliorated aspects of amyloid pathology in the 5xFAD mouse model at both early and late stages of disease. The absence of RXRα from either population reduced plaque burden, curtailed inflammatory gene transcripts, and abrogated neuritic dystrophy. Deletion of RXRα in microglia diminished apolipoprotein E expression and suppressed genes associated with microglial neurodegenerative phenotype in young animals. Remarkably, RXRα-deficiency in blood monocytes reduced parenchymal plaque load and brain inflammation, even though prior studies demonstrated that inflammatory monocytes do not invade the brain in the 5xFAD mouse model of AD. Additionally, RXRα gene expression was upregulated in human AD brain samples, pointing to a potential role in neurodegenerative diseases. Our study reveals the importance of further understanding the nuanced actions nuclear receptors exert in various myeloid subsets during AD progression.

SEX-SPECIFIC DIFFERENCES IN HIPPOCAMPAL BLOOD FLOW AND CEREBROVASCULAR REACTIVITY IN AGED DAHL SALT-SENSITIVE RATS TREATED WITH AN ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITOR LISINOPRIL

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Background: Increase in pulse wave velocity (PWV), a marker of central arterial stiffness (CAS), adversely affects cerebral microvasculature and can lead to reduced brain blood supply, neuronal degeneration and cognitive impairment. Dahl salt sensitive (DSS) rats have a compromised renin-angiotensin system and develop CAS and hippocampal memory decline with age on a normal salt diet. Thus, they represent a model of vascular dementia. We hypothesize that there is a sex difference in the association of PWV and systolic blood pressure (SBP) with hippocampal cerebral blood flow (CBF) and cerebrovascular reactivity (CVR) in response of these parameters to the treatment with an ACE inhibitor, lisinopril (L).
Methods: Female (F; n=23) and male (M; n=32) DSS rats were fed a normal salt diet (0.5% NaCl). Rats were administered L (15mg/kg BW/day in drinking water) or vehicle (control; C) beginning at age 6mo for 6mo (F: n=12/11; M: n=18/14; L/C). At age 12mo, SBP by plethysmography, PWV by echocardiography, hippocampal CBF and CVR by continuous arterial spin labeling MRI were assessed. Data was analyzed using 2-way ANOVA and presented as mean±SE.
Results: Aged F-C and M-C had high SBP (188±9 and 182±7 mmHg) which was reduced by L in F-L and M-L (116±4 and 117±3 mmHg; p=0.00, C vs. L). L decreased PWV in both sexes (F: 4.4±0.3 vs. 5.7±0.4 m/s; p=0.06; M: 4.3±0.1 vs. 5.9±0.3 m/s; p<0.00; L vs. C). CBF was similar in F-C and M-C (189±7 and 179±10 mL/100g/min). L did not affect CBF in F or M. CVR was similar in F-C and M-C (36±10 and 25±4 mL/100g/min). F-L had higher CVR vs. M-L (72±26 vs. 36±10 mL/100g/min; p=0.04) but L did not affect CVR in both sexes. A significant correlation was found between PWV and CBF (r=0.56, p=0.05) and PWV and CVR (r=-0.77, p=0.00) in males only.
Conclusion: Lisinopril effectively ameliorated age-associated midlife hypertension and CAS but did not affect CBF in both sexes of DSS rats, and numerically increased CVR in females only. The association of PWV, but not SBP, with CBF and CVR was found in males. Effect of age on CBF and CVR showed different trends for males and females. Thus, downstream effects of CAS on cerebral microvasculature may vary between sexes in DSS rats. We will analyze neuronal density level and other brain MRI parameters to assess changes in cerebral morphology and their relation to CAS in DSS rat model of vascular dementia.

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EFFECT OF IL-10 AND ITS ANTAGONISM IN MOUSE MODELS OF NEURODEGENERATIVE DISEASES

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Neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, are characterized by the presence of activated glial cells suggesting that immune activation may play a critical role in these diseases. However, recent advances utilizing painstaking single cell analysis have uncovered that this process is complex, with both inflammatory and anti-inflammatory signaling molecules being possibly involved in the neurodegenerative cascade. Previously, we have shown that overexpression of the canonical anti-inflammatory cytokine Interleukin-10 exacerbated Alzheimer-type amyloidosis by altering Apolipoprotein E directed phagocytosis. On the other hand, while Il-10 expression promotes neurodegenerative features, it did not accelerate neurofibrillary tangle formation in a mouse model of tauopathy. In recent data from our lab, we have uncovered a similar neurodegenerative role of Il-10 in a seeded model of synucleinopathy which recapitulates stereotypical disease progression in Parkinsonism. We show that overexpression of IL-10 leads to accelerated death in this mouse model of synucleinopathy and this phenotype is amplified by a natural variant of Il-10 that has predominantly anti-inflammatory properties. The mechanism by this the Il-10 variant seems to exaggerate synuclein pathology and shorten lifespan is by altering glial activation and autophagic pathways. To understand whether biological antagonism of the Il-10 pathway would have an effect on neurodegenerative pathways, we designed a decoy receptor strategy based on mouse Il-10 receptor, referred to as soluble or sIl-10R. We observed that expression of sIl-10R in amyloid models leads to reduced plaque load in the early phase of amyloidosis in the TgCRND8 model. However, the overall effects of sIl-10R expression is only modest in the tauopathy model. Interestingly, preconditioning the CNS with sIl-10R extends lifespan in the seeded model of synucleinopathy. Our results indicate that sIl-10R is potentially a therapeutic that can broadly alter immune function and have disease modifying effects on two independent models of neurodegenerative diseases. Future experiments will focus on understanding the mechanistic basis of this protective strategy and how to optimize the window of opportunity for such therapies.

IMAGING THE INVADERS IN MULTIPLE SCLEROSIS: PET IMAGING OF CNS-INFILTRATING PERIPHERAL MYELOID CELLS

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Increasing evidence highlights the critical role of myeloid cells (e.g., macrophages, microglia, neutrophils) in the initiation and progression of multiple sclerosis (MS). Hence, the presence, spatiotemporal dynamics and activation state of these cells have the potential to serve as clinically meaningful biomarkers for MS. However, existing imaging strategies for detecting myeloid cells in vivo lack specificity and cannot distinguish between beneficial and toxic immune responses. We identified triggering receptor expressed on myeloid cells 1 (TREM1) as a promising biomarker of pathogenic peripheral myeloid cells and subsequently created the first positron emission tomography (PET) tracer permitting in vivo imaging of this target. Our aim was to validate TREM1 as an imaging biomarker of innate immune responses in the experimental autoimmune encephalomyelitis (EAE) mouse model of MS. We also explored TREM1 as a clinical biomarker of active MS lesions via immunostaining of rare brain biopsy tissue. Flow cytometry revealed selective expression of TREM1 on peripheral myeloid cells in EAE mice, with negligible expression on microglia, astrocytes, neurons, endothelial cells and lymphoid cells. Moreover, TREM1+ myeloid cell infiltration was dramatically increased in the CNS of EAE mice compared to naïve and TREM1 knockout mice. TREM1-PET imaging revealed elevated tracer binding in the brain, spinal cord, spleen and femur of EAE mice (vs. naïve and TREM1 knockout mice), even prior to disease symptoms. Ex vivo analysis of tissues confirmed increased tracer binding in these regions. Treatment with a TREM1 decoy receptor peptide known to attenuate TREM1 signaling, ameliorated EAE severity compared to saline-treated mice. Finally, substantial TREM1 staining was identified in human MS brain biopsy tissue compared to non-MS control brain tissue. To our knowledge, this is the first in vivo imaging strategy for detecting peripheral CNS-infiltrating myeloid cells and the first evidence of TREM1+ cells in human MS brain. Thus, TREM1-PET has high potential for clinical translation and to meaningfully impact early-stage diagnosis and therapy monitoring in MS patients.

SIGNIFICANCE OF IL-1β IN REGULATING MONOCYTES/MACROPHAGES INFILTRATING THE BRAIN AFTER TAIL AMPUTATION IN ZEBRAFISH

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Background: Increasing lines of evidence demonstrate that systemic immune responses triggered by either sterile or infectious inflammation can activate neuroimmune responses, resulting in developing Alzheimer's pathology, such as phosphorylation of tau protein. Systemic inflammation can be a risk factor for developing cognitive dysfunctions and even Alzheimer's disease. We have been using a laboratory murine model, laparotomy, to demonstrate that systemic immune responses stimulate production of cytokines and activation of microglia in the brain. However, it is still unclear how systemic monocytes/macrophages responses after sterile inflammation. As zebrafish embryo has transparent body that suitable for imaging monocytes/macrophages, we aim to use zebrafish and tail amputation for investigating which factor can affect the influence of systemic inflammation in the brain.
Methods: Zebrafish was used as experimental model. Tg(coro1a:DsRed) transgenic fish was used to monitor microglia and macrophages. Tg(mpx:mCherry) was used for monitoring neutrophils, Tg(mepg1:GFP) was used for monitoring macrophages. We have used TALEN method to make knockout or mutation of IL-1β in zebrafish. Quantitative PCR and immunohistochemical staining were used for examination. Acridine orange and TUNEL were employed for labeling apoptotic cells. Tail amputation was performed at day 3 of embryos under anesthetization with tricaine.
Results: Tail amputation recruited accumulation of monocytes/macrophages into the wound site in zebrafish tail. Surprisingly, increased number of macrophages and microglia were also found in the brain, which was tissue specific as no other organs showed similar phenomenon. Expression of mRNA for different cytokines was examined in the brain. Among which, IL-1β was significantly increased. Therefore, we prepared IL-1β knockout and mutated zebrafish. After amputation, the number of macrophages in the brain did not show any significant increase. To further understand why monocytes/macrophages migrate into the brain after peripheral wound injury, we found increased number of apoptotic cells in the brain after wound injury. IL-1β had a key role in regulating migration of monocytes/macrophages.
Conclusions: Our study demonstrated that zebrafish is a good animal model to investigate how peripheral wound injury triggers neuroimmune responses. IL-1β is a key player to regulate migration of monocytes/macrophages into the brain.

AN ENDOGENOUS RETROVIRUS DRIVES THE ACCUMULATION OF TDP-43 PROTEINOPATHY AND INTER-CELLULAR PROPAGATION OF NEURODEGENERATION IN A *DROSOPHILA* TDP-43 MODEL

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Intercellular spread of pathological protein aggregates is a hallmark of neurodegenerative diseases (NDs), and is hypothesized to underlie the progressive propagation of NDs through neural tissue. In amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), pathology is often associated with aggregation of TAR DNA Binding protein 43 (TDP-43). TDP-43 is normally a nuclear protein, but under pathological conditions it is cleared from the nucleus and accumulates as inclusions in the cytoplasm. Although aggregated TDP-43 protein has been found to move between cells, it is not clear whether and how this movement propagates the degenerative effects. We have recently established a *Drosophila* model of human TDP-43 to investigate mechanisms of focal onset and pathological spread. By initiating toxic expression of human TDP-43 focally within small groups of glial cells, we were able to observe the impact on surrounding tissue. We found that the gypsy endogenous retrovirus (ERV) actively replicates within the glial cells that exhibit TDP-43 pathology. We also previously demonstrated that DNA damage is detected within the specific glial cells in which gypsy ERV replication takes place and this is followed by apoptotic cell death. At the same time, this focal glial onset kills adjacent neurons. Surprisingly, this spreading death is caused by action of the gypsy ERV within the glia, which leads to subsequent DNA damage and death in adjacent neurons.
The above findings demonstrated that TDP-43 protein pathology drives activation of gypsy expression and replication, along with DNA damage-mediated apoptosis that spreads to nearby cells. We now show that this cascade actually involves a feedback amplification loop in which gypsy expression also drives TDP-43 protein pathology. We demonstrate that silencing gypsy ERV expression is sufficient to reduce accumulation of TDP-43 proteinopathy in the cytoplasm. We also find that the gypsy ERV is capable of both an inter-cellular and intra-cellular replication cycles. Together, these findings suggest a possible mechanism by which ERVs, and related retrotransposons, may contribute to both an intracellular amplification of TDP-43 proteinopathy and to an inter-cellular spread of this pathology and progression of the disease through neural tissue.

SEED-COMPETENT TAU MONOMER APPEARS EARLY IN DISEASE AND ITS ACTIVITY IS INDEPENDENT OF PHOSPHORYLATION

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Tau aggregation into ordered assemblies underlies a series of neurodegenerative diseases. We have previously reported an alternative conformation of monomeric tau isolated from Alzheimer's disease (AD) patient brain that can drive tau aggregation in vitro and in cells. However, it is unclear what mediates the conversion of normal tau, which cannot aggregate, into seed-competent tau in disease. While hyperphosphorylation of tau has long been associated with pathological tau assemblies, it is not clear if these modifications play a role in early stages of disease prior to the development of pathology. We used a biochemical approach to isolate tau monomer from a tauopathy mouse model (PS19) at ages ranging from 0-48 weeks and revealed that the seeding monomer appears at 4 weeks of age, months prior to the appearance of insoluble tau fibrils. We used mass spectrometry to identify phosphorylation patterns on tau monomers derived from our PS19 mice and compared them to human AD and age-matched control samples. Surprisingly, we do not observe strong correlations between phosphorylation patterns and seeding activities in tau monomer isolated from PS19 mice. Consistent with this observation, AD-derived seeding monomer showed a higher similarity in phosphorylation patterns to control brains than to AD fibrils. Consistent with a lack of a phosphorylation signature between seeding and non-seeding samples, phosphatase treatment did not alter seeding activity of tau monomer derived from PS19 mouse or AD brains. Our work reveals that the seed-competent tau monomer is the incipient species that drives disease and that phosphorylation patterns are independent of seeding activity. These findings suggest novel approaches to detect disease prior to the deposition of insoluble tau aggregates.

INVESTIGATION OF EPIGENETIC MACHINERY GENES IN BRAIN TISSUE OF ALZHEIMER'S DISEASE.

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Alzheimer's disease (AD) is an irreversible and progressive neurodegenerative disease, responsible for most cases of dementia. Several studies have demonstrated differential expression and regulation of ribosomal DNA (rDNA) in AD, an integral gene associated with protein synthesis. Moreover, alterations of gene expression in cerebellum have been recently associated to AD pathology, including the downregulation of rDNA expression. Epigenetic abnormalities, such as DNA methylation, play a role in gene expression regulation, and have been associated with AD pathogenesis. Hence, the aim of this study is to investigate the contribution of the methylation machinery enzymes - MBD1-4, MeCP2, DNMT1 and DNMT3A - to the regulation of the rDNA expression in cerebellum of AD patients. Our results showed a significant correlation between expression of all the methylation machinery enzymes ($p< 0.05$) and between the methylation of the rDNA promoter and upstream region of the rDNA promoter ($p< 0.05$) in AD. We failed to observe any significant correlation between methylation of rDNA and expression of any of the methylation machinery enzymes ($p> 0.05$) in AD. Thus, although the methylation machinery enzymes are present in the cerebellum tissues of AD samples, they may not be acting at the rDNA promoter region or in the upstream region to the rDNA promoter. In conclusion, we suggest that alternate epigenetic mechanism might be acting in rDNA expression regulation in AD, such as histones modifications. Moreover, the results reinforce the importance of investigating the upstream region of rDNA promoter, as a possible region that regulates rRNA expression in AD.

ENHANCING MITOCHONDRIA INTEGRITY TO RESTORE NEURONAL FUNCTION IN PARKINSON'S DISEASE

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Mitochondrial dysfunction has been identified as a key contributor in the development of multiple neurodegenerative conditions including Parkinson's Disease (PD). The mechanism by which dysfunctional mitochondria alters cellular function and contributes to PD pathology is under active investigation. Here, we characterize the pathways activated in response to mitochondrial dysfunction, typical of many neurodegenerative diseases. As a model system we used the knockdown of the mitochondrial fusion protein, OPA1, in post-mitotic neurons. We show that mitochondrial dysfunction leads to the activation of the integrated stress response and ATF4. This leads to an impairment in neuronal function characterized by alterations in synaptic proteins and neuronal firing. Given that our lab and others have shown that mitochondrial dysfunction can be rescued using the anti-apoptotic protein, Mcl1-Matrix, we asked if enhancing mitochondrial integrity through upregulation of Mcl1-Matrix could attenuate the stress response and restore neuronal function. Our preliminary data reveal that enhancing mitochondrial function by expressing Mcl1-Matrix under stress conditions can reduce neuronal damage and maintain integrity under stress conditions. These data suggest that novel strategies aimed at enhancing mitochondrial integrity may provide effective approaches to mitigate neurodegeneration and maintain function in PD.

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PROBING AND TARGETING MOTOR NEURON SUBTYPE DIFFERENTIAL VULNERABILITY IN ALS VIA MIR-17~92

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Selective motor neuron (MN) degeneration is the hallmark of amyotrophic lateral sclerosis (ALS). Among degenerated MNs, lateral motor column motor neurons (LMC-MNs) innervating limbs are one of the most vulnerable types. Previously, we reported that the deletion of mir-17~92 in MNs leads to selective apoptosis of LMC-MNs in embryos by promoting PTEN nuclear import. Here, we further revealed that mir-17~92 expression is sustained in the adult MNs, and a reduction in mir-17~92 expression, with concomitant nuclear PTEN accumulation, is manifested in spinal MNs before disease onset in SOD1G93A ALS mice. Using a novel double-transgenic reporter system in embryonic stem cells (ESCs), we uncovered down-regulation of mir-17~92 and increased nuclear PTEN in ALS-linked degenerating LMC-MNs, whereas non-LMC-MN subtypes remained relatively unaffected. This dysregulation axis of mir-17~92/nPTEN hallmark is recapitulated in the human ALS SOD1+/L144F iPSC-derived MN system. Finally, we demonstrate that overexpression of mir-17~92 can significantly rescue human SOD1+/L144F iPSC-derived MNs and improve motor deficits as well as survival in the SOD1G93A mouse model. These findings envisage mir-17~92 to be a potential prognosis marker for MN degeneration and a promising candidate for therapeutic targets in ALS.

TO DEVELOP ALS BIOSENSORS BY TUNING THE LOW-COMPLEXITY DOMAIN OF TDP-43

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TDP-43 belongs to the heterogeneous nuclear ribonucleoproteins (hnRNPs) family and it plays numerous role of RNA regulation. This RNA-binding protein comprises 414 amino acids (43kDa), including a folded N-terminal domain, tandem RRMs (RNA-recognizing motifs), and a disordered C-terminal domain. TDP-43 dominantly locates in the nucleus. However, cytoplasmic accumulation of TDP-43 is one of the pathogenic markers of amyotrophic lateral sclerosis (ALS) and frontotemporal lobe degeneration (FTLD). Intriguingly, the C-terminal domain of TDP-43 was clinically characterized with lots of genetic variants, such as point mutations, alterative splicing, and post-translational modifications (PTMs). The C-terminal repetitive sequences of polar and aromatic residues form a low-complexity domain (LCD) which is prone to self-assembly during neurodegeneration. In this study, we built a high-throughput screening platform to develop synthetic biosensors for TDP-43 aggregates. Specifically, we applied randomized mutageneses for direct evolution of prion-like LCD as biosensors. We currently focus on tuning LCD for familial ALS mutations (A315T), phospho-mimicking PTMs (SS409-410DD), and splicing alternatives (TDP-S6) as a proof of concept. These approaches are expected to deepen our understanding of TDP-43 modification and aggregation that underlie ALS and to open up possibilities for discovering new therapeutic strategies.

TDP-43 INTERACTS WITH AMYLOID-β, INHIBITS FIBRILLIZATION, AND WORSENS ALZHEIMER'S DISEASE PATHOLOGY

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TDP-43 inclusions are found in many Alzheimer's disease (AD) patients presenting faster disease progression and greater brain atrophy. Previously, we showed full-length TDP-43 forms spherical oligomers and perturbs amyloid-β (Aβ) fibrillization. To elucidate the role of TDP-43 in AD, here, we examined the effect of TDP-43 in Aβ aggregation and the attributed toxicity in mouse models. We found TDP-43 inhibited Aβ fibrillization at initial and oligomeric stages. Aβ fibrillization was delayed specifically in the presence of N-terminal domain containing TDP-43 variants, while C-terminal TDP-43 was not essential for Aβ interaction. TDP-43 significantly enhanced Aβ's ability to impair long-term potentiation and, upon intrahippocampal injection, caused spatial memory deficit. Following injection to AD transgenic mice, TDP-43 induced inflammation, interacted with Aβ, and exacerbated AD-like pathology. TDP-43 oligomers mostly colocalized with intracellular Aβ in the brain of AD patients. We conclude that TDP-43 inhibits Aβ fibrillization through its interaction with Aβ and exacerbates AD pathology.

PRION INFECTION IS ASSOCIATED WITH REDUCED RAB7 ACTIVATION AND IMPAIRED VESICULAR TRAFFICKING.

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Prion diseases are fatal and transmissible neurodegenerative diseases in which the cellular form of the prion protein 'PrP^{c}' misfolds into an infectious and aggregation-prone isoform termed PrP^{Sc}, which is the primary component of prions. Infections, mutations in the gene encoding PrP^{c} as well as spontaneous misfolding can trigger the conversion of PrP^{c} into PrP^{Sc}. This results in the accumulation of PrP^{Sc} aggregates mainly in the neurons and eventually leads to neuronal death in the brain. Many cellular impairments are observed in response to prion infection, including impaired lysosomal maturation. PrP^{Sc} conversion is thought to happen in the late endosomes which implies a relationship with the impaired lysosomal maturation and consequently, reduced EGFR and PrP^{Sc} degradation. Furthermore, prion infection results in elevated cholesterol levels in the neurons. The goal of our research is to shed light on the molecular mechanism behind these cellular impairments, focusing on the role of Rab7. Rab7 is a protein critical for lysosomal maturation and vesicle trafficking including the transport of low-density lipoprotein (LDL). A significant reduction in the PrP^{Sc} levels was attained by the over-expression of a constitutively active mutant of Rab7 in persistently prion-infected neuronal cells. We also show that the amount of GTP-bound Rab7 is reduced in prion-infected cells, indicating a defect in Rab7 activation. By deploying pulse-chase experiments with confocal microscopy, we observed impaired trafficking of LDL from early endosomes to lysosome as well as a delayed retrograde transport of cholera toxin B. In summary, we conclude that a defect in Rab7 activation in prion-infected cells is causally linked to the impaired endosomal transport and, as a consequence, elevated cholesterol levels. Hence restoring the normal neuronal physiology by the over-expression of active Rab7 could be one promising remedy in prion diseases, for which currently no cure exists.

STUDYING BRAIN TUMOR INVASION WITH CEREBRAL ORGANOIDS FROM HUMAN PLURIPOTENT STEM CELLS

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Novel technical innovations have now allowed for the generation of cerebral organoids (COs) from human pluripotent stem cells (PSCs), providing access to the human brain for disease modeling with fewer ethical and practical concerns. We exploited this system as a microenvironment surrogate to test the growth of patient-derived brain tumor cells in three-dimensional human 'mini-brains'. Brain tumor growth is normally modeled by tumor xenografting in immunocompromised mice, but as this is a cross-species system, species-specific features of tumor growth cannot be examined. Here we examined the importance of SMPD3, a gene that controls the biogenesis of extracellular vesicles (EVs), on the growth of a human oligodendroglioma cells (ODG) cell line, (BT088) cells. A survey of the TCGA dataset revealed that ODG patients that have higher SMPD3 expression levels survive longer. Accordingly, we found that when SMPD3 is knocked-down in BT088 cells, these cells grow faster in vitro. We next used cerebral organoids to assess tumor growth in a human 'mini-brain'. We used classical free-floating cerebral organoids generated from human embryonic stem cells using a modified Lancaster protocol and a spinning bioreactor (spin-Ω). Cerebral organoids at day 30 contain neural tube-like rosette structures that resemble the developing brain. In a cerebral organoid-tumor co-culture, we recapitulated the enhanced proliferative phenotype of sh*SMPD3* BT088 cells grown *in vitro* and in mouse xenografts. Notably, the sh*SMPD3* BT088 knockdown cells localized to the organoid periphery, where $SOX2^+$ neural rosettes localize. Thus, the CO-tumor co-culture system provides an excellent model system for assaying brain tumor growth, and provided important proof-of-concept work for the importance of SMPD3 in regulating ODG growth.

MICROGLIAL DEPLETION REDUCES ADENO-ASSOCIATED VIRUS MEDIATED TAU PROPAGATION FROM THE ENTORHINAL CORTEX TO THE DENTATE GRANULAR CELLS IN HUMAN APP^{NL-G-F} KNOCK-IN MICE WHILE INCREASING AMYLOID BURDEN.

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Microglia have an emerging role in neuropathogenesis of neurodegenerative disorders, highlighted by the propagation of tau pathology in the brain. They alter their phenotype from homeostatic to disease-associated/neurodegenerative microglia (MGnD/DAM) in the presence of amyloid pathology in the brain. We hypothesize that activated MGnD/DAM microglia may accelerate tau propagation via enhanced phagocytosis and secretion of misfolded tau protein via extracellular vesicle (EV) release. To test this hypothesis, humanized APP^{NL-G-F} knock-in (APP KI) mice, which develop amyloid plaques by 2 months of age, and wild type mice were treated with colony stimulation factor 1 receptor inhibitor PLX5622, which depletes microglia in the brain, at 4 months of age for 2 months. Mice underwent stereotaxic injection of adeno-associated virus expressing P301L tau mutant (AAV-P301L) into the medial entorhinal cortex (MEC) starting at 5 months of age. While we observed phosphorylated-tau (p-tau) accumulation in the granule cell layer (GCL) region of the dentate gyrus in both WT and APP KI mice resulting from tau propagation from the MEC, APP KI exhibited over 10-fold increase of p-tau accumulation compared to WT group. Strikingly, PLX5622 treatment, which depleted ~ 99% of microglia, showed 74% and 87% reduction of tau propagation in placebo-dieted WT and APP KI groups, respectively. Interestingly, both the area of amyloid plaques and plaque-associated pTau+ dystrophic neurites were increased upon depletion of associated with C-type lectin domain family 7 member A (Clec7A)-positive DAM/MGnD microglia, which surround amyloid plaques in APP KI mice. Moreover, we found that Tumor susceptibility gene 101 (Tsg101), an exosome-specific ESCRT-I molecule, was mostly co-expressed in Clec7A+ microglia around thioflavin-S+ amyloid plaques, which was significantly reduced by microglial depletion. To investigate the propensity of DAM/MGnD to hyper-secrete EVs in vivo, we developed a novel lentivirus (pLV- mEmerald -CD9) to specifically express mEmerald fused to CD9, an exosomal marker, in microglia. Stereotaxic injection of pLV-mEmerald-CD9 into the MEC of WT and APP KI mice shows that DAM/MGnD release over 3-fold more mEmerald-CD9+ EVs compared to homeostatic microglia in WT and APP KI mice. These findings corroborate the idea that plaque-mediated activation of microglia may supply tau seeds through EVs.

ALZHEIMER'S DISEASE: HISTONE POST-TRANSLATIONAL MODIFICATION AMONG AMYLOID-BETA ISOFORMS

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Alzheimer's disease (AD) is the 5th leading cause of death in the United States in patients 65 years of age and above. At present, an estimated 5.8 million Americans are living with AD, with over 122,000 recorded AD related deaths in 2018. Approximately 5% of all AD cases are familial, while the other 95% are termed sporadic, as these patients have no familial history of the disease. The exact causes leading to the disease are poorly understood, which has also lead to a lack of proper early-stage diagnostic tools as well as treatment options. A hallmark symptom of the disease is neuronal death in the cortex of the brain. This cell death is associated with two key features; the formation of neurofibrillary tangles formed by the Tau protein, as well as inclusions formed by the proteolytic cleavage of the amyloid β precursor protein (APP). Imprecise cleavage of APP leads to various amyloid-beta (Aβ) peptide lengths, with Aβ40 and Aβ42 being the most common in the cerebral cortex of AD patient samples. These peptides then form aggregates called amyloid plaques between neurons, which in turn induce synaptic loss and neuronal death. Traditional genetics fails to explain the origins of AD hence, epigenetic mechanisms might allow for the discovery of novel markers of disease propensity and progression. Presently, studies in the epigenetics of AD focus mainly on DNA methylation. Another form of epigenetic modulation are histone post-translational modifications (PTMs) such as methylation, acetylation, and phosphorylation. An AD yeast model overexpressing, and Aβ43 peptides provides a great tool for studying the effects of Aβ aggregation by specific Aβ peptide lengths. Here, we exploit this yeast model to reveal what histone modifications are associated with Aβ aggregation. Our findings can lead to the discovery of novel treatment targets as well as markers of disease progression.

SEX- AND REGION-DEPENDENT MICROGLIAL MORPHOLOGY RESPONSE TO ALZHEIMER'S DISEASE PROGRESSION

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Microglia reactivity depends on the local environment, in which they are embedded, as well as on environmental factors. To estimate their functional state, microglial morphology is often analyzed and compared across conditions selecting features such as total process length or number of intersections across subjective distances from the soma. These methods are suboptimal as the feature selection might result in substantial information loss. This makes it challenging to identify subtle changes as they occur during disease trajectories like Alzheimer's disease. Here, we developed a strategy to apply topology and generated persistence images of several thousand microglia across different brain regions and sex for the Ckp25 and 5xFAD Alzheimer disease models. As expected, we found that microglia in the hippocampus of Ckp25 show the strongest morphology changes. Interestingly, this occurs already 1 week after induction suggesting that microglia adapt already before neuronal effects occur. In addition, we observed that the disease trajectory is recapitulated in the frontal cortex and that a strong sex-specific effect was observed in the hippocampus starting from 2 weeks after the onset. On the other hand, the microglia in the cerebellum lack such a response. We are now comparing the disease trajectory of Ckp25 with the 5xFAD Alzheimer's model, which causes amyloid plaque disposition in the brain. Our method provides a novel strategy to identify subtle changes in microglia response, which is not possible with current standard techniques. This will be indicative for investigating the success of early drug intervention targeted towards microglia.

SINGLE-CELL EPIGENOMIC ANALYSES IMPLICATE CANDIDATE CAUSAL VARIANTS AT INHERITED RISK LOCI FOR ALZHEIMER'S AND PARKINSON'S DISEASES

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Genome-wide association studies (GWAS) in neurological diseases have identified thousands of variants associated with disease phenotypes. However, the majority of these variants do not alter coding sequences, making it difficult to assign their function. Here, we present a multi-omic epigenetic atlas of the adult human brain through profiling of the chromatin accessibility landscapes and three-dimensional chromatin interactions of seven adult brain regions across a cohort of 39 cognitively healthy individuals. Single-cell chromatin accessibility profiling of 70,631 cells from six of these brain regions defines broad cell classes and diverse neuronal cell types and identifies 359,022 cell type-specific regulatory elements, capturing the regulatory diversity of the adult brain. We developed a machine learning classifier to integrate this multi-omic framework and predict dozens of functional single nucleotide polymorphisms (SNPs) for Alzheimer's disease (AD) and Parkinson's disease (PD), nominating target genes and cell types for previously orphaned GWAS loci. These predictions both inform well-studied disease-relevant genes, such as *BIN1* in microglia for AD and reveal novel gene-disease associations, such as *STAB1* in microglia and *MAL* in oligodendrocytes for PD. Using CRISPR-based tools we identified functional regulatory effects for a subset of these loci. Moreover, we dissected the complex inverted haplotype of the *MAPT* (encoding tau) PD risk locus, identifying putative ectopic regulatory interactions in neurons that increase *MAPT* expression and may mediate this disease association. This work greatly expands our understanding of inherited variation in AD and PD and provides a roadmap for the epigenomic dissection of noncoding causal regulatory variation in disease.

SMALL MOLECULE SHC BLOCKERS AMELIORATE A-BETA TOXICITY AND APOE4-DEPENDENT MITOCHONDRIAL DEFICITS AND INSULIN RESISTANCE.

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Background and Prospects-Shc as a neuroprotective drug target. 25 years of an 'A-beta dissolution' therapeutic hypothesis has met with limited clinical success--new therapeutic targets for AD are needed. Mice with genetic deficiency of Shc protein resist AD in the PSAPP mouse model. Shc reduction in PSAPP mice improves cognition, memory and survival with *no reduction in amyloid burden*, thus Shc inhibition neuroprotects *downstream* of A-beta deposition, and mediates a metabolic improvement *in vivo* (PMID: 27431297).**To isolate small molecules that mimicked genetic Shc reduction**, Buto's repurposing screen identified 7 Shc binding chemical scaffolds. WithChemDiv's 3-dimensional analogging technology we identified ~70 3D isomers per scaffold. These ~500 molecules were screened using Bio-Layer Interferometry (BLI) for binding to p52Shc protein,resulting in 100 superior p52Shc binders and blockers. These 100 were prioritized by growth factor sensitization potency, and from the 50 most potent biological actives 400 New Chemical entities were made and verified for novelty. Of 100 best, each molecule was screened 20X for potency to confer resistance to aged Amyloid-beta toxicity to N2A neural cells. Top-scoring neuroprotectors were further screened with in silico algorithms for Lipinski rules, LogP, LogD, ADME, tox, rigidity and predicted BBB penetrance. The output was 40 NCE Shc blockers with affinity in the 2-300nM KD range on diverse scaffolds. All 40 were tested for their potency to engage p52Shc in vivo and block Shc phosphorylation, in vivo, as a combined measure of pharmacokinetics, protein binding and pharmacodynamics, resulting in 5 'most potent' Shc blockers on 5 scaffolds. **Properties of top Shc blockers.** Thus, NCEs have been identified that bind p52Shc protein, and block its access to phosphotyrosines on the insulin receptor. These molecules protect N2a cells from A-beta, sensitize them to insulin, rescue ApoE-4 dependent defects in mitochondrial glucose oxidation, and engage the p52Shc target in vivo. They reduce the A-beta Oligomer- and LPS-dependent inflammation of microglial cells. Pharmacokinetics of these molecules are being evaluated and some are clearly brain-penetrant.**Summary and Prospects**. Several New Chemical Entity Shc inhibitors have been synthesized that engage the Shc target in vivo. The protect from A-beta stress, apparently through improvement of insulin sensitivity and mitochondrial metabolism. Two of the most active molecules are clearly brain-penetrant and we are gearing up to test them in 5XFAD and ApoE4 mouse models of Alzheimer's disease. We believe Shc represents a new neurotherapeutic target in Alzheimer's Disease.

MULTI-TISSUE PROTEOMIC SIGNATURES OF GENETICALLY-DEFINED ALZHEIMER DISEASE CASES: A WINDOW INTO PRECISION MEDICINE

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Alzheimer disease (AD) is the most common cause of dementia characterized by the presence of brain amyloid-beta (Aβ) and tau pathology. Autosomal-dominant AD (ADAD) is caused by pathogenic mutations in the APP, PSEN1, or PSEN2 genes. Several rare variants inTREM2 tat strongly increases the risk of developing AD have been reported. Here, we aim to elucidate the downstream effect of genes and functional mechanisms leading to the disease by analyzing the proteome profile of AD, ADAD and TREM2 carriers in multiple relevant tissues. Deep proteomics profiling was obtained on brain (n=450), cerebrospinal fluid (CSF, n=1,301), and plasma (n=648) tissue. Comprehensive clinical information about AD pathology and cognition was available for all samples. We identified 12, 117, and 26 AD-specific proteins (with Bonferroni-corrected statistical significance) in the brain, CSF, and plasma, respectively. Twenty-seven of the proteins identified in CSF showed also differential levels in brain and plasma ($P < 0.05$) and lead to prediction model with higher accuracy than the well-accepted pTau/Aβ42 ratio (AUC=0.87 vs. 0.81, P=4.1×10-4) regardless APOE status. We also identified 27, 38, and 69 TREM2-specific proteins in the brain, CSF, and plasma. Twenty-three of the proteins identified in plasma showed differential levels in brain and CSF. A prediction model of these 23 proteins was able to discriminate TREM2 carriers from controls (AUC=0.94) and other AD cases (AUC=0.91). We identified 371 ADAD-specific proteins in the brain, among which 225 proteins were nominally associated with AD neuropath traits. Furthermore, 54, 89, and 85 proteins showed differential levels in sporadic AD vs controls in the brain, CSF, and plasma tissue, respectively, at nominal significance ($P < 0.05$). Pathway analysis of AD-specific proteins are involved in angiogenesis (P=9.2×10-8), hemostasis (P=1.6×10-12) and growth factor signaling (P=9.5×10-10). TREM2-specific proteins are associated with growth factors including VEGF (P=1.9×10-9), PDGF (P=2.9×10-6), EGF (P=2.8×10-12) and immunological response. ADAD-specific proteins converge in immunological response pathways (P=1.1×10-40) including cytokine-mediated signaling (P 6.2×10-37) and DAP12-mediated pathway (P 6.2×10-21). This is the first high-throughput multi-tissue proteomics study for genetically defined AD cases and TREM2 risk variant carriers, as well as AD cases in general. Multiple proteins significantly associated with status for each AD group were found in CSF, brain, and plasma. These findings will not only help create novel prediction models but also point to specific pathways implicated in AD, elucidating the functional mechanisms underlying the genetic architecture of this neurodegenerative disease.

HUMANISED AND PHYSIOLOGICAL MOUSE MODELS OF ALS

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) constitute a spectrum of fatal neurodegenerative disorders with only very limited treatment options available. By working with genetic mouse models of human disease we can unravel disease mechanisms and start to produce specific therapies to modulate neurodegeneration. However, most transgenic models overexpress proteins of interest, and may produce phenotypes unrelated to the disease itself. Furthermore, the biochemistry of some human proteins, and of some human mutations, may not be modelled in mouse. Here we discuss our humanised, knock-in mouse models that we are engineering and studying, created to dissect the physiological mechanisms that underpin genetic forms of ALS/FTD, including C9orf72, FUS, TDP43 and SOD1 models. In addition to humanisation of the wild type human gene variants, we are generating further lines harbouring pathogenic mutations via CRISPR/Cas9 editing, thus far including FUS-P525L and SOD1-A4V. Using novel recursive cloning and CRISPR/Cas9 techniques, we have also completed engineering of a seamless, uninterrupted $(G4C2)_{1000}$ C9orf72 repeat expansion within a humanisation targeting construct, which we are now preparing for ES cells targeting. By design, these models express human genes from endogenous loci, with endogenous expression and human splice patterns. Thus, we envisage these models can also be utilised for translational studies targeting human DNA, RNA, or protein.

MITOCHONDRIAL DYSFUNCTION IN THE BRAIN ALTERS THE HOMEOSTASIS OF A NEWLY IDENTIFIED POPULATION OF MITOCHONDRIA-DERIVED EXTRACELLULAR VESICLES

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Objectives: Mitochondrial damage and oxidative stress are well-established players in the pathophysiology of several neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease, and Down syndrome (DS). Using a novel high-resolution density step-gradient to isolate and fractionate subpopulations of extracellular vesicles (EVs) from the brain parenchyma, we investigated the effect of mitochondrial dysfunction on the number and content of EVs in DS brains.
Methods: We isolated EVs from murine and human DS and diploid control post-mortem brains or from cell media. EVs were analyzed by nanoparticle tracking analysis, cryogenic electron microscopy, Western blotting, mass spectrometry, and qPCR.
Results: We found that the extracellular matrix of the brain contains a newly identified population of metabolically active, double-membrane, electron-dense EVs of mitochondrial origin that we have named 'mitovesicles'. In vitro study revealed that oxidative stress enhances mitovesicle release in a mitophagy-independent fashion. In wild-type brains, we revealed that mitovesicles are low in number and encapsulate a specialized subset of mitochondrial constituents that reflects only partially the composition of intracellular mitochondria. Conversely, in human and murine DS brains, mitovesicle number is higher when compared to controls. The content is also modified, as the amount of the pro-inflammatory mitochondrial DNA in mitovesicles was higher in DS compared to controls.
Conclusions: Brain mitovesicles are tightly regulated in normal conditions but are modified in DS, suggesting that mitovesicles are a previously unrecognized player in mitochondria quality control and may have a yet undiscovered role in the inter-cellular response to oxidative stress and neuroinflammation.

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A PUR-BASED PEPTIDE OF RATIONAL DESIGN BINDS AND ALTERS G-QUADRUPLEX SECONDARY STRUCTURE PRESENT IN THE EXPANDED RNA REPEAT OF *C9ORF72* ALS/FTD

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PURA, the gene encoding Pur-alpha, is a member of an ancient nucleic acid-binding protein family with mammalian neurological functions. *PURA* knockout mice die shortly after birth with effects on brain and hematopoietic development. In neurons, Pur-alpha protein accompanies mRNA transcripts to sites of translation in dendrites. Microdeletions in the *PURA* locus have been implicated in several neurological disorders. *De novo PURA* mutations have been related to a spectrum of neurological phenotypes, indicating a potential *PURA* syndrome. The G-rich Pur-alpha binding element is amplified as expanded polynucleotide repeats in several brain diseases, including fragile X syndrome and a genetic form of amyotrophic lateral sclerosis/frontotemporal dementia (ALS/FTD). Throughout evolution the Pur-alpha protein plays a critical role in survival, based on conservation of its nucleic acid binding properties. Increased Pur-alpha protein levels in animal models alleviate certain cellular symptoms of the disease spectrum ALS/FTD. Pur-alpha, like other family members, contains a repeated signature PUR domain of 60–80 amino acids, which enables guanine-rich polynucleotide binding. Pur-alpha prefers the binding element $(G_{2\text{-}4}N_{1\text{–}3})_n$, where N is not G. We have employed a synthetic peptide, TZIP, similar to a Pur domain, but with sequence alterations based on a consensus of evolutionarily conserved Pur family binding domains and having an added transporter sequence. A major familial form of ALS/FTD, *C9orf72* (C9), is due to a hexanucleotide repeat expansion (HRE) of (GGGGCC), a Pur binding element. We show by circular dichroism that RNA oligonucleotides containing this purine-rich sequence consist largely of parallel G-quadruplexes. TZIP peptide binds this repeat sequence in both DNA and RNA. It binds the RNA element, including the G-quadruplexes, with a high degree of specificity versus a random oligonucleotide. In addition, TZIP binds both linear and G-quadruplex repeat RNA to form higher order G-quadruplex secondary structures. This change in conformational form by Pur-based peptide represents a new mechanism for regulating G quadruplex secondary structure within the C9 repeat. TZIP modulation of C9 RNA structural configuration may alter interaction of the complex with other proteins. This Pur-based mechanism provides new targets for therapy, and it may help to explain Pur-alpha alleviation of certain cellular pathological aspects of ALS/FTD.

MODELING HEREDITARY CEREBRAL AMYLOID ANGIOPATHY WITH CEREBRAL ORGANOIDS.

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Dutch-type cerebral amyloid angiopathy (D-CAA) is a rare autosomal dominant disease characterized by cerebrovascular deposition of the amyloid beta (Aβ) peptide. Aβ is produced after the enzymatic cleavage of amyloid precursor protein (APP). The disease is caused by a missense mutation on chromosome 21 in the APP gene, resulting in a glutamine for glutamic acid substitution (Glu693Gln). The loss of a negatively charged amino acid causes accelerated Aβ oligomerization together with impaired clearance, leads to Aβ deposition mainly around the cerebral vessel walls making the blood vessels more prone to breakage.
For many rare diseases there are no appropriate animal models available, thus making the translation from preclinical studies into clinical application difficult. Emerging iPSC technologies make it possible to reprogram patient fibroblasts to induced pluripotent stem cells (iPSCs) that can be later differentiated into neurons. However, two-dimensional neuronally induced iPSCs cannot provide as much information as a three dimensional human brain tissue. Developed from iPSCs, cerebral organoids are a new, 3D in vitro model of brain disorders.
We have generated cerebral organoids from 4 patient and 4 control iPSC lines, including an isogenic pair of cell lines that was created with CRISPR/Cas9. Cerebral organoids were collected at three time points. Half of them were fixated and cryosectioned for subsequent immunofluorescent analysis, whereas the rest half was used for qPCR analysis. For the immunofluorescent analysis a set of different antibodies specific for various cell types was used in order to validate the quality of the samples (βTubbIII, CTIP2, PAX6, FOXG1, brachyury and Iba1). To investigate possible phenotypic differences between control and D-CAA cerebral organoids APP- and Aβ- specific antibodies were used. For qPCR analysis, various genes were selected based on results of RNA-sequencing studies in patient and control post-mortem brain tissues previously done in our group.
Our preliminary data show that all cerebral organoids exhibit similar morphology and are positive for all the antibodies tested. The most interesting aspect though is the phenotypic difference we observed between control and D-CAA cerebral organoids, when comparing the Aβ staining. Already from day 55, D-CAA cerebral organoids exhibit Aβ aggregates and controls not. On day 110, both D-CAA and control organoids show Aβ aggregate formation, however the amount of Aβ in D-CAA organoids is elevated. Aβ aggregate quantification as well as qPCR analysis are currently ongoing procedures. Having developed an in vitro model of D-CAA, future plans involve testing of an APP RNA-targeting therapy, that has been already through iv vitro and in vivo testing by our group, to observe amelioration of the phenotype.

OPTIC NERVE ALTERATIONS IN A RAT GLAUCOMA MODEL OF SUBACUTE OCULAR HYPERTENSION

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Glaucoma is a neurodegenerative disease characterized by optic nerve injury and degeneration of retinal ganglion cells (RGCs). It is the major cause of irreversible blindness in the world. Pathophysiology and progression of disease is not completely understood, reflecting on limited treatment strategies. An important risk factor is high intraocular pressure (IOP). It might contribute to a stress injury in the optic nerve head, including axonal transport blockage, ischemia, oxidative stress and reactive gliosis, which leads to neurodegeneration. However, there is no certainty about the responses and role of glial cells on axon degeneration in glaucoma.
In this work, we evaluated axonal degeneration and the responses of glial cells in the optic nerve in a glaucoma model based on limbal plexus cautery (OHT), recently developed in our laboratory.
OHT was induced in adult (2-3 months) pigmented Lister-Hooded rats anesthetized by ketamin (75 mg/kg) and xylazine (5 mg/kg), as approved by the Ethics Committee from The Health Sciences Center of the Federal University of Rio de Janeiro (#083/17). Some animals received and intravitreal injection of cholera Toxin Subunit B (CTB) Alexa Fluor™ 555 Conjugate (Invitrogen, C22843) 3 days before euthanasia.
For analysis of axonal degeneration, cross sections of eye plus optic nerve were immunostained for Tubb3. A decrease in immunoreactivity was detected in the optic nerve at 14 days of OHT, progressing at 28 and 56 days. Degeneration occurred mainly distally to the glial lamina. Tubb3- regions also did not contain CTB anterograde marker. Astrocyte morphology was evaluated in transversal sections of the optic nerve. Morphological alterations were identified in GFAP+ astrocytes at 14 days after OHT, and specially at 28 and 56 days. In respect to microglia, Iba1+ cell density was quantified in transversal optic nerve sections. There was no difference in early time points of 1, 3 and 7 days post-OHT. There was an increase at 14 (38148±8347 cells/mm^3; p= 0.0092; n=8), 28 (43301±7130; p= 0.0023; n=6) and 56 (52479±6242; <0.0001; n=6) days after OHT compared to naïve (8368±386.0; n=4). Expression of CD68 marker in Iba1+ cells was also increased in those late time points.
Limbal plexus cautery led to axonal degeneration and glia alterations, specially at late time points after OHT induction. Correlation of glia activation and axon degeneration in this model suggests a role for glia in axonal damage in glaucoma. Furthermore, characterization of optic nerve alterations after limbal plexus cautery is important for future analysis of neuroprotective strategies in this model.

PLASMA MARKERS OF BIOLOGICAL AGE SUGGEST ACCELERATED PROTEOMIC AGING IN ALZHEIMER'S DISEASE AND ALS.

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Background: Alzheimer's disease (AD), Parkinson's Disease (PD), and Amyotrophic lateral sclerosis (ALS) are debilitating neurodegenerative diseases (ND) with limited prognostic biomarkers and therapeutic options. A recent study found that plasma proteins that were altered later in life were enriched for AD-related proteins, suggesting that the age-related proteome remains an untapped source of information for biomarkers of ND.
Objectives: The following questions were asked: Is aging a pre-ND state which increases risk for a superimposed degenerative process, or are NDs a form of "accelerated aging" where normal changes are seen sooner? Can we use plasma markers of normal aging to make predictions in disease states?
Methods: Age-associated proteins were identified in plasma samples from 147 neurologically normal control (NC) individuals ages 39-91 from three clinical sites. Somalogic aptamer-based technology was used to quantify >1000 proteins per plasma sample and, after quality control measures, 940 proteins were investigated for an association with age using multiple regression. Stability selection was performed using elastic net with repeated 10-fold cross-validation, and the final linear predictor of age was chosen based on performance across multiple hold-out test sets within the NC group. Biological age was then predicted across three ND cohorts, consisting of 311 PD, 25 AD, and 59 ALS individuals.
Results: We identified 101 age-associated proteins (FDR < 0.05) in NC individuals. From this initial pool of proteins, a 21-parameter model (consisting of 20 proteins and sex) was developed to predict age in the NC group, with excellent performance in cross-validation (Pearson's cor=0.894). Applying this predictor to PD, proteomic age did not significantly differ from chronological age. However, for the AD and ALS groups, proteomic age was significantly greater than the chronological age ($p<0.05$). Moreover, in ALS individuals, proteomic age showed a trend towards correlation (Spearman's rho=-0.258, p=0.0504) with functional status as measured by the ALS Functional Rating Scale, whereas chronological age did not.
Conclusions: AD and ALS individuals exhibit an accelerated proteomic age, whereas PD individuals do not. Furthermore, in ALS, proteomic age seems to better predict functional status than chronological age, with implications for prognostic utility. Future directions include validating these aging signatures with alternate protein-measurement methods, in larger patient cohorts.

GAINING INSIGHTS INTO THE MOLECULAR MECHANISMS UNDERLYING X-LINKED DYSTONIA-PARKINSONISM (XDP) WITH FEMALE CARRIER iPSC-DERIVED STRIATAL ORGANOIDS

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X-linked Dystonia-Parkinsonism (XDP) is an inherited adult-onset movement disorder affecting males with female carriers generally unaffected. XDP is caused by a SVA retrotransposon insertion within intron 32 of TAF1, core member of the TFIID complex coordinating RNA pol II promoter initiation. While it is unknown if XDP is caused by TAF1 loss of function or pathological gain of function, patients have progressive degeneration specifically in the caudate nucleus. Disease cellular models are associated with aberrant intron retention and decreased TAF1 expression. Studies of X-linked disorders, such as XDP, can take advantage of the natural process of X-chromosome inactivation (XCI). Female cells transcriptionally inactivate one of the two X chromosomes, maintaining this status during further cell proliferation and differentiation. Thus, genetically matched induced pluripotent stem cell (iPSC) lines can differ only in whether the wild type or mutant X chromosomes are expressed.
Here, we characterized the XCI status of iPSC lines derived from multiple XDP female carriers. We observed a striking clonal difference in XIST expression, maintained throughout differentiation. We successfully identified a pair of isogenic XDP carrier iPSCs. Surprisingly, we found a sex specific effect of TAF1 expression in iPSCs. While decreased TAF1 expression is specific to male XDP, TAF1 is also decreased in all carrier lines and replicated in HipSci lines from 301 healthy individuals. This indicates that decreased TAF1 expression alone is not sufficient to cause XDP phenotype.
To recapitulate the mature and complex XDP cellular system, we developed a novel protocol to differentiate iPSCs into striatal organoids. Mutant female carrier-derived iPSCs and relative iPSC-derived striatal organoids showed prominent TAF1 intron retention. By harnessing the XCI and using a new 3D cellular model, this study aims to shed light upon novel insights of XDP etiology.

CASY-1/Calsyntenin protects against axon degeneration caused by loss of mitochondria

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Mitochondrial defects are tightly linked to neurodegeneration. We used the *C. elegans ric-7* mutant to model axon degeneration induced by loss of axonal mitochondria. Using an unbiased screen, we found that *casy-1*/Calsyntenin is required for degeneration in this model. Axons in *casy-1* mutants do not degenerate, even though they lack mitochondria.
CASY-1 is a single-pass transmembrane(TM) protein with extracellular cell adhesion domains and intracellular kinesin binding sites(KBS) [1,2]. We found that CASY-1 functions cell autonomously to promote degeneration, and that the TM and KBS domains are sufficient for this function. Mutations of two CASY-1 interactors, kinesin light chain 2(KLC-2) and LIN-10/X11L, also suppress degeneration.
We also discovered a key role for *unc-43*/CaMKII in axon degeneration induced by loss of axonal mitochondria. Loss-of-function mutations in CaMKII makes axons more susceptible to degeneration, whereas active CaMKII protects axons from degeneration in the absence of mitochondria. Genetic analysis indicates that *unc-43*/CaMKII is downstream of *casy-1* and *lin-10*. Our working model is that CASY-1 is transported from the soma to the distal axon by the kinesin motor, where it regulates CaMKII localization or activity via LIN-10 and its interactors.
We generated a human iPSC cell line that lacks all three human calsyntenins (TKO) by CRISPR. We differentiated the iPSCs into neurons to test their resistance to mitochondria damage. Preliminary results suggest that the TKO neurons are more resistant to mitochondrial stress (complex III inhibition) which suggests an evolutionarily conserved role for calsyntenins in a neurodegenerative response to mitochondrial dysfunction.
Our findings reveal a novel role for calsyntenin and CaMKII in axon degeneration caused by loss of mitochondria, and identify this axis as a potential target for intervention in neurodegenerative diseases.

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TRANSCRIPTOME ANALYSES OF 7-DAY-OLD ZEBRAFISH LARVAE POSSESSING A FAMILIAL ALZHEIMER'S DISEASE-LIKE MUTATION IN *PSEN1* INDICATE EFFECTS ON OXIDATIVE PHOSPHORYLATION, ECM AND MCM FUNCTIONS, AND IRON HOMEOSTASIS

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Early-onset familial Alzheimer's disease (EOfAD) is promoted by dominant mutations, enabling the generation of EOfAD-like mutations in animal models for the study of Alzheimer's disease (AD) pathogenic mechanisms. In a previous study, we generated an EOfAD-like mutation, *psen1*$^{Q96\_K97del}$, in zebrafish and performed transcriptome analysis comparing entire brains from 6-month-old wild type and heterozygous mutant fish. As zebrafish larvae can be used for screening of chemical libraries in drug discovery, we aimed to determine whether the disease transcriptomic signatures seen in 6-month-old mutant brains occur in 7 day post fertilization (dpf) zebrafish larvae that might be exploited in screening of chemical libraries to reverse their presumably pathological transcriptome state to normal. Therefore, we generated clutches of wild type and heterozygous *psen1*$^{Q96\_K97del}$ 7 dpf larvae using a paired-mating strategy to reduce extraneous genetic variation before performing a comparative transcriptome analysis. 228 differentially expressed (DE) genes were identified, and Goseq analysis and gene set enrichment analysis (GSEA) were used to predict cellular functions. Although the correspondence between DE genes identified in heterozygous *psen1*$^{Q96\_K97del}$ mutant 7 dpf larvae and in heterozygous mutant 6-month-old brains was lacked, our analyses still predicted similar affected cellular pathways between the larvae and the brains, including oxidative phosphorylation, mitochondrial function, lysosomal acidification, and iron ion transport although the larvae lacked detectable enrichment of the prevalence of transcripts containing iron responsive elements (IREs) in their 3'UTRs. Also, some apparently larva-specific effects of the mutation were identified. The dysregulation of minichromosome maintenance protein complex (MCM) genes strongly contributed to predicted effects on DNA replication and the cell cycle and may explain earlier observations of genome instability due to PSEN1 mutation. The upregulation of crystallin gene expression may be a response to defective activity of mutant Psen1 protein in endolysosomal acidification. Genes related to extracellular matrix (ECM) were downregulated, consistent with previous studies of EOfAD mutant iPSC neurons and postmortem late onset AD brains.

TRANS-SYNAPTIC TRANSMISSION OF MUTANT HUNTINGTIN AGGREGATES IS MEDIATED BY DRAPER-DEPENDENT PHAGOCYTOSIS IN *DROSOPHILA* BRAINS.

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Huntington's Disease (HD) is an inherited neurodegenerative disorder caused by expansion of a trinucleotide repeat in exon 1 of the huntingtin (Htt) gene. Expansion of this region beyond a pathogenic threshold of 37 CAG nucleotide repeats results in synthesis of mutant Htt (mHtt) proteins with expanded N-terminal polyglutamine tracts that prevent proper folding. Misfolded mHtt proteins self-assemble into insoluble aggregates that are visible as dense, proteinaceous inclusions in neurons and glia in HD patient brains. An emerging body of evidence supports the hypothesis that mHtt aggregates and pathogenic aggregates associated with other neurodegenerative diseases (e.g. Alzheimer's disease, frontotemporal dementia, Parkinson's disease, and amyotrophic lateral sclerosis) spread between cells in a manner similar to infectious prions—the aggregates transfer from cell to cell and nucleate the aggregation of normally-soluble, cognate proteins. Utilizing *Drosophila* genetics, we have demonstrated that mHtt protein aggregates transfer from a population of presynaptic olfactory receptor neurons (ORNs) to their partner postsynaptic projection neurons (PNs), entering the PN cytoplasm and templating the prion-like conversion of wild-type Htt (wtHtt) proteins. We observed that mHtt transmission between synaptically-connected neurons is inversely correlated with neuronal activity and absolutely dependent on expression of Draper, a MEGF10 homolog responsible for clearance of apoptotic cells and neuronal debris. These findings suggest that phagocytic glia mediate the prion-like spreading of mHtt across synapses. To test this hypothesis, we tracked the movement of mHtt aggregates between ORN axons, glia, and PN dendrites, and found that Draper-expressing glia were an obligatory intermediate in mHtt aggregates transferring across ORN-PN synapses. Ongoing studies suggest that intracellular proteins involved in recycling endocytosis also play a role in ORN-to-glia-to-PN aggregate transmission. Together, our findings suggest that phagocytic glia play neuroprotective (i.e., by clearing pathogenic aggregates from neurons) and neurotoxic (i.e., by enhancing spread of aggregates to other cells) roles in the brain and thus, expand our understanding of glia as "double-edged" players in neurodegenerative disease progression.

GENOME-WIDE DETECTION OF EXPRESSION QUANTITATIVE TRAIT LOCI SUGGESTS REGULATION OF T CELL GENES BY TAU VARIANTS IN ALZHEIMER'S DISEASE PATIENTS

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Recent genomic and functional studies have shed light on important interactions between the innate immune system and the central nervous system (CNS) in Alzheimer's disease (AD). While microglia, the resident immune cells of the CNS, have received particular attention, comparatively little is known about the contribution of T and B cells of the adaptive immune system. However, single-cell sequencing of immune cells from aged individuals and AD patients has suggested that T cells become senescent and dysregulated in these conditions, often driving pathological neuroinflammation. Here, we profile the transcriptomic signature of naive and memory CD4+ and CD8+ T cells from AD patients and age-matched controls in the Religious Orders Study/Memory and Aging Project (ROSMAP). We also correlate RNA sequencing with genomic data to detect both cis and trans expression quantitative trait loci (eQTL). Significant cis-eQTLs at FDR-corrected p-value<0.05 were found in naïve CD4+ T cells (n=69), memory CD4+ T cells (n=37), naïve CD8+ T cells (n=53) and memory CD8+ T cells (n=36). None of the cis-eQTLs were found in candidate AD or PD loci. However, several trans-eQTLs were observed in AD and PD loci. In particular, expression of several genes was regulated by single-nucleotide polymorphisms (SNPs) in MAPT, the gene coding for the AD-associated protein tau, across all four cell types. Key pathways enriched among the trans-eQTLs include mitosis, GTPase activity and mitochondrion organization. Our study implicates a possible role of adaptive immune T cells in AD genetic susceptibility. Ongoing studies are focused on dissecting the role of AD-associated T cell genes in vivo using animal models of AD.

IDENTIFYING KEY REGULATORY MECHANISMS OF TDP-43 NUCLEAR EXPORT USING A PERMEABILIZED CELL ASSAY

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TAR DNA-binding protein-43 (TDP-43) is a DNA- and RNA-binding protein that stabilizes RNA and represses cryptic exon splicing. In healthy cells, TDP-43 is mostly localized to the nucleus where it binds GU-rich nuclear RNAs. However, over 97% of amyotrophic lateral sclerosis (ALS) are characterized by mislocalization of TDP-43 to the cytoplasm. Of the 10% of ALS cases that are familial, a small subset is linked to mutations in TDP-43, supporting a causative role for TDP-43 disruption in neurodegeneration.

Though TDP-43 had been previously thought to exit the nucleus through receptor-mediated active export mechanisms, recent work has shown that the putative nuclear export signal (NES) in TDP-43 is nonfunctional and that TDP-43 export from the nucleus is independent of the nuclear export receptor, exportin-1. Further studies posit that TDP-43 primarily exits the nucleus by passive diffusion across the nuclear pore and show that this egress is size-dependent.

In order to understand the key factors regulating TDP-43 export, we developed a TDP-43 nuclear export assay in digitonin-permeabilized HeLa cells. Following permeabilization, we manipulated reaction conditions including temperature and ATP/GTP levels, and tested the effect of RNase and delivery of short GU8 vs. A16 oligomers.

We observed rapid export of TDP-43 within 15 minutes post-permeabilization, as assessed by immunofluorescence. Low temperature conditions halted TDP-43 export, typical of an active, energy-dependent process. However, RNase promoted rapid export regardless of temperature, suggesting that TDP-43 can passively diffuse from the nucleus once liberated from nuclear RNA binding partners. Consistent with this, delivery of GU8 but not A16 oligomers also triggered passive TDP-43 nuclear export. These data suggest that nuclear RNA binding critically regulates TDP-43 availability for export by passive diffusion.

AAV-MEDIATED GENOME EDITING AMELIORATES AMYLOID-ASSOCIATED PATHOLOGIES IN FAMILIAL ALZHEIMER'S DISEASE MOUSE MODELS

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Familial Alzheimer's disease (AD) is caused by autosomal dominant mutations in the genes encoding amyloid precursor protein (APP), presenilin 1 and presenilin 2, which result in excessive production of amyloid-beta (Aβ) peptides. Despite considerable advances in our understanding of the genetic causes of familial AD, there are currently no effective disease-modifying treatments for the disease. It has been demonstrated that CRISPR/Cas9-mediated genome editing has the ability to disrupt familial AD mutations and reduce Aβ production *in vitro*. However, development of CRISPR/Cas9 as a targeted genome-editing approach for disease-modifying AD therapies requires addressing major technical obstacles such as limited efficiency of genome editing and inefficient delivery of CRISPR/Cas9 into the brain. Here, we show that using intahippocampal delivery of CRISPR/Cas9 system to delete the mutant allele can rescue both Aβ-mediated pathologies and neuronal loss in two different familial AD transgenic mouse models with the *APP* Swedish (*APPswe*) mutation. Of note, this CRISPR-mediated rescue effect is effective in transgenic mice at an age when Aβ pathology is obvious and can persist for at least 6 months after a single virus administration. Thus, our results provide strong evidence that an AAV-mediated CRISPR/Cas9-based strategy can selectively and efficiently edit a familial AD mutation *in vivo* and ameliorate AD-associated pathologies. Our findings also provide important insights for the development of disease-modifying treatments for familial AD as well as other brain diseases caused by dominant mutations.

LYSOSOME-MEDIATED STRUCTURAL AND FUNCTIONAL CHANGES IN NEURONAL NUCLEUS IN PATHOPHYSIOLOGY OF ALZHEIMER'S DISEASE: A POTENTIAL TREATABLE TARGET

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Alzheimer's disease (AD) remains an incurable illness and is on the verge of becoming a major global health issue. Increased oxidative stress, deficient autophagy, accumulation of Amyloid beta (Aβ) plaques and phosphorylated tau containing neurofibrillary tangles (NFT) have been the target of intensive research. Recent studies suggest the involvement of nuclear damage and aberrant gene expression in pathophysiology of AD. A compromised antioxidant capacity in the brain of AD victims has been shown by decreased Thioredoxin levels (Trx). We have discovered that inhibition of Trx initiates nuclear lamin degradation through activation of caspase 6. In this study, using a mouse model of AD and human brain autopsy samples we have identified yet another novel mechanism of nuclear lamina damage that is associated with significant upregulation of lysosomal cathepsins.

To examine the implication of cathepsins in nuclear lamina damage, we employed a model of Aβ42 cytotoxicity in human neuroblastoma (SH-SY5Y) cells, rat primary hippocampal neurons, and genetically engineered mouse embryonic fibroblasts (MEF) cells. We show that activation of cathepsins can induce nuclear lamina damage. These results were further substantiated using an animal model of AD (3xTg mice) and human AD brain tissues. The observed nuclear lamina damage resulted in induction of structural changes detected using 3D Structured Illumination Microscopy (SIM) and immunohistochemistry. We observed that Aβ42 toxicity induces a robust conformational change in heterochromatin/euchromatin distribution. Granulometry analysis showed significant increase in average DNA particle size and DNA free space in Aβ42 treated cells; indicating an enhanced chromatin compaction. This was associated with an altered pattern of histone acetylation and methylation in Aβ42 treated cells that are reported in AD pathophysiology. Application of specific cathepsin inhibitors or genetic deletion was partially effective in alleviating these changes. Our study identifies a novel aspect of lysosomal contribution to pathophysiology of AD. This proteolytic system responsible for nuclear lamina damage in AD can be used for better understanding the neurotoxicity mechanisms and for developing new therapies.

SEQUENCING THE PREVIOUSLY UNSEQUENCABLE USING AMPLIFICATION-FREE TARGETED ENRICHMENT POWERED BY THE CRSIPR-CAS9 SYSTEM

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Introduction: Genomic regions with extreme base composition bias and repetitive sequences have long proven challenging for targeted enrichment methods, as they rely upon some form of amplification. Similarly, most DNA sequencing technologies struggle to faithfully sequence regions of low complexity. This has especially been trying for repeat expansion disorders such as Fragile-X disease, Huntington disease and various Ataxias, where the repetitive elements range from several hundreds of bases to tens of kilobases.

Methods: We have developed a robust, amplification-free targeted enrichment technique, called No-Amp Targeted Sequencing, that employs the CRISPR-Cas9 system. In conjunction with SMRT Sequencing, which delivers long reads spanning the entire repeat expansion, high consensus accuracy, and uniform coverage, these previously inaccessible regions are now accessible. This method is completely amplification-free, therefore removing any PCR errors and biases from the experiment. Furthermore, this technique also preserves native DNA molecules, allowing for direct detection and characterization of epigenetic signatures. The No-Amp method is a two-day protocol that is compatible with multiplexing of multiple targets and multiple samples in a single reaction, using as little as 1 µg of genomic DNA input per sample.

Results: We have successfully targeted a number of repeat expansion disorder loci including *HTT*, *FMR1*, *C9orf72* as well as built an Ataxia panel which consists of 15 different disease-causing repeat expansion regions. Using the No-Amp method we have isolated hundreds of individual on-target molecules, allowing for reliable repeat size estimation, mosaicism detection and identification of interruption sequences with alleles as long as >2700 repeat unites (>13 kb). In addition to multiplexing several targets, we have also multiplexed at least 48 samples in one experiment making the No-Amp Targeted Sequencing method a cost-effective option.

Conclusions: Combining the CRISPR-Cas9 enrichment method with Single Molecule, Real-Time Sequencing provided us with base-level resolution of previously inaccessible regions of the genome, like disease-causing repeat expansions. No-Amp Targeted Sequencing captures holistically in one experiment all aspects of repeat expansion disorders which are important for better understanding the underlying disease mechanisms.

THE PATHOLOGICAL AGGREGATION AND FUNCTIONALITY OF *IN VITRO* MODIFICATIONS WITHIN TDP-43

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One of the fundamental mechanisms associated with neurological and neurodegenerative disease progression is protein pathogenesis, which is attributed to the abnormal aggregation of misfolded proteins. Many proteins have been identified as pathological goldmines, inducing the progression of Amyotrophic Lateral Sclerosis (ALS), including transactive-response DNA-binding protein of 43 kDa (TDP-43). TDP-43 43 is a protein that functions in transcriptional repression, modulation of RNA metabolism, and stress granule formation. However, this protein is post-translationally modified into aggregated and mutated structures in the cytoplasm and nucleus, including phosphorylation, acetylation, and cleavage. Many of these alterations may represent key pathological features in ALS and similar diseases. Since pertinent research has only recently been established on the protein, the pathogenicity and functionality of TDP-43 in ALS have yet to be determined, thus providing us with a critical knowledge gap in TDP-43's functions. Thus, systematic in vitro studies must be carried out to characterize TDP-43's aggregation and its toxicity and will provide critical insight towards the early detection of protein pathogenesis and its treatment. The hydrophobicity of its structure along with its poor solubility in vitro has prevented the structure of TDP-43 to be fully elucidated, leading us to discover more about aggregate formation and what primarily drives this process. Our research, therefore, has proved TDP-43 protein as an inherently aggregation-prone protein, with several in vitro conditions applied to induce misfolding of the protein followed by Thioflavin T (ThT) fluorescence assays to monitor β-sheet formation. Transmission electron microscopy (TEM) allowed for visualization of the structural morphologies of these TDP-43 aggregates. As a result, data indicate that this TDP-43 aggregates under specific environmental conditions and results in a ThT-positive yield. Furthermore, preliminary results have proved the feasibility of using casein protein kinases to phosphorylate this protein, specifically casein kinase II (CK2). Using these kinases will allow us to further determine TDP-43's role when mutated, thus potentially revealing key details about the proteins' functions. These in vitro analyses of TDP-43 aggregation are a valuable model for further protein studies and provide a preliminary platform for screening therapies towards a viable treatment for ALS.

NEUROPROTECTIVE EFFECT OF LEVETIRACETAM IN LRRK2 RELATED PD EXPERIMENTAL MODELS

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Parkinson's disease (PD) is a neurodegenerative disorder currently incurable. The most common genetic cause of PD is the mutation in Leucine-Rich Repeat Kinase 2 (LRRK2) which physiological and pathological function is still debated. However different experimental evidence based on LRRK2 cellular localization and LRRK2 protein interactors suggest that LRRK2 may be part and regulate a protein network modulating vesicle dynamics/trafficking. SV2A is part of this protein complex and it is the binding site of the Leviteracetam (LEV), a compound largely used in human therapy for epilepsy treatment. The binding of LEV to SV2A reduces the neuronal firing by the modulation of vesicle trafficking although by an unknown molecular mechanism. We have analyzed the interaction between the LRRK2 and SV2A pathways by LEV treatment. Interestingly LEV significantly counteracts the effect of LRRK2 G2019S pathological mutant expression in three different cellular experimental models. LEV rescues the negative effect of LRRK2 pathological mutant expression in the differentiation of primary neurons or PC12 cells. Furthermore, LEV rescues the effect of LRRK2 pathological mutant expression on dopamine receptor D2 (DRD2) localization/trafficking. We are currently investigating the molecular mechanism of LEV action by analyzing LRRK2 localization, phosphorylation and the LRRK2 interaction with other protein partners. Our data strongly suggest that LEV treatment may have a neuroprotective effect on LRRK2 pathological mutant toxicity and that LEV repositioning could be a viable compound for PD treatment.

THE ROLE OF THE CYTOSKELETON IN MODULATING NUCLEAR PORE FUNCTION IN ALS/FTD

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Synapse loss, dendrite retraction, and axonal degeneration are common phenotypes observed in ALS/FTD and other neurodegenerative diseases. These morphological and functional abnormalities have been attributed to widespread changes in mRNA transcription, splicing, and translation caused by the disfunction of RNA and DNA binding proteins such as TDP-43 and FUS. A substantial body of evidence points to the failure of the nuclear pore complex (NPC) and nucleocytoplasmic transport (NCT) as major contributors to this disruption. Our studies show that the actin cytoskeleton is a novel regulator of NPC stability and function. We found that disruption of actin homeostasis is associated with structural and functional defects to the NCT, such as decreased rates of nuclear import. Importantly, we show that positive modulation of the actin cytoskeleton and its association with the nuclear envelope rescues nuclear import defects in multiple cellular models of familial ALS.

CHARACTERIZATION OF NETWORK ACTIVITY AND RESPONSE TO NEUROTOXINS IN iPSC DERIVED MOTOR NEURONS

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There is a growing demand among the scientific community and industries for in-vitro cell culture platforms that recapitulate human CNS cells and their responses to the external cues, including environmental neurotoxins. The desired models should follow the same response pathway when exposed to the toxins and could predict better response than animal models for toxicology studies in humans. To this end, we evaluated human iPSC-derived motor neurons (iCel$^{l®}$ Motor Neurons) which offer a developmentally and physiologically relevant in-vitro model of human spinal motor neurons. iCell® Motor Neurons are highly pure neurons as measured by ISL 1/2 and Tuj1 positive staining and express all the genes and proteins that are necessary for making functional synapses and secretion and release of acetylcholine. Furthermore, we confirmed that iCell® Motor Neurons can develop functional networks using microelectrode array (MEA) and display spontaneous Ca^{+} oscillation measured by Ca^{+} binding dyes. We investigated the co-culture of motor neurons with iCell® Astrocytes and found that in the presence of astrocytes, motor neurons formed better networks indicated by earlier and enhanced synchronous bursting compared to monoculture. More importantly, these cultures respond to various neurotoxins including Botulinum (BoNT/A), Bungarotoxin, glutamate receptor blockers (both *NMDA* and *AMPA*) and glycinergic receptor antagonists, in a degree that is consistent with the reports from rodent motor neuron explants. Our results demonstrated that iCell® Motor Neurons (in the presence of astrocytes or as mono-culture) can provide an effective model system and alternative for animal models for drug safety assessment and toxicology studies.

DEFINING THE METABOLIC SIGNATURE OF ALZHEIMER DISEASE TO IDENTIFY NOVEL THERAPEUTIC TARGETS

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Alzheimer disease (AD) is a multifactorial pathology for which we do not have yet a clear understanding of its mechanisms and causes. There are three main AD etiologies with diversity in phenotype, onset and progression of clinical symptoms: autosomal dominant (ADAD), early onset (EOAD) and late onset (LOAD). Yet, AD is ultimately characterized by the deposition of Aβ and ptau protein aggregates in the brain. Genomic studies have proposed several molecular mechanisms that are being further examined by multiple omic techniques. Among those, metabolomics represents the ultimate biochemical consequence of genomic abnormalities. Metabolomic decline is one of the first symptoms detected in patients with wild cognitive impairment and studies using plasma have identified lipid and energy metabolism as hallmarks of AD. We aim to interrogate the metabolomic and lipidomic signatures of AD brain donors to profile each of the three AD etiologies.
We have generated metabolomic and lipidomic data from parietal brain tissue from donors to the Knight ADRC and DIAN cohorts (ADAD=25; EOAD=101; LOAD=221; controls (CO)=42) using the Metabolon global metabolomics platform. We tested association of 627 metabolites that passed our QC process against disease etiology using linear regression models correcting for sex, age, and post-mortem interval. Benjamini-Hochberg multiple test correction was applied.
We identified 190 metabolites associated with disease status (FDR q-value<0.05). Of those, 158, 2 and 4 were exclusive of ADAD, EOAD and LOAD respectively. We observed an inverse relationship between lipids and amino acid metabolism, being ADAD enriched in lipids followed by EOAD and LOAD. Nine metabolites were common across ADAD, EOAD and LOAD: glycerophosphoinositol (b=-0.184, -0.114, -0.102) representing the phospholipid metabolism, beta-citrylglutamate (b=-0.204, -0.162, -0.140) and N-acetylglutamate (b=-0.214, -0.121, -0.098) representing glutamate metabolism, N-acetylasparagine (b=-0.154,-0.145,-0.129), N-acetylhistidine (b=-0.659,-0.328, -0.342), retinol (b=-0.276, -0.146,-0.138), serotonin (b=-0.454,-0.341,-0.292), ergothioneine (b=-0.25, -0.191, -0.198) and sacharopine (b=0.449, 0.189, 0.203).
The glutamate metabolism has been implicated in the pathogenesis of AD and these analysis identify novel targets in this pathway. Similarly, phospholipids have been previously reported as major contributors to AD pathology, and recently immune cells, another major player of AD pathology, have emerged as targets of glycerohosphoinositol. These preliminary analysis identify novel contributors to known AD pathological pathways that we are currently following up in independent datasets (ROSMAP, ADNI). In addition, our findings suggest that the analysis of the metabolome can identify novel therapeutic targets for the continuum of the disease, but also, specific of disease etiology.

CLUSTERIN MITIGATES DISPERSION OF ALPHA-SYNUCLEIN AND ER STRESS IN A CELL MODEL OF PARKINSON'S DISEASE.

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Introduction and Objective

Alpha-synuclein (a-syn) is a protein involved mainly in synaptic transmission between neurons. Aggregates of a-syn (Lewy Bodies) are associated with Parkinson's disease (PD) which impair proper cell physiology. A-syn is also secreted to the extracellular compartment and may spread to surrounding cells. Clusterin (CLU), also known as apolipoprotein J, is an extracellular chaperone involved in homeostasis, proteostasis and inhibition of cell death pathways. The role of CLU in PD is still a matter of investigation, however genetic variations in CLU gene have been associated to Alzheimer's Disease. Considering the a-syn cytotoxicity and its pathological roles in dopaminergic neurons, the purpose of the present study is to analyse the role of CLU on a-syn dispersion, cell viability and the levels of proteins associated to ER stress in SH-SY5Y-derived dopaminergic neuron-like cells.

Material and Methods

SH-SY5Y neuroblastoma were differentiated into neuron-like cells during 11 days with all-trans retinoic acid and transfected with the following plasmids: Empty Vector (EV) and a-syn Wild Type (WT), A30P and A53T mutants (associated to familial PD). CLU (1mg/ml) was added to culture medium simultaneously to transfection (during 48 hours). Soluble and insoluble protein fractions as well as medium of SH-SY5Y neuron-like were collected and subjected to Western Blot to evaluate the levels of insoluble a-syn, GRP-78, CHOP and XBP-1 to evaluate ER stress, Calcein-AM assay and caspase-3 to evaluate cell viability and co-immunoprecipitation to analyse the association between CLU and a-syn in the extracellular medium. Data were analyzed by Two Way ANOVA, $p<0.05$ indicates significant differences. N=5 cultures/experimental group.

Results

Alpha-syn A53T expression significantly increased the levels of insoluble a-syn by 400% compared to EV or a-syn WT and A30P. The presence of CLU in culture medium blocked this increase in insoluble a-syn. Co-immunoprecipitation results demonstrated that CLU and a-syn interacted in the extracellular medium, indicating that a-syn A53T was trapped in the extracellular medium preventing its invasion to surrounding cells. Expression of alpha-syn A30P and A53T promoted a significant decrease in cell viability, while CLU treatment prevented that. Proteins associated to ER stress were increased in A30P and A53T a-syn expressing cells (BiP 50%, ATF6 30% and XBP-1 50%) compared to control groups, whilst CLU treatment prevented that.

Conclusions

Clusterin prevented the spread of a-syn proteinopathy and improved cell viability, which is related to mitigation of ER stress. These data suggest that CLU might be a possible therapy to prevent cell death in PD.

CHARACTERIZATION OF iPSCs-DERIVED HUMAN MICROGLIA FROM A FAMILY WITH NASU-HAKOLA DISEASE.

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Microglia are the resident immune cells of the brain and express the triggering receptor expressed on myeloid cells 2 (TREM2), an innate immune receptor of the immunoglobulin superfamily. The receptor can also be cleaved in a soluble form (sTREM2). Mutations in the TREM2 gene are associated with Nasu-Hakola disease (NHD). NHD is a rare leukodystrophy characterized by polycystic osseous lesions and early-onset dementia. However, the mechanisms by which mutations in TREM2 alter microglia function remain unclear. To begin to address these unanswered questions, dermal fibroblasts were collected from siblings who were positive for the TREM2 mutation (NHD; Q33X/Q33X) or normal controls (WT/WT). Fibroblasts were then reprogrammed into induced pluripotent stem cells (iPSC). Fully characterized iPSCs were then differentiated into microglia like cells (iMGLs). TREM2 Q33X results in an early stop mutation, leading to nonsense mediated decay; thus, as expected, sTREM2 was undetectable in NHD iMGLs. NHD iMGLs exhibited a lower survival rate compared to related control iMGLs. Additionally, NHD iMGLs were less reactive under homeostatic conditions and displayed an exaggerated reaction in the presence of LPS. The soluble form of TREM2 may enhance microglia survival and proliferation. Thus, we are currently investigating whether sTREM2 is sufficient to rescue the phenotype observed in NHD iMGLs. Together, our findings begin to clarify how TREM2 Q33X impacts human microglia and provides a platform for testing novel therapeutic interventions.

APOLIPOPROTEIN E VARIANT ALTERS CYTOKINE SECRETION PATTERNS IN ASTROCYTES FOLLOWING AMYLOID-B STIMULUS

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Alzheimer's disease (AD) is a neurodegenerative disease characterized by amyloid-β plaques, neurofibrillary tau tangles, and neuroinflammation that currently affects more than 5.6 million Americans over the age of 65. The ε4 variant of apolipoprotein E (APOE4) is the strongest and most common genetic risk factor for AD, however, it is not fully understood how APOE genotype affects the neuroinflammation cascade in different neural cell types. The purpose of our experiment was to elucidate the differences in cytokine signatures expressed by APOE4 astrocytes as compared to astrocytes carrying the more common ε3 variant (APOE3) when stimulated by varying concentrations of amyloid-β, as well as how neurons react to these signatures. Cytokine signaling is extremely complex and thus, multivariate analysis is necessary to take into account the covariation and dependence between levels of these molecular analytes. Using partial least squares discriminant analysis, we found that amyloid-β-stimulated APOE4 astrocytic activation and subsequent impact on neurons is distinct from the APOE3 astrocytic reaction. Specifically, APOE4 astrocytes treated with amyloid-β secrete more of the pro-inflammatory cytokines IL-6 and LIF, while APOE3 astrocytes treated with amyloid-β secrete more of the neuroprotective factors IP-10/CXCL10, VEGF, and KC/CXCL1. Additionally, we found that despite exposure to higher levels of pro-inflammatory cytokines, APOE4 neurons treated with the APOE4 astrocyte-conditioned media had significantly higher survival rates than APOE4 neurons exposed to amyloid-β alone, suggesting that the pro-inflammatory signature may in fact be activating downstream defensive mechanisms within APOE4 neurons. Our results highlight the importance of disentangling cell type-specific reactions to AD stimuli and communication between cell types in the face of disease insults. Future studies will define the downstream neuronal mechanisms underlying the protective effect of astrocyte-secreted cytokine cues in APOE4 vs. APOE3 systems.

HUMAN iPSC-ASTROCYTES RECAPITULATE A1 REACTIVE NEUROTOXICITY IN VITRO

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Astrocytes are the most abundant macroglial cell type in the central nervous system (CNS) and perform critical functions such as providing neuronal support, maintaining brain homeostasis, and modulating the immune response. Their increasing implication in neurodegenerative diseases makes them an appealing iPSC-based model system to investigate disease mechanisms. We generated human induced pluripotent stem cell-derived astrocytes and identified CD49f as a novel surface marker for astrocyte purification. hiPSC-derived CD49f+ astrocytes display highly heterogeneous morphology, express canonical markers, perform glutamate uptake, and transcriptionally resemble primary human astrocytes. Importantly, we show that these human astrocytes can be activated to exhibit the A1 neurotoxic phenotype recently described in rodents. Human iPSC-derived A1 astrocytes show reduced phagocytic capacity, impaired glutamate uptake, and are toxic to neurons. Bulk and single-cell RNA sequencing analysis reveals several dysfunctional pathways in A1 astrocytes vs. unstimulated, and suggests that A1 reactivity varies with astrocyte developmental stages. Taken together, this work establishes a novel, human, patient-specific in vitro model ideal for deciphering astrocyte-related pathogenic mechanisms of neurodegeneration, which we are now applying to the study of Alzheimer's disease and multiple sclerosis.

INVESTIGATION OF MECHANISMS INVOLVED IN APOLIPOPROTEIN E AUTOPHAGIC DEGRADATION AND ENDOCYTOSIS

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The Apolipoprotein E4 allele (APOE4) is the greatest genetic risk factor for late-onset Alzheimer's disease (AD). Levels of APOE4 protein are reduced in human tissue, possibly due to rapid degradation, but the mechanism of degradation is unknown. We have found that APOE is degraded by the lysosome, and have investigated autophagic mechanisms that may be involved. Lysosomal de-acidification with bafilomycin A1 (Baf) or disruption of the golgi apparatus with Brefeldin A cause APOE accumulation, and prevent lysosomal trafficking of pH-sensitive dual tagged APOE-mCherry-SepHluorin. To investigate the mechanism of APOE lysosomal degradation, we knocked down autophagy proteins Lamp2, Atg7, Stx17, and non-canonical autophagy protein Rubicon. In mouse brain tissue, Lamp2 knockout results in accumulation of mouse APOE in vivo. In transfected St14a cells, APOE3 and APOE4 significantly accumulate with Lamp2A knockdown, but APOE2, which is protective for AD, does not. Staining and lysosomal immunoprecipitation suggest that APOE accumulates around the periphery of Lamp2A knockdown lysosomes. In human immortalized hepatic HepG2 cells, knockdown of Lamp2A, Atg7 or Stx17 all significantly increase endogenous APOE3 levels. Atg7 knockdown also impairs endocytosis of APOE, as does knockdown of Rubicon. Endocytosis of APOE3 and APOE4 significantly increase LC3 lipidation in St14a and HepG2 cells, and GABARAPL1, another member of the Atg8 family, co-localizes with endocytosed APOE3 following chloroquine treatment. Imaging of the endocytosis of fluorescent APOE suggests that APOE is endocytosed in an isoform-dependent manner, with APOE4 endocytosed more robustly than APOE3, and APOE2 endocytosed the least. In conclusion, our data suggest that APOE is degraded by the lysosome through autophagy that may require Lamp2A, Atg7, and Stx17, and may enter the cell through LC3-dependent endocytosis. APOE4, the AD risk allele, appears to be degraded by the lysosome through autophagy and to be endocytosed more robustly than APOE2, which may contribute to AD pathogenesis. Understanding how APOE traffics through the endolysosomal system may lead to insight into mechanisms that contribute to AD, and may be targeted to develop novel therapies.

ALS-LINKED CYTOPLASMIC INCLUSIONS ALTER DNA DAMAGE RESPONSE ACTIVATION

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The vast majority of all Amyotrophic Lateral Sclerosis (ALS) cases are marked by the accumulation of cytoplasmic inclusions containing TDP-43 in patient motor neurons and predisposing mutations fall in TDP-43 and FUS genes. It has been reported that ALS motor neurons fail to efficiently repair DNA damage. Scattered evidences propose that both FUS and TDP-43 play a role in DNA repair under physiological conditions. Instead, whether TDP-43 and FUS pathological aggregation may affect DDR activation is still unknown. Thus, we analysed DDR activation in cells experiencing cytoplasmic inclusions containing TDP-43 or ALS-linked mutant FUS-P525L. We observed that acute induction of cytoplasmic inclusions of both TDP-43 and mutant FUS, rapidly induce the aberrant hyper-activation of DDR apical kinase ATM, which is nevertheless defective in coordinating the cell response to exogenous DNA damage. Intriguingly, block of ATM activation by different means is able to revert retina neurodegeneration in a D. Melanogaster TDP-43 ALS-model system. We observed that FUS cytoplasmic inclusions determine an accumulation of the autophagic-cargo protein p62 that sequester the DNA repair factor RNF168 into cytoplasmic granules. Importantly, p62 inactivation restores RNF168 nuclear level and ameliorates DDR defects. Similarly, RNF168 overexpression in cells with FUS cytoplasmic inclusions reduces DDR aberrant activation. These results indicate a novel mechanistic link between FUS and TDP-43 cytoplasmic inclusions and DDR mis-regulation leading to DNA repair defects.

IMPAIRMENT IN PS1 ACTIVITY IN MICROGLIA LEADS TO PRO-INFLAMMATORY PHENOTYPE AND NEUROTOXICITY

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Alzheimer's disease (AD) is the most common type of dementia affecting more than 20 million people worldwide. Currently, there is no cure for the disease and no effective treatment that slows down the disease's progression. Amyloid beta (Abeta) and neurofibrillary tangle exhibit synergistic effects that finally lead to an acceleration of neurodegenerative mechanisms involved in metabolism, cellular detoxification, mitochondrial dysfunction, and energy deficiency which results in the formation of neuritic plaques. Microglia activation in AD is considered a double-edged sword suggesting that on one hand, it has the ability to clear the amyloid load and on the other hand, it can cause an increase in neuronal pruoining that may lead to neurodegeneration. The majority of the early cases of AD genetic cases (75%) relate to presenilin 1 (PS1), located at chromosome 14. PS1 is considered important determinants of the γ-secretase catalytic site that processes amyloid precursor protein (APP) to Abeta toxic isoforms and is essential for the activation of signaling important for cell activity such as TREM2. We show that PS1 regulated microglia inflammatory activity. We found that an inhibition of gamma secretase activity impaired microglias ability to clear and uptake Abeta. It has been reported that a mutation in PS1, affiliated with AD, reduces its processing and results in impaired gamma secretase activity. We discovered that Alzheimer's disease mutated PS1 in microglia increases secretion of pro-inflammatory cytokines and neurotoxicity. Furthermore, mutated PS1 show a deficiency to uptake Abeta that is linked with an impairment in expressing scavenger receptors. Further research of the pathways in which PS1 regulates microglia activity may lead to identifying new candidate targets for therapeutic intervention.

GENETIC DEPLETION OF GPNMB DOES NOT ALTER SYNUCLEIN-RELATED PATHOLOGY

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Genomic variants near the gene GPNMB are associated with both elevated Parkinson's disease risk and higher expression of this gene. Furthermore, GPNMB is a "DAM" gene, elevated in microglia in many mouse models of neurodegenerative disease as well as in human patients. This suggests that inhibiting GPNMB activity might be protective in Parkinson's disease. We tested this hypothesis in three different mouse models of neurological diseases. We found that Gpnmb deletion did not rescue histological, cellular, behavioral, neurochemical or gene expression phenotypes in (1) the lysolecithin model of demyelination, (2) the pre-formed fibril model of spreading alpha-synucleinopathy, or (3) a dopaminergic lesion model induced by AAV-delivered mutant alpha-synuclein. Therefore, these endpoints, in these mouse models, are not suitable for studying the role of Gpnmb in the development or progression of Parkinson's disease.

RATIONALLY DESIGNED NEUROPROTECTIVE ANTIBODIES AGAINST PRIONS

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Antibodies to conformational epitopes of the prion protein PrP^{C} can trigger neurotoxicity mimicking prion infections. Here we show that the toxic antibody POM1 induces the H-latch, an intramolecular R208-H140 hydrogen bond within PrP^{C} that rigidifies its epitope while flexibilizing its α2-α3 and β2-α2 loops. Cerebellar slices expressing a PrP^{R207A} mutant unable to form the H-latch were resistant to POM1 toxicity. Conversely, expression of a PrP^{2Cys} mutant mimicking the H-latch was constitutively toxic. We therefore engineered “pomologs” antibodies retaining the POM1 epitope specificity yet unable to induce the H-latch. These antibodies were innocuous when injected intracerebrally, conferred resistance to POM1 toxicity, and repressed neuro-degeneration of prion-infected cerebellar organotypic cultures, suggesting that they prevented the docking of infectious prions to PrP^{C}. Finally, we developed phage-displayed antibody fragments differentially binding wild-type PrP^{C}, but not PrP^{2Cys}. These reagents conferred protection against prions and, similarly to innocuous pomologs, were unable to induce the H-latch. These findings identify the H-latch and the related changes in PrP^{C} as important for prion neurotoxicity, and enable the rational design of drugs interfering with the toxicity of prions.

INHIBITION OF α-SYNUCLEIN FIBRILLOGENESIS BY DIPHENYL TRIAZINE HYBRIDS: DESIGN, SYNTHESIS AND IN-VITRO EFFICACY STUDIES

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Parkinson's disease (PD) is the 2nd most important neuro-degenerative disease after Alzheimer's disease. Aggregation of α-synuclein has been found to be one of the central events in PD and has been reported in the Lewy bodies and Lewy Neurites in the brain regions of the Substantia Nigra. Therefore, its inhibition and fibril disaggregation is one of the approaches for the treatment of PD. In literature, it has been reported that the various 1,2,4-triazine compounds have the ability to cross the BBB and found to be neuroprotective in several models of PD. Here, we design and accomplished docking studies of synthesized di-phenyl triazine hybrids against α-synuclein binding. We did in-vitro efficacy studies of these hybrids against α-synuclein fibrillogenesis and also for disaggregation. Further, to test these compounds in various in-vivo models, we also performed cytotoxicity studies. We observed that out of nine compounds, five of them (A1, A2, A4, A8 and A9) have a substantial binding affinity with the essential residues of α-syn having binding energy values -6.0, -7.0, -6.3, -6.6 and -6.7 Kcal/mol respectively. These inhibitors also have a potential inhibitory activity as observed by α-synuclein fibrillogenesis confirmed by Thioflavin-T assay and fluorescence microscopy. Further, A2 has been found to be a good disaggregator in the pre-aggregated form of α-synuclein confirmed by fluorescence microscopy. Further, the cell viability results have shown that none of the compounds were found to be toxic except A2. We also observed that cells incubated with aggregated α-synuclein in the presence of A4 and A8 compounds exhibited a protective role. These findings suggest that di-phenyl triazine based compounds can be further investigated for synucleinopathies and Lewy-body dementia diseases.

INTRAVASCULAR DELIVERY OF NEURAL PRECURSORS IMPROVES MEMORY IMPAIRMENT IN MOUSE MODELS OF ALZHEIMER'S DISEASE

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Background: Currently available treatments for Alzheimer's disease (AD) are essentially symptomatic and not disease-modifying, positioning it as a serious global health problem with the increase of the elderly population. This disease is clinically characterized by progressive memory impairment and cognitive dysfunction. The neuropathological hallmarks of AD are the accumulation of extracellular β-amyloid (Aβ) peptide in senile plaques, intracellular deposition of hyper-phosphorylated tau as neurofibrillary tangles (TNF), neurodegeneration, synaptic loss throughout the brain, and a neuroinflammatory process governed by the activation of glial cells. Recently, stem cell therapy has provided great potential in treating AD patients. However, there is an urgent need to replace the conventional intracerebral stem cell therapy for a less invasive method to avoid some of the technical challenges. Our recently published results indicated that peripheral treatment with neural precursors (NPs) ameliorates clinical symptoms by reducing the disease-associated neuroinflammation in a mouse model of Parkinson's disease. Therefore, we hypothesize that intravenous administration of NPs and their released neurotrophic factors can be used as a non-invasive therapy to ameliorate memory impairment in mouse models of AD. **Methods:** In this pre-clinical study, NPs derived from mesenchymal stem cells (MSC-NPs) and induced pluripotent stem cells (iPSC-NPs) were intravenously injected into APP/PS1 and P301S mice at 3 and 6 months old (before and after brain pathology is stablished). Before treatment and at the age of 7 months old, experimental and control (PBS) animals were subjected to Barnes maze task, novel object recognition and rotarod test. **Results:** NPs treated mice displayed an amelioration in memory dysfunction compare to the PBS-injected animals. In addition, P301S mice injected with NPs showed improved motor function to rotarod coordination test in comparison to the control group. **Conclusion**: Peripheral inoculation using NPs can be used as a treatment to reduce AD-related clinical signs.

APOE4 DISRUPTS INSULIN SIGNALING IN N2A NEUROBLASTOMA CELLS

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Background: Despite over 25 years of Alzheimer's Disease (AD) research and therapeutics aimed at Aβ plaques, there are no disease-modifying treatments available. ApoE-ε4 is the single biggest genetic risk factor for sporadic AD. ApoE-ε4 inheritance confers a decreased cerebral glucose metabolism that is obvious years before AD signs and symptoms. Also, Type 2 Diabetic individuals bearing one ApoE-ε4 allele have a much higher risk to develop AD, supporting the hypothesis that central insulin resistance contributes to AD pathophysiology. Furthermore, ApoE4 was recently shown to impair neuronal insulin signaling in mice in vivo. If ApoE4 inheritance confers neural insulin resistance, this could result in a metabolic-bioenergetic deficit in aged neural cells that could contribute to AD pathophysiology.

Question. What is the impact of ApoE2, -E3 and -E4 on insulin sensitivity on neural N2A cells?
Methods. N2A neuroblastoma cells were stably transfected with vectors containing GFP (control), ApoE2, -E3, or -E4. These cells were dosed with insulin ranging from 0.02nM to 400nM and p-Akt, Akt, p-Erk and Erk levels were measured to generate dose response curves. Insulin-stimulated mitochondrial metabolism was also measured by Agilent Seahorse. Furthermore, the physical interaction of ApoE isoforms with insulin receptor was measured in vitro via co-immunoprecipitation (co-IP) and biolayer interferometry (BLI).

Results. First, we observed that each of the ApoE alleles increased the rate and final extent of insulin dependent p-Akt response, more than the no-ApoE control. Second, we observed that the greatest rate and final extent of insulin-dependent p-Akt response was: ApoE3>ApoE4>ApoE2. Third, the ApoE4 isoform has a statistically significant lower basal response, lower slope, and lower maximal insulin-induced p-Akt response compared to ApoE3. We expect that this lower insulin sensitivity will confer a lower glycolytic rate and mitochondrial glucose oxidation, to be measured by oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). We have begun BLI-based ApoE-Insulin Receptor binding experiments to determine if the decreased insulin sensitivity of ApoE4-bearing neural cells is the result of altered binding of ApoE4 to Insulin Receptor.

Interpretation. These data support the hypothesis that ApoE4 contributes to metabolic dysfunction of neurons through impairment of insulin signaling relative to ApoE3. These results could help to explain the cerebral hypometabolism of glucose in ApoE4 carriers that exists many years before cognitive defects.

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HUMAN PLURIPOTENT STEM CELLS AS A RESEARCH TOOL FOR ELUCIDATING THE ROLE OF GLIAL CELLS IN ALZHEIMER´S DISEASE

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Background:
Alzheimer's disease (AD) is characterized by presenting a complex pathology, not fully resolved yet. This fact, together with the lack of reliable models, has impeded the development of effective therapies. Recently, several studies have shown that functional glial cell defects have a key role in the pathology of AD. However, this glial dysfunction, currently, cannot be correctly modeled using the available animal models, so we hypothesized that cells derived from Alzheimer's patients can serve as a better platform for studying the disease. In this sense, human pluripotent stem cells (hPSC) allow the generation of different types of neural cells, which can be used for disease modeling, identification of new targets and drugs development.
Methods:
We have a collection of hiPSCs derived from patients with sporadic forms of AD. We have differentiated these cells towards neural lineage to obtain neurons and astrocytes. For the generation of oligodendrocytes (OLs), we have developed a fast and robust protocol to generate mature OLs in just 22 days.
Results:
We have generated neural precursors from all the lines tested. In the case of OLs, the cells generated resemble primary OLs and can myelinate neurons in vivo and in vitro using a screening compatible platform. This platform is being transferred for the generation of the other glial cells.
Conclusions:
This methodology can be used to elucidate the pathogenic pathways associated with neurodegeneration and to identify new therapeutic targets susceptible to modulation, contributing to the development of new effective drugs against AD.
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QUANTITATIVE ANALYSIS OF EARLY ENDOSOME PATHOLOGY IN YOUNG AND AGED TS65DN MICE FOLLOWING MATERNAL CHOLINE SUPPLEMENTATION (MCS) USING 3D RECONSTRUCTED Z-STACKS

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Accurate assessment of changes in intracellular vesicles is crucial for the rigorous study of neurodegenerative disease. Defects within the endosomal-lysosomal (E-L) system and autophagy pathways have been described in many disorders, including Alzheimer's disease (AD) and Down syndrome (DS). Prior studies have performed vesicular quantitation using confocal microscopy images taken on a single plane of focus. However, important aspects of E-L pathobiology may be missed by analyzing only a single image of the perikaryon, as endosomes and lysosomes are distributed throughout the cell body and neuronal processes. In order to perform quantitative morphometry on vesicle populations, we compared 2 analysis programs for quantification of immunolabeled early endosomes (EEs) using 3D reconstructed z-stacks: ImageJ and Imaris (Bitplane). Parallel quantitation using both ImageJ and Imaris within transmitter-identified basal forebrain cholinergic neurons (BFCNs) revealed Imaris was significantly more adept at accurately quantifying individual EEs in densely packed clusters within BFCNs. To further test Imaris' capacity for precise quantification of vesicular pathology, we assessed the effects of an inexpensive treatment modality, maternal choline supplementation (MCS), on the EE pathology observed within a murine model of DS, the Ts65Dn mouse. This trisomic mouse model recapitulates several key aspects of DS and AD pathology, including cognitive dysfunction, loss of BFCNs, and dysregulation of the E-L system. To explore the extent to which early choline delivery can affect EE phenotype and BFCN survival, we compared Ts65Dn mice at two time-points: 3-4 months of age (MO) (pre-BFCN degeneration) and 10-12 MO (post-BFCN degeneration). Quantitative analysis of endosomal compartments using Imaris indicate 11 MO Ts65Dn mice had significantly more Rab5-immunoreactive EEs per choline acetyltransferase (ChAT)-identified BFCN compared to disomic (2N) littermates when these offspring were fed a normal choline diet. No significant differences in EEs were found between Ts65Dn and 2N littermates at 3-4 MO. MCS administered during the perinatal period decreased the average number of EEs per BFCN in both aged Ts65Dn and 2N mice, irrespective of genotype and underlying endosomal pathology. Stereological data suggests increased survivability of BFCNs within the medial septal nucleus, indicating that early-life MCS treatment may confer lasting neuroprotective benefits on vulnerable neuronal populations.

CAN ZEBRAFISH (*DANIO RERIO*) PRODUCE AMYLOID BETA PEPTIDE? EXPRESSING TAGGED FORMS OF APPA AND APPB *IN VIVO*

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Alzheimer's Disease (AD) has confounded decades of effort into finding effective therapeutic interventions. A long-standing theory of disease pathogenesis, the Amyloid Cascade Hypothesis, has remained popular despite conflicting evidence. Although it is unclear whether or not amyloid beta (Aβ) plaques and tau neurofibrilliary tangles are drivers of disease progression, Aβ remains an important piece of the Alzheimer's puzzle. A 2014 study argued that the β-secretase cleavage sites in the APP-orthologous proteins of zebrafish (Appa and Appb) are not conserved (Moore et al. Mol Biol Evol. 31:696). Therefore, zebrafish may not produce the Aβ peptide. This has important implications for the interpretation of results from zebrafish AD models such as those used in our laboratory. However, the ability or not of zebrafish to form Aβ has never been demonstrated experimentally.

We designed constructs containing the protein-coding sequences for human APP and zebrafish Appa and Appb with FLAG tags added at both the N and C termini to facilitate detection of these proteins on western immunoblots. We subcloned these constructs into expression vectors optimised for use in zebrafish and expressed the tagged forms of Appa and Appb in zebrafish embryos by injection as mRNA. We will also co-inject mRNAs coding for human and zebrafish forms of BACE1 as well as observing the effects of BACE inhibitors. In preliminary experiments, low-level expression of our constructs has been detected as specific bands at the expected sizes. Experiments are ongoing to optimise expression of the constructs. Evidence of whether or not zebrafish can produce Aβ will provide valuable context for the study of Alzheimer's disease in zebrafish, for interpretation of the Amyloid Cascade Hypothesis, and for AD research as a whole.

SEXUAL DIMORPHISM IN VESICULAR ACETYLCHOLINE TRANSPORTER (VACHT) REGULATION OF PLAQUE PATHOLOGY IN ALZHEIMER'S DISEASE KNOCK-IN MOUSE MODELS

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Cholinergic deficiency is a characteristic of many neurodegenerative disorders including Alzheimer's disease (AD). Decreased levels of the vesicular acetylcholine transporter (VAChT) have been detected in AD patients and imaging data suggests that VAChT levels can predict human pathology, however, whether changes in VAChT are associated with plaque formation is unknown. To test for a causal relationship between VAChT levels and amyloid beta (Abeta) plaques, we crossed a humanized APP knock-in (KI) mouse, carrying 2 AD-associated familial mutations, with mice lacking VAChT in forebrain neurons. We quantified the area covered by plaques in the cortex and the levels of insoluble Abeta in 9 and 12-month-old males and females. We found that elimination of VAChT increased plaque area and Abeta levels. Remarkably, these changes were seen only in males, while females remained unaffected. Also, to study whether increased VAChT regulates pathology, we crossed APPKI mice carrying 3 AD mutations with mice overexpressing VAChT. We analyzed plaque pathology in 2, 3 and 6-month-old males and females and we found a decrease in plaque area and Abeta levels in 2 and 3-month-old VAChT-overexpressing males; females were not affected. Interestingly, at 6 months VAChT regulation of plaque pathology is lost, suggesting that VAChT influences early stages of Abeta deposition in these mice. These results indicate a causal relationship between VAChT and plaque pathology in a humanized AD mouse model and point to a sexually dimorphic response in mice due to changes in VAChT, a topic that should be explored in humans in the future.

LOSS OF LONG ISOFORM OF hnRNP R IMPAIRS DNA DAMAGE RESPONSE IN MOTONEURONS: A NEW PATHOPHYSIOLOGICAL MECHANISM FOR SMA AND ALS

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Neurons critically rely on the functions of RNA-binding proteins to maintain their extensive polarity and resistance to environmental and aging-related neurotoxic stresses. The RNA-binding protein hnRNP R has a diverse range of post-transcriptional regulatory functions and is important for neuronal development by regulating axon growth. hnRNP R interacts with the wild-type SMN protein, but not with the mutant forms found in disease, and hnRNP R expression was reduced in motoneuron axons upon SMN deletion. In patients with FTLD-FUS, hnRNP R has been found as a component of neuronal cytoplasmic and intranuclear inclusions suggesting that hnRNP R dysfunction might contribute to the underlying disease pathogenesis. *Hnrnp*r pre-mRNA undergoes alternative splicing of exon 2 to produce transcripts encoding two protein isoforms: a full-length protein (long isoform), and a shorter form lacking the N-terminal acidic domain (short isoform). While the neuronal defects produced by total hnRNP R depletion have been investigated before, the individual functions of each hnRNP R isoforms are unknown. To investigate such functions we generated a hnRNP R knockout mouse (*Hnrnpr*$^{tm1a/tm1a}$) with selective loss of the full-length hnRNP R isoform. Motoneurons cultured from *Hnrnpr*$^{tm1a/tm1a}$ mice did not show any obvious RNA transport or axonal growth defects suggesting that the short hnRNP R isoform can compensate for the loss of the long one for these processes. However, mutant mice show an accumulation of double-strand breaks and an impaired DNA damage response, indicating a potential role of the hnRNP R long isoform in DNA damage repair. Proteomic analysis of the hnRNP R interactome revealed the multifunctional Y-box binding protein 1 (YB-1) as a top interacting partner. Motoneurons depleted of YB-1 also exhibit an accumulation of double-strand breaks and we provide evidence for a mechanistic link between hnRNP R, YB-1, and impaired DNA repair. Our findings thus suggest a role of hnRNP R in maintaining genomic integrity and highlight the function of its N-terminal acidic domain in this context.

MISLOCALIZED EXPRESSION OF THE NUCLEAR PORE COMPLEX PROTEINS NUP153, -93, AND -214 IN ALZHEIMER'S DISEASE BRAINS

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Alzheimer's disease (AD) is a progressive, neurocognitive disorder characterized by memory dysfunction. The presence of neuropathological aberrations, namely amyloid plaques, and neurofibrillary tangles, although key characteristics of this disease, have shown to be poor prognostic indicators. As such, clinical trials targeting AD neuropathology have largely been unfruitful, necessitating new research into novel mechanisms of the underlying pathways mediating cognitive decline. Nuclear pore complexes (NPCs) are the main conduits for molecular exchange across the nuclear envelope in eukaryotic cells. The NPC contains approximately 30 distinct nucleoporins (NUPs) which form a selective channel that supports the factor-mediated shuttling of cargo through the NPC. Mutations in nucleoporin genes have been linked to various human diseases including neurodegenerative diseases. Tau is the major component of neurofibrillary tangles in (AD), and a recent study has suggested a role in NPC deterioration and thus nuclear-cytoplasmic defects. This study however was not an extensive one and only investigated four nucleoproteins from one layer of the NPC structure. In this study, we have targeted all three major components of the NPC structure, by analyzing gene expression of representative NUPs from homogenate brain tissue and neuronal data in AD brains. Three significantly differentially expressed NUPs (NUP-214, -93, -153), representing different parts of the NPC structure (cytoplasmic filaments, inner ring structure, nuclear basket), were selected for validation by immunohistochemistry and Western blotting in postmortem human hippocampal sections. Bioinformatic analysis revealed widespread differential NUP gene expression across multiple brain regions in AD. These results were reflected in immunohistochemistry and immunoblotting results, which revealed quantity and localization changes of the selected NUPs in AD. Our findings revealed mislocalization of cytoplasmic-facing nucleoporin NUP214 in the cytoplasm in AD and nuclear localization of inner ring and nuclear basket nucleoporins NUP93 and NUP153 in hippocampal CA1 neurons. These results and this research, represents one of the first attempts to categorize differential changes throughout the entire structure of the NPC in AD. Future studies will explore the hierarchical relationship between neuropathological hallmarks of AD and NPC aberrations to better understand the etiology of impaired nucleocytoplasmic transport in neurodegeneration.

MODELING KIFA LOSS-OF-FUNCTION *IN VITRO*: UNDERSTANDING THE RELEVANCE OF KIF5A-MEDIATED AXONAL TRANSPORT DEFECTS IN THE PATHOGENESIS OF ALS

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The regulation of cytoskeleton dynamics is of major relevance for maintaining motor neuron morphology and polarity. Growing evidence coming from genetic studies has positioned disruptions to cytoskeletal pathways as an emerging theme for the pathogenesis of motor neuron diseases, including Amyotrophic Lateral Sclerosis (ALS). Recently, high throughput Whole-Genome Sequencing (WGS) approaches have identified mutations in *KIF5A*. This microtubule-binding motor protein is involved in the anterograde transport of a wide variety of cargos in mammalian cells. Interestingly, mutations in *KIF5A* have also been found in other forms of motor neuron disease such as Hereditary Spastic Paraplegia (HSP) and Charcot-Marie-Tooth Type 2 (CMT2). Even though these studies have been critical to constraint the hypothesis that cytoskeletal dynamics is a major process affected in the disease, how these genetic variants specifically contribute to compromised motor neuron function in ALS remains poorly understood. In the study where *KIF5A* was identified, the connection between the mutant genotype and an *in vitro* cellular phenotype is still lacking. The location of these mutations in the C-terminal cargo binding domain of the protein suggests a possible molecular mechanism whereby decreased loading to the motor might eventually lead to disrupted transport of specific cargos that are important for motor neuron survival. Some of the cargos known to be trafficked by KIF5A in mammalian neurons are cytoskeletal elements such as neurofilaments, mitochondria and granules containing RNA-binding proteins (RBP) among others. However, the transport or localization of which of these cargos is impaired in the context of KIF5A mutations is unclear. Here, we combined the application of CRISPR/Cas9 genome editing technology in human motor neuron models to generate and characterize KIF5A$^{-/-}$ mutant motor neurons. Specifically, we find KIF5A protein to be highly enriched in human derived motor neurons, and its transcript to be upregulated in older neurons. Preliminary characterization of the mutant lines shows that depletion of *KIF5A* results in significant deficits in neurite outgrowth and axonal repair capacity, but no changes in neurofilament protein expression and localization. Although further functional examination of these mutants is required, taken together, these phenotypes harbor plausible connections to motor neuron pathology.

G PROTEIN-COUPLED RECEPTOR KINASES ARE LINKED WITH ALZHEIMER'S DISEASE PATHOLOGY

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Alzheimer's Disease (AD) is primarily characterized by the extracellular deposition of amyloid-β in amyloid plaques and the intracellular aggregation of hyperphosphorylated tau in neurofibrillary tangles (NFTs). Phosphorylation is a vital cellular process associated with protein homeostasis and disease. Tau, for instance, has more than 80 putative phosphorylation sites, which regulate both physiological and pathological functions of this protein. Although several tau kinases have been identified to play causative roles in tau pathogenesis, kinase-targeted therapies for AD have been unsuccessful thus far. Critically, the kinases responsible for several putative tau phosphorylation sites remain unknown, bearing tremendous therapeutic potential. G protein-coupled receptor kinases (GRKs) are a family of seven kinases (GRKs 1-7) that are implicated in numerous peripheral and brain pathologies. Nevertheless, a comprehensive characterization of the expression and distribution of GRKs in the non-demented and AD human brain has not been previously performed. To address this fundamental gap in knowledge, we hypothesize that determination of the GRK expression profile and pattern in the human brain will provide insight into the putative involvement of GRKs in AD pathobiology. We performed a comprehensive immunohistochemical and biochemical analysis of the four ubiquitously expressed GRKs (i.e., GRKs 2, 3, 5, and 6) in postmortem human brain tissue from the hippocampus and entorhinal cortex of control subjects and AD patients. We report that levels of the GRKs are specifically decreased in the CA1 region of the AD hippocampus relative to the CA3, dentate gyrus, subiculum, and entorhinal cortex. Moreover, the GRKs display unique cell type-specific expression patterns in neurons, astrocytes, and microglia. Biochemical analysis indicates that the GRKs are differentially associated with total, soluble, and insoluble tau in the AD brain, potentially regulating distinct pools of tau in AD pathogenesis. In accordance with these findings, we show that the GRKs differentially co-localize with total tau, phosphorylated tau, and late-stage NFTs. Strikingly, GRKs 3 and 5 also co-localize with amyloid-β plaques, suggesting a putative role of these kinases in modulating and possibly linking the pathways that lead to development of the two main pathological hallmarks of AD. Collectively, our findings strongly implicate the GRKs in the pathological phosphorylation of tau and accumulation of both tau and amyloid-β in AD, providing a compelling foundation for future mechanistic studies to further understand the involvement of GRKs in AD pathophysiology.

MicroRNAs TARGETING NEURONAL NMDA RECEPTORS

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N-Methyl-D-Aspartate Receptors (NMDARs) are glutamate-gated calcium channels, which play a major role in synaptic plasticity and synaptic pruning during development. Dysregulation of NMDAR is associated with several neurodegenerative and neuropsychiatric disorders. The NMDAR subunits GluN2A and GluN2B, which are majorly associated with neurological disorders, have a development associated expression profile (Cathala L. et al, 2002, J.Neurosci.,20:5899). We found that GluN2B expression is high in primary neuronal cultures maintained for 7-9 days in vitro (DIV 7-9) and then it starts to gradually decrease. GluN2A expression becomes substantial by DIV 7-9 in cultures and the level is maintained subsequently.
Drug candidates that target NMDAR often cause major side effects resulting in failure during the clinical trials and hence alternate, subtle yet stronger strategies are required to manipulate NMDAR. The differential expression of microRNAs (miRNAs) in neurological disorders is being widely explored (Wang W. et al, 2012, Learn. Mem.,19:359). Our objective is to understand the mechanism of action of some miRNAs involved in NMDAR-mediated synaptic plasticity that are also differentially expressed in disease conditions. Certain miRNAs that are altered in schizophrenia, Huntington disease and autism were predicted to interact with NMDAR subunits by bioinformatics analysis. Luciferase assays showed that miRNAs such as miR-223 and miR-129 interacted with the subunits of NMDAR. Expression levels of the target proteins in primary cortical/hippocampal neurons and in neuronal like cell lines after transfection of miRNA/s are being investigated. Data so far indicate downregulation of GluN2B. We also plan to study these miRNAs in the MK-801 model of schizophrenia. We have injected rats with MK-801 for five days followed by a five day washout period and subjected them to open field test, object recognition test, objection location test and Morris water maze test. It was found that the treated animals exhibit high level of anxiety and have major cognitive impairments. Expression of some of these miRNA/s and their target proteins in these animals is under investigation. These studies may unravel novel mechanisms, which can be of therapeutic potential.

NUCLEAR EXPORT INHIBITOR KPT-350/BIIB100 FOR THERAPY OF *C9ORF72* ALS/FTD

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A hexanucleotide expansion mutation (GGGGCC) in intron 1 of C9orf72 is the most common genetic cause of both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) (C9-ALS/FTD). Loss of C9orf72 protein function, repeat-containing RNA gain-of-function and the production of proteins by repeat-associated non-ATG (RAN) translation have been proposed as possible disease mechanisms for C9-ALS/FTD. Nucleopore protein abnormalities have also been reported in C9-ALS/FTD patient brains and nuclear transport abnormalities are found in patient-derived iPSC neurons. Previously published data show that enhancing nuclear import by overexpressing importin or suppressing nuclear export by using RNAi to inhibit exportin rescued degenerative eye phenotypes caused by G4C2 repeat expansion in C9-ALS/FTD flies. In addition, inhibition of the nuclear export protein exportin1 (XPO1) with the small molecule drug KPT-276 rescues degenerative eye phenotypes in C9-ALS/FTD flies. A similar small molecule nuclear export inhibitor, KPT-350/BIIB100, also shows a neuroprotective role in an inflammatory demyelination mouse model.

We tested the efficacy of KPT-350 in C9orf72 bacterial artificial chromosome (BAC) transgenic mice developed in the Ranum lab. Repeat length matched cohorts of C9-BAC transgenic (C9+) and their non-transgenic (NT) littermates were treated with KPT-350 or vehicle twice a week beginning at 6 weeks of age and monitored for changes in the molecular, behavioral, and neurodegeneration features of the disease. We observed increased KPT-350 levels in C9+ mouse brains and demonstrated direct interaction of an alkyne derivative of KPT-350 with XPO1 using click chemistry and proximity ligation assay in T98 cells. C9+ mice treated with KPT350 showed improvements in 5 out of 8 gait deficits found between C9+ vehicle and NT vehicle treated animals at 55 weeks. At 80 weeks of age poly-GA aggregates were reduced in KPT-350 vs. vehicle treated mice. Additionally, reduced levels of microglial activation were observed by Iba1 staining, suggesting an anti-inflammatory role of KPT-350. In summary, KPT-350 treatment decreased GA levels and improved neuroinflammation and gait abnormalities in C9-BAC transgenic mice. Understanding the mechanisms of how KPT-350 mitigates disease will provide further in-sight into the therapeutic potential of KPT-350 for C9orf72 ALS/FTD.

INVESTIGATION OF THE GENETIC REGULATION OF TFEB AND ITS ROLE IN LYSOSOMAL STORAGE DISORDERS

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Lysosomal dysfunction is associated with more than 70 rare monogenic, largely neurodegenerative disorders caused by the accumulation within lysosomes of macromolecules that cannot be degraded, classified as lysosomal storage disorders (LSDs). TFEB is a master regulator of lysosomal activity whose overexpression has been shown to increase lysosomal function and clear storage in cellular models of LSDs. Our goal is to better understand the genetic regulatory network of TFEB and determine how the network is perturbed by LSDs in order to identify pathways that could potentially be modulated to improve lysosome function and ameliorate storage. We used a genome wide CRISPR knock out screen to expand our knowledge of the genes and pathways that regulate TFEB activity. For this, we generated a cell line expressing blasticidin antibiotic resistance (BSD) driven by a TFEB responsive promoter containing Coordinated Lysosomal Expression and Regulation (CLEAR) elements. BSD-CLEAR cells were infected with the GeCKOv2 lentiviral knock out library and then split into blasticidin treated and untreated groups. Cells were grown for 10 days before pooling and extracting genomic DNA. Knock out library sgRNA sequences were enriched from total genomic DNA by PCR and sequenced. MAGeCK analysis of our preliminary screen confirmed several pathways already known to regulate TFEB such as intracellular calcium and AKT signaling. Additionally, our data show that TFEB activity is regulated by numerous pathways of amino acid metabolism, which are likely related to mTOR activity. In parallel, we performed RNAseq to understand how lysosomal storage affects the transcriptome during human fetal brain development. For this, we evaluated brain tissue from human fetuses affected with Tay Sachs disease (TSD), a prototypic LSD featuring GM2 ganglioside storage and progressive neurodegeneration. We sequenced total RNA extracted from brain sections of 17-week TSD fetuses and two age-matched controls. The results showed that transcriptome signatures of TSD fetuses differed significantly from controls and were especially pronounced in the brainstem. We also identified areas of overlap between TFEB regulatory pathways and differentially expressed pathways in TSD fetal brains, and studies are underway to validate those genes and pathways. We expect the results of these studies to increase our understanding of lysosome function and identify new therapeutic targets for LSDs and other neurodegenerative disorders.

A NOVEL DISEASE MODIFYING THERAPEUTIC AND BIOMARKER FOR VASCULAR CONTRIBUTIONS TO COGNITIVE IMPAIRMENT

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Vascular contributions to cognitive impairment and dementia (VCID) and Alzheimer's disease related dementias (ADRD) are strongly correlated with increases in systemic and brain inflammatory cytokines, increased reactive oxygen species (ROS) and decreased brain blood flow all of which accelerate the progression of VCID and ADRD. Our research team has developed, optimized and completed in vitro and in vivo preclinical development of our novel synthetic glycopeptide derivative of Angiotensin-(1-7), known as PNA5, that has outstanding brain penetration, improved half-life, reverses cognitive impairment in our preclinical model of VCID, inhibits VCID induced microglia activation and brain cytokines and inhibits endothelial ROS production. The purpose of the present study was to 1) Determine if blood-based measurements of serum neurofilament light protein (NFL), an indicator of neuronal injury and/or neuronal degeneration in many neurological condition, is increased in both our preclinical model of VCID as well as in patients with diagnosed VCID and 2) Determine if treatment with PNA5 modifies NFL and is correlated with levels of brain inflammatory cytokines and cognitive dysfunction. In our preclinical model, C57Bl/6J adult male mice were subjected to myocardial infarction to induce heart failure (HF) and VCID. Control animals had surgery but no infarction. After 5 weeks of HF, 3 groups (10-15/group) of animals were treated with daily subcutaneous injections of either 1) Control saline treatment, 2) 50 micrograms/kg/day PNA5, or 3) 500 micrograms/kg/day PNA5, for 21 days. After the end of treatment, animals were sacrificed, and serum and brain samples obtained. Data were analyzed by one-way ANOVA. HF-VCID resulted in cognitive impairment as measured by novel-object recognition (NOR) and significant 126% increase in NFL ($p=.02$) and this increase in NFL was prevented by PNA5 treatment at all doses. The NFL levels were significantly correlated with novel-object recognition scores ($R2 = 0.18$, $p=.03$) and with levels of brain cytokines (composite brain cytokine z-score $R2= 0.41$, $p=.0002$). NFL levels were then assessed in humans diagnosed with VCID (n=25). Plasma levels of NFL were significantly increased by 858% in samples from VCID patients as compared to age-matched controls. These data suggest that NFL may serve as a biomarker in both experimental and clinical studies of VCID and putative VCID-modifying treatments. We will be developing NFL as a biomarker for patient stratification for treatment with PNA5 to decrease cognitive impairment in patients with VCID and ADRD. (supported by NIA to MH).

THE LINKS BETWEEN ALZHEIMER'S DISEASE AND NIEMANN-PICK TYPE C DISEASE

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Alzheimer's disease (AD) and Niemann-Pick type C (NPC) disease are progressive neurodegenerative diseases with very different epidemiology and etiology. AD is a common cause of dementia with a complex polyfactorial etiology, including both genetic and environmental risk factors, while NPC is a very rare autosomal recessive disease. However, the diseases have important converging molecular pathways, including abnormal lipid metabolism, endolysosomal dysfunction and involvement of amyloid-β (Aβ) and tau pathology. Our recent studies have shown enhanced cleavage by β-secretase (BACE1), a key enzyme in the pathogenesis of AD, in NPC1 disease mouse brains and in NPC1 mouse primary neurons. Indeed, we detected increased proteolysis of APP and also additional, recently identified BACE1 substrates, seizure protein 6 (Sez6) and seizure 6-like protein (Sez6L), which are primarily cleaved by BACE1. Furthermore, immunocytochemistry analysis revealed more punctuate staining of Sez6 and Sez6L in NPC1 *vs*. wt neurons, suggesting their accumulation within endosomal vesicles. We hypothesized that endosomal accumulation of BACE1-substrates in NPC1 cells is due to dysfunction of retromer transport. Subcellular and regional distribution of retromer proteins Vps26, Vps35 and receptor sorLA, and their levels were analyzed by immunocyto(histo)chemistry and Western blotting in: Chinese Hamster Ovary wild-type (CHOwt) and CHO *NPC1*-null cells; hippocampi, cerebella and cortices of wt and NPC1 mice and in primary neurons from wt and NPC1 mice. Altered trafficking of retromer proteins was observed in *NPC1*-null vs. CHOwt cells. In NPC1 mouse brains we detected decreased SorLA immunostaining that was accompanied with Vps35 accumulation in the neuronal soma at early disease stage. In primary neurons retromer was sequestered in axons in NPC1 vs. wt neurons. Our studies indicate that altered BACE1-mediated proteolysis and retromer function are additional common features between AD and NPC. Thus, the reversal of BACE1 and/or retromer dysfunction may be considered as targets to ameliorate and/or develop treatments against still untreated and devastating neurodegenerative disorder NPC. Further studies of similarities and differences between AD and NPC will increase our understanding of both these devastating neurological diseases.

THE NEUROTOXIC EFFECT OF AMYLOID-BETA OLIGOMERS ON SLEEP AND NEURONAL INTEGRITY IN RODENTS

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Synapse loss and ensuing neuronal death are the best predictors of memory deficits in Alzheimer's disease (AD). Hippocampus-dependent memory for recent facts and events (explicit memory) is the first type of memory that is affected in the disease because of the neurodegenerative process that takes place in the hippocampus of early AD patients. There is mounting evidence from recent studies that soluble low-molecular-weight amyloid-beta oligomers (Aβo), especially oligomers derived from Aβ1-42 peptides, are the most neurotoxic species and correlate extensively with memory deficits in AD patients and animal models. It is also well-established that sleep loss impairs the function of the hippocampus, and that sleep alterations are among the first clinical symptoms observed in AD.
The main objective of this project is to determine the impact of soluble Aβo-induced neurodegeneration on sleep architecture in rats. We also propose to identify molecular mechanisms underlying Aβo-driven hippocampal neurodegeneration.
We performed chronic hippocampal injections of soluble Aβ1-42 oligomers in rats and electroencephalographic (EEG) measurements were performed to assess sleep alterations. The effects of Aβo on signaling pathways were analyzed by Western blot and immunofluorescence.
Identifying the specific signature of hippocampal neurodegeneration on sleep features might serve as a non-invasive marker of early AD. A better understanding of the molecular mechanisms underpinning the effects of neurodegeneration and sleep disturbances on cognitive decline in AD could help developing novel treatments effective at the onset of the disease.

REVERSING Aβ FIBRILLATION AND TOXICITY IN PRIMARY NEURONAL CELLS USING AMPHIPHILIC DENDRONS

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Amphiphilic polyphenylene dendrons (APDs) with alternating negatively charged sulfonic acid and hydrophobic n-propyl peripheral groups disrupt amyloid-beta (Aβ) peptide assembly, which plays essential roles in Alzheimer´s plaque formation. The dendrons bind to the secondary structure of the Aβ aggregates, inhibiting Aβ oligomerization and fibrillation, and disassemble the already formed Aβ fibrils. In vitro experiments showed that APDs significantly reduced Aβ-induced cytotoxicity in primary murine neurons. For the treatment of neurological disorders such as Alzheimer's disease, the blood-brain barrier (BBB) represents a restrictive barrier, which is very challenging to overcome. The transport of known Aβ modulators or inhibitors through the BBB is often limited, which makes it challenging to achieve efficient delivery in vivo. The APDs reveal vesicular cellular uptake in endosomes as well as cell compatibility for endothelial and neuronal cells in vitro. We could demonstrate that they are transported into the brain after systemic application in mice, indicating their high potential to inhibit Aβ fibrillation in vivo due to their ability to cross the BBB. Moreover, APDs provide new opportunities for studying Aβ disassembly in vivo to explore their therapeutic potential for Alzheimer´s disease.

DEVELOPMENT OF TAU MISEXPRESSION SYSTEMS IN BRAIN ORGANOIDS FOR MODELING AD PROGRESSION AND THERAPEUTIC DISCOVERY

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Alzheimer's Disease (AD) affects approximately 5.8 million Americans and costs over $500 billion in care costs and informal care opportunity costs each year. Despite decades of research studying this disease, there are currently no effective cures or treatments that can do more than temporarily slow the disease. We believe that one of the most likely reasons extensive study has not yet yielded successful treatments is that the model systems being used are missing crucial pieces. Much of the work conducted thus far has been based on two-dimensional cell culture systems or mice engineered to exhibit features of the disease which do not normally occur in nature. These systems also have limitations in their ability to accurately model the structural and functional complexity of the human brain. Moreover, many studies have primarily focused on amyloid beta as the neurotoxic component of AD, while more recent research suggests that tau, a protein pathologically indicated in a variety of dementias, plays a crucial role in this process. Here, we will present new approaches for modeling the effects of tau in AD using pluripotent stem cell-derived brain organoids. To induce tau pathology, we have developed means to overexpress different tau forms in organoids. These methods result in foci of tau hyperphosphorylation which is an early step in the progression of AD and other tauopathies. We will present our progress applying these approaches to healthy and AD patient hPSC-derived organoids and investigating the resulting effects on neuronal health, glial response, synaptic stability, and overall neural network function. Ultimately, we aim to use this system as a means for investigating the pathogenesis of AD in human brain tissue and testing the efficacy of new tau aggregation blockers to halt disease progression.

TAU PATHOLOGY SPREADS BETWEEN VULNERABLE AND ANATOMICALLY-CONNECTED REGIONS OF THE BRAIN AND IS PREDICTED BY NETWORK MODELING

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Tau pathology is a diagnostic feature of Alzheimer's disease (AD). Tau pathology is progressive and correlates with cognitive decline. While neuropathological staging studies have suggested that tau spreads through the brain, it is unclear if this spread is mediated through neuroanatomical connections, spatial proximity, or regional vulnerability. We utilized seed-based models of tauopathy and quantitative pathology to capture spatiotemporal patterns of tau pathology spread in mice. Following the injection of AD tau into the brains of non-transgenic mice, tau pathology spreads progressively through the brain in a spatiotemporal pattern that is well-explained by anatomical connectivity in both antero- and retrograde directions. Network models based on diffusion along anatomical connections were then used to predict tau spread, estimate regional vulnerability to tau pathology, and investigate gene expression patterns related to regional vulnerability.

Tau pathology is also a prominent co-pathology in Parkinson's disease (PD), including genetic forms of PD with mutations in leucine-rich repeat kinase 2 (LRRK2). It is unknown how mutations in LRRK2 may modulate susceptibility to tau pathology initiation or spread. Therefore, we further investigated tau pathology spread in mice harboring a mutation in LRRK2 and found that while tau pathology spread is still constrained by anatomical connectivity, it spreads preferentially in a retrograde direction to regions that are otherwise resilient in wildtype mice. These studies provide insights on how tau pathology spreads along anatomical connections, the kinetics of spread, and allow us to use this platform to investigate the effect of genetic risk factors and treatments on the progression of tauopathies.

ELUCIDATING THE ROLE OF ADENOSINE MONOPHOSPHATE ACTIVATED PROTEIN KINASE IN ALZHEIMER'S DISEASE: A POSTMORTEM APPROACH

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Crucial metabolic pathways, such as insulin signaling, glycolysis, as well as mitochondrial function are all dysregulated in Alzheimer's disease (AD). Because AMPK regulates these cellular processes, it has been hypothesized to be a signaling node in AD. Despite this, neither (1) the expression of the regulatory AMPK isoforms nor (2) the activity of AMPK has been elucidated in the brain or in the context of human AD.

Using the PamGene12 Kinome Array, we observed a global decrease in peptide phosphorylation in pooled region-level dorsolateral prefrontal cortex (DLPFC) postmortem AD subjects that was decreased compared to Mild Cognitive Impairment (MCI) and control subjects. Deploying a novel bioinformatics workflow, we identified AMPK as a "hit" kinase between MCI and AD subjects, suggesting that AMPK may be implicated in the transition to AD from MCI.

To validate our bioinformatic findings, we biochemically validated AMPK in postmortem DLPFC. AMPK is an obligatory heterotrimer, composed of a catalytic (α) and two regulatory (β and γ) subunits. The transcripts of the two β isoforms were increased while the γ-1 isoform transcript level was decreased in AD. Transcripts for canonical upstream activators of AMPK (Tak1, LKB1, and OGT) were increased. Protein expression of LKB1 was increased in our AD cohort. We probed the transcript expression of key insulin signaling proteins: PTEN and Akt1, but not Akt2 or Akt3, were increased.

Inspired by the transcript changes observed for AMPK, we performed AMPK activity assays. AMPK activity was decreased in male and female AD samples. Moreover, we found lower AMPK activity in MCI vs controls, and even lower AMPK activity in AD vs MCI.

To mechanistically study AMPK, we knocked down AMPKα1 and AMPKα2 using siRNA (25 pmols) ± AICAR [1 mM], an allosteric AMPK activator, in primary murine astrocytes. Using the PamGene12 Kinome Array, we identified 15 peptides where the peptide phosphorylation was increased in the activated, control culture and decreased in the AMPK-KD culture. Subsequent examination of the phosphorylation of these peptides in our original AD Kinome Array showed decreased phosphorylation for 13/15 peptides in females, while 4/15 were decreased male AD subjects. Laminin-B1 (LMNB1) had increased phosphorylation in both sexes.

In summary, with a novel combination of bioinformatic and biochemical approaches, we have characterized AMPK in AD, providing novel pathophysiological insights.

MITOPHAGY-RELATED PROTEINS ARE REDUCED PRIOR TO TAU HYPERPHOSPHORYLATION IN THE HIPPOCAMPUS AND LOCUS COERULEUS OF A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Background: Loss of protein quality control and impairment of the autophagic pathway contribute to the cellular events that culminate in neurodegeneration. The purpose of the present study is to evaluate the cellular quality control and clearance system in genetically modified mice (Ts65Dn), used to study Alzheimer's disease related to triplicate copies of important genes. Levels of Pink1 and Parkin (PK), two proteins associated with mitophagy, and Rab7 and C9orf72, associated with autophagy traffic, were analyzed in the hippocampi (HC) and locus coeruleus (LC) of 2- and 5-months old mice, respectively before and after hyperphosphorylation of Tau.

Objectives: The objective of the study was to evaluate the cellular quality control system in Ts65Dn mice via measurement of Pink1, PK, Rab7 and C9orf72 levels in the HC and LC before and after Tau hyperphosphorylation.

Methods: Ts65Dn were genotyped as control group (down^{-}) and experimental group (down^{+}). The HC and LC of mice of each group were extracted. Protein extraction was then made, followed by quantification using the Bradford assay. Using SDS-PAGE and western blotting techniques, the proteins in each sample were quantified. Anti-Pink1, Anti-PK, Anti-Rab7 and Anti-C9orf72 antibodies were used to label the proteins. Anti-β-actin and Ponceau-S were used as the loading control. The data were submitted to two-way ANOVA and Bonferroni statistical analyses. All methods were approved by the ethical committee of the institution.

Results: Immunoblotting analyses revealed that the levels of Pink1 are reduced from 2-months control (2MC) to 2-months down (2MD) mice in the HC ($p<0.05$). The reduction is bigger when comparing 2MC to 5-months control and 5-months down (5MC; 5MD) ($p<0.001$). Between the two 5-months old groups, however, no significant change is observed. In the LC, Pink1 did not change significantly. PK also displays a reduction in the HC from 2MC to 2MD ($p<0.01$) and between 2MC and 5MC mice ($p<0.0001$). This is also observed in the LC of these animals, both from 2MC to 2MD ($p<0.05$) and from 2MC to 5MC ($p<0.05$). Rab7 does not show differences between the groups. The levels of C9orf72 were reduced in 5MD in comparison to 5MC mice in the HC ($p<0.05$).

Conclusion: We showed that expression of mitophagy-related proteins is reduced in the Ts65Dn mice in comparison to the control ones, indicating that there might occur a premature impairment of mitophagy. C9orf72 is also reduced from control to Ts65Dn mice, but only between 5-months old mice, indicating alterations later in the disease.

THE ROLE OF CO-TRANSLATIONAL INTERACTING FACTORS IN ALPHA-SYNUCLEIN BIOGENESIS

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Parkinson's disease (PD) is the second most common neurodegenerative disease and is rated as the 14th leading cause of death in the United States by the CDC. PD is part of a class of neurodegenerative diseases, referred to as Synucleinopathies, which are characterized by the presence of intracellular inclusions known as Lewy bodies. Lewy bodies are composed of aggregated protein alpha-Synuclein (aSyn). Understanding why aSyn is aggregating is a crucial step in developing preventative therapies for PD and other related Synucleinopathies. Protein misfolding and aggregation is a common cause for many human diseases. Our hypothesis is that alterations of aSyn interacting partners during translation leads to its misfolding and aggregation, causing disease. In PD, this alteration of interacting partners can be due to a mutation in aSyn itself (familial PD) or by defects in the interacting partners (sporadic PD). aSyn mutations (A30P, E46K, H50Q, G51D, A53E, and A53T) are all present within the first part of the protein, where early co-translational interaction events take place. The major goal of this study is to identify possible interacting partners during the translation of aSyn. Two complementary approaches were used in our studies: direct detection of the interacting factors by site-specific photo-crosslinking and an indirect candidate approach. Site-specific photo-crosslinking experiments, based on tRNA-mediated incorporation of a photo-probe into aSyn polypeptide nascent chain *in vitro*, revealed that aSyn interacts with a number of partners during its nascent chain elongation. The potential interacting partners for a candidate approach include proteins that are associated with the ribosome or polypeptide nascent chain during translation - signal recognition particle (SRP), chaperones Hsp70 and Hsp90, chaperonin TRiC/CCT, modifying factors, and others. We found that either depletion of the SRP subunit, SRP54, or TRiC/CCT subunit, CCT2, in cultured human cells affects both mRNA and protein expression of aSyn. Our data suggests that both the targeting factor SRP and chaperonin TRiC/CCT are involved in regulating aSyn biogenesis. Determining co-translational interacting partners of aSyn is key in discerning the causes of aggregation and developing therapies against it.

STUDY OF UPTAKE OF DNA NANODEVICES AND MULTIPLE ENDOCYTIC CARGOES IN SH-SY5Y CELLS AND DIFFERENTIATED NEURONS

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Alzheimer's disease (AD) is a neurodegenerative disease with a characteristic accumulation of extracellular amyloid-beta (Aβ) plaques and intracellular neurofibrillary tangles (NFT) of tau. Various in vitro cellular models such as primary neuronal cultures from mice or rats and neuroblastoma cell lines, e.g. rat PC12 and mouse Neuro-2A cells have been used to understand the origin and progress of these diseases. Understanding the cellular pathways involved in the uptake of such proteins is of prime importance to device-targeted therapeutics against these diseases. However, these models have unstable karyotypes and therefore, exclusive to particular species. Hence, a more relevant model for human neurodegenerative diseases is required to study various signaling and molecular pathway. We have built upon an existing method to differentiate human neuroblastoma cell line SH-SY5Y into homogeneous and viable populations of mature neurons. Further, we have studied the mechanisms of endocytosis of multiple cellular cargoes marking exclusively selective endocytic pathways viz transferrin to mark clathrin-mediated endocytosis and galectin-3 to mark clathrin-independent endocytosis. In parallel to exploring such endocytic pathways, we have designed various DNA nanodevices (Tetrahedron and Icosahedron) with varied geometries to interface with these differentiating neurons for targeted drug delivery of drugs and neuronal stimulators like neurotransmitters. We have monitored in-depth the uptake and dynamics of DNA cages of different geometries and interestingly we find a specific pattern and adaptability of the uptake of novel DNA devices with respect to specific endocytic pathways. This will indeed help us to design the smartly targeted biotherapeutics for neuronal targeting and diseases.

Keywords: Alzheimer's disease, DNA-Nano devices, DNA- cage, SH-SY5Y, Neurons

MICROGLIAL GQ SIGNALING MODULATES NEUROINFLAMMATION AND INDUCES IMMUNE MEMORY IN MICE

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Microglia, the resident immune cells of the central nervous system, play a critical role in maintaining brain homeostasis and responding to injury and disease. Microglia express a variety of G protein-coupled receptors (GPCRs) which allow them to survey their microenvironment and respond to signals from surrounding cells. Upon alterations to the central nervous system, microglia modify their actions which include phagocytosis of cellular debris and apoptotic cells, synaptic pruning, the production of neuromodulatory factors which influence synaptic plasticity, and the release of cytokines to modulate inflammation in the brain. Many GPCRs have been shown to modulate these microglial functions *in vitro*. However, the ubiquitous expression of these receptors across different cell types in the brain has made it difficult to investigate the role of microglial GPCRs *in vivo*. We generated mice expressing the hM3Dq Designer Receptor Exclusively Activated by Designer Drugs (DREADD) in microglia specifically to study the role of muscarinic M3-Gq signaling. In primary microglia derived from these mice, clozapine N-oxide (CNO) elevated intracellular calcium levels, suggesting that activation of hM3Dq does initiate Gq signaling. Furthermore, CNO treatment increased the phagocytosis of FluoSpheres *in vitro*, indicating that this receptor can be used to manipulate microglial activity. In mice, acute CNO treatment increased the synthesis of pro-inflammatory cytokine mRNA in the brain. In contrast, in mice that were chronically treated with CNO the levels of pro-inflammatory cytokines were not increased, suggesting tolerance to continued activation of hM3Dq. Interestingly, chronic CNO treatment prior to peripheral lipopolysaccharide (LPS) challenge attenuated LPS-induced cytokine mRNA changes in the brain. Similarly, unsociability triggered by LPS was decreased by preceding chronic CNO injections. No effect of CNO was observed in hM3Dq-negative mice. These results suggest that activation of M3 muscarinic receptors on microglia, and potentially other Gq-coupled GPCRs, can trigger an inflammatory-like response that preconditions microglia to decrease their response to further immunological challenges. Our results indicate that hM3Dq can be a useful tool to modulate neuroinflammation and study microglial immunological memory *in vivo*, which may be applicable for manipulation of neuroinflammation in neurodegenerative and psychiatric diseases.

INVESTIGATING NEURONAL TOXICITY AND THE SPREADING OF TWO AGGREGATE CONFORMERS IN A NOVEL MOUSE MODEL

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Protein misfolding and formation of neurotoxic protein aggregates are common hallmarks of various neurodegenerative diseases. Accumulating evidence argues that aggregates composed of disease-associated proteins act as seeds that spread throughout the brain in a self-perpetuating manner. So far it is unclear if the conformation of a given protein aggregate determines its target cell tropism and thus predefines its propagation route. Further, it remains to be solved whether neuronal toxicity results from a loss-of-function of the aggregated protein or from a gain-of-function of the newly-formed aggregates. To investigate the spread of different aggregate conformers and their potential toxic effects on the brain, we established a knock-in mouse model based on Sup35 NM, a *Saccharomyces cerevisiae* prion domain that has been successfully used in the past to study protein aggregate induction and intercellular spreading in mammalian cells. Mice expressing NM under the control of the Prnp promoter are viable and do not develop a spontaneous protein aggregation or motor phenotype during aging. We generated two structural variants of NM fibrils *in vitro* and characterized them biochemically and biophysically. Both fibril conformers will be injected into the hippocampus of adult NM mice. The spread of NM aggregates and associated pathologies such as neuroinflammation or synapse loss will be assessed biochemically and histochemically in various brain regions 1, 3, 5, and 8 months post injection. In addition, mice will be challenged in the RotaRod to reveal potential motor and coordination deficits. This approach will help to identify general mechanisms of protein aggregate toxicity and conformation-dependent spreading common to neurodegenerative diseases.

BIASED GPR3 SIGNALING REDUCES Aβ PATHOLOGY *IN VIVO*

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Selective G protein or β-arrestin signaling, i.e., biased signaling, has been shown to more precisely regulate the biological functions of G protein-coupled receptors (GPCRs), leading to the discovery of therapeutic drugs with superior efficacy and/or reduced side-effects in heart disease, pain management, and neuropsychiatric disorders. Abnormal accumulation and aggregation of amyloid-β (Aβ), which is derived from cleavage of the amyloid precursor protein (APP), and tau are pathological hallmarks of Alzheimer's disease (AD). We previously discovered that expression of GPCR GPR3 is elevated in AD patients and is involved in regulation of Aβ pathology and cognitive deficits in AD mouse models. Our *in vitro* evidence showed that β-arrestin (rather than classic G protein) signaling modulates GPR3-mediated Aβ accumulation. Here, we generated a G protein-biased GPR3 mouse model (*Gpr3*$^{HA\text{-}Ala}$) to investigate the physiological consequences of eliminating β-arrestin signaling *in vivo*. Our findings demonstrate that, in contrast to the phenotypes observed in *Gpr3*-deficient mice, *Gpr3*$^{HA\text{-}Ala}$ mice do not display memory deficits, anxiety-like behavior, or reduced fertility. Moreover, Aβ generation is reduced in a *Gpr3*$^{HA\text{-}Ala}$ AD mouse model. Recently, APP metabolism has been shown to affect tau proteostasis. As such, we determined that β-arrestin and tau levels are inversely correlated in AD patients. Significantly, tau levels, which are elevated in *β-arrestin2*-deficient AD mice, are unaffected in *Gpr3*-deficient and *Gpr3*$^{HA\text{-}Ala}$ AD mouse models. Collectively, these studies establish that *in vivo* G protein-biased GPR3 signaling reduces Aβ generation independently of tau and without side-effects. Therefore, we propose that biased GPR3 signaling is a viable AD therapeutic strategy.

THE PREVALENCE OF SPONTANEOUS ISOMERIZATION IN THE BRAIN IS HIGH IN ALZHEIMER'S DISEASE

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The involvement of lysosomal pathology in neurological disorders is common and has been well documented for Alzheimer's Disease (AD), although the relationship of this pathology to the disease is not completely known. Recently, a potential cause for lysosomal failure has been proposed in the form of spontaneous isomerization of amino acids in long-lived proteins. It has been demonstrated that isomerization inhibits proteolysis near modified residues, which could lead to accumulation of undigested material in a process similar to that observed when defective digestive enzymes are present in various lysosomal storage disorders. Herein, analysis of proteomics results obtained by data-independent acquisition from samples extracted from 44 human brains including both AD and control samples reveals that the isomerization of Tau is significantly more abundant in AD samples. In addition, reduced levels of PIMT (an enzyme which repairs spontaneous isomerization of aspartic acid) as well as lower levels of several proteins associated with autophagic pathways were found in AD samples. These results are consistent with a model where protein lifetime and degree of isomerization are connected to autophagic flux through a destructive cyclic pathway. Longer lifetimes lead to greater isomerization which reduces lysosomal capacity and autophagic flux, which leads to reduced protein synthesis and even longer protein lifetimes, etc. These results offer potential alternatives for therapy and prevention related to the frequency and intensity of autophagic pathways.

HARNESSING NEUROPROTECTIVE MICROGLIA FOR THERAPEUTIC BENEFIT IN *C9ORF72* FTD/ALS

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Microglia display altered function in multiple models of neurodegenerative diseases, and ablating them can profoundly affect neurodegeneration. Neuron-glia interaction is a key regulator for disease onset and progression. However, it remains unclear in which contexts neuron-glial interactions drive or rescue neurodegeneration, and studies suggest this may depend on the form or stage of dementia. Thus, there is a pressing need to understand how neuron-glia interactions modulate neurodegeneration. The repeat expansion in *C9ORF72* is the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), and it causes neurodegeneration through both loss- and gain-of-function processes. The prevailing view is that loss of C9ORF72 function activates inflammatory programs in microglia that promote neurodegeneration. However, no studies examined the effects of neuron-microglia interactions on neurodegeneration in the *C9ORF72* patient context. To study microglia in *C9ORF72* FTD/ALS, we established an induced microglia (iMG) model by using two microglial transcription factors and IL-34 to convert induced pluripotent stem cells into microglia. We verified that iMG possess phagocytotic ability, have similar transcriptomic signatures to primary human microglia, and respond appropriately to known activators such as lipopolysaccharide. We developed co-culture model using *C9ORF72* FTD/ALS patient iMG and induced motor neurons (iMNs). We found *C9ORF72* iMG significantly reduced the survival of healthy iMNs, indicating *C9ORF72* repeat expansion induces a default neurotoxic state in microglia. Surprisingly, *C9ORF72* iMG significantly increased *C9ORF72* iMNs survival. We also found *C9ORF72* iMG significantly lowered levels of neurotoxic, repeat expansion-derived dipeptide repeat proteins (DPRs) in neighboring neurons. In addition, we found that co-culture with iMNs from healthy individuals overexpressing DPRs, but not *C9ORF72*-deficient or sporadic ALS iMNs triggered microglia to be neuroprotective. Thus, *C9ORF72* gain-of-function processes in neurons induce microglia switching into neuroprotective state. From single-cell RNA seq, we found that co-culture with *C9ORF72* iMNs converted a subset of *C9ORF72* iMG into a neuroprotective state marked by reduced CSF1R expression and high TGFβ1 signaling. Flow-purified CSF1R-negative *C9ORF72* iMG activated secretion of IGF-1, a known autophagy stimulator in neurons, and rescued survival of *C9ORF72* iMNs. From single-nucleus RNA seq, we identified increased population of CSF1R-negative microglia with upregulated insulin-related signaling in *C9ORF72* FTD/ALS mice, compared to wild-type mice. In contrast to the prevailing view, our findings suggest that a subset of microglia switch from neurotoxic to neuroprotective in *C9ORF72* FTD/ALS. Converting microglia into neuroprotective state or eliminating neurotoxic microglia pharmacologically could delay the disease progression of *C9ORF72* FTD/ALS.

MODELING FAMILIAL ALZHEIMER'S DISEASE WITH HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CORTICAL SPHEROIDS

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Alzheimer's Disease (AD), the most common neurodegenerative disorder, is the only disease of the top ten most prominent in the United States that has seen significant increase in the number of affected people. There are currently no treatments that stop or prevent the onset of AD as the underlying mechanisms causing AD have not been determined. Genetic mutations in the presenilin1 gene (PSEN1) account for over 80% of all familial AD (fAD) cases. Although over 300 of these mutations have been reported, the mechanism by which these mutations cause fAD are still unclear. As the catalytic component of the gamma secretase, PSEN1 can have pleiotropic effects in the brain. Buildup of amyloid plaques are seen in all AD brains and are a result of PSEN1-mediated cleavage of the amyloid precursor protein (APP). PSEN1 also plays a large role in brain development through the Notch signaling pathway which governs cell fate decisions during neurogenesis. Our central hypothesis is PSEN1 mutations affect Notch signaling, APP processing, or both, to cause fAD. While there are many mouse models available for studying this gene, they fail to recapitulate the pathology and behavior seen in fAD and require several mutations to be induced and overexpressed in order to see pathological changes. Therefore, there is a need for a human-based model to study the cellular mechanisms that cause fAD as a direct result of mutations in PSEN1. Three dimensional human cortical spheroids (hCS) derived from patient induced pluripotent stem cells (iPSCs) are a valuable tool that can be used to study the effects of these genetic mutations in the human brain during development and disease. We have created hCS from three different CRISPR edited iPSC lines carrying genetic mutations in the PSEN1 gene, L435F, M146L, and D385A, that have been found in patients with fAD. We will be determining the effects of genetic mutations in PSEN1 in the Notch signaling pathway and subsequent proliferation, differentiation and cell death during the development of hCS. We will also be determining how mutations in PSEN1 affect APP processing and cause fAD-associated neuropathology. Together, the results of these experiments can give insight into how PSEN1 causes cellular changes in the brain to cause fAD and provide targets for future treatments to stop or reverse the progression of AD.

MITOCHONDRIAL DYSFUNCTION INDUCED INTEGRATED STRESS RESPONSE IMPAIRS NEURAL STEM CELL ACTIVATION

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Mitochondrial function is emerging as pivotal in regulating neural stem cells (NSC) and their fate decisions. Mitochondrial fragmentation is seen during aging and at an accelerated rate in neurodegenerative diseases. Presently, little is known regarding the role of mitochondrial dynamics in the regulation of adult NSCs and differentiation. As mitochondrial dysfunction is attributed to aging-associated neuronal pathology, we used Tamoxifen-inducible Opa1-knockout(KO) to model accelerated aging through aberrant ROS signaling and dysfunctional Oxidative phosphorylation in adult NSC-pool. We identified that mitochondrial dynamics is important to maintain stem cell state and that disruption induced by Opa1 deficiency impairs learning and memory. Histological analysis reveals that the number of NSCs remain similar while their proliferation and differentiation capacity was severely impacted. Single-cell transcriptome analysis from NSC lineages isolated from the adult hippocampus reveals that stem cell progenies are uniquely affected in Opa1-KO leading to impairments in NSC activation and differentiation. Unbiased transcriptional profiling suggested a metabolic shift in Opa1-KO that results in the activation of classic cellular stress response pathway genes (Atf4, Ddit3, and Ddit4). We propose that integrated stress response is required to maintain NSC survival, through maintenance of quiescence and dedifferentiation. Overall, our results suggest that metabolic alterations induced by mitochondrial dysfunction results in the induction of a cellular stress response to maintain NSC lineage survival under stress conditions. We also show that hypoxia-mediated metabolic rewiring can alleviate the stress response and partially restore neuronal proliferation and differentiation potential. These studies reveal how mitochondrial dysfunction typical of neurodegenerative diseases, alters NSC fate decisions.

CHARACTERIZATION OF TARGETED KNOCKDOWN OF α2-Na^+/K^+ ATPase WITH ANTISENSE OLIGONUCLEOTIDES IN SOD1*G93A MICE

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Amyotrophic lateral sclerosis (ALS) is the most common and debilitating motor neuron disease with progressive degeneration of motor neurons in the cortex, brainstem and spinal cord. We have previously found a role for a complex of the ion pump α2-Na^+/K^+ ATPase and cytoskeletal protein α-adducin in the pathogenesis of ALS. The levels of α2-Na^+/K^+ ATPase and α-adducin are increased in the spinal cord in familial and sporadic ALS patients. In vitro knockdown of α2-Na^+/K^+ ATPase or α-adducin by RNAi in SOD1*G93A primary astrocytes protects co-cultured motor neurons from death and dendrite degeneration. Further, inactivating one allele of α2-Na^+/K^+ ATPase in heterozygous mice substantially increases the mobility and lifespan of SOD1*G93A mice. These findings suggest that α2-Na^+/K^+ ATPase might represent a therapeutic target in ALS. Antisense oligonucleotides (ASOs) have emerged as a safe and effective method to modify gene expression in animal models and in the clinic. Here, we characterized the suitability of α2-Na^+/K^+ ATPase knockdown using locked nucleic acid (LNA) ASOs as a means to attenuate astrocyte-mediated neurotoxicity in SOD1*G93A mice, which have been extensively used as an experimental system for ALS. We screened five LNA ASOs targeting different sites in the 3'-UTR of *Atp1a2*, the mRNA encoding α2-Na^+/K^+ ATPase, in primary non-transgenic astrocytes and identified two ASOs that reduced *Atp1a2* expression by 50-70%. The two ASOs were used *in vivo* to test the impact on disease progression and survival. *Atp1a2*-specific or negative control LNA ASOs were administered via a single intracerebroventricular injection in presymptomatic SOD1*G93A mice, which were allowed to reach disease end stage. Surprisingly, we found that ASO-treated mice exhibited significantly earlier disease onset and shorter lifespan, suggestive of accelerated disease progression than animals receiving control LNA oligonucleotides. These results propose that reducing *Atp1a2* expression in adulthood with ASOs may not mimic the beneficial effects of gene knockout at birth in SOD1*G93A mice. Additional studies are required to understand the role of α2-Na^+/K^+ ATPase in ALS.

PATHOLOGICAL ALPHA SYNUCLEIN PROPAGATION THROUGH PROPRIOCEPTIVE PATHWAYS IN A MOUSE MODEL OF PARKINSON DISEASE

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Postmortem neuropathological studies on brains of Parkinson disease (PD) patients, and a significant number of observations in animal models of synucleinopathies support the prion-hypothesis of pathological alpha synuclein (aSyn) propagation in central nervous system (CNS). According to this hypothesis, aSyn misfolding is initiated in peripheral and/or central nerve terminals, and then propagates in spatiotemporal pattern into connected neuroanatomical tracts. In a transgenic mouse model of PD (overexpressing the A53T familial PD mutation in SNCA gene that encodes aSyn protein- the M83 line), PD like aSyn pathology is initiated by a single intramuscular injection of fibrillar aSyn in hindlimb femoris muscle (Sacino A.N. et al., PNAS, 2014). Using this mouse model of PD, we are studying the early neuronal populations in CNS that are affected by aSyn PD like pathology, and mechanisms of pathology propagation in relation with phenotype.

We have observed that the earliest observable phenotype in the M83 PD model is an abnormal performance in the tail suspension test (i.e., clasping behavior) around day 21 post-aSyn-injection (dpi 21). By dpi 45, this behavior is firmly established, and is followed by complete hindlimb paralysis around dpi 60-70 necessitating euthanasia. Intriguingly, immunohistochemical (IHC) analysis for aSyn pathology (phospho-aSyn S129) revealed that even before clasping phenotype is manifest (dpi 14), significant aSyn pathology is detected in lamina VI-IX of lumbar spinal grey which harbor cell bodies of motor neurons and proprioceptive pathways. By dpi 21 and 45, additional neuronal populations in lumbar cord, periventricular areas in brainstem, deep cerebellar nuclei and medial thalamus are affected. By dpi 45, significant aSyn pathology is also detected in the thoracic cord, which does not directly innervate the hindlimb, suggesting aSyn pathology propagation through proprioceptive intralaminar spinal tracts or possibly descending tracts from periventricular nuclei in brainstem. The significance of these observations to mechanisms in transynaptic aSyn pathology propagation and possible relevance to non-motor symptoms in PD are also discussed.

TRANSCRIPTION REGULATES snRNP PRODUCTION THROUGH INTERACTION OF 7SK WITH THE SMN COMPLEX: IMPLICATIONS FOR SPINAL MUSCULAR ATROPHY AND NEURODEGENERATIVE DISORDERS

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Spinal muscular atrophy is caused by deficiency of the Smn protein, a central constituent of the Smn complex that is essential for the assembly of spliceosomal snRNP particles. These snRNPs play an essential role in co-transcriptional processing of pre-mRNAs. While the functions of the Smn complex in snRNP assembly have been investigated in detail much less is known about the regulation of the activity of the Smn complex. Here we report an association of the Smn complex with the 7SK complex involved in transcriptional regulation. 7SK is a structured non-coding RNA that acts as a scaffold for the P-TEFb complex to regulate its activity in transcription activation. Beyond that, 7SK associates with hnRNP proteins in a transcription-dependent manner. We found that Smn interacts with the 7SK core components Larp7 and Mepce and specifically associates with 7SK subcomplexes containing hnRNP R. The association between Smn and 7SK complexes was enhanced upon transcriptional inhibition leading to reduced production of snRNPs. Thus, in addition to its canonical nuclear role in transcriptional regulation, 7SK has cytosolic functions in fine-tuning spliceosome production according to transcriptional demand. This regulatory mechanism might be of particular significance during neuronal development, which is accompanied by widespread alterations of transcription programs. Failure of appropriate snRNP assembly control might impede splicing of neuronal transcripts and render neurons susceptible to developmental stress and aging-related neurotoxicity exerted by aberrant protein aggregates.

PHAGOCYTOSIS DRIVES NAD^+ REDUCTION-INDUCED DENDRITE DEGENERATION IN *DROSOPHILA*

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Cellular nicotinamide adenine dinucleotide (NAD^+) level is a critical determinant of neuronal survival. Maintaining NAD^+ level suppresses axon degeneration in several *in vivo* models of neurodegenerative disease and peripheral neuropathy, including Wallerian (or injury-induced) degeneration. Injury results in NAD^+ depletion in severed dendrites or axons, which is believed to trigger neurite-intrinsic self-destruction. However, recent evidence suggests that severed neurites expose the "eat-me" signal phosphatidylserine (PS) on their surface downstream of NAD^+ reduction, raising the question of whether phagocytosis also contributes to Wallerian degeneration *in vivo*. In addition, it remains unknown whether PS-mediated phagocytosis is related to neurodegenerative pathologies linked to NAD^+ disruption. Using *Drosophila* sensory dendrites as a models system, we found that phagocytosis is the main driver of dendrite degeneration induced by both genetic NAD^+ disruptions and injury. Specifically, in uninjured dendrites, NAD^+ reduction resulting from Sarm activation causes PS exposure and phagocytosis-dependent degeneration. In injured dendrites, PS-mediated phagocytosis is sufficient but not required for dendrite breakdown due to the self-destruction pathway triggered by catastrophic NAD^+ depletion. Injured dendrites undergo severe membrane rupture during dendrite fragmentation, while neurons with mild NAD^+ reduction experience much milder disruptions of membrane integrity, likely due to dynamic repairing of membranes. Lastly, injured dendrites exhibit rhythmic calcium flashing prior to degeneration. This calcium dynamics depends on NAD^+ reduction and may promote dendrite self-destruction. These results reveal new mechanisms of dendrite degeneration and underscore the importance of phagocytosis in pathological neurite degeneration *in vivo*.

LOSS OF AN ER COMPONENT, MEMBRALIN, IN ASTROCYTES TRIGGERS NEUROINFLAMMATION

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During the progression of Alzheimer's disease (AD) and other neurodegenerative disorders, glial cells including astrocytes and microglia undergo morphological and physiological changes, indicating reactivity. However, how glial activation can contribute to disease pathogenesis is still unclear. Endoplasmic reticulum (ER) is the primary cellular organelle for the synthesis of transmembrane and secreted proteins. Previously, we identified an ER protein, membralin, as a novel component of the ER-associated degradation (ERAD) complex. Loss of membralin impaired homeostatic turnover of a key γ-secretase complex subunit, nicastrin, resulting in increased γ-secretase complex formation and activity, and aggravated AD pathology in TgCRND8 mice. Further, conditional deletion of membralin in astrocytes decreased expression of the excitatory amino acid transporter 2 (EAAT2) and induced excitotoxicity. Using adeno-associated viral (AAV) vectors to elevate membralin levels in the SOD1 G93A ALS mouse model can significantly extend the lifespan of these animals. Moreover, transcriptomic analysis of cortex from astrocyte-specific membralin knockout animals reveals the induction of reactive astrocyte profiles, plaque-induced genes (PIGs), and disease-associated microglia (DAM) signatures. Elevation of membralin in astrocytes can alleviate neurotoxic (A1) astrocyte induction. Together, these results suggest that modulation of astrocyte membralin levels can be promising as a neuroinflammatory suppressor in AD.

DETERMINANTS OF TAU AGGREGATION IN DISEASE

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Tau aggregation into ordered assemblies underlies myriad neurodegenerative diseases. We have previously reported that tau exists in two general monomeric conformational states. One is aggregation resistant and the other represents a converted conformation that is aggregation prone and has the capacity to act as conformational templates and can act as seeds in vitro and in cells. We have previously detected the aggregation resistant form in healthy control patient brain, and the aggregation prone in brains of tauopathy patients. However it has been unclear what is the precise relationship of the aggregation prone conformation to pathology as defined either by immunohistochemical abnormalities or development of detergent insoluble aggregates. Moreover, the post-translational modifications (PTMs), if any, that might play a role in the conversion of aggregation resistant states to aggregation prone states have not been elucidated. We studied a tauopathy mouse (PS19) based on transgenic expression of full-length (1N4R) tau containing a single disease-associated mutation (P301S) to determine when in the disease process monomeric aggregation prone forms first appear. We used established biochemical purification techniques to isolate seed-competent monomer from mice at ages ranging from 0-48 weeks of age. We tested for the presence of monomeric aggregation prone states using a cellular biosensor system that is based on expression of tau repeat domain (RD) containing two disease-associated mutations (P301L/V337M) fused to cyan and yellow fluorescent protein. We observed that the aggregation prone state first appears at 4 weeks of age, months before detergent-insoluble tau becomes apparent. At 4 weeks of age the dominant form of seed-competent tau is a monomer, but within 2 weeks many larger assemblies are detected. Post-translational analysis of the aggregation prone form over this time course revealed no clear phospho-residue patterns and indeed enzymatic removal of phosphorylation did not affect seeding capacity of the samples. Comparison of this dataset to seeding tau monomer isolated from three Alzheimer’s patients again revealed no clear patterns of modifications important for seeding. Our data support that phosphorylation may not be a direct driver of tau seeding capacity suggesting that other post-translational modifications or cofactor binding may underlie the structural origins of tauopathy.

EXPLORING THE CRITICAL ROLE OF PROTEIN CONTEXT IN TOXICITY FROM THE NEURODEGENERATIVE DISEASE PROTEIN ATAXIN-3

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Spinocerebellar Ataxia Type 3 (SCA3) belongs to the family of polyglutamine (polyQ) neurodegenerative diseases. Each disorder stems from abnormal lengthening of a glutamine repeat in a different protein. Although caused by a similar mutation, polyQ disorders are distinct, implicating non-polyQ regions of disease proteins as regulators of pathogenesis; in other words, protein context is a key determinant of polyQ toxicity. SCA3 is caused by polyQ expansion in the deubiquitinase, ataxin-3. Here, we sought to understand how non-polyQ regions of ataxin-3 regulate the toxicity of its expanded polyQ in SCA3, and to elucidate their mechanisms of action. We utilized new allelic sets of *Drosophila melanogaster* expressing strategically truncated or full-length versions of the human ataxin-3 protein with specific mutations in its domains. These lines provided a base to build a more comprehensive picture of the relative contributions from each domain of ataxin-3, starting with the truncated version containing polyQ alone and building domain by domain to the full length forms of the protein with key mutations in relevant domains. We used a combination of fly genetics, physiology and biochemistry to assess the importance and function of protein context in SCA3. We found that ataxin-3 pathogenicity is saliently controlled by the polyQ-adjacent, ubiquitin-interacting motifs (UIMs), which markedly enhance its aggregation and toxicity. The UIMs function in part by interacting with the HSP70 chaperone superfamily member, Hsc70-4, whose reduction diminishes SCA3 toxicity in a UIM-dependent manner. According to additional results, Hsc70-4 also enhances pathogenicity of other polyQ proteins. Our studies provide unique insight into the relative impact of non-polyQ domains of ataxin-3 in SCA3, identify Hsc70-4 as an enhancer of polyQ toxicity, and indicate pleiotropic effects from HSP70 chaperones, which are generally thought to suppress polyQ degeneration.

EARLY β-ADRENERGIC RECEPTOR DEPENDENT TIME WINDOW FOR LONG TERM FEAR MEMORY PERSISTENCE IN APPSWE/PS1DE9 MOUSE MODEL OF ALZHEIMER'S DISEASE

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In this study we demonstrate that two month old APPswe/PS1dE9 mice, a transgenic model of Alzheimer's disease, exhibited intact short-term memory in Pavlovian hippocampal - dependent contextual fear learning task. However, their long-term memory was impaired. Intra-CA1 infusion of isoproterenol hydrochloride, the β-adrenoceptor agonist, to the ventral hippocampus of APPswe/PS1dE9 mice immediately before fear conditioning restored long-term contextual fear memory. Infusion of the β-adrenoceptor agonist +2.5 h after fear conditioning only partially rescued the fear memory, whereas infusion at +12 h post conditioning did not interfere with long-term memory persistence in this mouse model. Furthermore, Intra-CA1 infusion of propranolol, the β-adrenoceptor antagonist, administered immediately before conditioning to their wildtype counterpart impaired long-term fear memory, while it was ineffective when administered +4 h and +12 h post conditioning. Our results indicate that, long term memory persistence is determined by a unique β-adrenoceptor sensitive time window between 0 - +2.5 h upon learning acquisition, in the ventral hippocampal CA1 of APPswe/PS1dE9 mice. Thus we conclude that, activation of learning dependent early β-adrenoceptor modulation is necessary to promote long-term fear memory persistence in APPswe/PS1dE9.

USE OF STEM CELL EXTRACELLULAR VESICLES AS A HOLISTIC APPROACH TOWARDS CNS REPAIR

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HIV-1 remains an incurable infection that is associated with substantial epidemiologic impacts. HIV-associated neurocognitive disorders (HAND) are commonly linked to HIV-1 infection; despite the development of combination antiretroviral therapy, HAND is still reported to affect at least 50% of HIV-1 infected individuals. It is believed that the over-amplification of inflammatory pathways, along with the release of toxic viral proteins, are primarily responsible for the neurological damage observed in HAND; however, the underlying mechanisms are not well-defined. Therefore, there is an unmet need to develop more physiologically relevant platforms for studying these pathologies. Recently, “3D” cultures of neurospheres derived from induced pluripotent stem cells (iPSCs) have been utilized to model the effects of different neurotropic viruses. Here, we report the generation of “3D” neurospheres from iPSC-derived neural progenitor cells (NPCs) and show that these cultures are susceptible to retroviral (e.g. HIV-1, HTLV-1) infection. We also examine the functional effects of stem cell derived extracellular vesicles (EVs) on HIV-1 damaged cells. In recent years it has been well-established that stem cell EVs play a critical role in the functionality associated with stem cells. The diverse biological cargo associated with these vesicles are proposed to mediate their effects and, to date, the reparative effects of stem cell EVs have been demonstrated in a wide range of cell types. While a high potential for their therapeutic use exists, there is a gap of knowledge surrounding their characterization, mechanisms of action, and how they may regulate cells of the central nervous system (CNS). We have isolated high yields of EVs from both iPSCs and mesenchymal stem cells (MSCs). Our EV characterization includes both phenotypic and biochemical assays. EV functionality has been assessed in vitro utilizing several cell-based assays related to cellular viability, migration, angiogenesis, and immunomodulation in cells that are relevant to the CNS. Consistent with the literature, our data suggests that stem cell EVs may modulate neuroprotective and anti-inflammatory properties in damaged cells. Collectively, these studies demonstrate the relevance of human “3D” neurospheres for modeling HIV-1 infection while also highlighting the potential of stem cell EVs for rescuing or partially reversing CNS-related cellular damage.

ADUCANUMAB: THE FIRST DISEASE MODIFYING THERAPY FOR ALZHEIMER'S DISEASE

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Alzheimer's disease is a progressive neurodegenerative disorder that is a major cause of dementia. Currently there is no cure. Anti-amyloid immunotherapies have shown promise in preclinical studies. However, translation into clinic has proven challenging until now. Aducanumab is a human anti-amyloid antibody that recognizes aggregated forms of amyloid beta. It is the first immunotherapy to slow cognitive decline in a dose-dependent manner in a recently completed Phase III clinical trial. Aducanumab is currently under review by FDA as a disease modifying therapy for Alzheimer's disease. However, its mechanism of action is unclear. Aducanumab was previously shown to clear amyloid plaques and restore neuronal calcium homeostasis disrupted in animal models of AD (Kastanenka et al., 2016; Sevigny et al., 2016). Growing evidence suggests that soluble Aβ oligomers are the toxic species that trigger the perturbation in neuronal calcium homeostasis observed in AD. Therefore, aducanumab's ability to target soluble amyloid beta oligomers and hence to normalize calcium homeostasis was evaluated. Aducanumab binds oligomeric amyloid beta in addition to amyloid plaques and thus restores neuronal calcium homeostasis. This antibody restores neuronal network function that possibly underlies cognitive deficits and is thus well-positioned to become the first disease modifying therapy for Alzheimer's disease.

PROTEIN QUALITY CONTROL AND AGGREGATION OF TDP43 PROTEIN FRAGMENTS

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The accumulation and aggregation of misfolded proteins is characteristic of many neurodegenerative disorders. During pathological conditions, neuronal proteins are cleaved to generate protein fragments (PFs) that are also aggregation-prone due to exposure of their hydrophobic regions. Previously, we found that the N-degron pathway of the UPS degrades several PFs, including those of the TAR DNA binding protein 43 (TDP43). Recently, we found that PFs of TDP43 accumulate and aggregate in cells lacking ATE1 gene function. In a follow-up study, we found that differences in the N-termini of otherwise identical PFs influence their degradation, aggregation propensity and aggregate morphology. Here, we report that BAG6, a molecular chaperone component of a cytosolic protein quality control complex, can recognize hydrophobic regions in PFs and prevent their aggregation. Also, we have identified a novel interaction between TDP43 fragments and RNF126, an E3-ubiquitin ligase, that may degrade the PFs when the N-degron pathway is defective.

INCREASED SUSCEPTIBILITY TO EXPERIMENTAL COLITIS, ALTERATION OF IMMUNE CELL POPULATIONS, AND BRAIN INFILTRATION OF PERIPHERAL IMMUNE CELLS IN THE ABSENCE OF PROGRANULIN

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Mutations in the progranulin gene (GRN) reduce levels of progranulin (PGRN) and granulins (GRNs) causing frontotemporal dementia (FTD), the most common form of early-onset dementia. PGRN loss in neurons is associated with lysosome dysfunction resulting in diminished cathepsin protease activity. PGRN is also highly expressed in brain-resident microglia and peripheral immune cells. Therefore, we asked if PGRN loss impacts immune cell profiles and communication in the gut-brain axis during chronic systemic inflammation. We performed multiplexed RNA & digital spatial protein profiling analyses, immunohistochemistry, and deep-immunophenotyping by flow cytometry on brain, spleen, and peripheral blood of Grn KO and Grn¬-suffficient mice aged 3-30 months. Furthermore, we exposed mice to experimental colitis to interrogate alterations in the gut-brain axis. Aberrant T cell and monocyte activation markers in Grn KO mice blood were evident with minimal increases in inflammatory cytokines in plasma. Aged Grn KO mice exhibited decreased microglia (CD45int, CD11bhi) in the brain relative to Grn-sufficient mice, with altered MHCII and CD68 expression. PGRN loss was also associated with increased brain infiltration of activated peripheral monocytes and vulnerability to experimental colitis. In summary, these novel findings suggest that PGRN plays key regulatory functions in innate and adaptive immune cell populations, in central-peripheral immune cell crosstalk and traffic to the CNS, and in regulating susceptibility to immune challenges that disrupt the gut-brain signaling axis.

IMMUNOHISTOCHEMICAL ANALYSIS OF LOCUS COERULEUS NEUROCHEMISTRY IN A TRANSGENIC RAT MODEL OF ALZHEIMER'S DISEASE

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The locus coeruleus (LC) is the brain's primary noradrenergic nucleus and is prone to developing hyperphosphorylated tau pathology at the onset of Alzheimer's disease (AD), often decades prior to the appearance of cognitive symptoms. TgF344-AD transgenic rats that overexpress mutant human amyloid precursor protein and presenilin-1 are the only known AD model that recapitulates endogenous tau pathology in the LC prior to other brain regions. We have previously shown that hyperphosphorylated tau is present in the TgF344-AD LC at 6 months and increases by 16 months, and that afferents and norepinephrine (NE) levels are reduced in the forebrain of transgenic rats at 16 months. However, it is unknown how the expression of markers for neurotransmitter function change in the LC itself with respect to the development and progression of tau. The current study sought to assess changes in molecules that regulate the LC neurochemical landscape in young (6 month) and old (16 month) TgF344-AD rats and wild-type littermates using immunohistochemistry. LC sections were sliced at 40 um and stained for dopamine β-hydroxylase (DBH) to proxy NE producing capabilities, NE transporter (NET) to assess NE uptake, monoamine oxidase A (MAOA) to evaluate NE metabolism, galanin to measure peptide co-transmitter levels, and GAD67 to investigate local inhibitory networks. Images were grayscaled, fluorescence was measured in ImageJ, and comparisons were made using two-way ANOVAs (genotype x age). Significant effects of age and genotype were found for NET, and a significant effect of age was noted for GAD67. Post-hoc analysis revealed that NET levels were significantly decreased in TgF344-AD rats at 6 months, while both genotypes showed similar reductions at 16 months compared to 6 month animals. GAD67 abundance was significantly lower in 16 month animals compared to 6 months, with no genotype differences. No main effects of genotype or age were found for MAOA, DBH, or galanin, although there was a trend for lower MAOA levels in 16 month TgF344-AD rats compared to wild-type littermates. Presented evidence suggests early LC dysfunction in TgF344-AD rats that coincides with the onset of tau pathology and worsens over time, recapitulating aspects of human AD. The next step will be to determine whether a causal link exists between pathological tau and LC abnormalities, as well as directly assessing LC activity during early and late stages of disease.

IRON-LOADING IS A PROMINENT FEATURE OF ACTIVATED MICROGLIA IN ALZHEIMER'S DISEASE PATIENTS

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Introduction: Many transcriptomic studies have showed microglia not only to be able to modulate Alzheimer's disease (AD), but also to undergo the biggest changes in response to pathology. Gene expression profiles indicate not only loss of homeostatic function and pro-inflammatory activation, but also dysregulated iron-metabolism, illustrated via upregulation of FTL (Mathys, 2019). Iron-accumulation itself has been reported to correlate with Aβ and tau spreading, and more importantly, to accelerate cognitive decline in Aβ+ AD patients. Therefore, in this study we aimed to research the possible link between iron accumulation and functionally activated microglia, and finally its relation with Aβ-plaques.
Methods: We created a multiplex immunofluorescence panel (mIF) with markers for P2RY12, TMEM119, Light-Chain Ferritin (FTL), Aβ, Iba1 and DAPI, and stained brain tissue of 12 AD and 9 control patients. Cells were automatically segmented, phenotyped, and spatially mapped for further analysis. Consecutive sections were stained for iron using enhanced Perl's.
Results: Automated segmentation allowed for evaluation of 69227 microglia, with great heterogeneity in its multi-marker expression profiles. Using unsupervised clustering we identified a microglia subset with increased FTL and Iba1 expression and decreased TMEM119 and P2RY12 expression, which was significantly more present in AD patients ($P = 0.0264$). Further investigation showed FTL+Iba1+-microglia to predominantly infiltrate Aβ-plaque plaques ($P < 0.0001$). These microglia reflected iron-accumulating microglia and showed advanced dystrophic morphology. Finally, these microglia were primarily present in subjects with high Aβ- and Tau-load, and were found to be more present and show more Aβ-infiltration in APOE4-carriers.
Discussion: mIF allowed for accurate multi-marker phenotype evaluation on single cell level, while preserving morphological and spatial information of microglia with relation to Aβ-plaques. We identified activated P2RY12-TMEM119-FTL+Iba1+ microglia, which reflect iron-accumulating microglia in AD, and predominantly infiltrate Aβ-plaques. These findings suggest iron to be taken up by microglia and to influence the functional phenotype, especially in conjunction with Aβ. This could provide a possible mechanism for how iron can act as potential disease modifier.

MUTANT HUNTINGTIN mRNA FORMS NUCLEAR CLUSTERS AND ARE RESISTANT TO OLIGONUCLEOTIDE-BASED SILENCING IN HD MOUSE MODELS

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Mutant mRNA and protein both contribute to the clinical manifestation of many repeat-associated neurodegenerative and neuromuscular disorders. The presence of nuclear RNA clusters is a feature shared amongst these diseases, such as C9ORF72/ALS and myotonic dystrophy 1/2 (DM1/2); however, this pathological hallmark has not been conclusively demonstrated in Huntington's disease (HD) *in vivo*. Investigations into HD – caused by a CAG repeat expansion in exon 1 of the huntingtin (*HTT*) gene – have largely focused on toxic protein gain-of-function as a disease-causing feature, with fewer studies investigating the role of mutant *HTT* mRNA in pathology or pathogenesis.

Here we report that in two HD mouse models, YAC128 and BACHD-97Q-ΔN17, mutant *HTT* mRNA is preferentially retained in the nucleus *in vivo*. Furthermore, we observed the early, widespread formation of large mutant *HTT* mRNA clusters (approximately 0.6 to 5 μm^3 in size) present in over 50-75% of striatal and cortical neurons. Affected cells were limited to one cluster at most. Endogenous wild-type mouse *Htt* or human *HTT* mRNA containing 31 or fewer repeats did not form clusters. These results suggest that multiple repeat-containing transcripts can coalesce to form a single cluster in a given cell. Treating YAC128 mice with antisense oligonucleotides (ASOs) efficiently silenced individual *HTT* mRNA foci but had limited impact on clusters. Our findings identify mutant *HTT* mRNA clustering as an early, robust molecular signature of HD, further supporting HD as a repeat expansion disease with suspected mRNA involvement.

INHIBITION AND DISRUPTION OF HUMAN LYSOZYME FIBRILS BY AN ANTIBIOTIC, LEVOFLOXACIN: NEW DIRECTION FOR ANTIBIOTICS IN THE ERA OF MULTI-DRUG RESISTANCE.

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Neurodegenerative diseases are a group of debilitating disease involving protein aggregation. Till date, all advances in neurodegenerative diseases' therapeutics help symptomatically but do not prevent the root cause of the disease, i.e., the aggregation of protein involved in the diseases. Antibiotics are increasingly becoming obsolete due to the rising multi drug resistance strains of bacterium. Thus, antibiotics if put to different use as therapeutics against other diseases could pave a new direction to the world of antibiotics. Hence, we studied antibiotic Levofloxacin for its potential anti-amyloidogenic behaviour in human lysozyme, a protein involved in non-systemic amyloidosis. Levofloxacin at sub-stoichiometric level was able to inhibit amyloid formation in human lysozyme as observed by various spectroscopic and microscopic methods. Thioflavin T assay confirmed that levofloxacin delays the lag phase at lower concentration and arrests the lysozyme fibrillation at lag stage in sub-stoichiometric concentrations. Structural and computational studies provided with mechanistic insight that levofloxacin stabilises the lysozyme in native state by binding to the aggregation-prone residues and thereby, inhibiting amyloid fibrillation. Levofloxacin also showed the property of disrupting amyloid fibrils into smaller polymeric form of protein which were less cytotoxic as confirmed by hemolytic assay. Therefore, we throw new light on levofloxacin as an amyloid inhibitor and disruptor which could pave way to levofloxacin as potential therapeutics against non-systemic amyloidosis and neurodegenerative diseases.

INVESTIGATING LYSOSOME DYSFUNCTION IN A ZEBRAFISH MODEL OF LYSOSOMAL STORAGE DISORDER, CLN2 DISEASE

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Lysosomal storage disorders (LSDs), a group of inherited genetic diseases, are often associated with early-onset neurodegeneration, highlighting the critical role of the lysosome in maintaining neuronal cell function and health. The study of LSDs can therefore generate insights into the interconnection between lysosomal dysfunction and neurodegeneration more broadly. Accumulating evidence from LSDs show that lysosome dysfunction can lead to impairment of autophagy, which is thought to contribute to disease pathogenesis. Here, we report on a model of CLN2 disease, an LSD caused by recessively inherited dysfunction of lysosomal serine protease Tripeptidyl Peptidase 1 (TPP1), leading to a characteristic accumulation of material in the lysosome. The mutant zebrafish larvae (*tpp1*$^{-/-}$) show phenotypes that resemble the human disease, including neuronal and retinal degeneration, seizures and premature death (Mahmood et al., 2013). We show that this model is suitable to investigate the relationship of impaired autophagy and lysosomal dysfunction. Specifically, we developed novel transgenic lines with autophagosome and lysosome markers LC3 and Lamp1 respectively, tagged by ZsGreen fluorescent protein. Using these lines, we were able to reliably quantify lysosomal number, size and morphology, as well as autophagic flux *in vivo*. This initial dataset highlights severe lysosomal abnormality in *tpp1*$^{-/-}$ mutants, alongside blocked autophagic flux and alterations in autophagosome position. Furthermore, we show a perturbation in mTORC1 and TFEB signaling in *tpp1*$^{-/-}$ mutants. Thus, this model provides a high-content imaging-based strategy to investigate the perturbation of autophagic pathways, and to explore the therapeutic potential of modulating lysosome and autophagy function in CLN2 disease, with potential implications for other neurodegenerative diseases.

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ALTERATIONS IN WWOX LEVELS IN AMYOTROPHIC LATERAL SCLEROSIS

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The exact molecular mechanisms leading to motor neuron degeneration in amyotrophic lateral sclerosis (ALS) are not yet fully elucidated. Therefore, unveiling the mechanisms involved and identifying disease-modifiers capable of altering the course of the disease are crucial for the development of new therapies. One such candidate is the WW domain-containing oxidoreductase (WWOX), a protein involved in DNA damage response, oxidative stress, neuronal differentiation, and neurodegeneration. In this study, we sought to verify whether alterations in WWOX signaling may be involved in ALS pathogenesis. Our results demonstrate a significant decrease in WWOX levels in a large cohort of ALS post-mortem motor cortex (mCTX). Genetic analysis also revealed several rare, genetic variants in WWOX in 4,366 ALS samples from Project MinE that were completely absent in gnomAD. Specifically, two of these mutations ($WWOX^{STOP261E}$ and $WWOX^{STOP353Q}$) were found in the short-chain alcohol dehydrogenases (SDR) domain of the protein, which is involved in regulating the mitochondrial electron transport chain (mtETC). Alterations in the mtETC have previously been reported in ALS and, similarly, our results revealed a significant decrease in the levels of the ATP synthase subunit alpha of complex V (ATP5A) and the cytochrome c oxidase of complex IV (COX II or MTCO2) in ALS mCTX. Furthermore, co-immunoprecipitation experiments revealed that, while there was no interaction between WWOX and COX II, WWOX interacts with ATP5A in ALS post-mortem tissue. In order to verify whether the two novel ALS mutations in WWOX may be linked to mitochondrial dysfunction, SH-SY5Y cells were treated with human recombinant $rWWOX^{WT}$, $rWWOX^{STOP261E}$, and $rWWOX^{STOP353Q}$ proteins. The results from the MTT assay revealed that treatment with both $rWWOX^{STOP261E}$ and $rWWOX^{STOP353Q}$ induced mitochondrial dysfunction, thus leading to a reduction in cell viability in SH-SY5Y cells. Additionally, we demonstrate that treatments with $rWWOX^{STOP261E}$ and $rWWOX^{STOP353Q}$ decrease the levels of several proteins involved in the mtETC in SH-5YSY cells. Lastly, treatment with $rWWOX^{STOP353Q}$ reduced mitochondrial length in SH-SY5Y cells. Collectively, our findings suggest that WWOX may be involved in mitochondrial dysfunction in ALS.

SEX AND AGING ALTER SECRETION OF BRAIN EXTRACELLULAR VESICLES: A POTENTIAL MECHANISM FOR MAINTAINING BRAIN HOMEOSTASIS

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Objectives
Upon aging, changes occur in the brain, including compromised communication between neurons, changes that are also affected by sex. Moreover, aging is a major risk factor for neurodegenerative diseases, including Alzheimer's disease, and females and males differ in the incidence of the disease. Extracellular vesicles (EVs) in the normal brain play a role in neuronal homeostasis, by removing intracellular accumulated material and regulating cell-to-cell communication. We investigated age- and sex-dependent differences in EV levels and content in the brain.
Methods
EVs were isolated and fractioned from the right hemibrains of 3, 6, 12, 18, and 24-month-old female and male C57BL/6 mice. Morphometric EV sizes and numbers were investigated by nanoparticle tracking analysis. EV constituents were characterized by Western blotting.
Results
Using biochemical analyses of brain EVs, we investigated the amount of the plasma membrane-derived microvesicles, late endosome-derived exosomes, and mitochondria-derived mitovesicles, recently identified in our laboratory. We found an age-associated increase in the number of microvesicles, exosomes and mitovesicles in the brain of both sexes. The number of these EVs was higher in the brain of females compared to males. Analysis of the EV content of the amyloid β precursor protein and its metabolites (APP-carboxyl-terminal fragments) revealed an increased load of β-CTF in exosomes with age in both sexes.
Conclusions
These findings reveal age-dependent altered generation and secretion of EVs into the brain extracellular space and a difference in exosome generation between females and males, likely a compensation mechanism that impacts successful brain aging and sex-dependent susceptibility to age-related neurodegenerative diseases.

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ALS-IMPLICATED PROTEIN TDP-43 SUSTAINS LEVELS OF STMN2, A MEDIATOR OF MOTOR NEURON GROWTH AND REPAIR

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Aggregation of the RNA-binding protein TDP-43 in vulnerable neurons is the diagnostic pathology for most patients with amyotrophic lateral sclerosis (ALS), which is characterized by the selective loss of motor neurons. Furthermore, mutations in the gene TARDBP, which encodes for TDP-43, is a cause of familial ALS. Although it has been proposed that these genetic and pathological perturbations disrupt normal RNA metabolism, the identity of the RNAs regulated by TDP-43 in human neurons remains poorly understood. Here, we used RNA sequencing to identify transcripts whose abundances in purified human stem cell-derived motor neurons (hMNs) were sensitive to reduced TDP-43 levels. We found that transcript levels of Stathmin 2 (STMN2), a regulator of microtubule stability and neurite extension normally highly expressed in motor neurons, were reproducibly and sharply decreased. This reduction was also the case in hMNs differentiated from patient-derived induced pluripotent stem cell lines with pathogenic TDP-43 mutations. STMN2 loss upon altered TDP-43 function was due to altered splicing, which is functionally important, as we demonstrate STMN2 is necessary for normal axonal outgrowth and regeneration. Although hMNs generated in vitro share key molecular and functional properties with bona fide hMNs, the in vivo validation of discoveries from stem cell-based models of ALS is a critical test of their relevance to disease mechanisms. To this end, we used ALS patient spinal cord tissues to provide in vivo evidence corroborating our disease modeling studies that TDP-43 dysregulation alters the expression of STMN2 through altered splicing. We further leveraged this molecular information of altered STMN2 splicing to develop a potential ALS biomarker assay, and we have identified compounds that can correct this splicing defect that could serve as an ALS therapeutic. In conclusion, findings from human stem cell-based models can be used to discover unique aspects of human biology underlying disease pathomechanisms and can illuminate potential therapeutic targets and disease biomarkers.

A SCREEN FOR GENETIC ENHANCERS OF ALPHA-SYNUCLEIN IN *DROSOPHILA*

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Alpha-synuclein is implicated in Parkinson's disease, dementia with Lewy bodies and multiple system atrophy, which are collectively referred to as synucleinophathies. Most cases of synucleinopathies have complex underlying mechanisms that cannot be ascribed to a single gene. To identify mutations that enhances neurodegeneration in the presence of alpha-synuclein, we performed a forward genetic screen on 3500 mutant fly lines and identified mutations in 16 genes. All 16 genes have human orthologs, two of which lie close to nucleotide polymorphisms that are significantly associated with Parkinson's disease in GWAS (Nalls et al., 2019). Proteins encoded by 6 of the human orthologs were found to be reduced in Parkinson's disease brains in two meta-analyses. Interestingly, we have found that enhancement of synuclein-induced neurodegeneration by *skd/MED13* can be partially suppressed by overexpression of phosphofructokinase. Since alpha-synuclein is known to disrupt mitochondria function (Devi et al., 2008; Guardia-Laguarta et al., 2014), our result suggests that a compensatory increase in glycolysis may be crucial for neuronal survival in this model of synucleinopathy.

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DYSREGULATION OF GENES ASSOCIATED WITH NEURODEGENERATIVE DISODERS IN NEURONS OVEREXPRESSING DNMT1.

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DNA methylation is an epigenetic modification of mammalian genomes that plays an important role in cell differentiation and normal development. This epigenetic modification is mediated by DNA methyltransferases (DNMTs) of which DNMT1 is a maintenance methyltransferase. Abnormalities in DNA methylation patterns as well as dysregulation of DNMTs have been reported in many disease conditions. In the context of Alzheimer's disease (AD), two recent studies showed increased levels of DNMT1 transcripts correlated with APOE polymorphisms and increased expression of DR4 that promotes apoptosis and neurodegeneration. However, it is not clear whether there is dysregulation of any other AD-associated genes due to DNMT1 overexpression. Towards this goal, we performed transcriptome sequencing of DNMT1-overexpressing neurons derived from Dnmt1tet/tet, a transgenic mouse embryonic stem cell line (ESC). We identified several dysregulated genes with multiple neurological disorders such as intellectual disability, schizophrenia, Alzheimer's disease, Epilepsy, Parkinson's disease, Bipolar disorder, autistic disorder, mental deficiency, multiple sclerosis and depressive disorder. Of the 2,463 dysregulated genes associated with neurological disorders, 939 were associated with AD and Parkinson's disease (PD). Sixty-six were common to both disorders and were studied further to understand the relationship between dysregulation and DNA methylation levels. Reduced Representation Bisulfite Sequencing (RRBS) analysis of the Tet/Tet neurons suggested that many of the dysregulated genes showed either no change in methylation or hypomethylation, but not hypermethylation, suggesting absence of clear correlation between methylation changes and gene expression. Bioinformatic analysis of the 939 genes revealed that signaling pathways regulating pluripotency, serotonergic synapse, HIF-1 and Axon guidance were affected in the Tet/Tet neurons. Importantly, the Tet/Tet ESCs showed defective neurogenic potential, a phenotype recently reported in AD and other neurological disorders. These results suggest that (i) DNMT1 overexpression is a potential etiological factor for neurodegenerative disorders such as AD and PD, and (ii) Dysregulation of AD- and PD-associated genes was independent of catalytic activity of DNMT1.

2’-5’-OLIGOADENYLATE SYNTHETASE 1 (OAS1) AS A NOVEL THERAPEUTIC TARGET FOR NEURODEGENERATIVE DISEASES.

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Neuronal damage from oxidative stress contributes to the pathophysiology of many forms of neurodegenerative disease. A major pathway in the neuronal response to oxidative stress involves poly(ADP-ribose) polymerase 1 (PARP1)-mediated bulk synthesis of poly(ADP-ribose) (PAR). Because PAR accumulation initiates the regulated form of necrosis known as parthanatos, PARP inhibitors have been proposed as neuroprotective therapeutic agents (1). However, these agents trap and inactivate PARP1 at sites of DNA damage, which impedes DNA repair. An alternative approach to limiting death-triggering PAR accumulation while allowing normal DNA repair is needed. Recently, we showed that 2’-5’-oligoadenylate synthetase 1 (OAS1) suppresses excessive PAR formation in response to pro-oxidative treatments by “capping” growing PAR chains with adenylates and thus terminating additional PAR synthesis (2). We propose that stimulating OAS1 activity is an alternative way of suppressing PAR accumulation and parthanatos, which could preclude additional DNA damage compared with direct PARP inhibitors. Therefore, OAS1 may be a novel therapeutic target for oxidative stress-associated neurodegenerative disease.

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TARGETING MS4A4A AS AN ALZHEIMER'S DISEASE THERAPEUTIC

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Membrane-Spanning Four-Domain 4A family member 4A (MS4A4A) is a transmembrane protein expressed on microglia in the brain and other tissue-resident macrophages in the periphery. It is thought to mediate myeloid cell signaling and polarization through interactions with other membrane-bound receptors. Multiple genetic studies have identified the MS4A gene family, including MS4A4A, as a top risk locus for Alzheimer's Disease (AD) susceptibility (*Naj 2011; Seshadri 2010*). Protective alleles of this locus decrease the likelihood of developing AD and increase the age of disease onset (*Lambert 2013; Kunkle 2019; Jansen 2019*).

In an effort to generate a therapeutic for Alzheimer's Disease, antibodies against MS4A4A were generated and analyzed for their ability to modulate primary human macrophage functions. Upon treatment with anti-MS4A4A antibodies, macrophages increase their expression of TREM2 on the cell surface, with a concomitant increase in levels of shed TREM2 in the culture supernatant. This is reminiscent of the effect of AD protective alleles of MS4A, which raise the level of soluble TREM2 in the cerebrospinal fluid (CSF). The mechanism for this increase is not transcriptional, as TREM2 mRNA is not elevated. Knocking out MS4A4A by CRISPR in the same cell type also elevated TREM2 and its downstream biomarkers, suggesting that anti-MS4A4A antibodies act by reducing MS4A4A function in these cells. Beyond modulating TREM2, anti-MS4A4A antibodies have other effects on myeloid cells. Specifically, they alter the metabolic state of macrophages, as measured by an increase in intracellular ATP levels. This effect appears to be TREM2-independent. They also increase the levels of several soluble biomarkers in the culture supernatants.

The in vivo consequences of treatment with anti-MS4A4A antibodies were examined in a non-human primate model. After treatment, TREM2 and other biomarkers were found to be elevated in the brains and CSF, indicating functional target engagement in the CNS. After treatment, transcriptomic and proteomic analyses reveal microglial changes, including enhanced viability and neuroprotective functions.

In summary, we have generated and characterized candidate therapeutic molecules targeting MS4A4A. These agents modulate the phenotype and function of macrophages and microglia in both TREM2-dependent and -independent fashions. Harnessing MS4A4A biology to regulate microglial function may provide us with a novel strategy for treating AD.

DEVELOPING AND TESTING AN ASO THERAPY IN CELLULAR AND iPSC-PATIENT MODELS OF DENTATORUBRAL-PALLIDOLUYSIAN ATROPHY (DRPLA)

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Dentatorubral-pallidoluysian atrophy (DRPLA) is a rare, progressive, genetic brain disorder that affects movement, higher cognition and emotional processing. DRPLA is caused by a CAG trinucleotide repeat expansion within the atrophin-1 gene, ATN1, with age of onset inversely correlating to the number of CAG repeats. The pathological hallmarks of the disease include development of atrophin-1 nuclear inclusions, astrogliosis and neuronal loss widespread throughout the CNS. There is currently no treatment for DRPLA and little is known about the consequence of ATN1 polyQ expansion on neuronal function. Antisense oligonucleotide (ASO)-based therapy, designed to reduce ATN1 levels through RNAse H knockdown of ATN1 mRNA, is a promising strategy for this disorder. A high throughput ASO screen in the BE(2)M-17 human neuronal cell line identified a number of ATN1 exon sequences efficiently targeted by 2'MOE RNAse H gapmer ASOs, resulting in mRNA knockdown. Subsequent microwalking through these sequence blocks, followed by validation studies in DRPLA patient induced pluripotent stem cells (iPSC)-derived neurons, was designed to define the most specific, efficient and safe ASO sequences for therapeutic knockdown. In parallel with ASO development, we have generated CRISPR/Cas9 ATN1 knockouts (KO) in human iPSCs, neuronal precursor cells (NPCs) and iPSC-derived neurons. We aim to compare the effect of ATN1 KO on cell survival, proliferation, differentiation and neuronal function at these three different developmental stages to: 1. improve our understanding of the function of ATN1 in the CNS, and 2. infer the safety of postnatal ATN1 knockdown in the adult CNS as a clinical strategy. Preliminary data on the functional impact of increased polyQ expansion on the ATN1 gene show that DRPLA patient-derived iPSCs demonstrate changes in autophagic flux and accumulation of intracellular p62 aggregates. Future studies will test for rescue of the observed phenotypes with either ASO-mediated ATN1 mRNA knockdown or CRISPR-mediated CAG copy number reduction.

HOW DO A11+ OLIGOMERS FROM NEURODEGENERATIVE DISEASES IMPAIR THE PROTEASOME?

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Accumulation of proteins such as amyloid-β and α-synuclein have been consistently recognized as a characteristic of neurodegenerative diseases. More recently, we learned that multiple neurodegenerative-associated proteins, despite their biochemical diversity, can adopt a common oligomeric conformation that inhibits the proteasome—a protein-degrading machine. Regardless of protein type, this inhibitory conformation can be recognized by a single antibody, A11. Moving forward, what remains in question is how the A11 conformation impairs the proteasome and whether restoring proteasome activity is sufficient to prevent and/or reverse the progression of neurodegeneration. We hypothesize that the inhibition by A11 oligomers occurs in neurodegeneration, thus contributing to the etiology of neurodegenerative diseases. As a first step in testing this hypothesis, we have identified where the oligomers bind to the proteasome through crosslinking experiments. With this information we plan to structurally study how the binding site regulates proteasome activity. We also plan to generate and express proteasome mutants that are resistant to oligomeric inhibition in models of neurodegenerative diseases to directly determine what role this inhibitory mechanism plays in neurotoxicity. In addition, to confirm and quantify oligomers-proteasome complexes in vivo, we have developed an AlphaScreen assay that can detect very low concentrations of these complexes in tissues lysates. We expect our efforts to demonstrate a novel molecular mechanism of proteasome impairment by disease-related oligomers that can be targeted for pharmacological treatment of neurodegeneration.

SMALL MOLECULE SARM1 INHIBITORS RECAPITULATE THE SARM1$^{-/-}$ PHENOTYPE AND ALLOW RECOVERY OF A METASTABLE POOL OF AXONS FATED TO DEGENERATE.

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Axonal degeneration is responsible for disease progression and accumulation of disability in many neurodegenerative conditions. The axonal degenerative process can generate a metastable pool of damaged axons that are still structurally and functionally viable, but fated to degenerate in the absence of external intervention. SARM1 is an NADase that depletes axonal energy stores upon activation and is the central driver of an evolutionarily conserved program of axonal degeneration. We identified a potent small molecule isoquinoline inhibitor of the SARM1 NADase that recapitulates the SARM1$^{-/-}$ phenotype and protects axons from degeneration induced by axotomy, and from mitochondrial dysfunction caused by rotenone. SARM1 inhibition post-injury with rotenone allowed recovery and rescued axons that had already entered the metastable state. We conclude that SARM1 inhibition with small molecules has the potential to treat axonopathies of the central and peripheral nervous system by preventing axonal degeneration and by allowing functional recovery of a metastable pool of damaged, but still viable, axons.

HUNTINGTIN MEDIATES THE RETROGRADE AXONAL MOVEMENT OF A RAB7 ENDOLYSOSOME: A NEW PATHWAY THAT LIKELY CONTRIBUTES TO EARLY DYSFUNCTION IN HUNTINGTON'S DISEASE

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Huntingtin (HTT), the protein involved in Huntington's disease (HD), moves bi-directionally within axons and loss/reduction of HTT was shown to cause axonal transport defects. Previously, we showed that reduction of HTT disrupted the retrograde movement of Rab7-containing vesicles and that HTT and Rab7 co-migrated together within axons *in vivo*. However, the identity of the moving HTT-Rab7 vesicle remains ambiguous. Using *Drosophila* genetics and *in vivo* microscopy, we found that the retrograde movement of the HTT-Rab7 vesicle is likely aided by accessory proteins RILP and/or HIP1 for associations with molecular motors kinesin-1 and dynein. Further, HTT and Rab7 co-migrated together with other components of endolysosomes (LAMP1, PI3P lipids) and autolysosomes (ATG8) within axons *in vivo*. Genetic reduction of SYX17, but not ATG5, disrupted the axonal movement of the HTT-Rab7 vesicle. Pharmacological disruptors of endolysosomes, Chloroquine and Bafilomycin-A1, impaired the retrograde movement of the HTT-Rab7 vesicle, but not the previously identified HTT-synaptotagmin vesicle. HTT and Rab7 co-immunoprecipitated from light membrane fractions isolated from mouse brains, which also contained molecular motors, accessory proteins, and endolysosomal proteins. Strikingly, expression of pathogenic HTT (HTT-138Q), sequestered Rab7 and LAMP1, but not ATG5 into HTT-138Q containing axonal accumulations. Together, our observations unravel a retrogradely moving HTT-Rab7 axonal endolysosomal cargo complex, whose motility is likely disrupted early in HD.

THERAPEUTIC EFFECT OF ANTISENSE OLIGONUCLEOTIDE TREATMENT IN YAC128 HUNTINGTON MICE

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In Huntington's Disease (HD), cellular toxicity is particularly caused by protein fragments of the mutant huntingtin (Htt) protein generated by proteolytic enzymes. Lowering the levels of these toxic Htt protein fragments is hypothesized to ameliorate the consequences of an expanded CAG-repeat in the Htt gene. To that end, an antisense oligonucleotide (AON) molecule has been developed in our group that targets huntingtin (Htt) RNA and induces skipping of exon 12 in Htt mRNA. As exon 12 contained the particular proteolytic cleavage sites, the resulting HttΔ12 protein can no longer be cleaved into its toxic fragments.

Preliminary data already showed successful in vivo generation of the HttΔ12 protein by exon skipping in the YAC128 mice. This mouse model of HD contains the full-length human Htt gene including 128 CAG-repeats and shows an early onset rotarod deficit and hypoactivity as well as striatal volume loss at 12 months of age. To determine whether skipping of exon 12 in Htt mRNA has a reversal effect on the YAC128 phenotype, mice will be treated and followed for 6 months afterwards. AON will be administered every other week intracerebroventricularly starting at 6 months of age until a total dose of 1500 µg has been reached. After treatment, motor behaviour will be assessed monthly by a rotarod and a straight swim test. PhenoTyper cages (Noldus) will be used to study (hypo)activity and anxiety behaviour. Cerebral volume, blood flow and connectivity will be assessed with MRI at the end of the study. Mice will be sacrificed at 12 months of age, whereupon Htt levels and neuropathology will be examined in brain tissue.

GENOME-WIDE POLYGENIC RISK SCORE IDENTIFIES INDIVIDUALS AT ELEVATED PARKINSON'S DISEASE RISK

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Parkinson's Disease (PD) is the second most common and fastest-growing neurological disorder. Polygenic Risk Scores (PRS) using hundreds to thousands of PD-associated variants support polygenic heritability. Here, for the first time, we apply a genome-wide polygenic risk score approach using 6.2 million variants to compute a PD genome-wide polygenic risk score (PD-GPRS) via the LDPred algorithm. PD-GPRS validation and testing used Accelerating Medicines Partnership – Parkinson's Disease (AMP-PD) and FinnGen Consortia genomic data from 1,654 PD Cases and 79,123 Controls. PD odds for the top 8%, 2.5%, and 1% of PD-GPRS were three-, four-, and seven times greater compared with lower percentiles, respectively ($p<1e-10$). PD age of onset and MDS-UPDRS motor scores also differed by PD-GPRS decile. Enrichment for phagosome, immune response, dopamine signaling, and neuronal signaling pathways was found for genes nearest high PD-GPRS variants identified by MAF analysis. PD-GPRS offers a promising screening tool to identify high-risk individuals for preventive lifestyle or new drug therapy trials.

TRACTABLE HUMAN STEM CELL-BASED MODELS FOR MODELING ADVANCED AGGREGATION PATHOLOGIES IN ALPHA-SYNUCLEINOPATHIES

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Parkinson’s disease (PD) is a synucleinopathy and the second most common neurodegenerative disease. Mutations and gene multiplication of alpha-synuclein can lead to misfolding and aggregation of the protein. A characteristic neuronal pathology in PD is the presence of Lewy bodies, which are intracellular inclusions containing aggregated alpha-synuclein. A key challenge to modeling PD using iPSc-derived neurons has been generating tractable models that capture advanced aggregation pathologies. Mature aggregates do not typically form in immature iPSc-derived neurons. Here, we describe a PiggyBac-based system that enables rapid, scalable and virus-free neuronal differentiation with mutant alpha-synuclein transgene expression. Using this system, we are characterizing two distinct models of alpha-synuclein inclusion formation: (1) the SNCA-3K mutant model in which expression of three E-to-K mutations result in spontaneous formation of vesicle-rich intracellular inclusions, and (2) the fibrillar model, in which neurons expressing the wild-type or mutant alpha-synuclein form neuritic and intracellular inclusions upon exposure to recombinant or patient brain-derived alpha-synuclein pre-formed fibrils. These iPSc-derived neuron models recapitulate markers of pathologic alpha-synuclein seen in other models (e.g., phospho-Ser129), but also recapitulate advanced intracellular pathologies found in postmortem synucleinopathy brains, as seen by electron microscopy. We are dissecting differences and similarities between these two types of inclusions, including examining their effect on neuronal survival through longitudinal live-cell tracking, and CRISPR/Cas9-based screens to identify genetic modifiers of cellular toxicity.

ACTIVATING IL-33-PU.1 TRANSCRIPTIONAL CASCADE PROMOTES MICROGLIAL CLEARANCE ACTIVITY IN ALZHEIMER'S DISEASE

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Impairment of microglial clearance contributes to Alzheimer disease (AD) pathogenesis. While transcriptome reprogramming of microglia directs functional modulation of microglia, how microglial transcriptome can be regulated to ameliorate AD pathology is largely unknown. Here, we show that interleukin (IL)-33 ameliorates AD pathology by inducing a microglial subpopulation with enhanced phagocytic activity. These IL-33–responsive microglia (IL-33RM) carry distinct transcriptome signature, highlighted by increased expression of major histocompatibility complex class II genes and homeostatic signature genes. Furthermore, epigenetic profiling revealed that IL-33RM induction is controlled by IL-33–induced remodeling of chromatin accessibility and PU.1 transcription factor binding. Disrupting PU.1–DNA interaction abolishes the induction of IL-33RM and Aβ clearance induced by IL-33. Thus, we define a PU.1-dependent transcriptional pathway that drives the IL-33–induced functional state transition of microglia, resulting in enhanced Aβ clearance.

GENERATING THE FIRST MOUSE MODELS FOR X-LINKED DYSTONIA PARKINSONISM

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X-linked dystonia parkinsonism (XDP) is a severe neurodegenerative disease endemic to the Philippines. XDP typically manifests with focal dystonia, which generalizes over time, and eventually co-exists or is replaced by parkinsonism. The disease primarily affects males, although a few female cases have been reported. Unfortunately, it appears to be completely penetrant and is currently incurable.

The disease-causing mutation is an SVA-type retrotransposon insertion in intron 32 of *TAF1*, the gene that encodes the largest subunit of the TFIID transcription factor complex. *TAF1* features a complex transcription pattern, with at least 20 coding and non-coding splice variants. The SVA, which contains a hexameric repeat expansion (range: 30-55), triggers the aberrant splicing of *TAF1*, resulting in decreased expression of certain *TAF1* transcripts. However, it is unclear how the SVA mediates splicing dysregulation or if reduced *TAF1* leads to neurodegeneration. Our ability to investigate disease mechanisms further is severely limited by the lack of XDP animal models. Here, we describe our efforts to generate three different mouse models for XDP.

The first model involves the full humanization of the *TAF1* gene. Because the introns of mouse and human *TAF1* are very sequence diverse, this humanization step is likely necessary for us to recapitulate the aberrant splicing patterns observed in human XDP, albeit in a mouse cellular environment. We are replacing mouse *Taf1* (~69kb) with the entire human *TAF1* gene (~166kb), and then knocking-in the XDP-SVA to obtain control and XDP *TAF1* humanized mice.

However, because knocking-in the ~166kb human *TAF1* gene is very challenging, we are also working on a second mouse model where only intron 32 and its flanking exons are human sequences within mouse *Taf1* (total knock-in size: ~32kb). Similarly, an XDP-SVA will be inserted to obtain control and XDP mouse lines.

For both humanization models, we aim to insert the XDP-SVA with the greatest number of hexameric repeats observed in patients (~55 copies). However, the repeat copy numbers have been notoriously difficult to maintain in bacterial culture. We successfully stabilized the SVA repeats by cloning it into a linear vector. We are also currently working on building 56 extra repeats by CRISPR/Cas9 cloning (doubling from 14-28-56) to increase the total number of repeats already in the plasmid.

Lastly, to explore possible loss-of-function mechanisms in XDP, we are generating a *Taf1* conditional knockout mouse.

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SYNDAPIN-2 MEDIATES AMYLOID-β TRANSCYTOSIS AT THE BLOOD-BRAIN BARRIER: IMPLICATION IN ALZHEIMER'S DISEASE?

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A faulty transport of amyloid-β (Aβ) across the blood-brain barrier (BBB), and its diminished clearance from the brain, contributes to neurodegenerative pathologies, including Alzheimer's disease (AD). At the BBB, Aβ efflux transport is associated with the low-density lipoprotein receptor-related protein 1 (LRP1). Recent evidence indicated that LRP1 transcytosis occurs by a tubulation mechanism mediated by a F-Bin/Amphiphysin/Rvs protein, syndapin-2, in which it was shown that avidity of the ligand-receptor interaction determines sorting and intracellular trafficking of LRP1 across the BBB. High avidity cargo biases LRP1 towards fast degradation, while mid-avidity augments the formation of syndapin-2 stabilised tubular carriers and a fast shuttling across the BBB. Here, we explore the involvement of syndapin-2 in the clearance of Aβ across the BBB.

Using an in vitro BBB model, we demonstrate that syndapin-2 is present as tubular structures within brain endothelial cells (BECs) in close proximity to LRP1. However, syndapin-2 is not associated with the LRP1-related vesicular endocytic proteins, such as EEA1, Rab5 and Rab7. In addition, downregulation of syndapin-2 in BECs triggers an increase in the association of LRP1 with classical endocytic proteins, which together with the low association of syndapin-2 with the endosomal proteins, suggests a balance between vesicular and tubular mechanisms, with BECs compensating the lack of syndapin-2 with an increase of endosomal proteins to drive trafficking of LRP1. Syndapin-2 colocalises with Aβ in BECs and, importantly, a downregulation of syndapin-2 impairs brain-to-blood Aβ transport across an in vitro BBB model. Colocalisation of syndapin-2 with LRP1 and Aβ was confirmed ex vivo in the brain blood vessels of healthy mice and in an AD mouse model (APP-PS1), in which syndapin-2 appears to be wrapped around Aβ within BECs. In the APP-PS1 mouse model, syndapin-2 levels are significantly reduced in the blood vessels and parenchyma of 12-months old brains compared to age-matched littermates, whereas endosomal Rab5 expression is significantly increased. Furthermore, syndapin-2 levels are reduced in ageing by comparing 4- and 12-months old APP-PS1 animals. Thus, in vitro and ex vivo downregulation of syndapin-2 appears to affect the transport of Aβ across the BBB.

Collectively, our data reveal that syndapin-2-mediated mechanism in BECs, and its balance with endosomal sorting of LRP1, are critical for the clearance of Aβ, and thus proposing syndapin-2 as a potential target for counteracting the build-up of brain Aβ.

TAU AGGREGATES ARE RNA-PROTEIN ASSEMBLIES ENRICHED IN snRNAs THAT MIS-LOCALIZE ESSENTIAL SPLICING SPECKLE PROTEINS

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Tau aggregates contribute to multiple neurodegenerative diseases including frontotemporal dementia and Alzheimer's disease (AD). Although RNA promotes tau aggregation in vitro, whether tau aggregates in cells commonly contain RNA is unknown. We demonstrate in cell culture and mouse brains that both cytosolic and nuclear tau aggregates contain RNA, with enrichment for snRNAs and snoRNAs. Nuclear tau aggregates co-localize with splicing speckles, which contain nascent RNA transcripts and splicing machinery. The essential splicing regulator, SC35, mislocalizes from nuclear splicing speckles to cytosolic tau aggregates in cells, mouse brains, and in patient brains with AD, frontotemporal dementia (FTD), and corticobasal degeneration (CBD). Formation of tau aggregates in cell models is sufficient to induce alterations in pre-mRNA splicing. This argues one consequence of tau aggregation is sequestration of both splicing-related RNAs and proteins with corresponding alterations in splicing, providing an explanation for the widespread splicing changes seen in AD brains.

FUNCTIONAL ROLE OF CALCIUM SENSORS IN ALZHEIMER DISEASE

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N-methyl-D-aspartate receptors (NMDARs) respond to glutamate to allow the influx of calcium ions and the signaling to the mitogen-activated protein kinase (MAPK) cascade. Both MAPK- and Ca^{2+}-mediated events are important for both neurotransmission and neural cell function and fate. Using a heterologous expression system, we demonstrate that NMDAR may interact with the EF-hand calcium-binding proteins calneuron-1, and NCS_1 but not with caldendrin. NMDARs were present in primary cultures of both neurons and microglia from cortex and hippocampus. NCS_1 in neurons, is necessary for NMDA-induced MAP kinase pathway activation. Remarkably, signaling to the MAP kinase pathway was blunted in primary cultures of cortical and hippocampal neurons and microglia from wild-type animals by proteins involved in neurodegenerative diseases: α-synuclein, Tau, and p-Tau. A similar blockade by pathogenic proteins was found using samples from the $APP_{Sw,Ind}$ transgenic Alzheimer's disease model. Interestingly, a very marked increase in NMDAR–NCS_1 complexes was identified in neurons and a marked increase of both NMDAR–NCS1 and NMDAR–CaM complexes was identified in microglia from the transgenic mice. The results show that α-synuclein, Tau, and p-Tau disrupt the signaling of NMDAR to the MAPK pathway and that calcium sensors are important for NMDAR function both in neurons and microglia. Finally, it should be noted that the expression of receptor–calcium sensor complexes, specially those involving NCS_1, is altered in neural cells from $APP_{Sw,Ind}$ mouse embryos/pups.

Keywords: Alzheimer's disease, calneuron-1, caldendrin, NCS_1, extracellular signal-regulated kinase, glutamate receptor, proximity ligation assay

EXPRESSION AND IMPLICATION OF GLIAL ADENOSINE A_{2A}-A_3 HETEROMER RECEPTORS IN NEUROINFLAMMATION.

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Adenosine triphosphate is the main energy-transfer molecule in Earth's life and adenosine is one of its main metabolites. Adenosine receptors belong to class A G-protein-coupled receptors (GPCRs) and there are four identified mammalian adenosine receptors whose cognate heterotrimeric G proteins are Gi (A_1R and A_3R) or Gs/Golf (A_{2A}R and A_{2B}R). There is evidence of heteromer formation within class A GPCRs and adenosine receptors are among the most studied as paradigm of heteromerization. In addition, studies on A_3R are limited despite the receptor is expressed in the central nervous system (CNS). In preliminary studies we have observed that A_{2A}R may form heteromers with A_3 receptors in an heterologous expression system and in natural brain sources. Accordingly, the first objective was to get insight into the structure and function of the A_{2A}-A_3 receptors heteromer.
A_{2A}R antagonists show promise in fighting neurodegenerative diseases such as Multiple Sclerosis. It is relevant that virtually all the selective A_{2A}R antagonists whose toxicity has been tested in animal models are very safe. The first A_{2A}R antagonist, istradefylline, has been approved for use in humans in the therapy of Parkinson's disease. The expression of adenosine receptors is altered in the course of any type of inflammation taking place in the brain. Interestingly, A_{2A}R expression in glial cells is low/negligible but we found that it was significantly increased in glial cells surrounding the neuropathological features found in necropsies from Alzheimer's disease patients. Adenosine may regulate microglial fate and activation, as shown from experiments using microglia activated by lipopolysaccharide (LPS).
Within this study, it has been observed that A_{2A}R may form heteromers with A_3R. When analyzing A_{2A}-A_3 receptor heteromer function, it has been detected that activation of A_{2A}R results in a blockade of the A_3R -mediated signaling. Consequently, A_{2A}R antagonists potentiate A_3 receptor activation. So, we demonstrated the presence of A_{2A}-A_3 receptor heteromers in an heterologous expression system (HEK-293T cells) and LPS-activated astrocytes. This new described heteromer shows potential for neuroprotection by using A_{2A}R antagonists that would potentiate the action of A_3R also inhibiting the pro-inflammatory action of the A_{2A}R receptor.

KEYWORDS: GPCR, receptor heteromer, A_{2A} receptor, A_3 receptor, CNS.

CELLULAR HETEROGENEITY OF SPORADIC AMYOTROPHIC LATERAL SCLEROSIS BRAINS REVEALED BY SINGLE-NUCLEI RNA-SEQUENCING

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Amyotrophic Lateral Sclerosis is a rapidly fatal neurodegenerative disorder with pathophysiological features of extreme cellular, molecular and genetic complexity, most of which are still poorly understood. Several studies have unravelled molecular mechanisms underlying the disease and pointed at multiple cell types that might be the cause and/or contributing to degeneration. However, which specific cell type might be affected by any of the mechanisms identified is still unresolved. In this study, we describe single-nucleus transcriptomic analysis of around 120,000 nuclei isolated from the frontal cortex of 22 individuals, affected by sporadic ALS or age-matched unaffected controls. This novel study provides an increased resolution of the complex cellular and molecular landscape in sporadic ALS and allowed us to transcriptionally classify specific disease-related molecular alterations in distinct subpopulations of cells. Notably, we identify a strong activation of cellular stress pathways previously described in the disease but now specifically associated with excitatory Cortico-Spinal Motor Neurons. Neuronal cellular burden is connected to a shift in oligodendrocyte cells from a myelinating to a neuronally supportive state. These changes are also accompanied by a clear reactive state of microglial cells. Overall, our findings indicate strong neuronal vulnerability in the disease, a possibly counterbalancing, neuro-supportive role of oligodendrocytes and microglial activation that might advance our knowledge of specific vulnerability and cellular responses in ALS.
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A PHENOTYPIC SCREEN USING PATIENT-DERIVED MOTOR NEURONS IDENTIFIES PIKFYVE AS A NOVEL THERAPEUTIC TARGET FOR DIVERSE FORMS OF AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a devastating and fatal neurodegenerative disease characterized by the degeneration of motor neurons. Given that >30 genes contribute to the disease pathogenesis and 80% of ALS cases have unknown mutations, we hypothesized that therapeutics that are effective in one form of ALS may not be effective in other forms and that screening patient-derived motor neurons may enable the identification of therapeutics that target points of convergence in multiple forms of ALS. To test this notion, we performed an unbiased phenotypic screen of 2,000 FDA approved drugs and 1800 tool compounds to identify compounds that rescue the survival of induced motor neurons (iMNs) generated from iPSCs derived from multiple ALS patients with known or unknown causal mutations. We found that targets whose perturbation is broadly efficacious across iMNs from many ALS patients are rare, providing experimental confirmation that patient-based target identification tools are critical. One broadly-efficacious target, PIKFYVE kinase, normally promotes autophagy. Surprisingly, inhibition of PIKFYVE rescues neurodegeneration mechanistically by blocking autophagosome-lysosome fusion, which induces secretory autophagy to robustly clear misfolded proteins that normally accumulate in the cytoplasm and drive neurodegeneration. Moreover, antisense-mediated suppression of PIKFYVE rescued motor deficits and extended lifespan in a mouse model of ALS, confirming the utility of this target in vivo. Our results demonstrate that convergent therapeutic targets in ALS are relatively rare, but one potential mechanism to broadly rescue ALS motor neuron degeneration includes the activation of secretory autophagy.

ALPHA-SYNUCLEIN INDUCES HIPPOCAMPAL COFILIN PATHOLOGY, THROUGH CXCR4/CCR5 AND PRPC/NOX PATHWAYS, LEADING TO SPINE IMPAIRMENT

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Parkinson's Disease (PD) is a neurodegenerative disorder which, although mainly characterized by motor dysfunction, often leads to cognitive impairment and dementia that are currently recognized as main complications with a devastating impact on patients. Alpha-synuclein (aSyn) is the major protein associated with PD, as its aggregation leads to the formation of Lewy Bodies (LB) which accumulate intraneuronally leading to neurodegeneration. In the case of PD with dementia, LB are highly enriched in the hippocampal region of the brain, and this was shown to be related to increased aSyn expression levels. Here, we investigated the effect of elevated levels of aSyn on primary cultures of hippocampal neurons, either by protein overexpression or by exogenous addition of pre-formed fibrils of aSyn (PFFs), and we observed the formation of cofilin-actin rods. These are neuropathology-related structures composed of bundles of cofilin-saturated actin filaments, which result from cofilin hyperactivation by dephosphorylation, and have been implicated in synaptic dysfunction and cognitive impairment in Alzheimer's disease (AD). aSyn-induced rod formation resulted from cofilin activation, as determined by decreased levels of phosphorylated protein in aSyn-expressing hippocampal neurons, which affected dendritic spine number and morphology. Mechanistically, cofilin pathology promoted by aSyn involved the CXCR4 and CCR5 chemokine receptors and the cellular prion protein (PrPC)-NADPH oxidase (NOX)-dependent pathways. In vivo, we validated hippocampal cofilin pathology in two PD mouse models, one based on the injection of aSyn PFFs in the substantia nigra, and the second one overexpressing human WT aSyn under the control of the neuronal Thy-1 promoter (Thy1-aSyn mice). Interestingly, cofilin dysregulation in Thy1-aSyn mice was observed at the age where mice present synaptic and memory impairments. Interestingly, we also detected rod formation in post-mortem hippocampal sections from PD patients where cognitive impairment was reported. Currently, we are evaluating the in vivo impact of cofilin pathology, as well as the effect of its blockage using chemokine antagonists to inhibit rod formation, on synaptic dysfunction and cognitive impairment. This work supports the innovative hypothesis that cofilin pathology occurs in brain regions relevant to cognitive decline in PD.

CNS DISEASE IN M83 MICE AFTER PERIPHERAL CHALLENGE WITH α-SYNUCLEIN AGGREGATES

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In synucleinopathies such as Parkinson's disease misfolding of α-synuclein, normally a cellular and soluble protein, leads to the accumulation of insoluble protein aggregates and to central nervous system (CNS) disease. Pathological α-synuclein shows some prion-like characteristics and therefore we wanted to investigate whether different routes of peripheral injection could lead to neuroinvasion of the CNS with pathological α-synuclein.
We investigated the spreading of pathological α-synuclein after intracerebral, intraperitoneal, intravenous, or oral inoculation of TgM83$^{+/-}$ mice overexpressing the A53T mutant of human α-synuclein with α-synuclein fibrils. Animals were sacrificed after they developed clinical signs of disease. We stained brain and spinal cord tissue sections with antibodies to pathological α-synuclein and confirmed these findings by biochemistry for phosphorylated α-synuclein in tissue homogenates and with a time-resolved fluorescence resonance energy transfer (TR-FRET) assay for oligomeric α-synuclein.
A synucleinopathy was confirmed in ten out of ten animals that developed clinical signs of disease in 133 ± 4 days post intracerebral, and 202 ± 35 days post intraperitoneal, and 208 ± 20 days post intravenous injection with 50 µg of α-synuclein fibrils. Oral challenge with 50 µg α-synuclein fibrils caused neurological disease in two out of eight mice in 220 and 350 days, and oral challenge with 500 µg α-synuclein in four out of eight mice in 384 ± 131 days. Diseased mice displayed aggregates of sarkosyl-insoluble and phosphorylated α-synuclein, which colocalized with ubiquitin and p62 and were accompanied by gliosis, indicative of neuroinflammation, throughout the CNS. In contrast, none of the control mice that were challenged with bovine serum albumin via the same routes developed any neurological disease or neuropathology.
Our findings show that aggregated α-synuclein behaves like a prion causing neuropathology and CNS disease, not only after intracerebral challenge but also by neuroinvasion after a single intraperitoneal, intravenous, or oral challenge.

CELLULAR AND MOLECULAR PHENOTYPES OF C9ORF72-ALS/FTD PATIENT-DERIVED HUMAN INDUCED PLURIPOTENT STEM CELL MICROGLIA

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The overlap of the C9orf72 (C9) mutation in amyotrophic lateral sclerosis (ALS) and frontotemporal degeneration (FTD) provides an opportunity to investigate common pathobiological mechanisms underlying the motor and cognitive dysfunction present in both diseases. Although neurons are affected in C9-ALS/FTD, persistent changes in glial physiological functions and intrinsic properties may contribute to disease progression and pathogenesis. The role of microglia in C9-ALS/FTD has only recently been explored. It's now known that C9 is highly expressed in microglial cells and the generation of C9 knockout mice revealed that altered immune responses are present in these cells. These findings suggest that inappropriate neural-immune interactions may contribute to neuronal dysfunction. Our goal is to determine if inherent properties of C9 mutant microglia actively contribute to neuronal degeneration or whether changes in microglia phenotypes are a consequence of a diseased cellular environment. We generated patient-derived human induced pluripotent stem cell differentiated microglia (hiPSC-MG) and examined the cellular and molecular phenotypes of the C9 hiPSC-MG mono-cultures. C9 ALS/FTD hiPSC-MG have a similar transcriptional profile compared to control hiPSC-MG, despite the presence of C9orf72-associated phenotypes including reduced C9orf72 protein levels and dipeptide-repeat protein translation. C9-ALS/FTD hiPSC-MG exhibit intrinsic dysfunction of phagocytic activity upon exposure to Aβ or brain synaptoneurosomes and display a heightened inflammatory response. C9 hiPSC-MG mono-cultures also revealed altered expression of endosomal marker early endosome antigen 1 and lysosomal associated membrane protein 1, which was confirmed in patient postmortem tissues. Our findings suggest that unstimulated C9 hiPSC-MG mono-cultures share a largely similar transcriptome profile with control microglia, alluding that a diseased environment triggers disease-specific microglia phenotypes. In summary, the hiPSC-MG culture system provides a validated model to study the role of microglia in disease pathogenesis and their contribution to neurodegeneration.

DISTINCT ROLES FOR SYNAPTIC VESICLE AUTOPHAGY IN EARLY AND LATE STAGES OF ALS IN MICE

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Plekhg5 regulates the autophagy of synaptic vesicles in axon terminals of motor neurons via its function as a guanine exchange factor for Rab26, a small GTPase that specifically directs synaptic vesicles to autophagosomes. Depletion of Plekhg5 in mice results in a motor neuron disease with late-onset characterized by swollen axon terminals of motor neurons with synaptic vesicle accumulations. Mutations in the human *PLEKHG5* gene have been linked to various forms of motor neuron disease. Besides, a variant in the *PLEKHG5* gene was identified as a potential disease modifier in a family with TDP43-linked ALS which had a rapidly progressing disease with early-onset, suggested that Plekhg5 might represent an unexplored modifier for motor neuron disease in general.
To examine the effect of disturbed synaptic vesicle autophagy on motor neuron disease, we depleted Plekhg5 in SOD1-G93A mice, a well-established mouse model for ALS. We found that Plekhg5 loss has opposing effects in the early and late stages of ALS in mice. Deletion of Plekhg5 in SOD1-G93A expressing mice prepones the disease onset, but decelerates the disease progression, resulting in prolonged survival. At early stages, loss of Plekhg5 results in remarkable vesicle accumulations at axon terminals of motor neurons, suggesting an impaired turnover of synaptic vesicles during maintenance of neuromuscular junctions. At late stages, depletion of Plekhg5 significantly reduced the muscular denervation, indicating that Plekhg5-mediated autophagy also contributes to the removal of synaptic vesicles during synapse elimination in SOD1-G93A mice. Besides, Plekhg5 loss also alleviated microglial neuroinflammation during disease progression.
Taken together, our data suggest that presynaptic autophagy needs to be tightly balanced for maintaining synaptic homeostasis and that dysregulation of this process triggers neurodegeneration. Thus, balancing the turnover of synaptic vesicles is a key mechanism for maintaining the function of neuromuscular junctions.

TRANSCRIPTOMIC PROFILING OF GPRC6A REPRESSION IN NEURODEGENERATION AND METABOLISM IN A MOUSE MODEL OF TAUOPATHY

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Tauopathies consist of a group of neurodegenerative diseases characterized by intracellular protein aggregates formed by tau. Tau neuropathology correlates closely to brain atrophy and cognitive impairment. Our previous work has revealed a relationship between tau and arginine metabolism associated to mechanistic target of rapamycin complex 1 (mTORC1) signaling. G protein coupled receptor (GPCR) family C, group 6 member A (Gprc6a) is a putative L-arginine extracellular sensor that signals to mTORC1. We further discovered that arginine, arginine producing enzymes and arginine sensors were increased in human Alzheimer's disease (AD) brains and tau transgenic PS19 (P301S+/-) mice. Therefore, we sought to decrease Gprc6a to inhibit mTORC1 activation and promote tau clearance by autophagy in PS19 mice. We measured mRNA from the hippocampus of non-transgenic/Gprc6a+/+ (nTg) mice, P301S+/-/Gprc6a+/+ (PS19) mice, nTg/Gprc6a+/- (Gprc6a het) mice, P301S+/-/Gprc6a+/- (PS19/Gprc6a het) mice, and performed transcriptomic analysis by using the nCounter Neuropathology Panel (770 genes) and Metabolic Pathways Panel (768 genes) (NanoString Technologies, lnc). Overall, the results indicated that Gprc6a repression in PS19 mice reversed several fundamental neurodegeneration and metabolic pathways compared to tau PS19 mice similar to that of nTg mice. Importantly, Gprc6a reduction in PS19 mice decreased cellular metabolism (mTOR, p53 Pathway) and biosynthesis and anabolic pathways (Glycolysis, Glutamine Metabolism), but also increased neurotransmission (Transmitter Release, Transmitter Response and Reuptake) and neuron-glial interaction (Trophic Factors). These data suggest that Gprc6a repression in models of tauopathy may impact alternate pathways that mitigate the tau phenotype. In summary, this is the first study to illustrate how Gprc6a repression impacts neurodegenerative and metabolic transcriptomic signature pathways in a mouse model of tauopathy. Pathways that mitigate human GPRC6A signaling may potentially provide new therapeutics for tauopathies and related dementias.

ENHANCING INHIBITORY INTERNEURON FUNCTION PROMOTES PRO-COGNITIVE BRAIN STATE AND TRANSCRIPTOME-WIDE CHANGES IN ALZHEIMER'S DISEASE MOUSE MODELS

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Alzheimer's disease (AD) is a complex disease characterized pathologically by beta-amyloid (Aβ) plaques, neurofibrillary tangles of hyperphosphorylated tau and neuroinflammation. Transcriptionally, gene co-expression meta-analysis of the human brain have also identified AD disease-associated modules and altered biological processes. AD causes aberrant brain oscillations, network hypersynchrony and is associated with decreased levels of the Nav1.1 subunit of voltage-gated sodium channels that are predominantly expressed in inhibitory interneurons. Inhibitory interneurons play a critical role in coordinating neuronal networks, in an activity- and brain state-dependent manner, that supports normal brain function. We hypothesize that functional deficits in inhibitory interneurons cause shifts in brain states that are sub-optimal for performing cognitive tasks and induce an imbalance between excitatory and inhibitory inputs leading to cognitive impairments and increased Aβ accumulation. Enhancing interneuron function by increasing Nav1.1 levels may restore brain oscillations and brain states, reduce Aβ plaques, decrease disease-associated transcriptional changes and improve cognitive function. Using long-term EEG recordings, we found that both J20 and APPswe/PS1dE9 mouse models of AD exhibited abnormal brain oscillatory activity that shifted the active encoding brain state towards resting state where high oscillatory frequencies are suppressed and low frequencies are increased. This shift towards resting brain state facilitated network hypersynchrony in the form of epileptiform discharges (EDs) and seizures and disrupted sleep-wake cycles. Furthermore, the phase-amplitude theta-gamma coupling that is associated with memory was significantly decreased during active brain states. Restoring interneuron function by enhancing Nav1.1 levels in both mouse models ameliorated aberrant oscillations, improved regulation of brain states and sleep-wake cycles, decreased EDs and seizures, increased theta-gamma coupling, and improved cognitive function. Transcriptomic analysis using the Nanostring mouse AD panel in the cortex of J20 mice showed significant alterations in modules identified from human AD brains and increasing Nav1.1 expression also reduced the AD-related gene expression changes. In conclusion, enhancing inhibitory interneuron function by increasing Nav1.1 levels could normalize brain oscillations and shift the brain state to a pro-cognitive state to improve cognitive function, reduce Aβ accumulation and diminish disease-related transcriptomic alterations to maintain an overall healthier brain.

NEURONAL CYTOSKELETON ALTERATIONS IN THE TTR A97S MOUSE MODEL OF FAMILIAL AMYLOID POLYNEUROPATHY

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Mutations in transthyretin (TTR) cause familial amyloid polyneuropathy (FAP), a neurodegenerative disease characterized by the deposition of TTR aggregates and amyloid fibers, particularly in the peripheral nervous system, which results in a dying-back axonopathy, culminating in neuronal death. Neuronal degeneration and death have been associated with disruption of the neuronal cytoskeleton in several neurodegenerative diseases. In the case of FAP, a genetic screen performed with a Drosophila model in which TTR Val30Met is expressed in the photoreceptor cells, revealed that Rho GTPases, major cytoskeleton regulators, are modulators of the TTR-induced rough eye phenotype. Here, using a FAP mouse model that carries the human TTR A97S mutation and presents sensory impairment (hTTRA97S), we explored the role of cytoskeleton disruption in the disease pathogenesis.
Our results showed that dorsal root ganglia (DRG) neurons from hTTRA97S mice present disrupted actin organization in the growth cone and a decreased F/G-actin ratio, when compared to control neurons from mice expressing human TTR WT (hTTRWT). These actin alterations preceded the in vitro neurodegeneration. To study the importance of Rho GTPases on hTTRA97S actin alterations, we silenced Rac1 and Cdc42 in DRG neurons from hTTRA97S mice, and were able to revert the disruption of actin organization in the growth cone. Additionally, pull down experiments in sciatic nerves of hTTRA97S mice showed an increase in activated Rac1 and a decrease in activated cdc42, pointing to Rho GTPases alterations in the hTTRA97S mouse model.
Having seen a disruption in actin organization in the FAP mouse model, we were interested in detecting whether microtubules were also altered, as these two cytoskeleton components are interconnected. We crossed hTTRA97S mice with Thy1-EB3 mice to analyse microtubule dynamics, and observed a decrease in the EB3 growth length in the peripheral root of hTTRA97S DRG explants, compared to controls. Moreover, the peripheral root of hTTRA97S DRG also presented mitochondrial trafficking defects, with decreased mitochondria speed and decreased motile mitochondria.
Our data shows impaired organization of neuronal actin and microtubules in the hTTRA97S FAP mouse model, with a potential key impact in FAP pathophysiology. We aim to further dissect the impact of cytoskeleton disruption for neurodegeneration in FAP, which can ultimately lead to the identification of novel therapeutic targets for this disorder.

JM and JE contributed equally to this work

DEFECTIVE PROTEOSTASIS IN INDUCED PLURIPOTENT STEM CELL MODELS OF FRONTOTEMPORAL LOBAR DEGENERATION

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Impaired proteostasis is associated with normal aging and is accelerated in neurodegeneration. This impairment may lead to the toxic accumulation of protein. In a subset of frontotemporal dementia cases, pathogenic mutations in the microtubule-associated protein tau (MAPT) gene are sufficient to cause tau accumulation and neurodegeneration. However, the pathogenic events triggered by the expression of the mutant tau protein remain poorly understood. We used human induced pluripotent stem cell (iPSC)-derived neurons and 3D cerebral organoids from patients carrying the MAPT p.R406W mutation and CRISPR/Cas9, corrected controls to evaluate proteostasis. We then measured molecular networks associated with proteostasis in brains from MAPT p.R406W carriers and neuropathology-free controls. Using iPSC-neurons from patients carrying the MAPT p.R406W mutation, we found that MAPT p.R406W was sufficient to induce morphological and functional deficits in the endolysosomal pathway. These phenotypes were reversed upon correction of the mutant allele with CRISPR/Cas9. Additionally, we observed a significant increase in total and phosphorylated tau in lysosomal vesicles in mutant neurons. Lysosomal defects were replicated in 3D cerebral organoids from MAPT p.R406W and CRISPR/Cas9-corrected isogenic controls as well as in molecular networks in brains from MAPT p.R406W mutation carriers. Our findings suggest that MAPT mutations are sufficient to cause impaired lysosomal function and tau accumulation, which may drive disease pathogenesis and serve as a cellular phenotype for drug screening.

THERAPEUTICALLY VIABLE GENERATION OF NEURONS WITH ANTISENSE OLIGONUCLEOTIDE SUPPRESSION OF PTB

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Huntington's disease (HD) is a fatal neurodegenerative disease with cognitive and movement dysfunction caused by a CAG trinucleotide repeat expansion in the gene encoding the Huntingtin protein. Intrathecal infusion of Anti Sense Oligonucleotides (ASOs) has been used to demonstrate that such designer DNA drugs can be delivered broadly and effectively throughout the mammalian nervous system, with one-time ASO injection producing reduction in mutant Huntingtin and producing partial disease reversal in multiple mouse models of HD. Similarly, mutant Huntingtin has been demonstrated to be reduced by intrathecal ASO injection in human trial. However, while suppression of mutant Huntingtin synthesis has promising clinical potential, it cannot replace neurons lost to the disease. Thus, new concepts in therapy development to reverse disease progression are crucial to discover. Development of methods to reprogram glial cells into neurons has opened the door to producing new neurons in the adult mammalian brain, and in particular for the replacement of neurons lost in neurodegenerative diseases. Recently, by transiently suppressing the expression of Polypyrimidine Tract Binding Protein 1 (PTB), our team, and colleagues have successfully converted astrocytes into neurons in disease mouse model of Parkinson’s Disease, with the new neurons partially reconstructing the affected nigral-striatal circuit and successfully reversing the disease course. Here, we use PTB-ASO's to generate new neurons in the adult mice brain, with the ultimate goal of identifying whether replacing neurons that are lost to the degenerative disease process may be a feasible approach for Huntington’s disease. First, we determine the efficiency of PTB-ASOs dependent astrocyte to neuron conversion in vivo in multiple brain regions and characterise the identities of induced Neurons (iNeurons) in healthy animal. We then Perceive if PTB-ASOs induced iNeurons can replace neurons lost in Huntington’s disease model. These efforts providing a novel approach for therapy in Huntington’s disease and possibly other neurodegenerative diseases.

INTEGRATIVE OMICS APPROACH IDENTIFIES NEW SIGNATURE OF MOTOR NEURON DISEASE –ALS

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease with a selective, highly progressive loss of both spinal and upper motor neurons. However, cranial motor neurons are much less sensitive and survive until the late stages of the disease. Despite extensive research, no single gene has been identified as the sole cause of ALS, suggesting a failure of the entire cellular system to maintain proteostasis. The inability of the cells to respond appropriately to Endoplasmic Reticulum (ER) stress is thought to be one of the major reasons for the ALS progression.

We exploited a unique cell model that uses highly pure populations of mouse stem cell-induced cranial and spinal motor neurons (iCrMN, iSpMN), which mirror the physiology of primary motor neurons. Concurrently with findings in primary neurons, iCrMN is more ER stress-resistant than iSpMN. Using this system, we quantified >8,200 mRNAs and proteins in their expression changes in response to ER stress in both the cell lines. While the expression profiles confirmed the high similarity between the motor neuron types, we identified many statistically robust, but often subtle expression differences at either or both the RNA and protein level. We found new pathway insights related to multiple protein turnover differences in these two neurons. Differential abundance points to a complex, system-wide communication between pathways, including the formation of endosomes and lysosomes, vesicle trafficking, and autophagy. Further, we investigated differences in the proteasomal pathway in more detail and accumulated evidence for non-canonical functions of the proteasome, consistent with recent findings on the neuroproteasome. We can show that a subset of the core proteasome localizes to the neuronal plasma membrane. Together, all these previously unknown differences between the two motor neuron types could explain their differential ability to respond to protein misfolding stress.

POLY ADP-RIBOSE BINDING BY HUNTINGTIN AND SIGNAL DYSREGULATION IN HUNTINGTON'S DISEASE

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Huntington's disease (HD) is an autosomal dominant genetic neurodegenerative disease caused by a CAG expansion in exon1 of the *HTT* gene, which translates to an expanded polyglutamine tract in the huntingtin protein. Age at disease onset is correlated to CAG repeat length but varies by decades between individuals. Genetic causes for this disease modification were linked to DNA repair, redox regulation, and mitochondrial health pathways by genome-wide association studies. The huntingtin protein responds to reactive oxygen species by moving to the nucleus where it localizes to sites of DNA damage and scaffolds DNA repair factors. We therefore sought to identify huntingtin-interacting proteins in the context of oxidative stress. We found a large degree of overlap with databases of proteins modified by poly ADP ribose (PAR), the moiety generated upon activation of poly ADP ribose polymerases (PARPs) by DNA damage. We subsequently identified a PAR-binding motif within the huntingtin protein capable of binding PAR *in vitro*. In cells, huntingtin exists in complex with proteins modified by PAR, including PARP1. Genetic ablation suggests that PARP1 and PARP2 may contribute to huntingtin chromatin recruitment, however enzymatic inhibition of PARP1 and PARP2 enhances huntingtin-PAR and huntingtin-chromatin interactions. In contrast to the elevated PAR levels previously reported in the cerebrospinal fluid from Parkinson's disease patients, HD mutation carriers had lower cerebrospinal fluid PAR levels than healthy controls, even at the premanifest stage. This may be explained in part by the reduced PARP activity we observed in fibroblasts derived from HD patients. While clinically tested PARP inhibitors are in use to treat ovarian, breast, and pancreatic cancers, our results argue against their repurposing as a therapeutic strategy for HD, and that caution should be considered when prescribing PARP inhibitors to treat cancer in HD patients.

SENOTHERAPEUTICS FOR NEURODEGENERATIVE DISEASES

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It is established that cellular senescence plays a causative role in ageing and age-related pathologies. Therefore, interventions targeting senescent cells is envisioned to limit ageing phenotypes. Age-related Macular Degeneration (AMD) is a neurodegenerative retinal disease and one of the leading causes of central blindness in elderly individuals for which there is currently no cure. We have previously reported that oxidative-stress induced senescence in retinal pigment epithelial cells (RPE) cells dysregulates the expression of factors linked to AMD progression. Here, we tested the senotherapeutic effect of caffeic acid (CAF), a bioactive polyphenol, on limiting the senescence associated secretory phenotype (SASP) and its paracrine effects. Cells were incubated with H_2O_2 to establish oxidative-stress induced senescent cultures. Senescent cells increased the expression of SASP markers which were reduced by CAF treatment. Senescent cultures showed a significant increase in inflammatory signaling pathways which were also inhibited by CAF. CAF treatment had no effect on senescent state. Conditioned medium from senescent cultures increased DNA damage and senescent markers in healthy cells. Conversely, conditioned medium from polyphenol treated cells showed reduced aggressiveness. We conclude that senescent RPE cells secrete factors that induce DNA damage and spread senescence in healthy counterparts. CAF treatment suppresses the SASP and consequently its bystander effects. Senotherapeutics based on CAF might limit senescence deleterious impact on AMD and other neurodegenerative pathologies.

VECTORED IMMUNOPROPHYLAXIS AS A THERAPEUTIC OPTION FOR TREATING ALZHEIMER'S DISEASE

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Alzheimer's Disease (AD), the most common form of dementia, is an incurable neurodegenerative disorder affecting nearly 50 million people worldwide. Excessive deposition of Aβ plays a pivotal role in AD etiology and progression. In neurons, Aβ is produced by the activity of two proteases, known as beta (BACE-1) and gamma secretase, responsible for the sequential cleavage of the amyloid precursor protein (APP). Hence, compounds able to inhibit or regulate the activity of these secretases could potentially be effective therapies for AD. A potentially ideal strategy is to use inhibitory antibody-based therapies, since antibodies are very stable molecules and have the major advantage of excellent binding selectivity. However, antibodies cannot generally enter the CNS from the systemic circulation, due to the shielding effect of the blood brain barrier (BBB). To overcome this issue, we have taken advantage of the ability of an adeno-associated virus (AAV)-based vector (AAV-PHP.B) to cross the BBB and deliver its genetic cargo to the CNS. Our novel PHP.B vector carries the coding sequence for a specific anti-BACE1 nanobody, known as B9, controlled by the CBA promoter. The vector was administered to AD murine models via tail vein injection. Vector administration led to a significant reduction in Aβ1-40 and Aβ1-42 levels in the cortex and hippocampus of injected animals, when compared to controls. These results prove the feasibility of using systemic vectored immunoprophylaxis as a therapeutic strategy for AD.

SLEEP DISRUPTION IN THE PROGRESSION AND TREATMENT OF ALZHEIMER'S DISEASE

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Sleep is an essential physiological behavior that supports brain health and cognitive function. In Alzheimer's disease (AD), a devastating neurodegenerative disorder, patients experience accelerated sleep loss which can be correlated with AD onset and contribute to AD progression. Tau is an axonal microtubule stabilizing protein that forms aggregates in AD and contributes to the cognitive decline, synapse loss and neuronal death. Tau mislocalization and aggregation at synapses may impair sleep and restorative sleep-dependent homeostatic plasticity. I hypothesize that sleep disruption occurs early in AD progression and subsequently drives further tau mislocalization and aggregation, and cognitive decline. Preliminary sleep behavior data using P301S (PS19) transgenic mice shows that differences in sleep behavior arise as early as 3 months in females and 6 months in males. Accumulation of tau becomes apparent between 6-9months. These results highlight sex differences in the onset of sleep disruption and support sleep disruption as an early-stage symptom. Endocannabinoids provide an intriguing avenue for therapeutic intervention because of their role in promoting sleep and anti-inflammatory signaling. Preliminary work shows that sleep disruption in PS19 mice can be acutely reversed by increasing the endocannabinoid anandamide. The objectives of this work are to investigate the link between sleep loss and tau aggregation and to identify the therapeutic window targeting endocannabinoid signaling during sleep in advance of tau pathology and cognitive decline. These studies will provide a deeper understanding of the behavioral and molecular changes that occur during abnormal sleep in AD and highlight endocannabinoids as a suitable signaling pathway for enhancing the restorative benefits of sleep.

DHA TREATMENT RESCUES RECOGNITION MEMORY BUT NOT ATROPHY OF SOME OLFACTORY BRAIN REGIONS IN A MURINE MODEL OF MCI

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Introduction: Olfactory dysfunction and atrophy of olfactory brain regions are observed early in Mild Cognitive Impairment (MCI) and Alzheimer disease (AD). Numerous studies have shown neuroprotective effects in MCI/AD with treatment of docosahexaenoic acid (DHA), an omega-3 fatty acid. However, there are very few studies regarding DHA and effects on the olfactory system deficits. Objectives: To determine if DHA treatment in a murine model of MCI rescues olfactory and cognitive deficits, and atrophy of olfactory brain regions. Method: Structural (Magnetic Resonance Imaging), functional (olfactory behaviour, novel object recognition and sucrose preference test) and molecular (markers of neurogenesis and inflammation) assessments of APOE4 and wild type mice (WT) +/- DHA treatment at 3 and 6 months of age have been performed. Results: There is a significant decrease in right-olfactory bulb weight ($p=0.03$), olfactory tubercle ($p=0.01$) and amygdala ($p=0.02$) volume in APOE4 mice compared to WT on the control diet at 3 months of age. No rescue of these regions was observed in APOE4 mice on the DHA diet. In the novel object recognition test, WT and APOE4 mice at 3 months of age, treated with the control or DHA diet, had a recognition index higher 0.5, showing that all these mice recognized the novel object. However, WT mice treated with control or DHA diet and APOE4 mice treated with DHA diet had a significantly higher recognition index compared to APOE4 treated with the control diet ($p=0.0001$). At 6 months of age, WT mice treated with control or DHA diet, and APOE4 mice treated with DHA recognized the novel object. However, APOE4 mice treated with the control diet did not ($p=0.0001$). In the olfactory behavior test (6 months), a significant decrease in the same sex's investigation time and in the opposite sex odor's investigation time was observed in APOE4 mice treated with control diet compared with WT mice treated with control diet ($p=0.005$, $p=0.0001$, respectively) which was rescued with DHA treatment. Conclusions: The olfactory bulb, olfactory tubercle and amygdala atrophy observed in the APOE4 mice at 3 months of age could be due to caspase activation and apoptosis and/or alterations in neurogenesis. Our results suggest that DHA treatment does not rescue these early deficits in APOE4 mice. The differences in the time spent to explore the odors between WT and APOE4 mice could be due to brain region atrophy involved in receiving, processing and relaying olfactory information in the APOE4 mice. The consumption of a diet rich in DHA may prevent the olfactory behavior and spatial reference memory deficits observed in the APOE4 mice, suggesting that high intake of DHA could rescue levels of brain DHA and prevent olfactory and recognition memory decline in E4 carriers.

VIRAL INFECTION ACCELERATES THE ONSET AND PROGRESSION OF ALS

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Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease, with a lifetime risk of approximately 1 in 350-400. It is a heterogenous condition characterized by progressive muscle wasting and paralysis, inevitably resulting in death. Presently, no cure exists, and current therapies marginally prolong life expectancy. ALS has a strong genetic basis, with a major genetic cause being toxic gain-of-function mutations in the *SOD1* gene, encoding Superoxide Dismutase 1 (SOD1). These mutations result in SOD1 misfolding, aberrant glial cell activation (gliosis), neuroinflammation, and ultimately motor neuron death. However, genetics alone cannot account for the immense heterogeneity observed in ALS, as individuals harboring the same genetic mutation can have vastly different clinical presentations. Thus, environmental factors, such as viral infections, likely play a key role in ALS development. However, most prior studies have focused on chronic/persistent and neurotropic viruses, whereas acute infections have been largely overlooked. Furthermore, whether these associations play a causative role in ALS onset has been controversial. Importantly, viral infections perturb many of the same pathophysiological pathways underlying motor neuron death in ALS. Therefore, we hypothesized that common, acute viral infections, such as those caused by influenza A virus (IAV), can accelerate the onset and/or progression of ALS. $SOD1^{G93A}$ mice were infected intranasally with a sublethal dose of IAV prior to the onset of ALS-like clinical signs. During acute infection, there were no differences in influenza-associated morbidity or viral replication when comparing wild-type (WT) and $SOD1^{G93A}$ mice. However, $SOD1^{G93A}$ mice that recovered from infection went on to experience significantly impaired motor function and reached endpoint more rapidly than uninfected $SOD1^{G93A}$ mice. This same acceleration of disease was evident in mice exposed to inactivated virus, indicating that the immune response stimulated by infection played a critical role in augmenting disease. Indeed, these mice showed signs of exacerbated gliosis in the lumbar spine relative to mice that had not been infected. Taken together, our data suggests that common, acute viral infections may accelerate ALS by triggering an inflammatory immune response that manifests in the CNS. This has important possible implications for the prevention and treatment of otherwise self-limiting infections in those at risk of developing ALS.

THYMOQUINON MODULATES THE EFFECT OF TAU AND AMYLOID BETA ON AUTOPHAGY GENE EXPRESSION IN DROSOPHILA MELANOGASTER MODELS OF ALZHEIMER'S DISEASE

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Alzheimer disease (AD) is one of the most common forms of neurodegenerative disorders associated with a progressive cognitive decline and memory loss. Neurofibrillary tangles of hyperphosphorylated Tau and amyloid plaques are two major hallmarks in AD pathology. Autophagy dysfunction, with a great impact on protein homeostasis, can result in enhanced levels of pathological aggregates in AD. Both pathological tau and amyloid beta, in turn, may affect autophagy function through increased levels of Reactive Oxygen Species (ROS). We have already shown that pathological tau and amyloid beta are, independently, involved in the autophagy gene dysregulation in Drosophila models of AD. Interestingly, this effect was distinctive for some of autophagy genes. Here, we investigated the effect of an antioxidant agent, Thymoquinon (TQ), on the levels of ROS and also altered expression levels of autophagy genes in transgenic Drosophila melanogaster models of amyloidopathy and tauopathy. We have shown that while TQ is able to modulate the expression of autophagy genes involved in nucleation and elongation, Atg6 and Atg8, it does not have any impact on the expression levels of Hook, which encode an adaptor protein. Moreover, TQ could decrease the levels of ROS and ameliorate the behavioral impairment in our transgenic flies. We also examined the effect of TQ, on Tau aggregation induced by formaldehyde, in vitro using several different assays. According to our in vitro results, TQ prevents the aggregation of Tau which could be probably through hydrophobic interactions with Tau molecules. Altogether, we concluded that TQ, as an antioxidant agent, may hamper the increase in the levels of ROS and therefore prevent autophagy gene dysregulation caused by pathological Tau or amyloid beta. Moreover, it seems that TQ plays a role in hampering aggregate formation, as well.

THE PHOSPHOLIPID CHANGES OF THE GLAUCOMATOUS OPTIC NERVE AND IMPLICATIONS FOR REGENERATION.

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Glaucoma refers to a group of progressive and irreversible blinding optic neuropathies. Prostaglandin analogs are the first line of treatment aimed to reduce elevated intraocular pressure and slow the disease progression, but no available treatment can restore the vision already lost. The aim of this research is to identify the optic nerve lipidome changes associated with degeneration in glaucoma and could be targeted to achieve a regenerative-permissive state. Cadaveric human optic nerves were obtained from Caucasian Control and Glaucoma donors with ages 72.3 +/- 5.9 and 70.3 +/- 10.5 years, respectively. Both groups contained both sexes. Optic nerve tissue was homogenized, and lipids were extracted using the Bligh and Dyer method. We performed the lipidomic studies using Q-exactive mass spectrometer coupled with an Accela HPLC system. Of the phospholipids, we found decreased phosphatidylserine (PS), decreased phosphatidylcholine (PC), and increased phosphatidylethanolamine (PE) in the glaucomatous optic nerve. This shift was verified by evaluating lipid metabolic enzymes expression and activity levels involved in the interconversion between PC, PS, and PE using Western Blot and ELISA analysis. We identified phosphatidylserine decarboxylase (PSD), responsible for the conversion of PS to PE, as aberrantly upregulated in both expression and activity levels. PSD, localized in the inner mitochondria membrane, influences mitochondrial dynamics and mitophagy, which are hallmark processes altered in neurodegeneration. Other lipid interconversion enzymes between these lipid classes showed no significant changes. Compared to previous work with human glaucomatous trabecular meshwork (TM) and Aqueous Humor (AH), there is consensus of the PS-to-PE shift and elevated PSD. These results were further verified using a DBA/2J Glaucomatous Mouse Model and evaluating the phospholipid changes within the optic nerve, TM and AH. Similar results were found with relative changes of PS to PE along with upregulated PSD. Collectively, these results suggest that the PS-to-PE shift and elevated PSD is a commonality with either glaucoma or elevated intraocular pressure. Future studies using normotensive Glaucoma samples are required.
Optic nerve regeneration is regulated by intrinsic and extrinsic factors that determine regeneration success. Intuitively, we expect that regeneration will have the opposite lipidomic shift of degeneration; however, this will be dependent on the pathways promoted in regeneration. We aim to compare the glaucomatous PS-to-PE shift and upregulated PSD expression/activity with the regenerating optic nerve lipidomic levels to guide therapeutics that will stop degeneration and support the transition to regaining lost vision.

SENOLYTIC DRUGS ATTENUATE DOWN SYNDROME INDUCED SENESCENCE PHENOTYPES OF NEURAL PROGENITORS

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Down syndrome (DS) is a genetic disorder driven by the triplication of chromosome 21 (T21) that is characterized by a wide range of neurodevelopmental and physical disabilities. Transcriptomic analysis of tissue samples from individuals with DS has revealed that T21 induces a genome-wide transcriptional disruption. However, the consequences of T21 on the nuclear architecture and its interplay with the transcriptome remain unknown. We find that unlike human induced pluripotent stem cells (iPSCs), iPSC-derived NPCs harboring T21 exhibit global 3D-genome architecture disruptions, altered heterochromatin distribution, and genome-wide chromatin accessibility changes in response to T21, consistent with the transcriptional and nuclear-architecture changes characteristic of senescent cells. Treatment of T21 harboring NPCs with senolytic drugs alleviates the transcriptional and cellular changes associated with the neurodevelopmental malformations observed in individuals with DS. Our findings indicate that senescence plays a key role in the neurodevelopmental pathogenesis of DS and we show that senolytics provide an exciting therapeutic avenue for treating individuals with DS by restoring NPC-dysfunctions induced by T21.

CERAMIDE METABOLISM ACROSS EVOLVING PHAGOSOMES

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Phagocytosis is a process by which foreign particles are engulfed by cells and enclosed in membrane-bound vesicles called phagosomes. It is an evolutionarily conserved process, predominantly involved in pathogen clearance and food particle uptake. Phagosomes undergo a process of maturation before they fuse with lysosomes, to perform their intended function. The proteomic changes across maturing phagosomes have been studied and are better understood than the underlying lipidomic changes. Our earlier studies on differential enrichment of lipids on Early and Late Phagosomes have led us to believe that enzymes - partaking in ceramide metabolism- and other lipases have a crucial role to play. Among them we have reported the role of ceramide synthase 2 and furthermore are exploring the roles of ceramidases and glucosylceramidases. Depletion of both these enzymes has been associated with Farber's disease and Gaucher's disease respectively, where neurotoxic levels of lipid accumulation are observed. We thus aim towards understanding the control of ceramide flux on phagosomes through biochemical assays and mass spectrometry based lipidomics and protein profiling techniques.

COEXISTANCE OF DIFFERENT MYELOID POPULATIONS IN THE FRONTAL CORTEX OF ALZHEIMER´S DISEASE PATIENTS

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Parenchymal microglia are the brain-resident immune cells capable of responding to damage and disease and has been postulated as a critical factor in the Alzheimer´s disease (AD) progression since the identification of several genetic risk factors related to their functions. Apart from microglia, CNS macrophages, like perivascular macrophages (PVMs), are also involved in neurodegeneration. However, the different phenotypes and the implication of myeloid cells in the human pathology have not been determined yet. Here, we analyzed the phenotypic profile displayed by these damage associated myeloid cells in the frontal cortex of AD brains. For this purpose, immunohistochemistry and image analysis approaches have been carried out in postmortem samples from non-demented controls (Braak II) and AD cases (Braak V-VI). Frontal cortex of AD patients showed strong myeloid activation similar to that observed in amyloidogenic mice. Microglial cells of Braak V-VI patients were observed forming clusters and exhibited, both plaque (Iba1+/Trem2+/TMEM119+/CD45high) and inter-plaque (Iba1+/Trem2-/TMEM119+/CD45high) damage-associated phenotype. Moreover, in these individuals the PVMs (Iba1+/CD45high/CD163+/ MCR1-) were localized in the parenchyma, predominantly located surrounding amyloid plaques. On the contrary, Braak II with mild amyloid pathology (CERAD B) cases presented only activated microglial cells, while, immunoreactivity of CD163 was absent (Iba1+/CD45high/CD163-).These strongly activated myeloid cells, could drive the AD pathology and, in consequence, could be implicated in the pathology progression.Taken together, these findings suggest the existence of two populations of myeloid cells associated with Aβ plaques in the frontal cortex in the advanced stages of the pathology and probably due to failures in the integrity of the blood-brain barrier. The differential contribution of these two myeloid populations to the pathogenesis of the disease remains to be elucidated. These results open the opportunity to design targeted therapies, not only to microglia, but also to the population of macrophages, in order to modulate amyloid pathology and provide a better understanding of the immunological mechanisms underlying AD progression.

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EVALUATING THE EFFECTS OF SYSTEMIC NK CELL DEPLETION IN A PRECLINICAL MOUSE MODEL OF PARKINSON'S DISEASE.

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Parkinson's disease (PD) is the second most common neurodegenerative disease and is characterized by dopaminergic degeneration within the substantia nigra and aggregation and accumulation of the alpha-synuclein (α-syn) protein into Lewy bodies (LBs). Natural killer (NK) cells comprise 10-15% of circulating lymphocytes, and as part of the innate immune system, serve as a first line of defense. NK cell numbers have been shown to be increased in the blood of PD patients, however, there has been limited investigation into their role in PD. Our lab has established a preclinical mouse model of PD which is induced by intrastriatal injection of pre-formed fibril (PFF) α-syn in M83 transgenic (Tg) mice overexpressing human A53T α-syn mutant protein. We have previously demonstrated that NK cells scavenge α-syn aggregates and systemic depletion of NK cells exacerbates motor deficits and synuclein pathology in the CNS, implicating a neuroprotective role of NK cells. In this study, we aim to investigate if systemic NK cell depletion aggravates peripheral pathologies, particularly within the gastrointestinal (GI) tract, as GI impairments and Lewy pathology in the GI tract are common in PD. To evaluate GI motility, we performed a gut motility assay and measured fecal water content. Our data showed that PFF α-syn mice expelled significantly fewer fecal pellets per hour compared to monomer α-syn control mice, while there is no difference in fecal water content. To characterize α-syn pathology within the enteric nervous system (ENS) immunohistochemistry experiments are being performed. The levels of phosphorylated serine 129 α-syn (p-α-syn) in the duodenum, jejunum, and ileum are evaluated. As aging is a risk factor for PD, here we investigated the effect of age on NK cell number and function in mice. Our results indicate that NK cell numbers are reduced and display hyper-responsiveness to IL-2 with age. Importantly, NK cells from aged females displayed impaired endocytosis of α-syn aggregates. Collectively, our study demonstrates a novel neuroprotective role of NK cells in the context of PD. Our work will provide evidence for utilizing NK cells as an immunotherapeutic target for age-related neurodegenerative diseases.

CELL-TYPE SPECIFIC TRANSCRIPTIONAL CHANGES IN DUTCH-TYPE HEREDITARY CEREBRAL AMYLOID ANGIOPATHY

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Dutch-type hereditary cerebral amyloid angiopathy (D-CAA) or hereditary cerebral haemorrhage with amyloidosis Dutch-type (HCHWA-D) is an autosomal dominant disorder clinically characterized by recurrent haemorrhagic strokes in the brain. The disease is caused by a mutation in the amyloid precursor protein (APP) gene which leads to aggregation of the APP cleavage product amyloid-beta around cerebral blood vessels. Previously we found that upregulated pathways in post-mortem brain tissue of D-CAA patients are related to extracellular matrix, mitochondrial dysfunction and inflammation, using bulk RNA sequencing. This method gives an average expression level of the brain tissue, but fails to provide details that could be derived from the expression profiles of individual cells. The aim of the current study is to use single nucleus RNA sequencing to investigate cell-type specific gene expression profiles in D-CAA.

Nuclei from brain frontal cortex of 8 D-CAA patients and 8 healthy controls were isolated and pooled into batches to reduce costs and batch-effects. The quality of the nuclear isolation was assessed by morphology and relative amount of nuclear RNA. Nuclei were captured in droplets using the 10X Genomics system, library preparation was done using the Chromium Single Cell 5' kit followed by sequencing on the Illumina NovaSeq6000. Data was filtered based on the number of reads and UMI's per nucleus using Seurat. Pooled nuclei from different subjects were demultiplexed using clustering algorithms based on allelic expression. All major cell types of the brain, including blood vessel related cells that are of particular interest in D-CAA, could be identified by gene expression based clustering. Relevant genes, such as APP-processing genes, inflammatory genes, and ECM-related genes were found to be expressed in a cell-type specific manner.

MODELING ALZHEIMER'S DISEASE PATHOGENESIS MECHANISMS USING 3D BRAIN ORGANOIDS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Apolipoprotein E4 (APOE4) is the leading genetic risk factor for sporadic Alzheimer's Disease (AD) which accounts for 95% of AD cases. AD is often associated with epileptic activity. Epileptic activity has been consistently observed in cognitively normal young and old APOE4 carriers preceding AD pathology, suggesting hyperexcitability of neural circuits may play a pathogenic role in AD. Hyperexcitability of neural circuits is a key feature of epileptogenesis, and results from either a decrease in inhibition from GABAergic neurons or an increase in excitation from glutamatergic neurons. APOE4 is 1 of 3 variants of APOE and is normally expressed in glia, although there is increased APOE expression in neurons in cellular and animal models in response to hyperexcitability. Therefore, we hypothesize that the APOE4 genotype might alter neuronal APOE expression and function contributing to hyperexcitability. Supporting this hypothesis, studies using human induced pluripotent stem cell (hiPSC) derived neurons show that homozygous APOE4 (APOE4/4) results in significant loss and dysfunction of GABAergic interneurons compared to the non-risk allele APOE3/3. To investigate the mechanisms underlying hyperactivity that preceded AD pathology, we used hiPSCs from APOE4/4 AD patients and APOE3/3 controls to generate cortical organoids patterned towards the excitatory neurons in the cortex or inhibitory neurons resembling the subpallium. GABAergic enriched subpallial organoids with APOE4/4 demonstrate an early and persistent reduction in organoid size. Immunohistochemistry of 30 DIV organoids suggests an increase in APOE expression, neuronal differentiation, and cell death in APOE4/4 organoids compared to APOE3/3. Our preliminary data demonstrate that 3D brain organoids may be used to investigate early cell-type specific changes that precede end-stage AD pathology.

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THE EFFECTS OF SEX, AGE AND GENOTYPE ON NEUROINFLAMMATION IN HUMANIZED TARGETED REPLACEMENT APOE MICE

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Neuroinflammation is implicated in the progression and pathology of several neurodegenerative diseases including Alzheimer's disease (AD). While AD presents differently in individual patients, advancing age and presence of the strongest known genetic risk factor, APOE4 (E4) genotype, have been shown to contribute greatly to the increased risk of AD. In addition, females have an increased risk of developing AD at a younger age which is modified by the APOE genotype. APOE protein is predominantly expressed in astrocytes and microglia; E4 glia from humans and mice have been consistently shown to have a more reactive phenotype compared to APOE3 (E3). We hypothesized that age, sex and APOE genotype modify the response to an inflammatory stimulus, potentially by inducing proinflammatory cytokine production and secretion in a cell-type, sex, and genotype-specific manner. We first sought to define the effects of an inflammatory stimulus on sex-specific E3 and E4 primary microglia (PMG) and astrocytes (PMA). Our findings indicate that both male and female E4 PMG produced at least a 65% increase in levels of media nitrite than E3 male and female PMG ($p<0.001$). Additionally, in the E4 PMG, a further increase of 25% was observed in females compared to males. E4 PMA isolated from females produced greater levels of nitric oxide, as well as a 2-fold increase in Il1b gene expression at 6h compared to female E3 PMA. To investigate our hypothesis *in vivo*, male and female humanized targeted replacement E3 and E4 mice at 3 or 16 months of age were injected with LPS (0.5 mg/kg) and sacrificed 4h later. LPS induced a higher expression of Il1b and Tnfa mRNA in both the frontal cortex and hippocampus of young and aged E4 mice compared to E3. Il1b expression increased in the hippocampus by ~30-fold in aged E4 males and ~40-fold in aged E4 females. In contrast, Il1b expression only increased ~15-fold in both, aged E3 males and females. Similar effects were observed in Tnfa and Il6 expression in the hippocampus ($p<0.001$). In the young cohort, no sex differences were observed, but Il1b and Il6 gene expression in both E4 males and females increased by 2-fold compared to E3. These data indicate that a peripheral LPS challenge induces a higher increase in proinflammatory cytokine mRNA expression in older E4 mice compared to E3 and this effect appears to be sex and region-specific. Taken together, these data demonstrate that multiple factors contribute to susceptibility to neuroinflammation and provide insight into the role of age, sex and genotype in this susceptibility.

INHIBITION OF THE TERMINAL COMPLEMENT PATHWAY PREVENTS NEUROINFLAMMATION

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Neuroinflammation plays a central role in a wide spectrum of neurodegenerative diseases. Injury of the central and peripheral nervous system as a result of an autoimmune, infectious or degenerative event causes secondary damage and this is a major determinant for outcome. Limiting neuroinflammation is suggested to be an important strategy for treatment.
We have identified the terminal pathway of the complement system as important determinant of outcome in several models for acute or relapsing neurological disease, like in the antibody-dependent EAE model of acute neuroinflammation, the chronic relapsing EAE model of relapsing neuroinflammation, the closed head injury model of TBI and the crush nerve injury model of acute peripheral nerve trauma.
We postulate that this effect is mediated by suppression of a membrane attack complex (MAC) mediated lytic and pro-inflammatory response. We previously found differences between inhibition of C5 and C6. C6 inhibition prevented relapse completely in chronic relapsing EAE, even after induction of disease, whereas, C5 inhibition only partially reduced disease severity. In addition, we recently found that inhibition of C6 after disease onset with the 7E5 primate-specific monoclonal antibody that blocks MAC, alleviated relapse in a “humanized” C6 rat, a transgenic rat deficient for rat C6 carrying a BAC clone containing the human C6 gene. Our RNA-seq data show that preventing MAC formation by C6 inhibition in models of acute or relapsing neurological disease downregulates key pro-inflammatory responses. In addition, it activates pathways of cholesterol biosynthesis promoting remyelination and repair of synaptic plasticity.
We propose that terminal pathway inhibitors, especially targeting C6, is a promising way to limit secondary damage and prevent neuroinflammation leading to neurodegeneration.

THE DNA REPAIR/REDOX PROTEIN APE1 IS INFLUENCED BY BIOLOGICAL SEX IN α-SYNUCLEIN FIBRIL-INJECTED MICE AND IN HUMAN LEWY BODY DISORDERS

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Lewy body disorders are characterized by proteostatic and redox disequilibrium, leading to deposition of α-synuclein in hallmark inclusions. Loss of redox homeostasis raises the risk of oxidative damage to DNA, as evidenced in Parkinson's disease. Emerging evidence suggests that α-synuclein is recruited to DNA damage sites, where it stimulates DNA repair. When diverted into cytoplasmic Lewy bodies and Lewy neurites, α-synuclein may fail to enter the nucleus to repair DNA. Furthermore, oxidized α-synuclein may promote DNA strand breaks. One common pathway for repair of oxidative DNA damage is base excision repair (BER), which involves the coordinated activity of several enzymes, including apurinic/apyrimidinic endonuclease 1 (APE1). We report that knockdown of APE1 with two independent shRNA sequences or inhibition of APE1 DNA repair activity increased inclusions bearing pathologically-phosphorylated α-synuclein (pSer129) in primary hippocampal cultures. Second, we examined APE1 expression in a mouse model of limbic-predominant α-synucleinopathy, in which α-synuclein fibrils are infused into the olfactory bulb/anterior olfactory nucleus (OB/AON). Six months later, we observed a fibril-induced decrease in APE1 expression in the brains of male mice and an increase in females. Third, we demonstrated that the loss of APE1 in fibril-infused male mice in vivo is mediated by oxidative stress, as APE1 loss was abolished by dietary administration of the antioxidant N-acetylcysteine. Fourth, OB/AON tissues harvested from fibril-infused male mice displayed higher cleavage of synthetic DNA lesions than tissues collected in parallel from fibril-infused female mice. However, OB/AON tissues harvested from fibril-infused female mice had higher DNA repair activity compared to males. Assessments of open field activity measures and sucrose preference revealed a negative behavioral impact of α-synuclein fibril infusions in male but not female mice. Finally, APE1 expression was quantified in the human olfactory bulb and amygdala harvested from control subjects or patients with Lewy body disorders. Similar to fibril-infused male mice, men with Lewy body disorders displayed lower APE1 expression compared to women with Lewy body disorders. These collective findings suggest that the impact of α-synucleinopathy on APE1 expression, DNA repair activity, and limbic behavioral outcomes may be modified by biological sex.

α-SYNUCLEINOPATHY IN SEROTONIN PATHWAYS EVOKES A DEPRESSIVE PHENOTYPE IN MICE. REVERSAL BY CONJUGATED ANTISENSE THERAPY

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Depression affects 40% of patients with Parkinson's disease (PD), often preceding the appearance of motor symptoms, and reducing health-related quality of life. However, mechanisms of depression in PD are not known in detail. Degeneration of serotonin (5-HT) neurons, which regulate mood and emotional pathways, occurs during the PD prodromal phase and contributes to a variety of non-motor symptoms. This study was designed to establish a mouse model of α-synucleinopathy in raphe nuclei, which could replicate early histopathological, neurochemical and neuropsychiatric features of human PD neuropathology. We show that AAV5 vector-induced overexpression of human wild-type α-synuclein (h-α-Syn) in vivo in the raphe nuclei produced h-α-Syn levels 3 times higher than the murine phenotype, which results in a progressive α-Syn phosphorylation, accumulation of oligomeric α-Syn forms, and axonal degeneration in connected brain regions over a period of 8 weeks. Mice overexpressing h-α-Syn showed a reduction of extracellular 5-HT concentration in caudate putamen and medial prefrontal cortex and of hippocampal brain derived neurotrophic factor (BDNF) expression, evoking a depressive state in the tail suspension and forced swim tests. Intracerebroventricular administration of an indatraline-conjugated antisense oligonucleotide targeting h-α-Syn (IND-1337-ASO, 100 μg/day) for 4 weeks reduced h-α-Syn production, improved 5-HT neurotransmission, increased BDNF levels and reversed the depressive phenotype. Our findings indicate that α-synucleinopathy in 5-HT neurons and their projections is enough to impair those brain regions that control mood and emotion, and that treatment with conjugated ASO may relieve the PD depressive symptomatology by reducing α-Syn expression, which restores 5-HT neurotransmission throughout the brain.

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ELUCIDATING THE MECHANISMS CONTROLLING TOXICITY OF PATHOLOGICAL TAU IN NEURONS

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Tau aggregation is a hallmark of many neurodegenerative diseases. The mechanisms by which tau aggregation causes neuronal dysfunction and death remain unclear. We have used iPSC-derived neurons with tau variants linked to familial neurodegenerative diseases to elucidate which cellular factors control the toxicity of disease-associated forms of tau. Using unbiased CRISPRi knockdown screens in iPSC-derived neurons, we uncovered that autophagy, glutamatergic signaling, and metabolism are key pathways that regulate neuronal vulnerability to tau toxicity. We aim to understand the molecular mechanism by which tau perturbs these pathways to ultimately uncover why tau aggregation causes neurodegenerative disease. Characterization of this mechanism will enable the development of disease-modifying therapies.

ALPHA-SYNUCLEIN ENHANCES NEURONAL LIPID DROPLET ACCUMULATION IN PARKINSON'S DISEASE

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Parkinson's disease is a neurodegenerative disorder characterized by accumulation of alpha-synuclein (αSyn) aggregates and by abnormalities in lipid storage. To investigate the potential pathophysiological consequences of interactions between αSyn and proteins that regulate the homeostasis of intracellular lipid droplets (LDs), we employed a transgenic Drosophila model of PD in which human αSyn is specifically expressed in photoreceptor neurons. We found that overexpression of the LD-coating proteins perilipin 1 and 2 (dPlin1/2) markedly increased LD accumulation in the neurons. Perilipins also co-localized with αSyn at the LD surface in both Drosophila photoreceptor neurons (dPlin2) and human neuroblastoma cells (PLIN3). Co-expression of αSyn and dPlin2 in photoreceptor neurons synergistically amplified LD content through a mechanism involving LD stabilization, independently of Brummer-mediated lipolysis or de novo synthesis of triacylglycerols. Accumulation of LDs also increased the resistance of αSyn to proteolytic digestion, a phenomenon associated with αSyn aggregation in human neurons. Our results suggest that binding of αSyn to PLIN-coated LDs stabilizes the LD structure and may contribute to the pathogenic misfolding and aggregation of αSyn in neurons.

MITOMETIN, A NOVEL DRUG CANDIDATE, WITH THE POTENTIAL TO SLOW DISEASE PROGRESSION AND PROLONG LIFE OF AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATIENTS.

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Background: Amyotrophic lateral sclerosis (ALS) is a rapidly progressive, invariably fatal neurodegenerative disease characterized by death of motor neurons in the brain and spinal cord. Mean survival after symptom onset is 36 months. Today there is no cure or effective therapies, which underpin the urgent medical need for better therapies. Metabolic pathways in connection with neurodegenerative diseases such as ALS have been overlooked in the past, though it seems timely to revise the universal view of these by considering a new generation of therapies. 2N Pharma have developed a ground-breaking treatment strategy by targeting mitochondrial metabolic dysfunction, which we have shown to be an underlying disease driver of ALS.

Objectives: To discover, develop and provide effective medicines for neurodegenerative diseases such as ALS by a addressing the extremely upregulated lipid metabolism often observed in patients with neurological conditions by shifting systemic cellular metabolism into glucose metabolism, thus prolonging the life and quality of life of ALS patients.

Methods: 2N Pharma has developed a new reversible CPT1 inhibitor, Mitometin, that is being optimized for clinical use. Preclinical data on the effect of blocking CPT1 have been obtained by using the SOD1 G93A animal model of ALS.

Results: Treatment with Mitometin decreases the disease progression and has life prolonging properties in SOD1 G93A mice. We found that downregulation of CPT1 activity by both pharmacological (first generation CPT1 blockers) and genetic methods results in amelioration of disease symptoms, inflammation, mitochondrial function and oxidative stress, whereas upregulation by initiating high-fat diet or corticosterone administration results in a more aggressive disease progression. Finally, we showed that downregulating CPT1 promotes shifts in the gut microbiota communities towards a protective phenotype in SOD1 G93A mice.

Conclusions: The findings reveal that mitochondrial metabolic dysfunction and specifically CPT1 plays a central role in the disease progression in the SOD1 G93A mouse model and shows CPT1 to be a promising future therapeutic target in ALS.

PHOSPHOPROTEOMICS IDENTIFIES MICROGLIAL SIGLEC-F INFLAMMATORY RESPONSE DURING NEURODEGENERATION

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Alzheimer's disease (AD) is characterized by the appearance of amyloid-β plaques, neurofibrillary tangles, and inflammation in brain regions involved in memory. Using mass spectrometry, we have quantified the phosphoproteome of the CK-p25, 5XFAD, and Tau P301S mouse models of neurodegeneration. We identified a shared response involving Siglec-F which was upregulated on a subset of reactive microglia. The human paralog Siglec-8 was also upregulated on microglia in AD. Siglec-F and Siglec-8 were upregulated following microglial activation with interferon gamma (IFNγ) in BV-2 cell line and human stem-cell derived microglia models. Siglec-F overexpression activates an endocytic and pyroptotic inflammatory response in BV-2 cells, dependent on its sialic acid substrates and immunoreceptor tyrosine-based inhibition motif (ITIM) phosphorylation sites. Related human Siglecs induced a similar response in BV-2 cells. Collectively, our results point to an important role for mouse Siglec-F and human Siglec-8 in regulating microglial activation during neurodegeneration.

ATLASTIN 1 MUTATIONS IMPAIR LIPID DROPLETS IN ASTROCYTES IN HUMAN STEM CELL MODEL OF HEREDITARY SPASTIC PARAPLEGIA

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Hereditary spastic paraplegias (HSPs) are a group of inherited neurodegenerative diseases with the common feature of a length-dependent axonopathy of the corticospinal axons, leading to spasticity of lower-limb muscles and gait abnormalities. SPG3A is the most common early-onset autosomal dominant forms of HSP caused by mutations in the *ATL1* gene that encodes atlastin-1, a membrane-bound, endoplasmic reticulum (ER)-localized dynamin-like GTPase. ER serving as lipid factory provides a bulk of lipids for cells. Previous studies show that atlastin-1 mutations decreased lipid droplets (LDs) size and increased LDs numbers in intestinal segment of *C. elegans* and *Drosophila* fat bodies. However, it remains unknown how the LDs in neural cells are affected by atlastin-1 mutations, especially in motor neurons and astrocytes. In this study, we generated isogenic human pluripotent stem cell (hPSC) lines for two *ATL1* missense mutations associated with SPG3A. In hPSC-derived cortical PNs, *ATL1* mutations resulted in reduced axonal outgrowth, impaired axonal transport, and accumulated axonal swellings, recapitulating disease-specific phenotypes. Next, we differentiated SPG3A hPSCs to both cortical PNs and astrocytes, and examined the lipid droplets in these neural cells. The mRNA expression of perilipin was significantly reduced in SPG3A glial cells but not in neurons. Strikingly, we found the presence of LDs in astrocytes; while there were very few, if any, LDs in enriched neuronal cultures. Further analysis of glial cell cultures revealed that the size of LDs was significantly reduced in ATL1-mutated astrocytes compared to that in wild-type control astrocytes. Finally, correcting the LD defects in SPG3A glial cells or co-culturing with normal glial cells rescued axonal defects of SPG3A cortical PNs. Taken together, *ATL1* mutations caused defects in lipid droplets, including altered LD gene expression, decreased LDs size and increased LDs numbers in human astrocytes, which are implicated in the degeneration of cortical PN axons in hereditary spastic paraplegias.

CATALYTIDE IMPROVES THE COGNITIVE DEFICITS BY INJECTING INTO THE CA1 OF HIPPOCAMPUS IN ALZHEIMER'S DISEASE MODEL MICE

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We recently reported about Catalytide (Catalytic peptide) that is the general name of the shorter synthetic peptide possessing the proteolytic activity, although it is generally accepted that enzymes are macromolecular to show the proteolytic activity. JAL-TA9 (YKGSGFRMI) derived from BoxA region of Tob1 protein is the first Catalytide and cleaved both soluble type and solid type of Aβ42 especially on the central region which thought to be the essential region that forms its β-sheet structure and causes oligomerization/aggregation [1-3]. In addition, ANA-TA9 (SKGQAYRMI) derived from BoxA region of BTG3 also showed the similar proteolytic activity [4]. In this study, we examined the effect of JAL-TA9 against Alzheimer's disease model mice (C57BL/6-APP) by Y-maze test. We injected 2 uL of JAL-TA9 (2.5 ug/uL) or saline into the both side CA1 of hippocampus directly. Before the injection, the alternation % are no differences between saline and JAL-TA9 injected group. After 5 days of injection, however, the alternation % of JAL-TA9 injected groups was significantly higher than that of the saline injected group, and this difference is lasted 22 days. Furthermore, the alternation % of saline injected group was showed any change. In contrast, the alternation % of JAL-TA9 injected group was steadily increasing. These results indicated that the spatial working memory of Alzheimer's model mice was improved by JAL-TA9. These data suggested that JAL-TA9 is an attractive candidate for the peptide drugs with a new strategy for Alzheimer's disease.

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EXPLORING COCKAYNE SYNDROME IMPACT ON BRAIN THROUGH NOVEL CELLULAR AND ORGANOID MODELS

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Cockayne Syndrome (CS) is a rare autosomal disease defined as an association of developmental impairment and premature aging, along with a devastating neurological dysfunction. The most observed characteristics are cachexia, cognitive disability, microcephaly, severe growth failure, visual and hearing impairment, cutaneous photosensitivity, recognizable facial appearance with deep sunken eyes, cerebral and cerebellar atrophy, brain calcification, ventricle enlargement, and demyelination. The syndrome prevalence is 2.7 cases per million births, and life expectance is usually less than 16 years old. Currently, there is no efficient treatment for the disease, mainly because its causes remain poorly understood.

CS is caused by mutations in ERCC8 or ERCC6 genes, which encodes for CSA and CSB proteins, respectively, constituents of the transcription-coupled nucleotide excision repair (TC-NER). NER is extensively known as a photo-lesion-solving pathway; however, ultraviolet light penetration does not penetrate more than 100 µm into the skin, while cranium bones have at least 0.5 cm of thickness. Therefore, DNA lesions induced by endogenous cellular processes, mainly oxidatively-generated, were proposed as the primary cause of the CS phenotypes. Contrarily to other DNA repair disorders, CS patients do not have an increased risk of cancer. Nonetheless, the linked to the profound neurological impairment and the type of DNA lesion involved remains unclarified.

This work is a pioneer study of induced pluripotent stem cells (iPSC) from CSB mutated patients and iPSC-derived neural progenitor cells (NPC), iPSC-derived mature neurons and astrocytes, and iPSC-derived brain cortex organoids to understand CS pathophysiology. Here, we have found that CS iPSC and NPC are more sensitive to the oxidizing agent potassium bromate using cell viability assays and caspase 3 activation. Moreover, CS NPCs accumulate more reactive oxygen species (ROS) inside mitochondria during genotoxic stress, and that these lesions are not adequately signalized by canonical DNA damage response. We demonstrated a lack of phosphorylation of the histone 2AX, a known marker for DNA damage. Besides, transcription on these cells is blocked by DNA damage caused by ultraviolet (UV) radiation but not by DNA oxidation. These indicate that CS's causes may rely more on DNA damage response than in transcriptional failure as it has been suggested. In CS brain cortex organoids – 3D structures that mimic some developmental steps of organogenesis -, we found evidence that neural stem cells, related to neural tube formation, are deeply affected in this disorder – suggesting exhaustion of these cells. Together these results point to possible novel targets for investigation and therapeutic development in CS.

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IDENTIFICATION OF REPEAT EXPANSION MUTATIONS USING NOVEL DEACTIVATED-CAS9-BASED TOOLS

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More than 50% of the human genome is made up of repetitive DNA, which comprises the repeatome. Among the various types of repetitive DNA microsatellite expansions containing repetitive stretches of 2-10 nucleotides have been intensively studied because they cause more than 50 neurological disorders. Microsatellite expansion mutations can cause protein loss-, protein gain- or RNA gain-of-function effects and a growing number of these expansions express both sense and antisense transcripts and up to six types of polymeric repeat-associated non-AUG (RAN) proteins. Despite recent advance in sequencing technology repetitive DNA remains difficult to detect and as a result the biology of human repeatome remains largely unexplored. Novel tools that allow the direct identification of repeat expansion mutations from patient genomic DNA samples are needed to accelerate research and to better understand the roles of repetitive DNA in biology and disease. To address this need we have developed a novel Cas9-based tool that enables the identification of novel microsatellite repeat expansion mutations directly from patient genomic DNA.
Our Cas9-based repeat enrichment and detection (dCas9READ) method, which uses *deactivated clustered regularly interspaced short palindromic repeat associated protein 9* (dCas9), enables the pull down of microsatellite expansion mutations with repeat motifs containing NGG motifs. dCas9READ works on the principle that repeat expansion mutations in genomic DNA from patients provide more binding sites for repeat-containing single guide RNA (sgRNA)-dCas9 complexes to assemble compared to shorter alleles in the general population. Repeat expansions and their flanking sequences are subsequently enriched by biotin-streptavidin pulldown and the unique flanking sequences at the expansion loci are identified by next-generation sequencing. In proof-of-concept experiments we show dCas9READ successfully enriched and identified the G4C2 and CCTG repeat expansion mutations that cause C9orf72 amyotrophic lateral sclerosis and frontotemporal dementia and myotonic dystrophy type 2 and their corresponding flanking sequences directly from the genomic DNA of all eight individual patient samples but none of the six expansion-negative controls. Additional control experiments performed without sgRNAs showed no enrichment of the C9 ALS/FTD locus establishing that sequence specificity of the pulldown is determined by the repeat containing guide RNAs. Our data establish dCas9READ as a novel tool that can be used to identify novel disease-causing repeat expansion mutations and to interrogate the function of human repeatome.

ALZHEIMER'S DISEASE (AD)-LIKE PATHOLOGY IN DOWN SYNDROME CEREBRAL ORGANOIDS: A PREVENTATIVE DRUG SCREENING AND PATHOLOGY PROPAGATION PLATFORM REVEALS BACE2 AS AD-SUPPRESSOR

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A population of >6 million people worldwide at high risk of Alzheimer's disease (AD) are those with Down Syndrome (DS, caused by trisomy 21 (T21)), 70% of whom develop dementia during lifetime, caused by an extra copy of β-amyloid-(Aβ)-precursor-protein gene. We report AD-like pathology in cerebral organoids grown in vitro from non-invasively sampled strands of hair from 71% of DS donors. The pathology consisted of extracellular diffuse and fibrillar Aβ deposits, hyperphosphorylated/pathologically conformed Tau, and premature neuronal loss. We found that T21, but not DupAPP, organoids secrete increased proportions of putative BACE2-θ-secretase (Aβ1-19) and BACE2-Aβ-degrading protease (AβDP or Aβ-clearance) products (Aβ1-20 and Aβ1-34) compared to isogenic normal controls. Increased ratios of BACE2-related to BACE2-unrelated anti-amyloidogenic cleavages were reproduced in CSF of people with DS, mirroring organoid secretions. We showed that BACE2 AβDP-cleavage at Leu34-Met35 is cross-inhibited by a clinically-trialled BACE1-inhibitor and detect its products intra-neuronally, and in large extra-cellular aggregates in AD-brain. CRISPR/SpCas9-HF1-reduction of BACE2 (3 to 2 copies) in T21-iPSC significantly decreased AβDP/amyloidogenic ratio and accelerated organoid AD-like pathology preventable by β-secretase and γ-secretase inhibitors. Our combined data demonstrate the role of BACE2 as a genetic-dose-dependent AD-suppressor in human brain, and organoid technology as a potential assay for screening for other protective genes and disease-preventive drugs, as well as an experimental platform to study the mechanisms of AD-like pathology propagation between human neurons.

I Alić, PA Goh, A Murray, E Portelius et al. **Mol Psychiatry** IN PRESS, PMID: 32647257

INTEGRATION OF ALZHEIMER'S DISEASE GENETICS, MYELOID CELL GENOMICS AND GENE REGULATORY NETWORKS REVEALS NOVEL DISEASE RISK MECHANISMS

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Genome-wide association studies (GWAS) have identified more than 40 loci associated with Alzheimer's disease (AD), but the causal variants, regulatory elements and genes remain largely unknown. In this study we show that AD risk alleles are specifically enriched in active enhancers of myeloid cells. To identify their targets, we utilized two complementary approaches. First, we integrated AD GWAS with myeloid epigenomic, chromatin interactions and expression quantitative trait loci (eQTL) datasets to identify putative functional links between active enhancers and their target genes. Second, we used a Mendelian Randomization framework to identify putative causal relationships between activity at myeloid enhancers, target gene expression regulation and AD risk modification. Using these approaches, we identify AD risk enhancers and nominate candidate causal genes among their likely targets (including *AP4E1*, *AP4M1*, *APBB3*, *BIN1*, *MS4A6A*, *RABEP1*, *SPI1*, and *ZYX*) in twenty loci. Fine-mapping of these enhancers nominates candidate functional variants that likely modify AD risk by regulating gene expression in myeloid cells. In the MS4A locus we identified a single candidate functional variant and validated it in human induced pluripotent stem cell (hiPSC)-derived microglia and brain. Importantly, we highlight the coalescence of candidate causal genes in the endolysosomal system of myeloid cells. To dissect the effects of AD risk genes on myeloid cell biology, we constructed myeloid single-cell gene regulatory networks to identify likely downstream targets of AD risk genes. The predicted targets of *SPI1*, a myeloid lineage-determining transcription factor and an AD risk gene, were enriched in lysosomal and endosomal cellular components in both mouse and human networks, highlighting the likely dysregulation of this pathway by AD risk variants affecting *SPI1* expression in myeloid cells. We validate these putative targets in *Spi1* knockdown and overexpression experiments in BV2 microglia. Taken together, this study explores the links between AD risk variants, myeloid enhancer activity, gene expression and subsequent network-level dysregulations that likely contribute to AD risk modification.

THE ROLE OF THE HSP110/HSP70 DISAGGREGATION SYSTEM IN THE PRION-LIKE PROPAGATION OF AMYLOIDOGENIC PROTEINS

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The accumulation and prion-like propagation of α-synuclein, Tau and other amyloidogenic proteins is associated with devastating neurodegenerative diseases. Molecular chaperones are known for their function in maintaining protein homeostasis by preventing the formation or promoting the disassembly of protein aggregates. Here we show that an ATP-dependent human chaperone network disaggregates not only α-synuclein, but also Tau fibrils *in vitro*. This function is mediated by the core chaperone HSC70, assisted by specific co-chaperones, in particular class B J-domain proteins and an Hsp110-type NEF. Recombinant fibrils assembled from all six Tau isoforms as well as Sarkosyl-resistant Tau aggregates extracted from cell culture were processed by the Hsp70 disaggregation machinery, demonstrating the ability of this machinery to recognize a broad range of amyloid substrates. Chaperone treatment released monomeric, and small oligomeric Tau species, which induced the aggregation of self-propagating Tau species in a Tau cell culture model. Moreover, we investigated the physiological consequence of chaperone-mediated disaggregation of amyloidogenic substrates *in vivo*. Using *Caenorhabditis elegans* models that exhibit pathological features of α-synuclein, such as misfolding, intercellular spreading and toxicity, we inhibited the Hsp70 disaggregase by depleting the crucial component HSP-110 and monitored the effect on α-synuclein related phenotypes. The knockdown of HSP-110 impaired Hsp70 disaggregation activity, prevented the resolubilization of amorphous aggregates, and compromised the cellular folding capacity. In stark contrast, HSP-110 depletion reduced α-synuclein foci formation, cell-to-cell transmission and toxicity. These data demonstrate that the Hsp70 disaggregation activity is a double-edged sword as it is essential for maintaining cellular proteostasis while participating in the generation of toxic seeding-competent protein species.

A NEW AGE-CONSERVING MODEL FOR ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a chronic neurodegenerative disease that affects over 50 million people worldwide. Despite numerous drug trials, no disease-altering treatment is available. A major challenge to the development of a therapeutic drug has been a lack of appropriate model systems to study AD. Although aging is the number one risk factor for AD, technical limitations have hindered the development of *in vitro* models of aging. For example, induced pluripotent stem cells derived from AD patient skin fibroblasts have been widely used to study different aspects of AD; however, studies show that markers of aging disappear when cells resume a pluripotent state. A new method to reprogram adult human dermal fibroblasts directly into neurons emerged a decade ago that bypasses the pluripotent state, thereby maintaining the aged signature of the original cell. Current protocols for direct differentiation convert dermal fibroblasts into neuronal subtypes by adding both microRNA and lineage-specific transcription factors. We aim to build upon current methods to directly convert AD patient-derived dural fibroblasts into neuronal populations that are specifically affected in AD, e.g., glutamatergic cortical neurons and cholinergic basal forebrain neurons. We hypothesize that, in comparison to skin fibroblasts, dural fibroblasts will display a higher conversion efficiency due to increased similarity in the transcriptomic profile between dural fibroblasts and neurons. Using this new model for AD, the goal of our work is to study the effect of known AD risk factors, e.g., APOE4 and familial AD-associated mutations, on the pathophysiology of AD. We believe this age-preserving model will allow us to understand the contribution of age to the onset of cellular dysfunction in AD.

H3K4ME3 MODIFIERS REGULATE AMYLOID TOXICITY IN *C. ELEGANS*

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Alzheimer's disease (AD) is characterized by the pathological deposition of amyloid-β (Aβ) plaques and neurofibrillary tangles, ultimately resulting in neurodegeneration. Aging is the greatest risk factor for this disease, and as a result, interventions that delay aging also slow the progression of age-associated diseases like AD. As the precipitating events of AD are thought to begin well before the onset of clinical symptoms, identifying processes early in life that influence aging may be key to significantly alter the trajectory of these age-related diseases. Our lab has recently discovered one such early-life event: naturally-occurring fluctuations in levels of reactive oxygen species (ROS) that are predictive of lifespan and stress resistance. These effects, identified in *C. elegans*, were found to be mediated by redox-dependent inactivation of the protein complex responsible for trimethylation of H3K4, the COMPASS complex. These data demonstrate that transient changes in the levels of H3K4me3 during development can have persistent effects on health and lifespan into adulthood. On this basis, we hypothesize that similar mechanisms could influence susceptibility to age-related diseases such as AD. In this study, we sought to determine if disruption of H3K4me3 modifiers influences amyloid toxicity using *C. elegans*. Reduction of H3K4me3 levels by knockdown of components of the H3K4me3 COMPASS complex *set-2* and *ash-2* in *C. elegans* strains expressing $A\beta_{1\text{-}42}$ reduced paralysis mediated by Aβ accumulation and aggregation. A similar delay in paralysis was achieved by transient exposure of Aβ-expressing animals to mild concentrations of the ROS-generator paraquat during development, or through pharmacological inhibition of the COMPASS complex with MM-401. Additionally, *C. elegans* strains expressing amyloidogenic polyglutamine repeats (Q40) characteristic of Huntington's disease were also protected from paralysis by *ash-2* knockdown, indicating that disruption of H3K4me3 levels can protect against multiple types of amyloidogenic proteins. Despite having a resistance to paralysis, polyglutamine-expressing animals with lower levels of H3K4me3 appear to have an increase in aggregation of the Q40 protein in both young and aged animals. We hypothesize that disruption of H3K4me3 may heighten the capacity of these animals to mount transcriptional responses to proteotoxic stress, and we are currently investigating the mechanisms that may confer this stress resistance.

GUANIDINOACETATE-CREATINE IN SECONDARY-PROGRESSIVE MULTIPLE SCLEROSIS

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Acute secondary-progressive multiple sclerosis (SPMS) is characterized by an escalating accumulation of neurological disability and limited disease-modifying therapeutic options. A 48-year old woman with acute SPMS being treated with interferon beta-1a and oral corticosteroids presented as a clinical outpatient with no disease-modifying effects after treatment. Her brain magnetic resonance (MR) imaging showed diffuse multinodular demyelination areas in cerebral cortex, midbrain and cerebellum, accompanied by impaired metabolism illustrated by low levels of creatine (4.51 mM), choline (1.05 mM), N-acetyl aspartame (NAA, 7.37 mM) and glutathione (0.13 mM), and high glutamate (8.35 mM) in gyrus cinguli, as evaluated with 1.5 T single-voxel MR spectroscopy. A decision was made to treat her with a combination of guanidinoacetate and creatine (4 g/d, 2:2 ratio) for 21 days. She made clinical progress at follow-up, with the intensity of fatigue dropped from severe to mild, while 1H-MR spectroscopy revealed increased brain choline, creatine, N-acetylaspartate, and glutathione, and a drop in glutamate levels. Patients with SPMS may benefit from guanidinoacetate-creatine treatment in terms of patient- and clinician-reported outcomes, and this requires additional study.

SLEEP IS BI-DIRECTIONALLY MODIFIED BY AMYLOID BETA OLIGOMERS

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Disrupted sleep is a major feature of Alzheimer's disease (AD), often arising years before symptoms of cognitive decline. Prolonged wakefulness exacerbates the production of amyloid-beta (Aβ), a major driver of AD progression. Previous studies have thus suggested that reduced sleep during AD may further accelerate Aβ accumulation and neuronal damage, creating a vicious cycle that leads to further neuronal dysregulation and increased sleep-wake cycle abnormalities (Roh et al., 2012). However, the mechanisms by which Aβ affects sleep are unknown. We demonstrate in zebrafish that Aβ acutely and reversibly enhances or suppresses sleep and brain activity as a function of oligomer length and independently of neuronal cell death. Genetic disruptions revealed that short Aβ oligomers induce acute wakefulness through Adrenergic receptor b2 (Adrb2) and Progesterone membrane receptor component 1 (Pgrmc1), while longer Aβ forms induce sleep through a pharmacologically tractable Prion Protein (PrP) signalling cascade. Consistent with a sleep-promoting role for amyloid beta, both gamma secretase inhibition and mutations in zebrafish App genes lead to reduced sleep maintenance at night. We propose that Aβ can trigger a bi-directional sleep/wake switch. This bi-directional Aβ modulation of sleep and wakefulness predicts that alterations to the relative concentrations of different Aβ oligomeric forms during healthy aging and AD disease progression will have opposing consequences on sleep and wake behaviour.

DISEASE-ASSOCIATED OLIGODENDROCYTE RESPONSES IN NEURODEGENERATIVE DISEASES

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Oligodendrocytes, the myelinating glial cells of the CNS, mediate rapid propagation of action potentials and provide metabolic support to axons. Accumulating evidence from animal models and clinical studies have implicated oligodendrocyte dysfunction and myelin loss in the pathogenesis and progression of neurodegenerative diseases. However, the spectrum of possible activation states of oligodendrocytes in different neurodegenerative contexts is not well understood. Using single-cell RNAseq datasets collected across diverse mouse models of Alzheimer's Disease (AD) and Multiple Sclerosis (MS), we derived a catalog of distinct activation states among the disease-associated oligodendrocytes, including subsets expressing immune signaling or cell cycle genes. We then analyzed single-cell RNAseq profiles from human neurodegenerative diseases and observed an elevated expression of some of these gene modules in MS patients. This catalog of disease-associated oligodendrocytes will inform our understanding of disease progression and help develop novel therapeutic interventions.

NEURONAL NLRP3 IS A PARKIN SUBSTRATE THAT DRIVES NEURODEGENERATION IN PARKINSON'S DISEASE

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Parkinson's disease (PD) is characterized by the intraneuronal accumulation of pathologic α-synuclein and subsequent preferential death of dopamine (DA) neurons in the substantia nigra pars compacta (SNpc). Microglial hyperactivation of the Nod-Like Receptor Protein-3 (NLRP3) inflammasome has been well-documented in various neurodegenerative diseases, including PD. We show here that loss of activity of the E-3 ligase Parkin in DA neurons results in NLRP3 priming and assembly leading to degeneration of DA neurons in familial and sporadic mouse models of PD. Moreover, we show that NLRP3 is a Parkin substrate and that Parkin normally inhibits inflammasome priming by ubiquitinating and targeting NLRP3 for proteasomal degradation. Loss of Parkin activity contributes to the assembly of the active NLRP3 inflammasome complex via mitoROS generation through the accumulation of the Parkin-interacting substrate (PARIS), ZNF746. Inhibition of NLRP3 inflammasome function prevents the degeneration of DA neurons. Strategies aimed at inhibiting the NLRP3 inflammasome activation through maintaining Parkin activity hold particular promise as a disease modifying therapy to treat PD. (This research is supported by a grant from the Maryland Stem Cell Research Fund (2017-MSCRFF-3838)

MODELING TAUOPATHY USING THREE-DIMENSIONAL CEREBRAL ORGANOID CULTURE SYSTEM

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Human tau is encoded by the microtubule-associated protein tau gene, *MAPT*, which contains 16 exons. Six isoforms of the tau protein expressed in the adult human brain result from alternative splicing, and these isoforms can be categorized depending on whether they contain three or four carboxy-terminal repeat domains (3R or 4R, respectively). Elevation of the 4R tau to 3R tau ratio is considered as a key for modeling tau-mediated neurodegeneration. However, the expression of 4R tau is relatively low in conventional two-dimensional (2D) culture system compared to 3D conditions, as alternative splicing of *MAPT* is developmentally regulated, and neurons grown in 2D conditions are less mature. To assess whether human cerebral organoids could express more 4R tau, and could serve as a model of tauopathy we applied a bioreactor culture system to generate 3D cerebral organoids from patients with familial frontotemporal dementia (FTD) harboring a tau P301L mutation, and from their isogenic, CRISPR-corrected tau wild-type controls. Compared to conventional 2D neuronal culture, we found that 3D cerebral organoids displayed an increase in 4R tau expression. Moreover, FTD organoids exhibited hyperphosphorylated tau and increased DNA damage, two pathological hallmarks of tauopathy. Together, our findings suggest that 3D cerebral organoid culture system is a valuable tool for modeling tauopathy and for elucidating new molecular mechanisms of tau-mediated neurodegeneration.

DIRECT NEURONAL REPROGRAMMING IN THE MOTOR CORTEX AS A NOVEL THERAPEUTIC STRATEGY IN A MOUSE MODEL OF ALS

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Amyotrophic lateral sclerosis (ALS) is a terminal neurodegenerative disease that results in a loss of motor neurons in the brain and spinal cord, leading to a deterioration of motor function and ultimately culminating in death. Currently there are no effective therapies for ALS. Our goal is to develop novel therapeutic strategies that replace or prevent the degeneration of upper motor neurons (UMNs) in the motor cortex of the brain. Our brain-centric approach is based on the postulated dying forward model, which suggests that UMN pathology precedes lower motor neuron (LMN) loss in the brainstem and spinal cord. Our **hypothesis** is that targeting ALS disease pathology in UMNs will delay or even prevent the progression of ALS to LMNs. For this purpose, we are evaluating the therapeutic efficacy of replacing lost UMNs on ALS disease progression using in vivo neuronal reprogramming, converting toxic astrocytes to new neurons. We use hSOD1G93A transgenic mice as an ALS model, which we crossed to a Cre-activated Rosa26-zsGreen reporter line to lineage trace cells that picked up our viral vectors. Our viral vector for gene therapy was an adeno-associated virus (AAV) 2/5-glial fibrillary acidic protein (GFAP) promoter-Achaete scute-like 1 (Ascl1)-SA6-v2a-Cre (AAV2/5-GFAP-Ascl1-SA6-v2a-Cre) and our control vector was AAV2/5-GFAP-Cre. We used a mutant form of Ascl1 (SA6), in which six serines in serine-proline (SP) sites were mutated to alanines to prevent phosphorylation, as this mutated gene enhances the capacity to promote neurogenesis. Our packaged viral particles were injected using stereotaxic coordinates into the motor cortex of hSOD1G93A;Rosa-zsGreen mice at a symptomatic (16 weeks) stage. Mice are currently being evaluated weekly using body weight, a neurological assessment score, and with motor behaviour tests (rotarod, grip strength, gait parameters). The efficacy of neuronal conversion will be monitored using neuronal markers at experimental endpoint. The results of these experiments will be presented.

APOLIPOPROTEIN E2 AFFECTS THE BRAIN ENDOSOME AND EXOSOME SYSTEM DURING AGING

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Introduction: The polymorphic apolipoprotein E (APOE) gene is the greatest genetic determinant of sporadic Alzheimer's disease (AD) risk: the APOE4 allele increases risk while the APOE2 allele is neuroprotective compared with the common risk-neutral APOE3 allele. We investigated APOE2's impact on brain exosomal and endosomal systems.

Methods: We performed pathway analyses following RNA sequencing of 12-month-old targeted-replacement APOE2 and APOE3 mice brains. Extracellular vesicles (EVs) were isolated from APOE2 and APOE3 mice brains at 6, 12, and 18 months of age. Exosome levels within EV-containing fractions were quantified using nanoparticle tracking and Western blot analyses. Following subcellular fractionation of 18-month-old APOE2 and APOE3 mice brains, early endosome levels were measured using Western blot analysis.

Results: Pathway analyses revealed differential regulation of brain endosomal pathways in 12-month-old APOE2 vs. APOE3 mice. Brain exosome levels were higher in 18-month-old APOE2 vs. APOE3 mice while remaining unchanged at younger ages. Early endosome levels were lower in endosome-containing subcellular fractions prepared from 18-month-old APOE2 vs. APOE3 mice brains.

Summary: Our findings revealed an aging-dependent APOE2-driven increase in brain exosome number and decrease in early endosome levels compared with APOE3 mice. We previously showed that impaired exosome production leads to detrimental accumulation of early endosomes, contributing to age-related APOE4-driven neuronal vulnerability. In contrast, our findings revealed that APOE2 supports clearance of endocytic pathways through robust exosome production. Given that functional interdependent endosomal and exosomal pathways are essential for catabolic cellular processes, we propose that APOE2 exerts its neuroprotective effects in part through the endosomal-exosomal system.

CELL-TYPE SPECIFIC VULNERABILITY TO ALZHEIMER'S NEURODEGENERATION IN A SUBCORTICAL LIMBIC CIRCUIT NODE

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The most striking clinically observed symptom in Alzheimer's Disease (AD) is impaired memory, but the neurobiological substrates leading to the deficit remain poorly understood. Although disruption of cortical and hippocampal functional activity during memory tasks is associated with AD, it still remains unknown whether subcortical structures contribute to the pathology and symptoms of the disease. The mammillary body (MB), a subcortical node of the medial limbic circuit, is one of the first brain regions to exhibit β-amyloid (Aβ) deposition in the 5XFAD mouse model, and amyloid burden in the MB correlates with pathological diagnosis of AD in humans. Using single-cell transcriptomic profiling, RNA in situ hybridization and electrophysiology approaches, we identify two neuronal subtypes in specific MB compartments, the lateral mammillary body (LM) and the medial mammillary body (MM). LM neurons are susceptible to neurodegeneration and neuronal dysfunction in both mice and humans with AD pathology. Slice electrophysiology revealed LM neurons were hyperactive several months before memory impairments emerged, and hyperactivity was exacerbated in the symptomatic stage of AD mice. Remarkably, bidirectionally modulating LM neuronal activity suggests LM hyperactivity is necessary and sufficient to drive memory deficits. By uncovering unique dysfunction of LM neurons in our taxonomy of MB heterogeneity, our findings suggest neurodegeneration is a result of genetically distinct, projection-specific cellular dysfunction and pinpoint dysregulated LM neurons to be causally linked to memory deficits in AD.

THE MULTI-DOMAIN ARCHITECTURE OF DNAJC7 MODULATES TAU AGGREGATION

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Many neurodegenerative diseases are attributed to brain deposition of misfolded proteins into amyloid fibrils. Tauopathies are the largest class of these diseases and are characterized by the amyloid deposition of the microtubule associated protein tau in the brain. However, the mechanisms by which the tau aggregation process is modulated in cells are currently unknown. Here we reveal that DnaJC7, a co-chaperone of the 40 kDa Heat Shock Protein (Hsp40) family, associates with tau and modulates its aggregation. We used cell-based tau biosensors to show that removal of DnaJC7 increases tau aggregation and attenuates the clearance of aggregates in cells. To validate our findings, we used biochemical and structural approaches to identify how DnaJC7 binds tau to modify its aggregation behavior. We found that a single tetratricopeptide repeat (TPR) domain in DnaJC7 preferentially recognizes the native-like collapsed conformation of tau over pathogenic expanded states. DnaJC7 binds to a structural element containing an amyloid motif to sub-stoichiometrically suppress aggregation of tau in vitro. Our findings highlight an exciting new mechanism for regulating tau aggregation distinct from those previously proposed for other chaperones. Understanding how molecular chaperones modulate protein aggregation has implications for harnessing cellular proteostasis mechanisms to mitigate protein aggregation diseases.

HYPERPHOSPHORYLATED AND MIS-LOCALIZED TAU IN AMYOTROPHIC LATERAL SCLEROSIS

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Although the exact molecular mechanisms leading to motor neuron loss in amyotrophic lateral sclerosis (ALS) are not yet fully understood, recent studies have described alterations in tau in both sporadic and familial cases of the disease. Interestingly, tau-induced alterations in cellular processes, such as mitochondrial dysfunction, synaptic loss, excitotoxicity, and impairments of the nucleocytoplasmic transport are also pathogenetic features of ALS. However, whether alterations in tau contribute to the pathogenic mechanisms underlying ALS remain to be elucidated. Therefore, we sought to determine whether tau is mis-localized and hyperphosphorylated in a larger cohort of ALS post-mortem motor cortex (mCTX). Our results demonstrate the presence of tau fibrils in both gray and the white matter of ALS mCTX together with an increase in axonal degeneration. Furthermore, we demonstrated that hyperphosphorylated tau at S396 and S404 (pTau-S396 and pTau-S404, respectively) is mis-localized to the nucleus and the synapses in ALS mCTX, reminiscent of Alzheimer's disease (AD). Interestingly, increases in pTau-S396 and pTau-S404 at the synaptic levels were independent of sex, genotype, and region of onset. Similar to AD, the non-receptor tyrosine kinase Fyn interacted with the post-synaptic protein 95 (PSD95) as well as with both pTau-S396 and pTau-S404 in ALS mCTX. Furthermore, we showed that the treatment of human neuroblastoma SH-SY5Y cells with synaptoneurosomes (SNs) derived from ALS mCTX increases pTau-S396 levels in vitro. Importantly, the treatment of SH-SY5Y cells with the selective tau degrader QC-01-175 not only reduced the increase in pTau-S396 levels induced by ALS SNs, but also reduced the interaction between pTau-S396 and Fyn in ALS SNs-treated cells. Lastly, we identified specific genetic variants in MAPT in ALS cases using the ALS Knowledge Portal (ALSKP) and Project MinE data browser. Taken together, our findings suggest that tau hyperphosphorylation and mis-localization may play a role in ALS pathogenesis and that targeting hyperphosphorylated tau with the novel degrader QC-01-175 mitigates these effects.

THE CONFORMATION-SENSITIVE SCFV ANTIBODY NUSC1 INHIBITS FIBRILLATION AND NEUROTOXICITY OF β-AMYLOID OLIGOMERS

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Soluble oligomers of the β-amyloid peptide (AβOs) are neurotoxins linked to memory deficits in Alzheimer's disease (AD). Because AβOs are heterogeneous in conformation and toxicity, antibodies sensitive to alternative conformations of Aβ aggregates are seen as promising therapeutic strategies to efficiently block AβO neurotoxicity in AD patients. Our group has selected a set of single-chain variable domain (scFv) antibodies by phage-display which distinguishes AβOs from both monomeric and fibrillary Aβ. One of these scFvs, named NUsc1, targets a subset of AβO larger than 50kDa. The NUsc1-targeted subset of AβOs is present in AD brain tissue and triggers oxidative stress and tau hyperphosphorylation in primary hippocampal neurons. Assuming that synaptic binding is the initial step by which extracellular AβOs induce major aspects of neuronal pathology, we aimed to investigate whether NUsc1 could inhibit AβO toxicity in differentiated human neuroblastoma cells. Cells of the SH-SY5Y lineage were differentiated into mature neurons and challenged with AβOs, NUsc1, or AβOs-NUsc1 mixture for 24h. AβO binding to the surface of SH-SY5Y cells, assessed by immunofluorescence, revealed a punctate binding pattern. The exposure to AβOs induced a reduction in cell viability, as measured by MTT assay. On the other hand, AβO treatment did not lead to increased cell death, quantified by the Live/ Dead assay. Importantly, MTT reduction viability was not affected when the culture was treated with the AβO-NUsc1 mixture. Motivated by the protective action of NUsc1 against AβO neurotoxicity, we have also evaluated amyloid fibril formation by AβOs in the presence or absence of NUsc1. Interestingly, we have found by both Thioflavin T fluorescence and transmission electron microscopy that NUsc1 completely inhibited the aggregation of AβOs into fibrils. In summary, we have demonstrated that the NUsc1 antibody selectively binds to a neurotoxic AβO conformation necessary to amyloid fibril formation. Furthermore, we found that NUsc1 neutralizes AβO-induced toxicity on differentiated SH-SY5Y cells, supporting its use as an *in vitro* neuronal-like model to study early molecular and cellular events underlying the neurotoxicity triggered by AβOs.

Keywords: Alzheimer’s Disease, β-amyloid oligomers, NUsc1, SH-SY5Y cells.

META-ANALYSIS OF GENE EXPRESSION CHANGES IN FAMILIAL AND SPORADIC FRONTOTEMPORAL DEMENTIA

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Frontotemporal lobar degeneration (FTLD), also known as frontotemporal dementia (FTD), results in a progressive decline in executive function leading to behavioral changes, speech problems, and movement disorders. FTD is the second most common cause of young-onset dementia affecting approximately 50-60,000 Americans. There are both genetic and sporadic forms of FTD, with mutations in the progranulin (GRN) and C9orf72 genes being the most common familial forms.

In this study, we compared the sporadic and familial transcriptome within the cerebellum, hippocampus, and frontal cortex of patients with FTD in order to identify shared biological pathways and genes.

A meta-analysis revealed 50 dysregulated genes in familial FTD and 95 genes dysregulated genes in sporadic FTD. Only 3 genes, F-Box, and leucine-rich repeat protein 8 (FBXL8), versican (VCAN), and sarcospan (SSPN) were dysregulated in both forms of FTD. Familial FTD genes centered on the Wnt and HIF-1 signaling pathways, adherents junctions, and diseases associated with loss of mental function. Sporadic FTD genes centered on MAPK signaling, platelet activation, metabolism, and psychiatric mood disorders. We also identified transcription factors and miRNA associated with the dysregulated genes, as well as the chemicals which may regulate the encoded proteins.

The results reveal that the genetic and sporadic forms of FTD share some pathways, suggesting common etiologies, and have unique mechanistic pathways. Also, valproic acid was identified as a putative therapeutic agent.

KEY GENE EXPRESSION CHANGES THAT LEAD TO THE TRANSITION FROM ASYMPTOMATIC TO SYMPTOMATIC ALZHEIMER'S DISEASE

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Alzheimer's Disease (AD) is a chronic, neurodegenerative brain disorder affecting millions of Americans that is expected to increase worldwide with the expanding aging population. Symptomatic AD patients show cognitive decline and often develop neuropsychiatric symptoms as a result of the accumulation of insoluble proteins that produce plaques and tangles that may be seen in the brain at autopsy. Unexpectedly, some clinically normal individuals also show AD pathology in the brain at autopsy. These individuals are referred to as asymptomatic AD (AsymAD) because it is thought that they would have eventually developed clinical symptoms if they had lived longer. The molecular mechanisms that lead to AD may be revealed by comparing gene expression changes between individuals who are AsymAD and AD and normal controls that show no AD pathology at autopsy. In this study, Switch Miner Software was used to identify key switch genes in the entorhinal cortex of the brain that lead to the development of AD.

Eighty-eight switch genes were identified that are differentially expressed in AD patients compared to controls that had no AD brain pathology. These genes are involved in inflammation, platelet activation, and phospholipase D and estrogen signaling. Peroxisome proliferator-activated receptor gamma (PPARG), zinc-finger transcription factor (YY1), sterol regulatory element-binding transcription factor 2 (SREBF2), and early growth response 1 (EGR1) were identified as key transcription factors that potentially regulate switch genes in AD.

A comparison of AsymAD individuals and AD patients revealed 58 switch genes. These genes were associated with 29 mechanistic pathways including platelet activation and phospholipase D signaling pathways. The transcription factor, PPARG, was identified as a key transcription factor that may be involved in the regulation of the switch genes. Chemical-protein interaction analysis revealed that valproic acid may be a therapeutic agent that could prevent AsymAD from progressing to AD.

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RETROMER COMBINATORIALS FOR GENE-THERAPY ACROSS A SPECTRUM OF NEUROLOGICAL DISEASES

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Endosomal trafficking is a biological pathway implicated in Alzheimer's and Parkinson's disease, and a growing number of other neurological disorders. For this category of diseases, the endosome's trafficking complex retromer has emerged as a validated therapeutic target. Retromer's core is a heterotrimeric complex composed of the scaffold protein VPS35 to which VPS26 and VPS29 bind. Unless it is deficient, increasing expression of VPS35 by viral vectors has a limited effect on other trimeric members and on retromer's overall function. Here we set out to address these constraints and, based on prior insight, hypothesized that co-expressing VPS35 and VPS26 would synergistically interact and elevate retromer's trimeric expression and function. Neurons, however, are distinct in expressing two VPS26 paralogs, VPS26a and VPS26b, and so to test the hypothesis we generated three novel AAV9 vectors harboring the VPS35, or VPS26a, or VPS26b transgene. First, we optimized their expression in neuroblastoma cell lines, then, in a comprehensive series of neuronal culture experiments, we expressed VPS35, VPS26a, and VPS26b individually and in all possible combinations. Confirming our hypothesis, expressing individual proteins failed to affect the trimer, while VPS35 and VPS26 combinatorials synergized the trimer's expression. In addition, we illustrate functional synergy by showing that only VPS35 and VPS26 combinatorials significantly increase levels of Sorl1, a key retromer-receptor deficient in Alzheimer's disease. Collectively, and together with other recent observations, these results suggest a precision-medicine logic when applying retromer gene therapy to a host of neurological disorders, depending on each disorder's specific retromer-related molecular and anatomical phenotype.

NEUROTOXIC ASTROCYTES SECRETED GLYPICAN-4 DRIVES ALZHEIMER'S TAU PATHOLOGY

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Apolipoprotein E4 (APOE4) is the most crucial genetic risk factor of late-onset Alzheimer's disease (AD). However, the mechanism through which APOE4 induces AD risk remains unknown. Here, we report the astrocyte-secreted protein glypican-4 (GPC-4), as a novel binding partner ofAPOE4, drives tau pathology. APOE4-carrying AD patients display more tau accumulation compared to APOE4-noncarring AD patients. GPC-4 is highly expressed in APOE4 AD patients, and is regulated by microglial factors via NF-κB signaling pathway. The astrocyte-secreted GPC-4 induced both tau accumulation and spreading in vitro and in vivo. Further, GPC-4 is required for APOE4-mediated surface trafficking of low-density lipoprotein receptor-related protein 1 (LRP1) and tau propagation. GPC-4 activates unfolded protein response (UPR) pathway IRE1α, and pharmacological inhibition of IRE1α with KIRA6 blocks GPC-4 induced tau propagation. Together, our data comprehensively demonstrate that the APOE4-induced AD risk is directly mediated by GPC-4, and that perturbing GPC-4 induced IRE1α pathway has therapeutic opportunities.

THE NUCLEAR GAPDH-HMGB CASCADE IN CORTICAL MICROGLIA REGULATES COGNITIVE FLEXIBILITY

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Glyceraldehyde dehydrogenase (GAPDH) is a key enzyme in glycolysis. Beyond its fundamental role in metabolism, GAPDH can get post-translationally modified in response to cellular stressors and subsequently translocate to the nucleus to exert transcriptional control [the nuclear GAPDH (N-GAPDH) cascade]. We hypothesized that this cascade might play a critical role linking stress and behavior. In particular, we studied the involvement of this cascade in the regulation of behavioral flexibility, an important behavioral construct impaired in neurodegenerative and neuropsychiatric disorders and highly affected by stress.
In this study, we discovered a novel mechanism that controls the response of microglia to stress through the N-GAPDH cascade, and regulates animal adaptive behavior. Specifically, activation of the N-GAPDH cascade solely in cortical microglia and cognitive inflexibility were observed in a Lipopolysaccharide (LPS)-treated mouse model. Such cognitive deficits were blocked through pharmacological treatment or microglia-specific genetic intervention targeting the initial steps of the N-GAPDH cascade. To understand how the N-GAPDH cascade in microglia causes the cognitive deficits, we performed a ChIP-Seq analysis against N-GAPDH on microglia isolated from LPS-treated mice. We found that N-GAPDH exerts the transcriptional control and upregulation of High-mobility group-box (HMGB) proteins that can be secreted from microglia and bind to N-Methyl-d-aspartate (NMDA) receptors on glutamatergic neurons, causing their hyperactivation which would turn to cognitive deficits.
We also searched for possible molecular based biomarkers to predict behavioral inflexibility in the clinic. Initially, we found that the N-GAPDH cascade was concomitantly activated in blood cells and microglia in the LPS-treated mouse model. Furthermore, the levels of autofluorescence in blood reflected the extent of activation of the N-GAPDH cascade. Interestingly, using a cohort of patients with schizophrenia and healthy controls we found that the levels of blood cell autofluorescence negatively correlates with behavioral flexibility.
In summary, in this study we define a new mechanism of microglia-neuron interaction through which stress-activated immune cells regulate neural system via a key metabolic molecule, GAPDH. Additionally, we propose a promising molecular based biomarker for behavioral inflexibility based on our preclinical findings.

CLEAVAGE BY BACE1 IS UPREGULATED IN MURINE PRIMARY NEURONS OF A RARE NEURODEGENERATIVE DISORDER NIEMANN-PICK TYPE C

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The β-site amyloid precursor protein cleavage enzyme 1 (BACE1), has been considered as a therapeutic target for the treatment of Alzheimer's disease (AD) as it initiates the proteolytic cleavage of the β-amyloid precursor protein (APP) to generate toxic amyloid-β peptides that accumulate in AD patients' brains. We have recently shown that BACE1-mediated proteolysis is enhanced in a rare, inherited lysosomal storage disorder Niemann-Pick type C (NPC) that shares several pathological features with AD. The analysis of NPC1-mouse brains revealed that enhanced cleavage by BACE1 in NPC involves APP and also additional, recently identified BACE1 substrates, seizure protein 6 (Sez6) and seizure 6-like protein (Sez6L), which are primarily cleaved by BACE1. The goal of this work was to characterize BACE1-mediated proteolysis in murine primary neurons of NPC1- vs. wt-mice and to test whether inhibition of BACE1 may have any beneficial effect(s) on the pathological features of NPC, including enlargement of early endosomes, tau hyperphosphorylation and cholesterol accumulation. Female and male NPC1$^{+/-}$ mice (BALB/cNctr-*Npc1*$^{N/+}$, the Jackson Laboratory, USA) were mated and the primary neuronal cultures were generated from the isolated brains of P0 pups. NPC1-genotyping was performed in parallel. We showed that BACE1-mediated proteolysis of Sez6 and Sez6L is enhanced in NPC1- vs. wt-neurons and that BACE1 inhibition blocked formation of the BACE1-generated sSez6/sSez6L fragments. Furthermore, immunocytochemistry analysis revealed more punctuate staining of Sez6 and Sez6L in NPC1- vs. wt-neurons, suggesting their accumulation within endosomal vesicles. BACE1-inhibition reversed the punctuate staining of Sez6/Sez6L in NPC1-neurons to that as in wt-neuronal cultures. These findings confirm that NPC1-neurons show enhanced proteolysis by BACE1, indicating that BACE1 may be involved in the pathogenesis of NPC disease and that inhibiting BACE1may ameliorate pathological feature(s) of NPC.

MINOCYCLINE AND INDOMETHACIN COTREATMENT REDUCES M1 MICROGLIA AND DYSKINESIAS IN MICE

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Dopamine replacement therapy with L-Dopa has been the frontline treatment for combating the motor symptoms for patients with Parkinson's disease (PD). However, up to 75% of patients develop debilitating L-Dopa induced dyskinesia (LID), abnormalities or impairments of voluntary movements. To date, the only FDA approved therapy to combat LID in PD patients is amantadine. Mechanistically, amantadine has long been thought to exert its beneficial effects through its weak NMDA receptor antagonism, while more recent research has identified additional anti-inflammatory properties. The latter are particularly interesting in the context of the growing body of literature implicating neuroinflammation, a well-known hallmark of PD, to the mechanistic development of LIDs. Accordingly, exploring the therapeutic potential of anti-inflammatories shapes as a promising option in pre-clinical and clinical drug development for LID. Here we show for the first time that anti-inflammatory treatment with minocycline and indomethacin can reduce LID in a mouse model of PD. Accordingly our study had 3 major findings. First, minocycline and indomethacin treatment reduced AIMs severity in mice with established LID after repeated treatment. Second, assessing the neuroinflammatory profile we found that minocycline and indomethacin had no effect of striatal microglia proliferation but reduced specifically M1 microglia activation. Lastly, these anti-inflammatory and anti-dyskinetic capabilities did not arise from any neuroprotective effects, as the 6-OHDA lesion severity was consistent in all animals that entered the study. Overall these results suggest that minocycline and indomethacin is a potent treatment to reduce dyskinesia and striatal microglia activation in a mouse model of PD.

NON-LUMENIZED LYMPHATIC ENDOTHELIAL CELLS IN THE VERTEBRATE LEPTOMENINGES INTERNALIZE AMYLOID BETA

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The vertebrate CNS is surrounded by the meninges, a protective barrier comprised of the outer dura mater and the inner leptomeninges, which includes the arachnoid and pial layers. Recently, we described in both mouse and human meninges a non-lumenized cell type we call Leptomeningeal Lymphatic Endothelial Cells (LLECs) based on their shared morphological, gene expression, and functional characteristics with zebrafish Brain/Mural Lymphatic Endothelial Cells or Fluorescent Granule Perithelial cells (muLECs/BLECs/FGPs). Both zebrafish BLECs and mouse LLECs internalize macromolecules from the cerebrospinal fluid, including a selective uptake of Amyloid-β, the toxic driver of Alzheimer's disease progression. More recently, we have found that zebrafish BLECs respond to changes in nearby neuronal activity. These novel cells are well positioned to participate in clearance of toxic molecules from the cerebrospinal fluid and are therefore a potential therapeutic target to combat neurodegenerative diseases.

LMNA-MEDIATED NUCLEOSKELETON DYSREGULATION IN ALZHEIMER DISEASE

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Nucleoskeleton dysfunction has been implicated in Alzheimer's disease (AD). Tubular invaginations of the nuclear envelope observed in AD brains are consistent with the accumulation of farnesylated prelamin A (encoded by the LMNA gene) that occurs in Hutchinson-Gilford Progeria Syndrome, a premature aging disorder caused by LMNA mutations. Proper function of the nuclear membrane is required for neuronal survival and for maintenance of genetic architecture. To determine whether dysregulated LMNA expression and prelamin A processing are responsible for nucleoskeleton dysfunction in AD, we performed differential gene expression and network analyses in human AD and age-matched control brains. In AD brains, we observed a significant increase in LMNA and a significant decrease in ZMPSTE24, which encodes the zinc metallopeptidase protein that defarnesylates prelamin A. We replicated these findings in laser capture microdissected neurons from AD brains, suggesting that the effect is neuronally driven. Thus, high levels of LMNA paired with low levels of ZMPSTE24 could result in the accumulation of farnesylated prelamin A and tubular invaginations in the nuclear membrane. LMNA-associated networks were also differentially expressed in AD brains. Genes within the dysregulated LMNA network were enriched in lysosomal and chromatin remodeling pathways. We propose that β-amyloid and tau accumulation disrupts prelamin A processing and downstream changes in the nuclear membrane. Alterations in the nucleoskeleton induce genomic instability, loss of proteostasis, and cellular senescence, which may accelerate AD pathogenesis.

HEREDITARY DIFFUSE LEUKOENCEPHALOPATHY WITH SPHEROIDS CAUSED BY R777W AND R782C MUTATIONS IN CSF1R: A REPORT OF FOUR CASES IN SWEDEN

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Background: Hereditary diffuse leukoencephalopathy with spheroids (HDLS) is a rare and devastating genetic disease that is caused by heterozygous mutations in the *CSF1R gene*. It is characterized by rapidly progressive neurodegeneration and variable behavioral, cognitive and motor disturbances as well as seizures.

Objective: To describe four cases of HDLS from three families with two different pathogenic mutations in the tyrosine kinase domain of *CSF1R* and to present clinical features with a variety of scoring scales in order to capture reproducibly inter-individual diversity of clinical presentations.

Methods: Each patient was evaluated with 8 standardized clinical rating scales, functional estimation scores (FES), to capture the diverging clinical phenotypes. Patients underwent lumbar puncture (LP) including cerebrospinal (CSF) analysis of biomarkers such as neurofilament light (NFL), Glial fibrillary protein (GFAP), tau, β-amyloid, and phospho-tau. Computed tomography (CT) and magnetic resonance imaging (MRI) were performed according to clinical routine and were systematically re-evaluated by an experienced radiologist. A genetic sequence analysis was performed as well as a functional phosphorylation assay in order to confirm the pathogenicity of the mutations found.

Results: Two mutations were identified, a missense variant c.2344C>T, p. (Arg782Cys), and a missense variant c.2329C>T, p. (Arg777Trp). A functional assay showed markedly reduced autophosphorylation in cells expressing the *CSF1R* mutations p.R777W and p. R782C, confirming the pathogenicity of these mutations. A radiological investigation revealed typical changes for HDLS in all of the cases. CSF analysis showed significantly elevated tau in Patient 1, while β-amyloid and phosphorylated tau were normal in all cases. The tau/phospho-tau ratio in Patient 1 was roughly 28 ng/L, which suggests relatively fast progression. GFAP in Patient 1 was markedly elevated. CSF NFL levels were significantly elevated, especially in Patient 1. FES (Table 1) illustrate the wide variability of the clinical picture in different time points along the disease course.

Conclusions: This case-series highlights the variability of *CSF1R*-related leukoencephalopathies, semi-quantitatively along the axes of frontal, motor neuron, and extrapyramidal disease. The present cases may serve as a nucleus of a future Swedish registry of this and similar disorders. Our ambition is to be able to increase awareness for genetic leukoencephalopathies among neurologists and psychiatrists who may encounter similar cases in their practices and encourage for genetic testing.

INTERCELLULAR PROTEIN TRANSMISSION FOR ATTENUATION OF AGGREGATION OF MUTANT HUNTINGTIN

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In Huntington's disease (HD), disease progression is associated with increased aggregation of mutant huntingtin with an expanded polyglutamine tract, leading to cell death. We have shown earlier that the helical peptide arising out of proteolytic cleavage of N-terminal fragment is able to inhibit the aggregation of proteins in vitro and in yeast cells (ACS Chem Neurosci doi:10.1021/cn400171d; FEBS J doi:10.1111/febs.14457), in a manner similar to 'holdases'. It is able to stabilize the client protein in an unfolded state till cellular chaperones complete the folding process.
A common feature of protein misfolding diseases is the transmission of protein aggregates to neighbouring cells, 'spreading' the diseased aggregates. Hence, in this work, we have investigated the effect of co-culturing cells expressing N-terminal mutant huntingtin with those expressing this helical peptide on aggregation of the former protein. The aim was to study whether the route of intercellular transmission could be used to introduce aggregation inhibitory proteins/peptides and slow down protein aggregation. Using the well-validated yeast model of HD, we show that co-incubation with cells overexpressing a chaperone-like protein, i.e. the helical peptide arising out of the N-terminal wild-type huntingtin, with cells expressing N-terminal mutant huntingtin which forms aggregates, reduces aggregation by 32%. Co-localization of the two proteins and FACS analysis suggests that intercellular transmission had occurred. This is accompanied by increased survival of the mixed cell population (0.213 generations/h) as compared to the population where cells expressing N-terminal wild-type huntingtin are absent (0.167 generations/h). Blocking dynamin-dependent endocytosis pathway has no effect on the intercellular transmission process, indicating that an unconventional pathway is at work. Our results imply that transplantation with tissue overexpressing chaperone-like protein(s) in transmissible protein misfolding diseases may be an approach worth exploring.

IDENTIFICATION AND CHARACTERIZATION OF MUTATIONS INFLUENCING AGGREGATION OF HUMAN Aβ PEPTIDE IN THE YEAST MODEL

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Alzheimer's disease (AD) is the most common form of dementia, that is linked to neuron degeneration and death. Heritable AD is caused by mutations, influencing production and/or oligomerization and amyloid aggregation of the Aβ peptide, while sporadic AD is associated with spontaneous oligomerization and amyloid formation by Aβ, and usually affects aged people. Understanding of the role of specific amino acid residues in oligomerization and amyloid formation by Aβ is therefore crucial for deciphering mechanisms, leading to the development of AD. We have employed the yeast-based assay for the large-scale screening for Aβ mutations influencing amyloid nucleation. This assay is based on phenotypic detection of amyloid aggregation, nucleated by the attachment of Aβ to prion domain of the yeast protein Sup35 in yeast (Chandramowlishwaran et al. 2018 J. Biol. Chem. 293:3436). Several dozen Aβ derivatives with single or multiple mutations, altering amyloid nucleation were uncovered by this screen. Some mutations occurred at sites previously linked to either heritable AD or amyloid formation *in vitro*, while other mutations marked new sites with previously unknown roles. Effects of most interesting mutations on biochemical and cytological parameters of Aβ aggregates, as well on the Aβ amyloid structure derived from experimental studies and/or predicted by computational algorithms have been investigated. Results of these experiments shed light on modes and pathways of Aβ oligomerization and aggregation.

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CHARACTERIZATION OF APOE PHENOTYPES IN HIPSC DERIVED-ASTROCYTES

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The apolipoprotein E4 (ApoE4) isoform is a genetic risk factor for many neurodegenerative diseases including Alzheimer's disease, Chronic Traumatic Encephalopathy, and Dementia with Lewy Bodies. Recent efforts have highlighted a fundamental role for apolipoprotein E in CNS lipid homeostasis, however, it is unclear how best to resolve the cellular dysfunction mediated by the ApoE4 isoform. We strived to establish ApoE-dependent assays in CNS-relevant cell types to test hypotheses generated by the unbiased phenotypic screening of Yumanity's yeast platform. ApoE in the brain is predominantly expressed by astrocytes, thus we used isogenically matched patient iPSC (induced pluripotent stem cells) expressing APOE3/3 or APOE4/4 to evaluate mitochondrial and endosomal phenotypes in astrocytes. Specifically, we differentiated isogenic iPSCs first into neural progenitor cells and then towards the astrocyte lineage, and characterized both the APOE3/3 and APOE4/4 lines for maturity and similarity. We evaluated the oxygen consumption rates using the Agilent Seahorse MitoStress test after the initiation of ApoE expression in early differentiating astrocytes. The spare respiratory capacity for APOE4/4 was increased relative to the APOE3/3 astrocytes, and correlated with steady-state dysregulation of mitochondrial complex levels. In more mature astrocytes, we observed a reduced number of early and recycling endosomes for the APOE4/4 genotype. These ApoE-dependent phenotypes within astrocytes provides a starting point to interrogate hypotheses around resolving ApoE4 dysfunction.

TARGETING TAU AND DRP1 TO MITIGATE MITOCHONDRIAL FISSION IN AMYOTROPHIC LATERAL SCLEROSIS

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Understanding the underlying pathogenic mechanisms leading to motor neuron loss in amyotrophic lateral sclerosis (ALS) is crucial for the development of new therapies. Accumulating evidence suggests that mitochondrial dysfunction is an early pathogenetic event in neurodegenerative diseases, including ALS. Similarly, deficits in bioenergetics and mitochondrial function have been described in ALS patient samples as well as in animal and cellular models of the disease. Interestingly, studies in Alzheimer's disease (AD) post-mortem brain and animal models have linked alterations in mitochondrial function to interactions between hyperphosphorylated tau and dynamin-related protein 1 (DRP1), the GTPase involved in mitochondrial fission. Here, we sought to verify whether the accumulation of DRP1 may lead to mitochondrial fragmentation and dysfunction in ALS and whether tau may contribute to those alterations. Our findings revealed a significant increase in both DRP1 and its active phosphorylated isoform at S616 (pDRP1-S616) in synaptoneurosomes (SNs) derived from ALS post-mortem motor cortex (mCTX). Importantly, the increase in DRP1 levels was independent of sex, region of onset, and genotype. Similar to AD, DRP1 interacts with tau phosphorylated at S396 (pTau-S396) in ALS mCTX, thus suggesting that tau hyperphosphorylation may contribute to mitochondrial dysfunction in ALS. Furthermore, the treatment of human neuroblastoma SH-SY5Y cells with ALS SNs, enriched in both pTau-S396 and DRP1, significantly increased mitochondrial fission, reducing mitochondrial length and volume and resulted in altered mitochondrial networks. Importantly, knocking down DRP1 using siRNA as well as reducing pTau-S396 levels with the specific tau degrader, QC-01-175, significantly mitigated alterations in mitochondrial length, volume, and networks induced by ALS SNs treatment. Lastly, we identified specific variants in DNM1L in ALS cases assessed through ALS Knowledge Portal (ALSKP) and Project MinE data browser. Collectively, our results suggest that increases in DRP1 and pTau may cause mitochondrial fragmentation in ALS, thus leading to an unfavorable energetic state and contributing to motor neuron loss. Importantly, targeting this molecular pathway may provide a novel therapeutic strategy to reverse bioenergetic deficits in ALS.

ALPHA-LIPOIC ACID REGULATES ER UNFOLDED PROTEIN RESPONSE VIA DOWNREGULATION OF PERK-ATF4 AND ACTIVATION OF IRE1-XBP1 PATHWAYS IN THE DROSOPHILA MODEL OF TAUOPATHY

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In human tauopathies, pathological aggregation of misfolded proteins particularly tau (MAPT, tau) is considered to be essential mechanisms that trigger the induction of endoplasmic reticulum (ER) stress. Here we assessed the molecular effects of natural antioxidant alpha-lipoic acid (ALA) in human tauR406W (htau)-induced ER unfolded protein response (ERUPR). In order to reduce htau neurotoxicity during brain development, we used a transgenic model of tauopathy where the maximum toxicity was observed in adult flies. Then, the effects of ALA (0.001, 0.005, and 0.025% w/w of diet) in htau-induced ERUPR and motor dysfunctions in the ages 20 were evaluated in Drosophila. Expression of ERUPR-related proteins involving activating transcription factor 6 (ATF6), inositol regulating enzyme 1 (IRE1), and protein kinase RNA-like ER kinase (PERK) were upregulated and locomotor function decreased in the model flies. Remarkably, the lower dose of ALA modified ERUPR and supported the reduction of behavioral deficits in model flies through enhancement of GRP87/Bip, reduction of ATF6, downregulation of PERK-ATF4 pathway, and activation of the IRE1-XBP1 pathway. Moreover, ALA significantly improved the locomotor function of model flies, in a dose-dependent manner. Taken together, based on our results we conclude that ALA has and neuroprotective effects on tau-induced ERUPR. Further molecular studies will warrant possible therapeutic applications of ALA in tauopathies.

MICROTUBULE STABILIZATION PROTECTS COGNITIVE FUNCTION AND SLOWS DOWN THE COURSE OF ALZHEIMER`S-LIKE PATHOLOGY IN AN AMYLOIDOGENIC MOUSE MODEL

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Cognitive decline in Alzheimer's disease (AD) is highly related to synaptic dysfunction and neuronal loss. In AD and other tauopathies, the hyperphosphorylation of tau compromises axonal transport and leads to the accumulation of autophagic/vesicular material and the generation of dystrophic neurites, contributing to synaptic impairment. In addition to phospho-tau, AD brains accumulate amyloid-beta (Aβ). The effect of microtubule stabilization has been successfully assessed on tau, but not on Aβ pathology. This study evaluated the effect of the brain-penetrant microtubule-stabilizing agent, Epothilone D (EpoD) in the progression of the disease in a double transgenic mouse model of amyloidosis.
Young APP751SL/PS1M146L mice (3-month-old) were weekly treated with intraperitoneal injections of EpoD (2 mg/kg) or vehicle solution for 3 months. Memory performance was tested using object-recognition tasks, Y-maze and Morris water maze. Levels of Aβ, APP-fragments, AT8 (phospho-tau), ubiquitin, and synaptic markers were analysed by Western/dot-blot, immunostaining and image analysis. Somatostin (SOM)-cell density was calculated by stereology. β- and γ-secretase activities were measured. APPswe-N2a cells were treated with EpoD 100 nM for 12/24 hours.
EpoD-treated mice improved their performance of cognitive tests, while hippocampal phospho-tau and Aβ levels, especially soluble oligomers, decreased significantly. β/γ-secretase activities were not affected by EpoD i*n vitro*. A significant amelioration of synaptic/neuritic pathology was found. Remarkably, EpoD exerted a neuroprotective effect on SOM-interneurons, a highly AD-vulnerable GABAergic subpopulation.
In conclusion, EpoD improved microtubule dynamics and axonal transport in an AD-like context, reducing tau and Aβ accumulation, and promoting neuronal and cognitive protection. These results underline the crosstalk between cytoskeleton pathology and proteinopathy. Therefore, microtubule-stabilizing drugs could be candidates for slowing AD progression at both tau and Aβ pathologies.
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APOE ALLELIC STATUS AFFECTS BOTH GLYCOLYTIC RATE AND MITOCHONDRIAL GLUCOSE OXIDATION IN N2A CELLS

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Background. ApoE4 inheritance is the single largest genetic attributable risk factor for Alzheimer's disease (1). ApoE4 inheritance causes defects in cerebral glucose oxidation, decades before cognitive deficits (2), the pathomechanism of which has not been determined.**Hypothesis.** Our hypothesis is that ApoE4 affects neural mitochondrial glucose metabolism and that this contributes to AD pathomechanism. **Methods.** We constructed N2a cells with stably transfected ApoEzero, (vector-GFP), ApoE2 (vector-GFP-ApoE2), ApoE3 (vector-GFP-ApoE3), and ApoE4 (vector-GFP-ApoE4). We tested the consequences of the stably-transfected alleles on mitochondrial glucose oxidation, and glycolytic rate as measured by ECAR (Extracellular Acidification Rate) in high 22mM glucose concentration in the Seahorse Xfe96. Basal, oligomycin-dependent, FCCP-dependent, and antimycin+rotenone dependent respiration measured at a sampling size of 20 well-repeats per ApoE allelic group.**Results. 1**. ApoE2, E3 and E4 each contribute a higher glycolytic rate than 'ApoE-zero' i.e GFP. As the 4 N2a cell lines were compared, there was a clear and obvious increase in Glyolytic rate (ECAR) in ApoE2, E3 and E4-transfected cells vs the empty vector GFP-only control. This increase was statistically significant after oligomycin-exposure, FCCP-exposure, and antimycin+rotenone exposure. **2. Apo E3** confers greater ability of mitochondria to oxidize glucose than E4, and E-zero. Bearing ApoE3 conferred a statistically significant rise in mitochondrial glucose oxidation vs. ApoE4 and ApoE-zero at basal (state 2) and FCCP stimulated rate (state 3). Also, ApoE4 was not statistically significantly different from ApoE-zero. Thus, while ApoE3 confers a significant benefit of mitochondrial glucose oxidation over other alleles and ApoE-zero, ApoE4 confers no significant mitochondrial glucose oxidation benefit over the lack of ApoE protein entirely.**Interpretation and Summary. Point 1**. We find that all 3 ApoE's increase the rate of glycolysis relative to the absence of ApoE, and this could potentially relate to a glucose transport function that has been attributed to ApoE alleles in astrocytes (3). Point 2. We find that while ApoE3 allele confers a significant benefit to mitochondrial glucose oxidation, ApoE4 does not and is not significantly different from the empty vector control ApoE-zero. One hypotheses that explain ApoE3's mechanism to increase mitochondrial glucose oxidation are 'pre-mitochondrial', i.e. that the presence of ApoE3 increases glucose uptake, intracellular transport or glycolysis as we observe in Result 1, and this hypothesis will be tested by relative glucose uptake and glycolytic enzyme expression. Other hypotheses that explain ApoE3's mechanism to increase mitochondrial glucose oxidation are 'mitochondrial', i.e. that the presence of ApoE3 post-glycolytic allocation of pyruvate to mitochondria or other regulatory sites, which will be investigated by PDK4 regulation in different ApoE allelic contexts.**Acknowledgement**. We acknowledge NIH/NIA support: P01AG062817

H3K27ME3 PROFILE AND DIFFERENTIALLY ENRICHED GENES IN ALZHEIMER'S DISEASE

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The Late-Onset Alzheimer's disease (LOAD) accounts for more than 95% of AD cases and is strongly influenced by environmental factors. Epigenetic mechanisms, such as histone modifications, have been suggested to play a significant role in LOAD. Epigenetic dysregulation can imbalance several pathways, affecting gene expression and chromatin remodeling, increasing the risk of Alzheimer's disease (AD). Trimethylation of histone H3 at lysine 27 (H3K27me3) is associated with transcriptional repression, acting mainly at promoter regions, and it is important for the proper function of cell cycle, differentiation and DNA damage repair. In order to evaluate the role of histone modifications in AD, we aimed to identify H3K27me3 pattern and genomic regions regulated by this epigenetic mark in three different brain regions – hippocampus, auditory cortex and cerebellum of AD patients compared to elderly controls. We performed ChIP-Seq in 6 samples from each brain region of both groups. Libraries were sequenced on Illumina HiSeq 2500. Reads were aligned to reference genome (GRCh38/hg38) using Bowtie2. Peak calling and differential analysis were undertaken by HOMER. Functional enrichment was performed considering Gene Ontology (GO) terms, using clusterProfiler package for R. We found 21, 799 and 4980 hypertrimethylated H3K27 peaks in hippocampus, auditory cortex and cerebellum, respectively. Auditory cortex and cerebellum presented 10 overlapped peaks. In contrast, hippocampus, auditory cortex and cerebellum showed 11, 24 and 2472 hypotrimethylated H3K27 peaks, respectively. Regarding the genomic location, approximately 25% of H3K27me3 peaks in auditory cortex and cerebellum and 12% in hippocampus are distributed at the promoter region of the nearest genes annotated. Differentially H3K27me3 included genes involved in vesicular trafficking and signal transduction, such as *EXOC7* and *PDE4B* in the hippocampus, whose imbalance could affect synaptic and cognitive functions. GO terms comprised synapse organization, neuron differentiation, axon development, ion transport and Wnt signaling pathway. These findings may contribute to the understanding of AD and provide support for future research and discovering targets for the treatment of AD.

CHOLINERGIC PRETREATMENT ABOLISHES HYDROMETHYLTHIONINE FACILITATION OF BRAIN ENERGY METABOLISM: LESSONS FROM A MOUSE MODEL OF TAUOPATHY

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Background: Currently available Alzheimer's disease (AD) treatments, such as the cholinesterase inhibitors and memantine provide limited symptomatic efficacy. The tau protein aggregation inhibitor hydromethylthionine (LMTM) was shown recently to have concentration-dependent pharmacological activity in delaying cognitive decline and brain atrophy in phase 3 clinical trials. However, for patients already receiving symptomatic therapies, the activity of LMTM was reduced by half. The methylthionine (MT) moiety has been reported previously to increase the clearance of pathological tau and to enhance mitochondrial activity, which is impaired in AD patients.

Aim: To explore mitochondrial function and substrate utilization for energy production as targets of drug interference, using a mouse model of tauopathy dosed with LMTM alone or LMTM added to chronic pretreatment with rivastigmine, a cholinesterase inhibitor.

Method: 5.5-month old, Line 1 (L1) overexpressing the core aggregation domain of tau, and control NMRI mice, were gavaged daily with rivastigmine (0.1/0.5 mg/kg) or vehicle for 5 weeks, followed by further 6 weeks of daily treatment during which LMTM (5/15 mg/kg) or vehicle was added. At the end of treatment, mice were tested in the problem-solving water maze task. Mice were anesthetized, perfused with saline solution, and the tissue was harvested, snap-frozen in liquid nitrogen and kept at -80° C for further analysis. Protein levels of subunits of the mitochondrial electron transport chain (ETC), activity of complexes I and IV (CI and CIV), levels of L-lactate and lactate dehydrogenase (LDH) subunits were measured. Brain concentration of the compounds was also measured.

Results: LMTM monotherapy reversed deficits in spatial learning in L1 mice. Chronic pretreatment with rivastigmine prevented LMTM-induced improvement of cognition. Similarly, in mitochondrial function, LMTM enhanced CI and CIV activities, but when administered after chronic pretreatment with rivastigmine, the LMTM dose-response increase of CI and CIV activity was abolished. From the analysis of L-lactate-related indicators, LMTM triggered a specific dose-dependent increase in the levels LDH-A that was blocked by prior rivastigmine. None of the treatments changed the levels of LDH-B. L-lactate levels were significantly increased in L1 mice compared to NMRI, and this was significantly decreased when both drugs were administered, but not by LMTM given alone.
Conclusion: LMTM monotherapy facilitated the use of substrates for energy production, in particular L-lactate, which is provided by astrocytes from the breakdown of local storages of glycogen, in different energy demanding situations. These may underpin several neuronal events related to memory formation. Chronic pre-treatment with rivastigmine abolished most of the LMTM-associated effects.

PHARMACOLOGICAL ANTAGONISM OF KAINATE RECEPTOR RESCUES DYSFUNCTION AND LOSS OF DOPAMINE NEURONS IN A MOUSE MODEL OF HUMAN PARKIN-INDUCED TOXICITY

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Loss of function mutations in the PARK2 gene encoding the protein parkin cause Autosomal Recessive Juvenile Parkinsonism (ARJP), a neurodegenerative disease characterized by early dysfunction and death of dopamine (DA) neurons in the substantia nigra pars compacta (SNc). ARJP is characterized by juvenile onset and slow progression. No neuroprotective therapy has been developed yet.
A previous study demonstrated that loss of parkin function or the expression of parkin mutants associated with ARJP lead to an accumulation of glutamate kainate receptors (KARs) and to an increase in postsynaptic kainate receptor-mediated currents in neurons. Because KARs contribute to excitatory synaptic transmission regulating neuron excitability, we hypothesized that such kainate receptor hyper-activation and accumulation at the post-synapse of ARJP DA neurons dysregulates neuronal excitability, thus leading to DA neuron death. Based on this hypothesis, we tested whether the pharmacological antagonism of KAR exerts a neuroprotective effect in the mouse model of human parkin toxicity parkinQ311X.
Mutant parkin expression in the parkinQ311X mouse model caused early SNc DA neurons dysfunction, accumulation of KARs and early dopaminergic neuron loss. The chronic administration of the KAR antagonist UBP310 prevented DA neuron loss. This neuroprotective effect was associated with rescue of the abnormal firing rate of SNc DA neurons and downregulation of the key KAR subunit GluK2.
The results of this study support KAR as a potential target in the neuroprotective therapy of ARJP.

PROBING THE FUNCTION OF ALZHEIMER'S DISEASE-ASSOCIATED GENES IN HUMAN iPSC-DERIVED MICROGLIA-LIKE CELLS BY COUPLING CRISPRi AND CRISPRa TO SINGLE-CELL RNA SEQUENCING

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Microglia have been implicated in a number of neurodegenerative diseases, in particular Alzheimer's disease (AD). However, we lack a systematic understanding of the mechanisms by which microglia contribute to the disease. An unbiased, systematic approach to uncover cellular mechanisms are functional genomics screens in cell based models. Human induced pluripotent stem cell (iPSC)-derived microglia provide a cellular model to dissect the role of microglia in health and disease.
Here, we introduce a scalable iPSC-derived microglia screening platform using the induction of transcription factors to differentiate iPSCs into microglia-like cells within eight days. After differentiation, these cells express key microglial markers, phagocytose various substrates, and exhibit a robust response to pro-inflammatory stimuli. Additionally, equipped with inducible CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) machinery, the platform achieves high CRISPRi/a activity, which enables large-scale genetic screens.
Utilizing our CRISPRi/a iPSC-derived microglia platform, we probed microglia transcriptomes using pooled CRISPR screening coupled to single-cell RNA sequencing (CROP-seq). We evaluated the consequences of modifying expression levels of over 50 genes in AD risk loci. Knockdown of a subset of AD risk genes lead to transcriptomic changes in proliferation and immune response, as well as functional changes in phagocytosis. Our CRISPRi/a-enabled iPSC-derived microglia-like cells open the door to systematically identifying the functional role of microglia in neurodegeneration and other diseases.

THE BRAIN AS THE LATENT HIV RESERVOIR: A PERSPECTIVE FROM THE INNATE IMMUNE SYSTEM

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BACKGROUND Strategies towards a cure of HIV require the elimination of its cellular reservoirs but this approach has been hampered due to a limited understanding of HIV persistence in distinct anatomical sites such as the central nervous system. The brain is an immunologically and pharmacologically privileged site and whether it is an anatomical site of HIV persistence during antiretroviral therapy is still unanswered. Brain myeloid cells such as perivascular macrophages and microglia have the potential to harbor HIV. However, the distribution and molecular characteristics regarding their gene expression profile and epigenetic landscape remain unclear.
The HIV reservoir and the myeloid cell populations in the brain are heterogeneous and a comprehensive analysis of the HIV reservoir in this compartment is currently lacking. To address this obstacle, we leveraged the potential of single cell ATAC-seq and RNA-seq to detect HIV DNA and RNA in myeloid cells and their respective epigenetic landscape and gene expression profile.
METHODS We isolated human myeloid cells by mechanical dissociation from fresh postmortem tissue from three virally suppressed persons with HIV (PWH) from the Last Gift Cohort. The Last Gift cohort enrolls altruistic, terminally ill PWH, who are closely followed until the time of death and donate their “whole-body” for HIV research. Blood and tissues are collected by rapid-autopsy and dissociated within 6h from death. Next, we performed single cell ATAC-seq and single cell RNA-seq using the 10x Genomics platform.
RESULTS Using single cell ATAC-seq, we detected HIV DNA in approximately 0.01 % of isolated myeloid cells suggesting that the number of HIV DNA containing cells is low under suppressive ART. HIV DNA mapped predominantly to the HIV env gene region. The epigenetic profile of the HIV DNA containing cells suggests that they are perivascular macrophages and microglia. Using single cell RNA-seq, we detected HIV RNA in a distinct subset of microglia.
CONCLUSIONS We were able to detect HIV DNA and HIV RNA in brain myeloid cells in virally suppressed individuals. Our findings show that perivascular macrophages are an important harbor for HIV in the brain and future “reservoir targeting” antiviral strategies need to take this cell type into account as these cells are located at the interface between systemic circulation and brain parenchyma.

TDP-43 REAL TIME QUAKING INDUCED CONVERSION REACTION OPTIMIZATION AND DETECTION OF SEEDING ACTIVITY IN CSF OF AMYOTROPHIC LATERAL SCLEROSIS AND FRONTOTEMPORAL DEMENTIA PATIENTS

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TDP-43 pathological deposition occurs in the majority (97%) of amyotrophic lateral sclerosis (ALS) and in around 45% of frontotemporal lobar degeneration (FTLD) cases. ALS and FTLD clinically overlap, presenting a continuum of phenotypes. Both ALS and FTLD lack treatments able to interfere with the underlying pathological process and early detection of TDP-43 pathology would facilitate the development of disease modifying drugs. The Real Time Quaking Induced Conversion reaction (RT-QuIC) showed the ability to detect prions in several peripheral tissues of patients with different forms of prion and prion-like diseases. Despite TDP-43 displays prion-like properties, to date the RT-QuIC technology has not yet been adapted to this protein. The aim of this study was to adapt the RT-QuIC technique for the TDP-43 substrate and to exploit the intrinsic ability of this technology to amplify minutes amount of misfolded proteins for the detection of pathological TDP-43 species in the CSF of ALS and FTLD patients. We first optimized the technique with synthetic TDP-43 preformed aggregates and with autopsy-verified brain homogenate samples and subsequently analyzed CSF samples from ALS and FTLD patients and controls. TDP-43 RT-QuIC was able to detect as little as 15 picograms of TDP-43 aggregates, discriminating between a cohort of subjects affected by ALS and FTLD and age-matched controls with a total sensitivity of 94% and a specificity of 85%. Our data give a proof-of-concept that TDP-43 is a suitable substrate for the RT-QuIC. TDP-43 RT-QuIC could be an innovative and useful tool for diagnosis and drug development in ALS and FTLD. CSF detection of TDP-43 pathological aggregates may be exploited as a disease biomarker for ALS and FTLD patients.

DNA DAMAGE AND DNA DAMAGE RESPONSE IN ALZHEIMER'S DISEASE.

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The pathogenesis of Alzheimer's disease (AD) is complex and involves several cellular pathways including redox balance, mitochondrial dysfunction and damage to macromolecules. Emerging evidences of the involvement of DNA damage in AD has recently been gathered in human and mouse model studies. Markers of activated DNA damage response (DDR) have been observed in the brains of AD patients and in AD mouse models. These observations support a role of DDR in the homeostasis of neurons, even though the molecular mechanisms of DNA damage generation and the causal role in the pathogenesis of DDR remain unclear. The administration of Abeta oligomers, the major component of amyloid plaques – a hallmark of AD-, to immortalized neuronal cells and mouse primary neurons, has been reported to be sufficient to induce DNA damage and DDR activation. We are now investigating DDR activation upon administration of Abeta oligomers in great depth in immortalized and primary neuronal cells. Thus, we analyze the molecular players of the DDR cascade such as gammaH2AX and mediators of DNA damage such as 53BP1, ATM and DDRNA/lncRNA which are novel RNA species discovered by our lab and involved in the activation of DDR. To accomplish a full characterization of DDR upon Abeta oligomers we study the potential cause of DNA generation induced by Abeta oligomers testing: the physical interaction of Abeta oligomers and DNA damage sites; the role of oxidative stress and the modulation of enzyme involved in DNA metabolism, such as topoisomerases that seem to be key players in neuronal homeostasis. We show here our results strongly supporting a link between Abeta oligomers and the activation of DDR markers.

DEVELOPMENT OF A LYST-DEFICIENT GLUTAMATERGIC NEURONAL MODEL OF CHEDIAK-HIGASHI SYNDROME

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Neurons are highly polarized cells due to the microtubule orientation in their axons that serves as tracks for the intracellular transport of organelles and macromolecular complexes. The lysosomal distribution, mobility, size and number are tightly regulated to maintain cellular homeostasis. Under pathological condition, such as Chediak-Higashi Syndrome (CHS), dysregulation of the lysosomal pathways occurs. CHS is a rare, lysosome-related organelle disorder associated with progressive neurological dysfunction, a bleeding diathesis, high susceptibility to infections and albinism. It is caused by bi-allelic mutations in the lysosomal trafficking regulator (*LYST*) gene that encodes a 429 kDa protein. To date, several cell and animal models of CHS have been investigated, but none of them consistently recapitulates the neurological phenotype seen in patients. Despite its name and presumed function, there is no clear evidence supporting the role of LYST in regulating lysosomal trafficking. In this study, we investigate the functions of LYST in lysosomal regulation using a neuronal cell model applying CRISPR/Cas-9 technology to knock-out *LYST* in induced pluripotent stem cell (iPSC). These cells have an inducible Neurogenin 2 expression cassette integrated into the AAVS1 safe-harbor locus, enabling the production of glutamatergic neurons. The perinuclear degradative lysosomes of LYST-deficient glutamatergic neurons are larger in size but fewer in number, indicating that LYST regulates lysosomal biogenesis. To further explore the dynamics of lysosomes in these neurons, we are using live cell imaging techniques to characterize the role of LYST in the lysosomal fusion/fission events and trafficking.
This model provides the first evidence that LYST-deficient glutamatergic neurons have lysosomal abnormalities and will help us to find the mechanism that explain the neurological phenotype seen in CHS patients.

EFFECTS OF AN ANTI-HYPERTENSIVE AGENT LISINOPRIL ON CARDIOVASCULAR AND COGNITIVE FUNCTION IN FEMALE DAHL SALT-SENSITIVE RATS

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Background: Dahl Salt-Sensitive (DSS) rats have increased blood pressure (BP) and aortic pulse wave velocity (PWV), a measure of central arterial stiffness (CAS), with age. These changes are accompanied by cognitive decline and brain structural changes, associated with CAS. Previously, ACE inhibitor lisinopril was effective in treating both cardiovascular and cognitive disorders in male DSS rats. The aim of this study was to determine whether these beneficial effects of lisinopril are applicable to female DSS rats.
Methods: Female DSS rats (n=23) were kept on a normal salt diet (0.5% NaCl) for the duration of the study. The measurements were performed at 6-mo of age (baseline, BL) and after 7-mo of lisinopril treatment through water (15mg/kg/day, n=12) or control treatment (n=11). Systolic BP (SBP) was measured using tail-cuff plethysmography, and PWV was measured by echocardiography. Open field test (OFT) was performed to assess anxiety-like behavior. At 13-mo of age, Morris water maze (MWM) was performed to assess hippocampal-dependent spatial memory. Statistical analyses were performed using 2-way ANOVA mixed effects model. Data are presented as mean ± SEM.
Results: At 6-mo of age, before treatment initiation, there was no difference between treated and control groups' SBP (150 ± 5.3 vs. 153 ± 6.0 mmHg) or PWV (4.6 ± 0.2 vs. 4.4 ± 0.4 m/s). After 7-mo of treatment, lisinopril treated animals had significantly lower SBP vs. their BL ($p<0.01$) and vs. control animals (116 ± 3.7 vs. 187 ± 9.5 mmHg; $p<0.01$). At 13-mo of age, PWV in the control group was higher vs. BL and vs. lisinopril treated animals (5.7 ± 1.0 vs. 4.4 ± 0.3 m/s; $p<0.05$). Treated rats demonstrated lower anxiety levels, i.e. increased relative activity in the center of the OFT (+25% vs. BL), compared to control rats (-32% vs. BL). On MWM trials in which the platform was hidden, treated animals had a cumulative distance to the platform of 6.5 ± 0.7 m, while the control group averaged 8.8 ± 0.9m ($p=0.039$). These results indicate better hippocampal memory in the treated group.
Conclusion: Increased SBP and CAS in aged female DSS rats were associated with spatial memory decline. Treatment with lisinopril reduced SBP and stabilized PWV in female DSS rats and was associated with better behavioral test performance, similar to the male DSS rats. CAS can affect brain function via its impact on structure and function of cerebral vasculature. Supported by the NIA/NIH Intramural Research Program.

A HIGHLY SELECTIVE MNK INHIBITOR RESCUES DEFICITS ASSOCIATED WITH FRAGILE X SYNDROME IN MICE.

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Fragile X syndrome (FXS) is the most common inherited source of intellectual disability in humans. This neurodevelopmental disorder is caused by mutations that trigger epigenetic silencing of the Fmr1 gene which encodes for the Fragile X Mental Retardation Protein (FMRP). Loss of FMRP results in increased activity of the Mitogen-Activated Protein Kinase (MAPK) pathway. An important downstream consequence is the activation of the Mitogen-Activated Protein Kinase Interacting Protein Kinase (MNK). MNK phosphorylates the mRNA cap-binding protein, eukaryotic Initiation Factor 4E (eIF4E) to initiate translation. A key consequence of increased eIF4E phosphorylation in FXS is aberrant protein translation. Excessive phosphorylation of eIF4E has been directly implicated in the cognitive and behavioral deficits associated with FXS. Pharmacological reduction of eIF4E phosphorylation is one potential strategy for FXS treatment. We demonstrate that systemic dosing of a highly specific, orally available MNK inhibitor, eFT508, attenuates numerous deficits associated with loss of Fmr1 in mice. eFT508 resolves a range of phenotypic abnormalities associated with FXS including aberrant dendritic spine formation, alterations in synaptic plasticity, and macroorchidism. Key behavioral deficits related to anxiety, social interaction, obsessive and repetitive activities, and impaired object recognition are ameliorated by eFT508. Collectively, this work establishes eFT508 as a potential means to reverse deficits associated with FXS.

HSPA8 KNOCK-DOWN INDUCES THE ACCUMULATION OF NEURODEGENERATIVE DISORDER-ASSOCIATED PROTEINS

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Heat shock protein 70 family was demonstrated to play a critical role in protein homeostasis, a process profoundly impaired in neurodegenerative disorders. Neurodegenerative diseases are characterized by the accumulation of different kind of proteins and the formation of insoluble aggregates which are toxic for neurons. To explore the role of heat shock protein family 70 (in particular HSPA8 and HSPA1A) in the accumulation of proteins implied in neurodegeneration pathogenesis, in this study we verified in human SH-SY5Y neuroblastoma cells how HSPA8 or HSPA1A knock-down can affect protein levels of tau, superoxide dismutase 1 and α-synuclein. We found HSPA8 and HSPA1A reduction caused an increase of tau, superoxide dismutase 1 and α-synuclein protein levels. We also noticed HSPA8 knock-down increased α-synuclein oligomeric forms and mRNA expression. Our results suggest HSPA8 can play an important role in the homeostasis of tau, superoxide dismutase 1 and α-synuclein and in the balance between α-synuclein oligomeric and monomeric forms.

TAU AS A NOVEL MODULATOR OF THE STABILITY OF THE ONCOSUPPRESSOR PROTEIN P53

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The hallmark of age-dependent neurodegenerative tauopathies is the neuronal accumulation of aberrantly modified forms of Tau, which drive neuronal dysfunction and cell death (toxic gain-of-function). Clinical evidence for the causative role of Tau in disease is the existence of inherited dominant mutations in the Tau-encoding MAPT gene leading to frontotemporal dementia with Parkinsonism (FTDP-17). On the other hand, the molecular mechanisms driving the switch from normal to aberrant Tau in the absence of MAPT mutations are still unknown. However, a risk factor for progressive neurodegeneration is aging, characterized by the slow accumulation of cellular insults such as e.g. DNA damage. In the healthy cell, a complex interplay of multiple cellular pathways - orchestrated by the "guardian of the genome" P53 - tightly regulates the management of a DNA damage. In contrast, aging-associated disorders such as cancer and neurodegeneration may share a pathomechanism of DNA damage accumulation and abnormal cell fate decision.
We examined this hypothesis in the context of tauopathies. For this, the response to an acute DNA damage was studied in neuroblastoma cells with depleted Tau, as a model of loss-of-function. Under these conditions, altered P53 stability and activity result in reduced cell death and increased cell senescence. This newly discovered function of Tau involves abnormal modification of P53 and its E3 ubiquitin ligase MDM2. Our current goal is to understand the molecular mechanism linking Tau to P53 stability and function. Considering the medical need with vast social implications caused by neurodegeneration and cancer, our study may reform the approach to disease-modifying therapies.

TARGETING NEUREGULIN-MEDIATED MICROGLIAL ACTIVATION IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by a gradual decline of memory and cognition that correlates with both neuronal and synaptic loss. Unfortunately, treatments that focus on ridding the brain of some of the pathologic hallmarks of AD, including amyloid plaques have not been clinically effective. Therefore, new approaches and mechanisms are desperately needed to stall the insidious and progressive neurodegeneration that underlies clinical symptoms of dementia. The gliotrophic factor neuregulin1 (NRG1) is a key neuronal communication signal critical for normal peripheral and central nervous system development. However, in the mature central nervous system NRG1 is reactivated in response to neuronal injury or degeneration producing an abnormal, pathogenic microglial activation. We have shown this in amyotrophic lateral sclerosis (ALS) patients, ALS animal models, and a nerve-injury mediated model of chronic pain. We have also shown that peripheral nerve injury produces a heightened inflammatory response in genetically predisposed ALS animals leading to significant synaptic loss of the spinal motor neurons. Blocking NRG1 signaling in both pain and ALS models prevents microglial activation, thereby preventing the development of chronic pain, and delaying disease onset and prolonging animal survival through reducing motor neuron loss in the ALS animal model.
Here, we show that NRG1 signaling from neurons to surrounding microglia promotes the local spread of AD pathology through microglial activation that leads to synaptic loss and neurodegeneration. In AD, microglia have mixed roles in disease progression by clearing Aβ and tau and releasing cytotoxic mediators. Aβ has been shown to activate microglia through innate immune receptors. We found that blocking endogenous NRG1 activity with NRG1 antagonist (HBD-S-H4) prevents microglial activation and Aβ plaque formation in early-stage and reduces microglial activation and Aβ plaque formation in progressive-stage disease. Consistently, intraventricular NRG1 augments microglial activation and Aβ plaque formation in early-stage 5XFAD mice in vivo and induces pro-inflammatory cytokine expression and promotes phagocytic activity in cultured microglia. HBD-S-H4 treatment works therapeutically by reducing NRG receptor activation on microglia and reduces the loss of dendritic spines. A small pilot study shows that human cerebrospinal fluid from AD patients has increased NRG1 activity compared to normal controls or patients with other neurodegenerative diseases. Our results suggest that blocking NRG1 signaling prevents and reduces AD pathology and supports the future use of our targeted therapeutic to slow disease progression in AD as it does in ALS.

EFFECTS OF BUSHEN YIZHI FORMULA IN ALZHEIMER'S DISEASE- RELATED APP/PS1 MICE

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Objective To investigate the effects of Bushen Yizhi Formula in Alzheimer's disease(AD)-related APP/PS1 mice.

Methods Eighteen APP/PS1 mice of 8 months old were divided into model and Bushen Yizhi Formula groups and 9 wild-type mice were used as normal control. After 8 weeks, behavioral functions were evaluated and then all mice were sacrificed for Immunofluorescence staining, Western blotting and Flow cytometry tests.

Results Compared with the control group, the abilities of learning, memory and spatial exploration in AD model group were declined, while the application of Bushen Yizhi Formula could ameliorate the changes. Compared with the model group, by Immunofluorescence staining and Flow cytometry tests we found Bushen Yizhi Formula treatment could significantly decrease the expression of characteristici markers in M1 microglia including iNOS and CD16/32 but increase the expression of characteristici markers in M2 microglia including Arginase-1 and CD206, obviously suppress the secretion of proinflammatory cytokines such as IL-6, TNF-α, IFN-Ɣ and IL-1β but promote the secretion of anti-inflammatory cytokine IL-10. Meanwhile, Bushen Yizhi Formula enhanced the expression of neurotrophic factors including GDNF, BDNF and NT3. In addition, by Western blotting we found Bushen Yizhi Formula could inhibit the activation of TLR4/MyD88/NF-KB signaling pathway in AD mice.

Conclusion Bushen Yizhi Formula could significantly improve cognitive function of AD mice. It could reverse harmful M1 microglia to beneficial M2 microglia and alleviate neuroinflammatory reaction, which may be achieved by inhibiting TLR4 / MyD88 / NF-KB signaling pathway.
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Keywords: Bushen Yizhi Formula; AD; APP/PS1 mice

ROLES OF MICROGLIAL NHE1 IN THE DISEASE-ASSOCIATED MICROGLIA TRANSFORMATION AND STROKE-INDUCED BRAIN INJURY AND COGNITIVE DEFICIT.

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Microglial activation plays an important role in white matter injury and tissue repair in Vascular Contributions to Cognitive Impairment and Dementia (VCID). Microglial dysfunction leads to impairment of debris clearance, remyelination, and synapse remodeling, collectively contributing to cognitive decline. New research reveal that disease-associated microglia (DAM) emerges as a key feature in neurodegenerative brains, which present significant elevations in functions of phagocytosis, lysosomal functions, and lipid metabolism. However, the mechanisms underlying the transformation from homeostatic to DAM are not well understood. Microglial NHE1 protein mediates H^+ efflux in exchange of Na^+ influx for regulation of the intracellular pH (pH_i) to maintain the optimal pH_i for sustaining NADPH oxidase activation. Our recent study showed that selective deletion of microglial *Nhe1* in the *Cx3cr1-CreER*$^{+/-}$*;Nhe1*$^{f/f}$ (cKO) mice reduced pro-inflammatory microglia polarization and increased restorative microglia population after ischemic stroke (transient middle cerebral artery occlusion model, tMCAO). Using bulk RNAseq transcriptomic analysis, here we report that the post-stroke cKO microglia also displayed significant elevation of transcriptomes for DAM hallmark genes (*Apoe, Trem2, Spp1,* etc.), as well as genes for phagocytosis (*C1qa, Cd68,* etc.), phagolysosomal function (*Ctsb, Ctsd, Lamp1,* etc.), and lipid metabolism (*Fdps, Agpat5,* etc.). Concurrently, the cKO mice exhibited increased microglial phagocytic activity, synaptic pruning, enhanced oligodendrogenesis and white matter remyelination, as well as improved cognitive functions during post-stroke recovery. Our findings reveal that microglial NHE1 function plays an important role in regulating DAM function for promoting white matter plasticity and synapse remodeling, which collectively contribute to accelerated sensorimotor and cognitive function recovery after brain lesion.

HIGH-THROUGHPUT QUANTIFICATION OF AQUAPORIN-MEDIATED WATER HOMEOSTASIS IN HUMAN ASTROCYTOMA CELLS

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Aquaporin 4 (AQP4) is a membrane-spanning water channel that is enriched at the end feet of astrocytes in the brain. Mounting evidence suggests that it contributes to water flow and waste removal across the blood-brain barrier [1]. In Alzheimer's Disease, AQP4 abundance, the ratio of its isoforms, the channel superstructures and localization are perturbed, suggesting it may contribute to the disease development [2]. Hence, to identify modulators of AQP4 function, we implemented a microscopy-based screening assay [3], which is based on the kinetic measurement of osmotically driven (de-)quenching of the cell-permeable dye Calcein-AM. We show that cell-based measurements yield a more sensitive and robust readout than a spectrophotometric (well-based) or image-based based approach. Running the optimized assay on human astrocytoma cells, we found that stable overexpression of the major *AQP4* isoforms promotes the intracellular buffering capacity in response to hyper- and hypo-osmotic insults. Currently, we are also evaluating the assay in primary astrocytes and we are testing a rationally selected drug library for their aquaporin-modulating properties. This way, we hope to identify compounds that may promote aquaporin-driven processes in the brain.

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THE BHMT–BETAINE METHYLATION PATHWAY EPIGENETICALLY REGULATES METHYLTRANSFERASE ACTIVITY IN OLIGODENDROCYTES

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Multiple Sclerosis (MS) is characterized by neurological dysfunction and demyelination of the central nervous system. Further, oligodendrocytes are killed off and myelin production is halted, with progenitor cells (OPCs) unable to differentiate. We have previously shown that there is a depletion of methyl donors in MS, and that activation of the betaine homocysteine methyltransferase (BHMT)–betaine pathway restores methylation and neurological deficits in the cuprizone and EAE models of MS. We have also found that betaine regulates histone and DNA methyltransferase activity and in neurons and oligodendrocytes. In the present study, we are investigating the role of BHMT in oligodendrocytes, hypothesizing that through betaine supplementation, the BHMT–betaine pathway locally contributes to SAM synthesis for methylation of DNA and histones in OPCs. We have found that BHMT is expressed in the nucleus of oligodendrocytes. In addition, we found that betaine enhances DNMT activity, and that BHMT is required for betaine to have an effect, suggesting a role for the BHMT–betaine methylation pathway in epigenetic regulation. We observed morphological changes in primary OPCs under oxidative conditions and betaine treatment throughout development. QRT-PCR was performed to determine the effects of oxidative stress and betaine on various OPC and oligodendrocyte differentiation regulators and we found that betaine enhances OPC differentiation marks. These data suggest that changes in methionine metabolism in MS may be linked to defects in oligodendroglial gene expression. Thus, activation of the BHMT-betaine pathway may provide epigenetic control required for oligodendrocyte differentiation.

THE ROLE OF RGG MOTIFS AND ARGININE METHYLATION IN FUS-DEPENDENT POST-TRANSCRIPTIONAL REGULATION.

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Fused in sarcoma (FUS) is an RNA-binding protein that functions in various RNA metabolic pathways, including the regulation of transcription, pre-mRNA splicing, mRNA stability and translation. A subset of patients with amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) present pathological redistribution and aggregation of FUS. Mutations in the FUS gene can cause familial ALS, and many of these disease-associated mutations occur within the Arginine-Glycine-Glycine (RGG) motifs of FUS. RGG motifs in RNA-binding proteins are increasingly recognized as sites of RNA and protein interactions and are often post-translationally methylated at arginine residues. Indeed, FUS is normally methylated on RGG motifs, however it is hypomethylated in FTD patients with FUS aggregates. Despite these links of RGG motifs and arginine methylation to disease, their functional relevance remains elusive.

Using pulldowns with methylated or unmethylated FUS and mass spectrometry, we now demonstrate that the methylation state significantly changes the protein interactions of FUS. In particular, proteins involved in pre-mRNA splicing, RNA stability, and translation showed a stronger association with FUS upon loss of methylation. In addition, we use electrophoretic mobility shift assays (EMSA) to show that the RGG motifs of FUS play a role in RNA binding, alongside the two canonical RNA binding domains of FUS. Further, we show that mutation of the arginines within the RGG motifs of FUS leads to cytoplasmic mislocalization in a subset of cells, a phenotype known to be associated with ALS/FTD pathology. Mutation of the arginines within the RGG motifs, but not mutations in the canonical RNA binding domains, diminishes the recruitment of FUS to stress granules, which are thought to be the precursors to pathological aggregates in ALS/FTD patients. This finding suggests that arginines in RGG motifs, but not RNA-binding, are important for recruitment of FUS to stress granules.

TAU SEEDING IN ALZHEIMER'S DISEASE REVEALS NOVEL REGIONS OF PATHOLOGY

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Introduction: Tauopathies are a heterogeneous group of neurodegenerative diseases defined by progressive accumulation of tau aggregates in the brain. Alzheimer's Disease (AD) is the most common, and is uniquely defined by coexistent amyloid beta and tau pathology. The severity of AD dementia correlates with the extent of postmortem tau pathology. The neuropathology of AD comprises intraneuronal pretangle, neurofibrillary tangle (NFT) pathology, and neuropil threads (NT), extraneuronal ghost tangles, threads, and amyloid beta plaques. Tau histopathology progresses in a defined and characteristic pattern that is proposed to involve transcellular propagation of self-amplifying "seeds," allowing the classification of AD stages. However, the relationship of these patterns of histopathology to tau seeding activity is unclear.
Methods: For decades the gold standard to detect and stage the disease has been immunohistochemistry with the anti-phospho-tau monoclonal antibody AT8. To detect tau seeding activity in biological samples, we previously developed a sensitive and specific cell-based "biosensor" assay. We subsequently optimized the biosensor cell line (TauRD(P301S)v2H) to increase its sensitivity approximately 300-fold. We used this assay to analyze seeding tau seeding in 25 brain regions across 20 individuals with and without known NFT pathology.
Results: We observed progressive accumulation of tau seeding within individuals with higher NFT stages. Seeding occurred in 4 steps (very early, early, intermediate, and late), and frequently preceded NFT pathology, e.g., in the amygdala and the substantia nigra. We observed seeding in brain regions not known to develop tau pathology, such as the globus pallidus, the cerebellar cortex, and the internal capsule, where mainly axons were affected. AT8 staining for brain regions with positive tau seeding also revealed tau pathology in unexpected cell types, e.g., in Bergmann glia of the cerebellar cortex, a finding not described previously.
Conclusion: Tau histopathology and seeding are two fundamentally different, yet complementary, methods to assess tau pathology. Specifically, sensitive tau seeding assays reveal pathology in regions where AT8 staining is negative. Our data suggest that tau pathology occurs beyond the brain regions that are traditionally included in NFT staging, and is highly variable between individuals. This may underlie variation in the clinical presentation and course of AD.

ENDURANCE EXERCISE AMELIORATES DISEASE PROGRESSION IN *DROSOPHILA* MODELS OF SPINOCEREBELLAR ATAXIA

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Endurance exercise is a powerful, low-cost intervention with substantial pro-healthspan effects proven to reduce disease incidence across species. While endurance exercise supports neural plasticity, enhanced memory and cognition, and reduced neurodegenerative decline, comparatively little is known about the effect of chronic exercise on the progression of movement disorders known as ataxias. Here, we focused on three different types of ataxias, known as Spinocerebellar Ataxias Type (SCAs) 2, 3 and 6, which belong to the polyglutamine (polyQ) family of degenerative disorders that also includes Huntington's disease. To examine a role of exercise in SCAs, we utilized the model organism, *Drosophila melanogaster*. The fly models of SCAs 2, 3 and 6 progressively lose motor function and accumulate toxic levels of polyQ protein. Excitingly, we observe dramatic protection of climbing speed and endurance in exercised SCA2 flies and modest protection in exercised SCA6 models, while no benefit is observed in SCA3 model flies. Importantly, accumulation of protein aggregates is also reduced in SCA2 flies after chronic exercise training, but not so for SCA3, linking protein levels to exercise-based benefits. Currently, we are focusing on the activation of exercise mimicking-genes in the neurons of SCA-model flies in order to define the mechanisms by which exercise preserves function in polyQ disorders. This study suggests differential responses of ataxia disorders to exercise, and emphasizes the potential for exercise-based therapies in the prevention of polyQ neurodegenerative progression more widely. Defining the mechanisms by which exercise prevents disease progression will inform disease targets driving individual polyQ disorders, opening the door for more effective treatment.

POLYGLUTAMINE EXPANSION CAUSES MITOCHONDRIAL FRAGMENTATION *IN VIVO*.

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Mitochondrial dysfunction, such as mitochondria fragmentation and mitochondrial oxidation, has been reported in Huntington's disease (HD) models including mice and human primary neuronal culture. However, it is unclear how mitochondrial dysfunction occurs in HD neuropathogenesis. Here we hypothesize that excess expression of pathogenic huntingtin (HTT) impairs mitochondrial homeostasis. We found that expression of pathogenic huntingin containing 138 polyQ repeats caused fragmented mitochondria compared to expression of normal HTT containing 15 polyQ repeats. However, HTT did not co-localize with mitochondria under normal or diseased conditions, and the amount of HTT aggregation within axons did not correlate with the extent of mitochondrial fragmentation, indicating that mitochondrial fragmentation is likely not due to HTT. To test if mitochondrial fragmentation was due to expansion of polyQ repeats we next expressed normal and expanded amounts of polyQ repeats alone or in the context of MJD. We found that expansion of polyQ repeats either alone or in the context of MJD caused mitochondrial fragmentation compared to expression of normal lengths of polyQ repeats, indicating that mitochondria fragmentation is likely due to expanded PolyQ. To further dissect the polyQ- mediated mechanism of mitochondrial fragmentation we next tested how proteins involved in fission and fusion affect mitochondria fragmentation. We found that excess of the mitochondria fusion protein Mfn or depletion of the mitochondria fission protein Drp1 rescued PolyQ-mediated mitochondria fragmentation. Taken together, our observations indicate that mitochondria fragmentation seen in HD is likely due to PolyQ expansion, and that PolyQ expansion likely affects mitochondria health. Further work will focus on isolating how polyQ-mediated mitochondrial fragmentation contributes to mitochondrial health using *in vivo* oxidation markers.

EXPERIMENTAL COLITIS PROMOTES SUSTAINED CD8 TCELL-DEPENDENT NEUROINFLAMMATION AND PARKINSONIAN NEUROPATHOLOGY IN MICE

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The etiology of sporadic Parkinson's disease (PD) remains uncertain, but genetic, epidemiological, and physiological overlap between PD and inflammatory bowel disease suggests that gut inflammation could promote dysfunction of dopamine-producing neurons in the brain. Mechanisms mediating this and interactions with sex and genetic and environmental factors are not well understood but may represent targets for therapeutic intervention in PD. To investigate this at the translational level, we compared the immune and inflammatory profiles of blood and colon from subjects with Parkinson's disease with those from age- and sex-matched healthy controls. We found high levels of NFκB p65 in colonic biopsies and other inflammatory mediators and reduced levels of Regulator of G-Protein Signaling-10 (RGS10) – a GAP identified previously by our group as a negative regulator of NFκB in myeloid cells. To evaluate directly whether this inflammatory profile could impact and/or increase vulnerability of dopaminergic pathways, we employed a RGS10-null mouse model challenged with experimental colitis. In male mice, colitis caused sustained CD8+ T-cell infiltration and interferon gamma gene expression in the brain which perturbed dopaminergic markers causing significant dopamine depletion. In both sexes, colitis potentiated effects of sub-threshold doses of the dopaminergic neurotoxicant MPTP. RGS10 deficiency increased baseline intestinal inflammation, colitis severity, and dopaminergic neuropathology. Consistent with a direct role in mediating inflammation-induced death, peripheral CD8+ T-cell depletion prevented colitis-induced reductions in dopaminergic markers. These novel findings elucidate mechanisms by which gastrointestinal inflammation could confer neurological vulnerability to Parkinson's in a sex-specific manner and suggest potential new avenues for therapeutic immunomodulatory interventions to delay or prevent progression of PD pathology responsible for motor symptoms.

THE BLOOD-EYE-BRAIN AXIS IN THE DEVELOPMENT OF AN EARLY DETECTION PLATFORM FOR ALZHEIMER

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Beta-amyloid (Aβ) plaque deposition is one of the distinctive neuropathological features in Alzheimer's disease (AD). Current evidences suggest that among the various Aβ assemblies, the intermediary Aβ oligomeric species (Aβo) is the most toxic to neurons and could potentially be detected at very early stages of AD. Therefore, targeting Aβo using antibodies is one of the most promising approaches to develop early diagnosis and treatment for AD. Blood-based identification of Aβo has been investigated extensively but remains at an experimental stage. Another alternative currently being investigated is the presence of retinal Aβo before clinical onset and establishment of AD neuropathology. Abnormal retinal changes are increasingly being recognized as one of the early pathological alterations associated with AD. Although, Aβo depositions have already been shown to accumulate in the retina of AD patients and AD animal models, it is not known whether the early retinal Aβo depositions precede their brain and blood accumulation. Here, we report that camelid-derived single domain antibodies targeting Aβ1-40 (PrioAD12) and Aβ1-42 (PrioAD13) oligomers were able to detect Aβo in the retina and blood but not in the cerebrum of 3-4-month-old APP/PS1 mice. We show that the majority of the retinal Aβo deposits were confined to the intraneuronal compartment with scattered extracellular accumulation. This study provides a very strong basis to develop and implement an 'eye test' for early detection of AD using camelid-derived single domain antibodies targeting retinal Aβ

CHOLESTEROL AND MATRISOME PATHWAYS DYSREGULATED IN HUMAN *APOE* ε4 GLIA

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Apolipoprotein E4 (*APOE4*) is the strongest genetic risk factor for Alzheimer's disease (AD), however, the impact of *APOE4* on human brain cell function remains unclear. Here we investigated the effects of *APOE4* on brain cell types derived from human induced pluripotent stem cells, post-mortem brains and *APOE* targeted replacement mice. Global transcriptomic analyses identified human specific *APOE4*-driven lipid metabolic dysregulation in astrocytes and microglia. Decoupled cholesterol metabolism in *APOE4* glia is due to lysosomal cholesterol sequestration; despite increased intracellular cholesterol leads to elevated de novo synthesis and decreased efflux. The most significant upregulated signal in post-mortem *APOE4* AD brains is matrisome associated with chemotaxis, glial activation and lipid biosynthesis, which derives from astrocytes, paralleled pathways uncovered in astrocytes when co-cultured with neurons. Further, *APOE4* astrocytes show enhanced chemokine/proinflammatory factors production when communicating with neurons. Thus, *APOE4* initiates human glia-specific cell autonomous and non-cell autonomous dysregulation that may increase AD risk.

EVALUATING iPSC-DERIVED MICROGLIA-NEURON CO-CULTURE PLATFORMS FOR CRISPR-BASED FUNCTIONAL GENOMICS

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Neuron-microglia signaling informs microglial surveillance, motility, phagocytosis, and activation. When microglial behavior appears aberrant, as in many neurological diseases, it is unclear if such behavior is causative in pathogenesis or responsive to abnormal extracellular cues. To approach this open question, we propose an iPSC-derived microglia-neuron co-culture system that supports high-throughput, CRISPR-based functional genomics. Here, we evaluate preliminary experiments for the 2D and 3D culture of iPSC-derived microglia and excitatory neurons. In 2D, microglia have been introduced to neuron cultures at various points during circuit maturation and at varying densities. Within these cultures, we characterize synapse maturity, cellular morphology, and circuit structure. In 3D, we have evaluated hydrogel materials including hyaluronic acid, gelatin, Matrigel, and N-cadherin functionalized gelatin methacrylate for co-culture viability.

BACH1 INHIBITION AS A NOVEL THERAPEUTIC APPROACH FOR PARKINSON'S DISEASE

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Parkinson's disease (PD) is a progressive neurodegenerative movement disorder characterized by the loss of nigrostriatal dopaminergic neurons. Mounting evidence accounts for Nrf2 to be a promising target for neuroprotective interventions in PD. However, canonical Nrf2 based drugs result in side-effects due to electrophilic chemical properties causing irreversible alkylation of cysteine residues on proteins. Bach1 is a known transcriptional repressor of the Nrf2 pathway. We report that Bach1 levels are upregulated in PD postmortem brains and preclinical models. Bach1 knockout mice were protected against MPTP-induced dopaminergic neurotoxicity and associated oxidative damage and neuroinflammation. Functional genomic analysis demonstrated that the neuroprotective effects in Bach1 KO mice was due to upregulation of Bach1 targeted pathways that are associated with both Nrf2 dependent ARE and Nrf2-independent non-ARE genes. Using a proprietary translational technology platform, a drug library screen identified HPPE as a novel Bach1 inhibitor that was validated as a non-electrophile. Oral administration of HPPE attenuated MPTP-neurotoxicity in pre- and post-treatment paradigms. HPPE-induced neuroprotection was associated with the upregulation of Bach1 targeted pathways in concurrence with the results from Bach1 KO mice. Our results suggest that genetic deletion and pharmacologic Bach1 inhibition by a non-electrophilic inhibitor is a promising therapeutic approach for PD.

AGED AND AMYLOID PLAQUE-LOADED BRAIN ENVIRONMENT CAUSES LOCAL EXUBERANT GRAFT INNERVATION

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Transplantation of fetal neurons aims to replace lost neurons and synapses in the injured or degenerated brain. Our lab showed that neurons transplanted into the mouse cortex after induced neuronal apoptosis are able to mature and integrate into the host brain circuitry by reaching similar connectivity rates to those lost (Falkner/Grade et. al, 2016). We asked here if integration of transplanted neurons in models like Alzheimer's disease and age-related neurodegeneration is equally successful, especially as preclinical mouse models do not show loss of neurons.

By transplanting embryonic mouse cortical neurons into double-transgenic APP/PS1 mice with broad amyloid plaque deposition at 8 months of age, and into 16 months-old aged WT mice, and into younger control mice, we tested whether those brain environments influence the rate of synaptic integration acquired by transplanted cells. To map the whole-brain connectivity of grafted neurons we used a rabies virus-based monosynaptic approach. Moreover, to reveal key molecules in accurate circuit integration we compared the proteome of these different conditions.

We observed in APP/PS1 and aged brains that transplanted neurons survived, integrated into the host brains, and received area-specific appropriate inputs, but with exuberant local connectivity compared to control brains. State-of-the-art deep proteome analysis using mass spectrometry (LC-MS/MS) revealed the composition of the brain environments promoting the excessive local input connectivity thereby providing important information on how different brain environments shape the input connectome of transplanted cells in the host brain.

IDENTIFICATION OF TRANS-ACTING FACTORS RESPONSIBLE FOR INDUCED ALTERNATIVE SPLICING OF α-SYNUCLEIN GENE BY PARKINSONIAN MIMETICS

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Aberrant alternative splicing events are being widely noticed in several neurodegenerative diseases including Alzheimer's disease (AD), muscular dystrophy, retinitis pigmentosa, neurofibromatosis etc. In Parkinson's disease (PD), α-synuclein (α-syn) happens to be the major causative agent leading to degeneration of dopaminergic neurons. Earlier reports from our group identified PD mimetics induced alternative splicing of α-syn gene leading to generation of 112-synuclein (112-syn). Skipping of exon 5 from α-syn gene has been shown to promote its aggregation propensity and altered membrane trafficking events. However, the factors responsible for skipping of exon 5 that leads to generation of 112-syn are not known yet. In this context, we aimed to identity the cis & trans-acting regulatory elements governing alternative splicing events by Parkinsonian agents (MPP^+) using minigene constructs. Initially, we have constructed minigene I (Mini-I) by trimming intron 4 region (from ~92kb to < 1kb) without altering exon 5 and branch point adenosine to preserve the splicing machinery. Treatment of cells with MPP^+exhibited exon 5 skipping leading to generation of 112-syn. In order to define the role of intron 5, minigene II (Mini-II) was constructed by trimming intron 5 region (from ~2.5kb to < 1kb) from Mini-I. Mini-II showed similar splicing pattern implying that the engineered intronic regions possess essential components responsible for exon 5 skipping. Further to identity regulatory elements in Mini-II, we have generated chimeric minigenes by replacing either 5' (Mini-III) or 3'(Mini-IV) flanking intronic regions of exon 5 with other intronic regions that are not responsive to MPP^+ induced alternative splicing. While Mini-IV exhibited MPP^+ induced exon 5 skipping, the same was not apparent in cells expressing Mini-III indicating that the 5' flanking intronic region (316 nucleotides) of exon 5 might possess cis-acting elements responsible for alternative splicing. We further investigated the trans-acting proteins that may bind to 5' flanking intronic region that govern exon 5 skipping. RNA-binding protein database (RBPD) and gene expression analysis identified four putative RNA binding proteins, namely, RBMX, MBNL, KHDRBS3 and SFRS1 that may bind to 316 nucleotides. The detailed analysis on the role of above RNA binding proteins on PD mimetic induced alternative splicing events will be discussed.

APOE4 INHIBITS MITOCHONDRIAL LIPID OXIDATION IN NEURAL CELLS.

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ApoE4. ApoE4 inheritance is the single largest genetic attributable risk factor for Alzheimer's disease, yet its pathomechanism is unclear, and understanding of its mechanism could lead to targeted therapy. ApoE4 allele results from a single amino acid exchange Cys to Arg at ORF position 112 relative to the most common ApoE3 allele.
Shc mutant mice.Mice with genetic reductions of Shc proteins are observed to have more ketogenic metabolism (Hagopian, Tomilov et al. 2012; Hagopian, Tomilov et al. 2015; Hagopian, Kim et al. 2016), and to resist Alzheimer's in the PSAPP model not through dissolution of amyloid plaques but through improved neuroprotection and increased mitochondrial metabolism (Derungs, Camici et al. 2017).
Hypothesis. Our hypothesis is that ApoE4 affects neural mitochondrial metabolism and that this contributes to AD pathomechanism. Our second hypothesis is that Shc inhibitors alter ApoE4 dependent defects in metabolism.
Methods. We tested the effect of ApoE3 and ApoE4 alleles in 3 different N2a-based cell models. ApoE3 and ApoE4 stable transfectants made in the Mahley laboratory (Chen, Ji et al. 2011), and ApoE2,E3,E4 both transiently and stably transfected in our laboratory.
Results. ApoE4 affects mitochondrial lipid oxidation. Among the 3 independently created lines, we observe significant mitochondrial defects only in the ApoE4-bearing cells. In examining the basis of this mitochondrial defect, we altered mitochondrial substrate fuel, substituting lipid (palmitate) for the usual glucose oxidative substrate. On palmitate, we observe that whereas ApoE2 and ApoE3 promote mitochondrial palmitate oxidation, ApoE4-bearing cells have a significant defect in palmitate lipid oxidation. Shc inhibitors rescue mitochondrial defects. Small-molecule inhibitors of Shc protein have been isolated, and were tested in the context of ApoE4 alleles, and appear to rescue mitochondrial defects in that context.
Interpretation. We hypothesize that ApoE4 specifically limits the availability of fat for neuronal mitochondrial oxidation. We speculate that this could be through alterations in apolipoprotein's extracellular transport function, or intracellular delivery to mitochondria and/or regulation.
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ASSESSMENT OF NEURODEGENERATIVE MICROGLIOSIS INDUCED BY ORAL PATHOGENIC BACTERIA

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Porphyromonas gingivalis (*P.gingivalis*) is a popular bacteria found in human oral cavity and they are not only responsible for chronic periodontitis development but also reported as a critical factor of Alzheimer's disease due to their ability to induce Aβ and tau protein accumulation in brains. Secondary metabolites produced during bacterial proliferation play an important role in their pathogenic features, which can trigger reactive astrocytes and neurodegeneration in a central nervous system. Our study hypothesizes that *P.gingivalis* produces pro-inflammatory mediators leading to neurodegeneration directly from bacterial conditioned media or indirect pathway via microglia. Bacterial conditioned media were collected during their proliferation to reach $7.62x10^{10}$ cfu/ml, followed by centrifuging and 0.2-μm membrane filtering to remove bacterial pellets. Bacterial conditioned media were utilized to demonstrate pro-inflammatory activity of microglia in single culture microglia while LPS (10 ng/ml) was considered as positive control. To illustrate microglia phenotype, we performed immunostaining against M1/M2 marker (CD86/CD206) as well as ELISA for multiple cytokine detection. Further, both bacterial and bacteria-stimulated microglial conditioned media were employed in a co-culture system of human neurons and astrocytes to examine the induction of astrogliosis and neurodegeneration leading to neural cells loss, by immunostaining against reactive astrocyte marker (GFAP) as well as neurodegenerative marker (pTau). We found significant morphological changes and increase of CD86 protein expression in human microglia cells treated with high concentration of bacteria condition media (BCMH), which was nearly 1.5-folds and 5-folds greater than LPS treated-microglia and control, respectively. Several pro-inflammatory cytokines, including IL-8, IL-18, MIF and Serpin, were detected in conditioned media of microglia treated with BCMH. The significant decreases in poppulations of neurons and astrocytes were found in both bacterial and bacteria-stimulated microglial conditioned media. In particular, treatment of microglial conditioned media led to about 90% neuronal cell loss while BCMH treatment resulted in almost 70% neuronal death. In addition, microglial-derived media showed nearly 9-times higher in GFAP compared to controls whereas BCMH treatment caused 7-times increased in GFAP. Consistently, neurons treated with microglial-derived media demonstrated almost 1.5-folds more pTau expression than the BCMH treatment, which provided more evidence to prove the effects of *P.gingivalis* on neuroinflammation leading to neurodegeneration. Our results suggest the crucial function of microgliosis triggered by *P.gingivalis* in induction of astrogliosis and neurodegeneration, which may contribute to understanding the mouth-brain axis in Alzheimer's disease.

Keywords: Alzheimer disease, microgliosis, neurodegeneration, *Porphyromonas gingivalis*

THE POLY ADP-RIBOSE RESPONSE IS AFFECTED DIRECTLY BY THE HUNTINGTIN PROTEIN IN HUNTINGTON'S DISEASE

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The huntingtin protein is a large 350Kda scaffold that is signaled via Casein Kinase 2 and ATM kinase phosphorylation to DNA damage in Base Excision Repair of adducts induced by age-onset reactive oxygen species. This is following signaling to the nucleus by reactive oxygen modification of a single methionine in huntingtin, thus defining huntingtin as sensor and effector of ROS-induced DNA damage. As a result of polyglutamine expansion, mutant huntingtin protein in Huntington's disease (HD) retains on chromatin to a greater degree than wild type protein. By interactome analysis under ROS stress, we noted a commonality of poly ADP-ribosylated or PARylated factors associated with huntingtin, as well as DNA repair factors such as XRCC1, FEN1 and APE1. Using homology to known PAR-binding motifs, and PAR-binding assays, we identified a classic PAR-binding amino acid motif in huntingtin, located on the surface of the 3D structure recently determined by Cryo electron microscopy.

In HD cerebral spinal fluid samples by an ELISA assay, we observed a significant reduction of PAR levels, in both those affected by HD as well as prodromal carriers. However, in HD fibroblasts, we see a robust PAR response to induced DNA damage. This suggests generation of PAR at DNA damage adducts is not affected by mutant huntingtin, but mutant huntingtin can irreversibly bind PAR chains, affecting assay readouts masking a hyper-PARylation as seen in AOA-ataxia and Parkinson’s disease. This indicates the proper dynamics of the PAR response is affected at the earliest stages of disease and that prodromal HD carriers have a chronic inhibition of the PAR response to DNA damage, years before predicted disease onset. Elevated DNA damage has been seen in clinical peripheral mononuclear blood cell samples at prodromal stage of HD.

By detailed UK registry analysis, there is significantly less cancer in the HD patient population. Thus, we hypothesize that the chronic inhibition of the PAR response in HD leads to chronic energy crisis in HD neuronal populations under highest ROS stress. We also hypothesize that the presence of a CAG expanded huntingtin allele may confer an evolutionary advantage with the avoidance of early onset cancers, due to chronic sub-optimal PAR response dynamics. This mechanism of PAR effect in HD is different than in Parkinson disease, suggesting a proper PAR response balance is critical to avoid neurodegeneration, but also outlines some potential drug targets in the modulators of the PAR response.

AMYLOID-β SEEDING AND PROPAGATION PROCESSES IN A hAβ-KI MODEL OF ALZHEIMER'S DISEASE

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Recent evidence indicates that Aβ can misfold and aggregate into seeds that structurally corrupt native proteins, mimicking a prion-like process. Several studies using FAD animal models have demonstrated that intracerebral infusion of brain extracts from APP-transgenic mice or AD patients induce Aβ deposition and cerebral amyloid angiopathy. To carry out most of these Aβ-seeding studies, APP-transgenic animal have been used. Nevertheless, it remains to be elucidated whether Aβ deposition can be induced by Aβ-seeds in a sporadic AD model that does not overexpress APP and produces wild type human Aβ.
We used an innovative model to better understand the amyloidogenic events that occur in sporadic AD. This hAβ-KI model, expresses wild-type human Aβ under the control of the endogenous mouse APP gene. Aβ-seeds from AD patients (stage C) from the AD Research Center (UCI) were administered into 7-8-month-old hAβ-KI and as positive controls 3xTg-AD mice were employed.
We demonstrated that amyloid seeds can stimulate Aβ aggregations in 3xTg-AD and hAβ-KI models. We found that Aβ aggregates occur earlier in the 3xTg-AD vs hAβ-KI and that a longer term of treatment is necessary to accelerate diffusible Aβ pathology in the hAβ-KI mice. Thereferoe, this hAβ-KI model represents an important step towards the development of next-generation animal models that will provide better predictive outcomes for human patients.

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A DISTINCT TAU CODE CONTROLLED BY HDAC6 IS LINKED TO TAU PATHOGENESIS

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The prion-like spread of tau pathology could underlie a spectrum of distinct clinical syndromes including Alzheimer's disease (AD). Although evidence indicates that tau is transmissible, it is unclear how pathogenic tau species evolve in neurons. In this research, we hypothesized that disease-associated tau seeds undergo distinct intracellular processing mechanisms which are directly associated with tau pathogenesis. Here, we analyzed fibrillar tau seeds comprising wild-type or disease-associated P301L tau using in vitro and neuronal-based assays. Unexpectedly, we show that P301L seeds are distinctly modified via post-translational modifications (PTMs) within the microtubule-binding region (MTBR). While these modifications do not alter tau seed trafficking or localization, acetylated tau showed accelerated tau aggregation kinetics, enhanced priming of nearby tau PTMs, and prion-like templating. To explain their susceptibility to acetylation, we show that P301L seeds undergo auto-acetylation and also preferentially inhibit the deacetylase HDAC6. We further identify a coordinated tau-HDAC6 signaling axis, as tau depletion activates HDAC6 by regulating a functional phosphorylation site on HDAC6 and globally alters the stress-responsive acetylation profile. Our study highlights the complex post-translational regulation of transmissible tau seeds and provides new insight into the biological properties of tau strains among diverse tauopathies including AD. In addition, genetic or pharmacological approaches to deplete tau and hence activate HDAC6 could be used to modulate the neuronal acetylation profile and provide new strategies to suppress neurotoxicity and cognitive decline in AD and related dementia.

C9ORF72 DIPEPTIDE REPEAT PROTEINS DISRUPT THE FORMATION OF GEM BODIES AND RESULT IN ABERRANT LOCALIZATION OF SURVIVAL OF MOTOR NEURON PROTEIN

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A G4C2 repeat expansion in the C9ORF72 gene is the most common genetic cause of amyotrophic lateral sclerosis (ALS), a devastating motor neuron disease. In the neurons of ALS patients, dipeptide repeat proteins (DPR) including poly(glycine-arginine) and poly(proline-arginine) are produced from the G4C2 repeats by an unconventional form of translation, which are thought to be toxic to cells. GEM bodies are nuclear structures that harbor survival of motor neuron (SMN) protein. They are essential for spliceosome integrity and we previously discovered that they are lost in the motor neurons of ALS patients. Here we show that DPR accumulation interferes with GEM formation and proper SMN localization in HeLa cells and in iPSC-derived motor neurons from an ALS patient with C9ORF72 mutation. Accumulation of poly(glycine-arginine) markedly reduced the number of GEM bodies and caused the formation of aberrant cytoplasmic RNA granules that sequestered SMN. Another arginine-rich DPR, poly(proline-arginine) did not alter GEM formation but significantly impaired the disassembly of stress granules. Taken together, DPRs produced from the C9ORF72 mutation impair GEM formation and proper SMN localization. Our findings might help to provide a mechanism to explain the abnormal RNA splicing seen in motor neurons of C9orf72-ALS patients.

NOVEL PATHWAY ENRICHMENT AND NETWORK ANALYSIS METHODS FOR INVESTIGATING CELL-TYPE SPECIFIC SELECTIVE VULNERABILITY TO PATHOLOGICAL TAU IN ALZHEIMER'S DISEASE

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Background: Region-specific neuronal subpopulations known to be selectively vulnerable and resistant to tau pathology, as characterized in our previous work (Fu et al., 2019), were identified across several public single-cell/nucleus RNA seq datasets and analysed using novel bioinformatic methods to better understand the factors underlying selective vulnerability.

Methods: In this poster, we show an analysis conducted using wild-type mouse SMART-Seq data from the Allen Brain Institute (Hodge et al. 2019) and human AD case/control 10X data from the Kampmann group (Leng et al. 2020). Following a standard preprocessing and exploratory analysis pipeline, we applied a custom differential pathway enrichment and network analysis pipeline that identifies features that covary with the vulnerability status of a cell-type.

Results: We show that using known marker genes, we were able to identify highly specific clusters of cells that correspond to cell-types that exhibit selective vulnerability. Our novel pathway/network analysis pipeline also recapitulates known endpoints of AD pathology, such as the robust downregulation of synaptic pathways and genes. Most importantly, however, we demonstrate that through trajectory inference across multiple datasets, we can identify putative pathways and genes that underlie selective vulnerability to said endpoints.

Conclusion: We present a novel pathway enrichment and network analysis pipeline and demonstrate its application in single cell/nucleus RNA seq data for uncovering pathways and genes that are associated with selective vulnerability in Alzheimer's Disease.

AN N-TERMINAL MOTIF IMPORTANT FOR THE AGGREGATION AND FUNCTION OF A-SYNUCLEIN

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Alpha-synuclein (αSyn) is known to be involved in the neurodegenerative disorder Parkinson's disease (PD) which affects 10 million people worldwide. Patients show multiple motor (e.g. tremor, lack of coordination) and non-motor (e.g. depression, anxiety) symptoms, as well as pathological symptoms which are characterised by the loss of dopaminergic neurons and the formation of aggregated αSyn-containing Lewy bodies in the brain. Understanding the process of amyloid formation from the intrinsically disordered monomer αSyn to the complex β-sheet rich fibrils is therefore crucial.

In order to understand the effect of the protein sequence on aggregation, we have engineered αSyn mutants which lack several amino acids in the N-terminal region that are shown in various aggregation/ hydrophobicity calculators to be part of highly aggregation-prone regions. We also created single point mutation variants within this region. ThT fluorescence aggregation assays and in vivo experiments in C. elegans have shown that these mutants significantly slow down amyloid formation and on account on this, the newly identified N-terminal motif plays indeed a critical role in aggregation. The importance of these regions was further analysed in molecular detail via paramagnetic relaxation enhancement (PRE) NMR experiments. Interestingly, the deleted N-terminal motif is shown to be involved in both intra- and intermolecular interactions and is therefore likely to play a key role in driving aggregation. Experiments on liposomes revealed that the N-terminal motif is crucial to fulfil αSyn's physiological function of membrane remodelling.

Conclusively, the analysis of this newly discovered N-terminal motif in αSyn gives an innovative insight in sequence determination of αSyn aggregation into amyloid. The results highlight the frustration of function versus aggregation in this intrinsically disordered protein and identify a motif that could be targeted to develop new drugs against PD or other neurodegenerative diseases.

EXTRACELLULAR VESICLES IMMUNE-PROFILING IN PLASMA AND CSF: A DIAGNOSTIC TOOL FOR PARKINSON'S DISEASE

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Objectives. Extracellular vesicles (EVs) play a central role in intercellular communication which is highly relevant for inflammatory processes implicated in neurodegenerative proteinopathies disorders, such as Parkinson's Disease (PD). Thus, we characterized and compared distinctive EV subpopulations from plasma and CSF of PD and atypical parkinsonisms (AP), with the aim to build a diagnostic model via artificial intelligence.
Methods. Plasmatic EVs were collected from 27PD, 19 matched healthy controls (HC), 9AP with multiple system atrophy (MSA) and 9AP with tauopathies (AP-Tau). CSF-derived EVs were collected from 4PD, 4MSA, 4AP-Tau. The expression of 37 EV-surface markers, related to inflammatory and immune cells, were measured by MACSPlex in plasm and CSF. Random forest (RF) diagnostic models based on EV markers expression were built via supervised machine learning algorithms.
Results. In plasma, 17 EV-derived markers resulted statistically different between HC and PD. Among these, 8 were overexpressed also in CSF of a subset of PD, 10 in a subset of MSA and 6 in a subset of AP-TAU. A RF model based on the 17 differentially expressed, EV-derived markers in plasma discriminates patients from HC with high sensitivity (100%) and specificity (83.3%). Integrating CSF-derived data a potentiated model was able to differentiate PD from not-PD patients with 96.6% of sensitivity and 88.0% specificity.
Conclusion. Multiple immune surface-markers profiling of EVs in plasma allows the stratification of patients with PD, MSA and AP-Tau. Furtherly it suggests a different immune dysregulation in PD and MSA vs. AP-Tau, to be confirmed by functional analysis in experimental models of disease.

MYELIN BASIC PROTEIN PHOSPHOLIPID COMPLEXATION LIKELY COMPETES WITH DEIMINATION IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS MOUSE MODEL

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Multiple sclerosis (MS) is a demyelinating disease with various components (immunologic, genetic and environmental). However, autoimmunogenicity against the host's myelin basic protein is a major contributor. Hyper-deimination (a post-translational modification) of myelin basic protein has been associated with MS. Protein-lipid interaction of myelin basic protein is an important part of maintaining a healthy myelin sheath, however this is an aspect of MS pathology that remains to be studied. Several biochemical methods, a capillary electrophoresis coupled system and mass spectrometry, were used in this study. These methods identified four specific phospholipids complexing with myelin basic protein. We demonstrate that lysophosphatidylcholine provides a robust competitive effect against hyper-deimination.

HDAC6 INHIBITION RESTORES TDP-43 PATHOLOGY AND AXONAL TRANSPORT DEFECTS IN HUMAN MOTOR NEURONS WITH TARDBP MUTATIONS

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TAR DNA binding protein 43 kDa (TDP-43) is the major component of pathological inclusions in most patients with sporadic and familial amyotrophic lateral sclerosis (ALS) and up to 50 % of patients with frontotemporal dementia (FTD). ALS patients suffer from progressive degeneration of motor neurons, while FTD is characterised by the progressive degeneration of cortical neurons in the frontal and anterior temporal lobes. Moreover, heterozygous missense mutations in the gene encoding TDP-43 are a rare cause of ALS. The aim of this study was to investigate whether mutant TDP-43-ALS iPSC derived motor neurons recapitulate aspects of TDP-43 pathology. We generated and characterized induced pluripotent stem cells (iPSCs) from ALS patients with different TARDBP mutations, as well as from three healthy controls and generated an isogenic control. In addition, we also created two different mCherry tagged cell lines starting from a mutant TDP-43 iPSC line, one with mCherry tagging the wild type TDP-43 and another with mCherry tagging mutant TDP-43. These iPSC lines were differentiated into motor neurons and we observed several changes in TDP-43 behaviour e.g. mislocalization to the cytoplasm, accumulation of insoluble TDP-43, C-terminal fragments and phospho-TDP-43. Furthermore, at functional level a defect in mitochondrial motility was noted in motor neurons with a TARDBP mutations compared to control lines. Pharmacological inhibition of histone deacetylase 6 (HDAC6) restored the observed TDP-43 pathologies and the axonal mitochondrial motility in patient-derived motor neurons linking TDP-43 pathologies with axonal mitochondrial transport defects, suggesting that HDAC6 inhibition may be an interesting target for neurodegenerative disorders linked to TDP-43 pathology.

LIPID-MEDIATED COUPLING OF MITOCHONDRIAL FUNCTION AND TAU DEGRADATION

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The early factors that drive accumulation of hyperphosphorylated Tau in Alzheimer's disease (AD) are poorly understood. Alterations in energy metabolism and mitochondrial function have been implicated as early pathogenic events in AD. Using high-throughput drug screening in human iPSC-derived neurons we have recently shown that excess cholesterol is an early, druggable, and Amyloid-beta independent driver of Tau accumulation in human neurons. Here we describe a novel pathway in which cholesterol acts as a metabolic switch that couples mitochondrial function and neuronal energy levels to proteasomal degradation of (phosphorylated) Tau. We show that in human neurons, mitochondrial energy production is tightly coupled to Tau turnover and that partial inhibition of mitochondrial respiration -by existing FDA-approved drugs- can be exploited to enhance Tau degradation both in *in vitro* and *in vivo* models of AD. Overall, our data provide novel mechanistic insight into the coupling of neuronal energy metabolism and Tau turnover in human neurons, while providing novel therapeutic candidate molecules for the treatment of AD.

A STUDY OF THE FUNCTIONAL PARTNERS OF MAX, A NEUROPROTECTIVE TRANSCRIPTION FACTOR IN RAT RETINAL GANGLION CELLS

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Introduction: Previous studies in our laboratory showed that the overexpression of Max protein into retinal ganglion cells was neuroprotective after optic nerve crush and subacute ocular hypertension model. Max is a member of the Myc/Max/Mad network that comprises a group of transcription factors whose distinct interactions result in gene-specific transcriptional activation or repression. There is also a Myc-Max family branch, composed of the Mlx-MondoA and Mlx-MondoB heterodimers, that act in the cellular metabolic adaptation. All members of this network form heterodimers with Max, permitting binding to specific DNA sequences. These heterodimers are present in different ratios depending on the cellular context, having a classical role in the proliferation or differentiation processes. The equilibrium between the Myc-Max, Mad-Max, Mlx-Mondo dimers is extremely important for the cellular gene expression context and its consequences. Additionally, Max also can form homodimers, however, formation of heterodimers among the other members of the family is more predominant. As a part of our neuroprotective study, it is important to understand the functional role of Myc/Max/Mad/Mlx network in the retinal ganglion cells in order to further understand which family members interact with Max to promote neuroprotection.
Aim: Describe functional partners of the transcription factor Max in the adult retinal ganglion cells.
Method: We used adult Lister Hooded rats (CEUA #083/17). We initially screened the adult retinal tissue for the expression of the 15 members of Myc-Max-Mad-Mlx family, using RT-qPCR. We collected rat retinas at 30 postnatal day, extracted the RNA to perform RT-qPCR for: c-Myc, N-Myc, Max, Mad1, Mad2, Mad3, Mad4, Mnt, Mga, Sin3A, Sin3B, Mlx, Mlx-ip e Mlx-ipl. For RT-qPCR data analysis we used 2-($\Delta\Delta$Ct) method. Based on RT-qPCR results, we used immunohistochemistry technique and confocal microscopy in order to localize the proteins in the different retinal cell types, with special attention to the ganglion cell.
Results and Conclusion: The expression of c-Myc, n-Myc, Mad3, MondoA e MondoB were almost undetectable. Only Mad2 e Mad4 had the expression level similar to Max. Mad1, MNT, MGA, Sin3A, Sin3B and MLX had the expression level below Max, but detectable, suggesting that these proteins might be expressed in the retina. Among the few antibody tested for immunohistochemistry, we were able to detect Sin3B, Mlx and Mnt in the ganglion cell layer, as observed with Max expression. Additional experiments are needed to determine the immunolocalization of Mad2 and Mad4, since they showed expression level similar to Max.

LONGITUDINAL ASSESSMENT OF TAU PET IMAGING AND ITS CORRELATION WITH NEUROPATHOLOGY AND CLINICAL SIGNS PROGRESSION.

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Alzheimer's disease (AD) and other associated dementias remain a consistent and unruly problem for the aging population and health. As the world's population increases, so does the prevalence of age-related dementias. The neuropathology of AD is characterized by the extracellular deposition of beta-amyloid protein (Aβ) and the formation of intraneuronal neurofibrillary tangles (NFT) composed of hyperphosphorylated tau (ptau), along with neuroinflammation and neuronal loss that ultimately induces to noticeable cognitive impairments. Abnormal ptau leads to the formation of insoluble, beta-sheet rich amyloid aggregates in tauopathies such as AD. Positron emission tomography (PET) imaging is a promising avenue that may identify tau aggregates in vivo cross-sectionally and longitudinally in various dementia conditions. The goal of this study is to characterize the longitudinal assessment of the tau tracer 18F-THK5351 by in vivo tau PET imaging concomitantly to behavior and tau pathology by histology and biochemistry from 6 to 12 months of age in tau transgenic P301S mice, a mouse model of tauopathies. Our results demonstrate an augmentation of overall gross brain tau pathology by in vivo PET imaging in P301S mice compared to age-matched wild-type (WT) animals accompanied by P301S-model associated pathological tau and phenotypic and behavioral deficits. This longitudinal study provides new insights on the relationship between imaging diagnostic tools, the in vivo neuropathological temporal pattern and the clinical signs observed in animal models of AD that could benefit early disease diagnosis.

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ENTORHINAL CORTEX WOLFRAMIN-1-EXPRESSING NEURONS PROPAGATE TAU TO CA1 NEURONS AND IMPAIR HIPPOCAMPAL MEMORY

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In early stages of Alzheimer's Disease (AD), phosphorylated tau propagates from layer II of the entorhinal cortex (ECII) to the CA1 in the hippocampus. Previous animal models, however, primarily exhibited tau spread to the dentate gyrus (DG) in the hippocampus, where tau pathology does not appear until later stages of AD. This is due to the expression of P301L MAPT mutant in neuropsin-1$^+$ stellate neurons in ECII, which connect to the DG via the perforant path. Wolframin-1-expressing (Wfs1$^+$) pyramidal neurons in ECII connect to the CA1 region via temporo-ammonic pathway directly or indirectly via interneurons in the stratum lacunosum-moleculare. We hypothesized that Wfs1$^+$ neurons in ECII could mediate tau propagation to the CA1 and mimic early stages of tau pathology in AD. Wfs1-Cre mice at 4-6 months of age were injected with Cre-inducible AAV2/6-Flex-P301Ltau expressing human P301L tau mutant or AAV2/6-Flex-TdTomato in ECII to specifically express mutant tau in Wfs1$^+$ neurons. At 4 weeks post-injection, the mice were euthanized for immunohistochemistry, electrophysiology and electron microscopy or underwent trace fear conditioning behavioral test. The functional effect of tau on these connections was assessed by multielectrode array to evaluate light-evoked CA1 neuronal firing responses after optogenetic stimulation of Wfs1$^+$ ECII axons and by measuring the chemogenetic activation of CA1 pyramidal neurons. Wfs1-Cre mice injected in ECII with AAV2/6-Flex-P301Ltau displayed significant human tau positivity in CA1 pyramidal neurons but not in DG at 4 weeks post-injection. Electron microscopy revealed a synaptic connection between ECII Wfs1$^+$ axons and CA1 dendrites, and revealed the presence of human tau in pre- and post-synaptic elements. Whole cell patch clamp recordings of CA1 pyramidal neurons showed reduced measures of excitability. Multielectrode array recordings of optogentically stimulated Wfs1$^+$ axons resulted in a reduced CA1 neuronal firing and chemogenetic activation of CA1 neurons showed a reduction in c-fos$^+$ cells in CA1 after AAV2/6-Flex-P301Ltau injection. Trace fear conditioning revealed deficits in trace and contextual memory in the AAV2/6-Flex-P301Ltau injected mice. Expression of P301Ltau in Wfs1$^+$ neurons in ECII spreads tau specifically to CA1 pyramidal neurons, and is accompanied by several measures of neurophysiological impairment, including reduced neuronal excitability and deficits in contextual memory.

ENDO-LYSOSOMAL SORTING AND TRAFFICKING PATHWAYS IN AXONS DRIVE THE BIOGENESIS OF MISFOLDED PRION PROTEIN ENDOGGRESOMES

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Neurons are vulnerable to intra-axonal protein aggregate formation, a pathological feature of neurodegenerative diseases. Understanding how such aggregates are formed is key to delineating neuronal vulnerability. Previous work identified aggresomes, IPODs and JUNQ as structures formed by cytosolic misfolded/aggregated proteins. However, how misfolding-prone proteins that transit within the endomembrane system aggregate, remains unexplored.

By analyzing the trafficking of a mutant GPI-anchored prion (PrP) that misfolds and is involved in human prion disease, we uncovered novel endo-lysosomal trafficking pathways that impose different fates on mutant PrP in neurons: one pathway drives mutant PrP for immediate lysosomal degradation in the soma, the other shuttles mutant PrP into the axon for aggregation in late endosomal structures which we've termed endoggresomes. We uncovered the mechanisms of selective endoggresome formation in axons: the small GTPase Arl8b associates with post-Golgi vesicles harboring misfolded mutant PrP, and recruits kinesin-1 and the homotypic fusion and protein sorting (HOPS) complex onto these vesicles, marking them for axonal entry, homotypic fusion, and aggregation via a mechanism we call **a**xonal **r**apid **e**ndosomal **s**orting and **t**ransport-dependent **a**ggregation (ARESTA). Axonal endoggresomes persist due to impaired retrograde transport and decreased axonal degradative capacity, resulting in calcium intake defects and accelerated neuronal death. Remarkably, endoggresome formation can be circumvented by depletion of the ARESTA component kinesin-1, revealing a dual role of transport in the biogenesis, as well as the maintenance of these aggregates. Our findings identify the endo-lysosomal system as a key player in the discriminating itinerary of misfolded mutant PrP that drive opposing forces in the same neuron: to protect the soma but also to render axons susceptible to endoggresomes formation, a new type of aggregate structure that forms uniquely inside endosomal compartments and is associated with neuronal toxicity. The identification of the Arl8b/kinesin-1/HOPS ARESTA pathway provides an actionable anti-aggregation target, as it can modulate neuronal dysfunction in prionopathies. As many other aggregation-prone proteins transit through the endomembrane system, we posit that endoggresomes and ARESTA are a common feature of various proteinopathies, a hypothesis under investigation.

AGED MICE CARRIERS OF A RECESSIVE NEURODEGENERATIVE DISORDER NIEMANN-PICK TYPE C SHOW TAU HYPERPHOSPHORYLATION, ENDOLYSOSOMAL DYSFUNCTION AND NEUROINFLAMMATION

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Niemann-Pick type C disease (NPC) is a rare, recessive, fatal, lipid storage disorder caused by mutations in *NPC1* or *NPC2* genes. *NPC1/NPC2* code for cholesterol transport proteins. Their dysfunction leads to accumulation of free cholesterol and other lipids within late endosomes/lysosomes. NPC disease is characterized by progressive neruodegeneration (of primarily Purkinje neurons in the cerebellum) and neuroinflammation (activation of astrocytes and microglia). The carriers of NPC disease are considered healthy, assuming that heterozygous *NPC1/NPC2* mutations do not cause any symptoms. The goal of this work was to analyze whether the brains of *NPC1* heterozygous mice show any pathological features of NPC disease, including accumulation of cholesterol, tau hyperphosphorylation, neurodegeneration and neuroinflammation. We hypothesized that the aged carriers of *NPC1*-mutant allele present several pathological features of NPC disease. To test this, we used the BALB/cNctr-*Npc1*$^{N/+}$ mice (NPC1$^{+/-}$, Jackson Laboratory, Bar Harbor, Maine, USA). Female and male NPC1$^{+/-}$ mice were mated to generate NPC1$^{+/+}$ (wt, control) and to maintain NPC1$^{+/-}$ mice. The brains (cortex, hippocampus and cerebellum) of 40-, 60-, 80- and 100-weeks old NPC1$^{+/-}$ and NPC1$^{+/+}$ mice were isolated and analyzed by western blotting and immunohistochemistry. Our results show that aged (60- and 100-weeks old) NPC1$^{+/-}$ mice show several key pathological features of NPC disease compared to aged-matched controls, including altered endolysosomal pathway, neuroinflammation and differences in tau hyperphosphorylation pattern. The analysis of younger NPC1$^{+/-}$ mice is underway to identify the earliest changes upon loss of single *NPC1* allele. Our findings indicate that carriers of the autosomal recessive NPC disease may not be considered healthy, suggesting that human heterozygous *NPC1* mutation may be a risk factor for neurodegenerative disorders in the aged population.

LONGITUDINAL ANALYSIS OF CSF FOR AMYOTROPHIC LATERAL SCLEROSIS BIOMARKERS

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A majority of ALS clinical trials fail, presumably due to the highly complex disease etiology resulting in a heterogeneous patient population that may not respond to drugs that target a specific pathogenic mechanism. Enriching these patients into subsets would aid in identifying those that could benefit from specific therapeutic approaches. Many studies have used clinical measures of disease progression based on the change in the ALS functional rating scale revised (ALSFRS-r) to segregate patients into fast progressing (FP) and slow progressing (SP) ALS, and then used biofluid samples to identify candidate biomarkers. However, the majority of these studies are cross-sectional and therefore a temporal response of many biomarkers has not been explored. In this study, we sought to identify pathways and candidate biomarkers that distinguish FP versus SP ALS over time. We employed shotgun mass spectrometry based proteomics on longitudinally collected cerebrospinal fluid (CSF) obtained from 13 ALS patients. Overall, we identified 1148 proteins in the CSF of all ALS patients in this study. Proteins related to acute phase responses, complement, and coagulation cascades were significantly higher in FPs as compared to SPs including complement C3, C4 and C5, coagulation factor F12, RBP4 and members of the SERPINA family. Proteins related to synaptogenesis were significantly higher in SPs including and ephrins and neurexins. Longitudinal analysis revealed a panel of 59 candidate markers that could segregate FP and SP ALS. Based on multi-variate analysis, we determined three biomarkers, F12, RBP4, and SERPINA4, as optimal candidates that segregate ALS patients based on disease progression rate and these proteins are currently being examined in a separate validation cohort. Overall, we identified pathways and candidate biomarkers that can segregate the patient population based on disease progression rate. These biomarkers also demonstrate distinct pathways that are modulated in FP versus SP patients and new therapeutic targets.

NOX-DEPENDENT ZNRF1 ACTIVATION INITIATES NEURITE DEGENERATION

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Oxidative stress is a well-known inducer of neurite degeneration. Nicotinamide adenine dinucleotide phosphate (NAPDH) oxidase (NOX) is an enzyme complex with a function of producing reactive oxygen species (ROS). Of the NOX subunits, p47 plays a central role as the organizer and translocator of the subunits for activation. We previously showed that the E3 ubiquitin ligase ZNRF1 promotes Wallerian degeneration by degrading AKT via the ubiquitin proteasome system. We also found that NOX activity is required for the EGFR-dependent phosphorylation-induced activation of ZNRF1. We herein demonstrate that phosphorylation of p47 is a molecular switch which initiates neurite degeneration. We found that phosphorylation of p47 is a key step required for its interaction with ZNRF1, and overexpression of non-phosphorylated p47 decreases injury-induced NOX-dependent ROS production in degenerating neurites and significantly delays neurite degeneration. These findings suggest the pathophysiological significance of molecular interaction between ZNRF1 and p47 in the regulation of neurite degeneration.

ASSESSING STIMULATION-DEPENDENT CHANGES IN LRRK2 AND GCASE EXPRESSION/ACTIVITY AND CONVERGENCE AT THE LYSOSOME IN CRYOPRESERVED MONOCYTES

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Both LRRK2 and GCase are promising targets for the treatment of Parkinson's disease (PD). Evidence suggests that both proteins are involved in similar biological pathways. For example, both are highly expressed in monocytes and have been implicated in both immune and lysosomal. Given this high degree of overlap, there is now substantial interest in determining if both enzymes may converge on the same biological pathways, specifically the lysosomal pathway, to mediate PD risk. However, studies to date have largely investigated the enzymes in isolation and any relationship between LRRK2 and GCase remains unclear.
Recent data suggest that it is the response to immunological challenges that is altered in PD and that circulating baseline cytokine levels are not useful diagnostic or predictive biomarkers in PD. Taken together, this project aims to optimize a blood collection and cryopreservation protocol to facilitate the collection and study of monocytes from PD patients and healthy controls, with a particular focus on measuring stimulation-dependent changes in LRRK2 and GCase protein activity levels and lysosomal function.

Given that the vast majority of biorepositories and future biomarker studies will need to utilise cryopreserved patient peripheral blood mononuclear cells (PBMCs), we assessed the effects of cryopreservation on LRRK2 and GCase expression/activity levels both at baseline and in a stimulation-dependent manner in healthy controls. Despite cryopreservation having a small effect on these read-outs at baseline, both fresh and cryopreserved monocytes gated from total PBMCs exhibited comparable changes in response to IFN-γ. Interestingly we observed that LRRK2 kinase inhibition decreases cathepsin activity in monocytes. However, this is only observed in monocytes co-treated with IFN-γ. This is coupled with increased LRRK2 expression and alterations in immune cell activation with LRRK2 kinase inhibition. Similarly, an increase in GCase activity levels were observed in monocytes upon IFN-γ treatment.

These results show that cryopreservation of PBMCs doesn't effect our ability to measure stimulation-dependent changes in both LRRK2 and GCase expression/activity levels. These assays are therefore to be repeated in idiopathic PD PBMCs and compared to those from healthy controls. Furthermore, these results suggest a fundamental role of LRRK2, specifically LRRK2 kinase activity, in lysosomal function in immune cells. Concomitant changes in both lysosomal activity and immune cell activation suggests that these two pathways may intersect, with LRRK2 regulating the two. Given the parallel increases in GCase activity, future research aims to investigate how these two enzymes interact and converge on biological pathways regulating inflammation and the lysosome in patient monocytes.

DROSOPHILA GBA IS EXPRESSED IN GLIA WHERE IT METABOLIZES GLCCER GENERATED BY ACTIVE NEURONS

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Disrupted sphingolipid metabolism has been implicated in lysosomal storage diseases (LSD). Gaucher disease (GD), the most prevalent LSD, is caused by recessive variants in *GBA*. *GBA* encodes a lysosomal enzyme that hydrolyses glucosylceramide (GlcCer) into glucose and ceramide. However, the role of GlcCer in the pathogenesis of GD has not been well characterized.

To study the function of fly homolog *Gba1b* in *Drosophila*, we used CRISPR-cas9 technology to insert an *SA-T2A-GAL4-polyA* cassette in the 3rd intron of the *Gba1b* locus (*Gba1b*$^{T2A\text{-}Gal4}$). The *polyA* arrests the transcription of *Gba1b* and generates a severe loss of function allele, which was confirmed by RT-PCR. Interestingly, GlcCer accumulates mainly in glia in an activity-dependent manner in *Gba1b*$^{T2A\text{-}Gal4}$ mutants. To assess the expression pattern of *Gba1b*, we used *Gba1b*$^{T2A\text{-}Gal4}$ to drive *UAS-mCherry.NLS*. Surprisingly, *Gba1b* is expressed in glia, but not in neurons. This is consistent with human transcriptome data that *GBA* is expressed more in astrocytes than neurons. The glial specific expression of *Gba1b* was confirmed by G-Trace, a technique used to trace a gene expression throughout development. Also, glial specific knockdown of *Gba1b* causes activity-dependent loss of synaptic activity in photoreceptor neurons, yet no loss is not observed upon neuronal knockdown. Moreover, ultrastructural studies revealed that loss of *Gba1b* leads to vacuolized glia that precede the demise of neurons. Furthermore, neuronal, but not glial knockdown of *GlcT*, a gene that produces GlcCer, reduces GlcCer levels, suggesting a cell non-autonomous process in which GlcCer is synthesized in neurons and transported to glia for lysosomal degradation. Finally, neuronal knockdown of homologs of the human GlcCer transporters, *ABCA12* or *ABCC1*, reduces GlcCer levels, suggesting that these ABC transporters are involved in GlcCer transport from neurons to glia.

Based on our data, we propose a model in which *Gba1b* is primarily expressed in glia. Upon activation of neurons, GlcCer is synthesized in the neurons by *GlcT*. The excess amount of GlcCer is then transported by ABC transporters to glia, where it is hydrolyzed by *GBA/Gba1b*. Loss of *GBA/Gba1b* causes the accumulation of GlcCer in glia and neurons and impairs the function of glia to support neuronal survival. This in turn leads to the activity-dependent demise of neurons.

ROLE OF IL-33/ST2 SIGNALING IN HOMEOSTATIC SYNAPTIC PLASTICITY IN THE HIPPOCAMPUS

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Hippocampal synaptic plasticity is important for learning and memory. In particular, homeostatic synaptic plasticity is induced upon prolonged changes in neuronal activity to maintain network homeostasis. Dysfunction of homeostatic synaptic plasticity might contribute to cognitive impairment in neurodegenerative diseases such as Alzheimer's disease. Emerging evidence suggests that immune molecules are important modulators of synaptic development and plasticity. Interleukin (IL)-33 is a cytokine that mediates both innate and adaptive immunity. We previously reported that IL-33 rescues synaptic plasticity deficits and cognitive impairment in an Alzheimer's disease transgenic mouse model. However, the mechanisms that underlie the beneficial effect of IL-33 on the hippocampal synaptic dysfunction in AD are not well understood. Here, we combined immunochemical and electrophysiological techniques to examine the physiological role of IL-33 in hippocampal synaptic plasticity. We found that blockade of neuronal activity by tetrodotoxin (TTX) triggered the release of IL-33 from cultured hippocampal cells. Moreover, inhibition of IL-33 signaling attenuated activity blockade-induced homeostatic plasticity in cultured hippocampal neurons, as indicated by the abolishment of TTX-induced increase in excitatory synaptic transmission in neurons treated with soluble ST2 (sST2), a decoy receptor for IL-33. Furthermore, IL-33 administration increases excitatory synapses and neurotransmission, while knockdown of IL-33 receptor complex, ST2, or IL-1RAcP, abolished this enhancement, indicating that IL-33 mediates homeostatic plasticity via signaling dependent on ST2/IL-1RAcP in hippocampal neurons. These findings demonstrate that IL-33/ST2 signaling is essential for synaptic homeostasis in the hippocampus.

THE ROLE OF ASTROCYTES IN SMA MOTOR NEURON SYNAPTIC DEFECTS

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Spinal muscular atrophy (SMA) is a neurodegenerative disease primarily characterized by the loss of lower spinal motor neurons. Evidence suggests that survival motor neuron (SMN) protein deficiency in other cell types, including astrocytes, also contributes to SMA pathology. We and others have previously demonstrated that astrocytes derived from patient induced pluripotent stem cells (iPSCs) show SMN-dependent intrinsic defects. In addition to providing neurotrophic support, astrocytes play an important regulatory role in synapse development and function. Motor neuron peripheral and central synaptic defects have been previously described and restoration of SMN expression in motor neurons can improve synaptic integrity, but only to a certain extent. It remains to be fully determined how human glial cells contribute to SMA synapse pathogenesis.

Differential expression analysis of our iPSC-derived astrocyte RNA seq data demonstrates a significant downregulation of genes associated with synaptic transmission and plasma membrane cell projections in patient-derived samples. Using the SurfaceGenie prediction tool, we verified many of these downregulated genes are likely to encode cell surface proteins. A large number of genes are associated with regulation of ion gradients (K+ ion channels, Ca2+ regulatory channels), neurotransmitter release (glutamate receptors and transporters) and synaptic formation and integrity (ephrins, cell adhesion proteins). We therefore hypothesize that SMA astrocytes may lack important synaptic-related cell surface genes and their encoding proteins, which could contribute towards motor neuron synaptic defects. We will further characterize abnormal cell surface protein expression in SMA astrocytes using cell surface capture mass spectrometry and investigate the downstream molecular and functional implications in our in vitro co-culture system using super resolution microscopy and multi-electrode array (MEA) approaches. Preliminary MEA data from SMA iPSC-derived motor neuron cultures already suggests an intrinsic defect in mean firing rate and we will investigate if SMA astrocytes could further compound this synaptic deficit.

CELL TYPE-SPECIFIC HISTONE ACETYLATION PROFILING OF ALZHEIMER'S DISEASE SUBJECTS AND INTEGRATION WITH GENETICS

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We profile genome-wide histone 3 lysine 27 acetylation (H3K27ac) of 3 major brain cell types from hippocampus and dorsolateral prefrontal cortex (dlPFC) of subjects with and without Alzheimer's Disease (AD). We confirm that single nucleotide polymorphisms (SNPs) associated with late onset AD (LOAD) show a strong tendency to reside in microglia-specific gene regulatory elements. Despite this significant colocalization, we find that microglia harbor more acetylation changes associated with age than with amyloid-β (Aβ) load. In contrast, we detect that an oligodendrocyte-enriched glial (OEG) population contains the majority of differentially acetylated peaks associated with Aβ load. These differential peaks reside near both early onset risk genes (APP, PSEN1, PSEN2) and late onset AD risk loci (including BIN1, PICALM, CLU, ADAM10, ADAMTS4, SORL1, FERMT2), Aβ processing genes (BACE1), as well as genes involved in myelinating and oligodendrocyte development processes. Interestingly, a number of LOAD risk loci associated with differentially acetylated risk genes occupy H3K27ac peaks that are specifically enriched in OEG. These findings implicate oligodendrocyte gene regulation as a potential mechanism by which early onset and late onset risk genes mediate their effects, and highlight the deregulation of myelinating processes in AD. More broadly, our dataset serves as a resource for the study of functional effects of genetic variants and cell type specific gene regulation in AD.

DNA DAMAGE REPAIR IN ALZHEIMER'S DISEASE

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Alzheimer's Disease (AD) is an incurable neurodegenerative disease characterized by toxic amyloid beta (Aβ) oligomers and neuronal cell loss. The ultimate cause of neuronal loss has not been determined, but has been correlated to studies showing extensive oxidative damage in specific brain regions where Aβ oligomers are abundant. These Aβ oligomers have been shown to reduce levels of critical DNA repair proteins and inhibit their recruitment to DNA damage sites. The accumulation of unrepaired DNA damage has been detected extensively in AD brains. However, the method of this accumulation has not been demonstrated. Here we utilized a human neuronal progenitor cell line (ReN GA2) to investigate whether oxidative DNA damage, specifically DNA double strand breaks (DSBs), lead to Aβ production, contributing to dysregulation of DNA damage repair. We found that treatment with the topoisomerase II inhibitor etoposide, which induces DNA double strand breaks, led to significantly increased secretion of Aβ40 and Aβ42. These novel results indicate that direct DNA damage leads to the increased production of neurotoxic Aβ secretion, contributing to the DNA repair dysregulation, accumulation of unrepaired DNA, and eventual neuronal loss characteristic of AD.

LOSS OF TMEM106B POTENTIATES LYSOSOMAL AND FTLD-LIKE PATHOLOGY IN PROGRANULIN DEFICIENT MICE

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Heterozygous mutations in the progranulin (GRN) gene cause frontotemporal lobar degeneration (FTLD) with TAR DNA-binding protein-43 (TDP-43) positive inclusions, while a total loss of progranulin (PGRN) protein results in neuronal ceroid lipofuscinosis (NCL), a neuronal lysosomal storage disease. Aged mice with complete loss of PGRN expression recapitulate features of both FTLD and NCL.
In FTLD-TDP cases, particularly in patients with GRN mutations, TMEM106B has been identified as a major disease modifier. TMEM106B is an endo-lysosomal protein and is crucial for the regulation of lysosomal size, morphology, acidification and subcellular trafficking.
We and others could show that, in mice, TMEM106B deficiency results in impaired lysosomal acidification, reduced lysosomal enzyme activities and altered axonal transport accompanied by an appearance of LAMP1 positive vacuoles. Together, this caused an accumulation of lipofuscin and p62 positive autophagosomes in neurons.
How PGRN and TMEM106B are synergistically linked and if a gain or a loss-of-function of TMEM106B is responsible for the increased disease risk of patients with GRN haploinsufficiency is still unclear.
We therefore compared behavioral features, gene expression, lysosomal activity, protein aggregation and TDP-43 pathology in single and double Grn and Tmem106b knockout animals as well as Grn+/–/Tmem106b–/–. Grn–/–/Tmem106b–/– mice showed a strongly reduced life span and an early onset of massive motor deficits. Gene expression analysis revealed an upregulation of molecular signatures characteristic for disease associated microglia and autophagy dysregulation. Accelerated microgliosis and astrogliosis occurred exclusively in young Grn–/–/Tmem106b–/– mice. A dysregulated maturation of lysosomal hydrolases as well as an accumulation of ubiquitinated proteins and widespread p62 deposition suggest an impairment in proteostasis. Moreover, while single Grn–/– knockouts only occasionally show TDP-43 pathology, the double knockout mice exhibit deposition, processing and phosphorylation of TDP-43. Thus, a loss-of-function of TMEM106B may enhance the risk for GRN-associated FTLD by a reduced protein turnover in the lysosomal/autophagic system.

WESTERN DIET ACCELERATES NEUROINFLAMMATION AND AMYLOIDOGENESIS IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is an aging-dependent, irreversible neurodegenerative disorder and the most common cause of dementia. The prevailing AD hypothesis points to the central role of formation of toxic amyloid-β (Aβ) and hyperphosphorylated tau deposits in the brain. The lack of efficient AD treatments stems from incomplete knowledge on AD causes and environmental risk factors. The role of lifestyle factors, including diet, in neurological diseases is now beginning to attract considerable attention. One of them is western diet (WD), which leads to many serious diseases that develop with age.

WD is a pattern of nourishment characterized by ultra-processed foods with a combination of simple carbohydrates, saturated fatty acids, and cholesterol. WD induces such metabolic disorders as obesity, hypercholesterolemia, diabetes and non-alcoholic fatty liver disease (NAFLD). All these systemic alterations cause a whole-body inflammatory state, which impacts brain functions.

The aim of the study was to investigate whether WD-derived metabolic and inflammatory systemic impairments accelerate the brain neuroinflammation and amyloidogenesis at the early stages of AD development.

To verify this hypothesis, transgenic mice expressing human APP with AD-causing mutations (APPswe) were fed with WD from the 3rd month of age. These mice were compared to APPswe mice, in which low-grade systemic inflammation was induced by injection of lipopolysaccharide (LPS) and to untreated APPswe mice. To follow the sequence of mechanisms leading to diet-induced neurodegeneration, all experimental subgroups of animals were subsequently analyzed at 4-, 8- and 12- months of age. APPswe mice 4- and 8-months old represent earlier pre-symptomatic stages of AD, while 12-months animals represent later stages of AD, with visible amyloid pathology.

Just short time of WD feeding induced in 4-months old animals such brain neuroinflammation events as enhanced astrogliosis, to a level comparable to that induced by the administration of LPS, and microglia activation in 8-months old mice. Also, WD feeding accelerated Aβ production and tau phosphorylation, observed already in 8-months old animals. These brain changes corresponded to diet-induced metabolic disorders, such as hypercholesterolemia in 4-months, and hyperglycaemia and NAFLD in 8-months old mice.

These results indicate that the westernized pattern of nourishment is an important modifiable AD risk factor, and a healthy, balanced diet may be one of the most efficient AD prevention methods.

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USING TRACER METABOLOMICS TO INVESTIGATE THE EFFECTS OF APOE ON CEREBRAL GLUCOSE METABOLISM.

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Background: Apolipoprotein E, encoded by the APOE gene, is present in humans as three main isoforms (E2, E3, and E4). E4 carriers face up to a 15-fold greater risk for developing late-onset Alzheimer's disease (AD), while E2 carriers are protected. A hallmark of AD is cerebral glucose hypometabolism as defined by decreased 18FDG-PET signal. Interestingly, a similar pattern of glucose hypometabolism is also observed in young, cognitively normal E4 carriers. Although this phenomenon has been well described over the past two decades, information regarding the specific cell types responsible for this reduction and the intracellular fate of cerebral glucose in the E4 brain are unclear. We hypothesized astrocytes are primarily responsible for reduced glucose uptake associated with E4 and suspect mitochondrial dysfunction impairs glucose metabolism.
Methods: Mitochondrial respiration was measured in immortalized astrocytes expressing human E2, E3, or E4 using Seahorse XF96 Glycolysis Stress Test. Glucose metabolism was measured in vitro using tracer metabolomics via a [U-13C] glucose supplemented growth media. In vivo brain metabolism in human APOE mice (E2, E3, or E4) was examined using an oral gavage of [U-13C] glucose. Cell culture and brain metabolites were analyzed using mass spectrometry to detect 13C enrichment.
Results: E4 astrocytes show significantly less oxygen consumption relative to E2 or E3. E4 astrocytes exhibit decreased glucose flux into early glycolysis (G6P and F6P) relative to E2 or E3. The increased fraction of partly labeled G6P and F6P in E4 indicate contributions from the pentose phosphate pathway (PPP). Conversely, glucose flux into pyruvate, lactate, and TCA cycle were increased in E4. TCA metabolites reflect more pyruvate carboxylase (PC) activity relative to pyruvate dehydrogenase (PDH) in E4, opposing E2 or E3. De novo synthesis of nucleotides, glutathione and phospholipid species were also increased in E4. Finally, cortical tissue from E4 mice demonstrate similar decreases in glucose flux through glycolysis and increased TCA cycle enrichment.
Conclusion: The alterations in glucose utilization by E4 may be driven by decreased efficiency of the TCA cycle. Increased TCA enrichment suggests carbon is not being oxidized and is instead incorporated into amino acids for biosynthesis of nucleotides, glutathione, and de novo lipid synthesis. Higher PC:PDH activity in E4 replenishes TCA cycle carbon lost to amino acid synthesis. Increased PPP flux in E4 provides precursors for biosynthesis pathways while also generating reducing equivalents to compensate for the lack thereof from the TCA cycle. Identifying how E4 affects brain metabolism may illuminate specific enzymes as potential targets for development of preventative therapies to mitigate the risk of AD for E4 individuals.

AGE-RELATED NEUROPROTECTION BY DIETARY RESTRICTION REQUIRES OXR1-MEDIATED RETROMER FUNCTION

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Dietary restriction (DR) is the most robust method to delay aging and the onset of neurodegenerative disorders across multiple species, though the mechanisms behind this phenomenon remain unknown. To elucidate how DR mediates lifespan extension, we analyzed natural genetic variants that associate with increased longevity under DR conditions in the Drosophila Genetic Reference Panel (DGRP) strains. We found that neuronal expression of a gene called mustard (mtd) in Drosophila, whose ortholog is known as Oxidation resistance 1 (OXR1) in other organisms, is necessary for DR-mediated lifespan extension. Neuronal RNAi for OXR1 also prevented the DR-associated slowing of age-related visual decline but not physical activity decline, arguing for a specific role of OXR1 in specific forms of neurodegeneration. Further, overexpression of the TLDc domain in human OXR1 is sufficient for extension of lifespan in a diet-dependent manner. Studies from Accelerating Medicines Partnership – Alzheimer's Disease (AMP-AD) network show that OXR1 protein levels are reduced in brains of patients with Alzheimer's disease (AD), and we found that overexpression of human OXR1 is protective in AD fly models, as well as in neuronal stem cells from Huntington's disease (HD) patients. In seeking the mechanism by which OXR1 protects age-related neuronal decline, we discovered that it provides a necessary function in regulating the neuronal retromer complex, which is essential for the recycling of transmembrane receptors. We discovered that OXR1 deficiency can be rescued by genetic or pharmacological enhancement of retromer protein expression. Understanding how OXR1 functions could help uncover novel mechanisms to slow neurodegeneration and extend healthspan across species.

APOLIPOPROTEIN E TARGETS CYTOPLASMIC LIPID DROPLETS IN RESPONSE TO LIPID ACCUMULATION IN ASTROCYTES

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Apolipoprotein E (ApoE) is the principal vehicle of intercellular lipid trafficking in the central nervous system. Astrocytes secrete ApoE-containing lipoprotein particles carrying cholesterol and unsaturated phospholipids. Neurons take up these lipoproteins via endocytosis and use the lipids provided by astrocytes to build vast membranes and synapses. ApoE can also transport lipid peroxides from neurons under oxidative stress and shuttle them back to astrocytes, where they accumulate in cytoplasmic lipid droplets (LDs), as a neuroprotective mechanism. The importance of ApoE in the central nervous system is underlined by its association with Alzheimer's Disease (AD). The APOE gene is the strongest genetic risk factor for sporadic late-onset AD. Individuals with the APOE4 variant have an increased risk of developing AD later in life compared to those who possess the APOE3 variant. However, little is known about the trafficking of ApoE in astrocytes or how it impacts intracellular lipid metabolism. We have discovered that, in response to lipid accumulation in astrocytes, ApoE targets to the cytoplasmic surface of lipid droplets (LDs) rather than progressing through the secretory pathway. Live-cell imaging and fluorescence recovery after photobleaching (FRAP) experiments demonstrate that ApoE protein translocates from the ER lumen to LDs at ER-LD contact sites. The C-terminal domain of ApoE is responsible for LD binding, while the N-terminal domain is necessary for efficient targeting of ApoE to the cytosolic compartment. Mutations of positively charged residues in the N-terminal domain of ApoE, including the rare AD-protective Christchurch variant of ApoE, increase targeting to LDs. Together, these results indicate that ApoE can target LDs within astrocytes instead of being secreted and may play a role in regulating the balance between lipid secretion and intracellular lipid storage and metabolism.

TREM2 INTERACTS WITH TDP-43 AND MEDIATES MICROGLIAL NEUROPROTECTION AGAINST TDP-43-RELATED NEURODEGENERATION

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Triggering receptor expressed on myeloid cell 2 (TREM2) is a surface receptor that, in the central nervous system, is exclusively expressed on microglia. TREM2 variants have been linked to increased risk for neurodegenerative diseases, but the functional effects of microglial TREM2 remain largely unknown. To this end, we used a mouse model of TAR-DNA binding protein 43 kDa (TDP-43)-related neurodegenerative disease via viral-mediated expression of human TDP-43 protein (hTDP-43). We found that TREM2 deficiency impaired microglia phagocytic clearance of pathological TDP-43, and enhanced neuronal damage and motor function impairments. Mass cytometry analysis revealed that hTDP-43 induced a TREM2-dependent subpopulation of microglia with high CD11c expression. We further demonstrated an interaction between TDP-43 and TREM2, in vitro and in vivo, in hTDP-43-expressing mouse brains and in patient tissues, and computationally identified the region within TDP-43 that interacts with TREM2. Our data reveal that the interaction between microglial TREM2 and TDP-43 mediates a protective function of TREM2 in microglia in TDP-43-related neurodegenerative disorders.

THE ROLE OF RNA IN SYNAPSE PHYSIOLOGY AND NEURODEGENERATION IN MODELS OF PARKINSON'S DISEASE

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Synaptic dysfunction is an early alteration in multiple neurodegenerative disorders. Parkinson's disease (PD) is characterized by the accumulation of α-synuclein (αsyn) in pathological inclusions known as Lewy bodies and Lewy neurites. αsyn is involved in synaptic vesicle trafficking, and SNARE complex formation at the nerve terminals. In pathological conditions, it is associated with alterations of synaptic function. Interestingly, αsyn also occurs in the nucleus where it induces epigenetic changes. RNA-mediated processes contribute to synaptic remodelling by RNA translocation to the synaptic compartment. This is particularly relevant for microRNAs that can regulate mRNA expression by complementary binding. Here, we sought to identify microRNAs associated with synaptic processes that may contribute to synapse degeneration.
We performed RNA- and small RNA-Seq of the midbrain of 6 month-old transgenic mice expressing A30P mutant αsyn, present in familial forms of PD. Gene ontology (GO) functional annotation and pathway analysis of differentially expressed genes and microRNAs revealed several deregulated biological processes linked with the synaptic compartment. A negative correlation between deregulated microRNA and gene targets highlighted the top interacting microRNAs and identified mir101a-3p as a prominent regulator of synaptic plasticity. Mir101a-3p was validated by qPCR in the transgenic mouse midbrain and in the cortex of Dementia with Lewy Bodies patients. Confocal imaging of primary cortical neurons overexpressing mir101a-3p showed reduced dendritic length and altered spine morphology. Further correlation with synaptic plasticity was provided by wild-type mice exposed to enriched environment which showed reduced levels of mir101a-3p. Finally, exposure of primary cortical neurons to recombinant αsyn species showed a direct effect of αsyn on mir101a-3p levels.
Our data support the emerging role of specific microRNAs as key regulators of gene expression alterations associated with αsyn. Identification of RNA based processes leading to synaptic compromise may reveal novel targets for therapeutic intervention in synucleinopathies, and may also result in the development of novel biomarkers.

EFFECTS OF PREBIOTIC SUPPLEMENTATION ON CHRONIC MILD TRAUMATIC BRAIN INJURY RECOVERY

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Background: The CDC reports that around 5.3 million people live with a permanent disability after TBI. Recently, it was found that gut dysbiosis occurs acutely after traumatic brain injury and literature suggests manipulation of the gut microbiome may be actionable to reduce chronic symptoms after mTBI. Prebiotic fibers are known to beneficially alter the gut microbiome and increase metabolites such as short chain fatty acids (SCFA). The gut microbiome and associated metabolites also play a role in the regulation of brain vascular and metabolic integrity which are important in recovery following mTBI. The goal of this study was to assess the effects of inulin during the chronic time period after mTBI.
Methods: Injury was administered at four months of age. An electromagnetic impactor was used to deliver a single controlled mid-line cortical impact. Fecal samples were collected at 5 m.p.i. Shotgun metagenomic sequencing was done by CosmosID. SCFA analysis was done by Metabolon, Inc on blood and cecal samples. Cerebral blood flow (CBF) was measured using MRI-based pseudo-continuous arterial spin labeling. Magnetic resonance spectroscopy was used to assess brain metabolism. MRI was conducted at 9 months of age (5 m.p.i). Inulin and cellulose diets were provided by TestDiet.
Results: We see trends to indicate that inulin increases putatively beneficial bacteria such as *Bifidobacterium pseudolongum, Akkermansia muciniphila* and *Eubacterium spp*. We also see decreases in putatively harmful bacteria such as *Dorea sp. 5-2*. SCFA levels reflect these increases as *B. pseudolongum* and *A. muciniphila* produce acetate, which is higher in the cecum and blood of mice fed inulin. Butyrate is higher in the cecum of mice fed inulin and is produced by *Eubacterium spp.*. Inulin also proved to be beneficial in the brain by increasing CBF in both the thalamus and hippocampus, and decreased Glycerophosphocholine (GPC) in the hippocampus.
Conclusion: The increase in beneficial bacteria and SCFA may tie back to the improved cerebral blood flow and reduced GPC. Butyrate is known to increase tight junction protein expression in the intestine and the brain. This could be indicative of the higher CBF in inulin fed mice as a more intact brain barrier is protective of CBF. Inulin also decreased harmful bacteria such as *Dorea sp. 5-2* which has been positively correlated with intestinal permeability. GPC is a marker of inflammation and chronic time points, increases in choline metabolites indicate glial proliferation. As inulin decreases the GPC levels this could be indicative of reduced glial proliferation and inflammation. It is clear that even when administered in the chronic phase of injury, inulin exerts beneficial effects that aid recovery.

INTEGRATIVE MULTI-TISSUE MULTI-OMICS FOR BIOMARKER AND THERAPEUTIC TARGET DISCOVERY IN ALZHEIMER DISEASE AND OTHER NEUROLOGICAL TRAITS

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Background: Alzheimer disease (AD) is a neurodegenerative disease, affecting around 5.8 million people in the United States as of 2020. It has been widely studied, but there is still no effective treatment. Using large-scale Genome-wide association studies and whole genome/exome sequencing, researchers have identified common and rare variants associated disease risk that can be used to build prediction models. However, there are limited studies that use high-throughput genomic approaches to identify novel biomarkers beyond genetic variations.
Method: We profiled 1,305 proteins using SOMAscan platform for 971 CSF, 636 plasma, and 458 parietal lobe from participants of the Knight-ADRC. We aim to identify the genetic architecture that govern protein levels and how do these proteins modulate AD risk, age at onset, rate of progression, and additional neurodegenerative traits. To do so, we first identified protein quantitative trait loci (pQTLs) from CSF, plasma, and brain tissues. Next, we inferred causality between proteins with significant pQTLs and AD and neurodegenerative traits employing Mendelian Randomization (MR) approaches.
Result: We generated a genomic atlas of protein levels in multiple neurologically relevant tissues, by profiling thousands of proteins (713 CSF, 931 plasma and 1079 brain) in a large and well-characterized cohort. We identified 274, 127 and 32 protein quantitative loci (pQTL) for CSF, plasma and brain respectively. We successfully replicated several reported pQTLs from CSF (e.g. rs2673908 associated with the protein Siglec-9) and plasma (e.g. rs12740374 associated with the protein Granulins). To infer the causality between proteins and AD or other neurological diseases (Parkinson Disease (PD) risk, amyotrophic lateral sclerosis (ALS) risk and frontotemporal dementia (FTD) risk), we performed MR analysis by using pQTLs as instrumental variables. Overall, we processed MR framework using proteins with significant pQTLs within each tissue on six neurological-related traits. Known protein-disease relationships were replicated. For example, plasma Siglec-3 (CD33) increases the AD risk, consistent with a larger plasma-proteome MR analyses by Zheng and colleagues in 2020. We also uncovered the novel proteins associated with other diseases. As for PD risk, 13 CSF, 12 plasma and 23 brain proteins were likely to be the cause. Among these proteins, plasma IDUA was prioritized as it was encoded by a risk locus for PD and as a drug target for chondroitin sulfate, reported to treat osteoarthritis.
Conclusion: Taken together, these findings prioritized the proteins for the functional validation study. Thus, the findings helped identify potential biomarkers for AD or other neurological traits.

AN INTEGRIN RECEPTOR COMPLEX MEDIATES FILAMENTOUS TAU-INDUCED ACTIVATION OF PRIMARY ASTROCYTES

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Protein aggregates formed by microtubule-associated protein Tau can be transmitted in a stereotypic pattern in human brains, which correlates with the progression of several neurodegenerative diseases collectively termed Tauopathies. This process requires Tau, released from neurons, to interact with a cell surface receptor on a target cell, but little is known about the underlying mechanisms and the downstream pathophysiological consequences particularly for Tau that engages glial cells. Using a spatially resolved proteomic mapping strategy, we identify integrin αV/β1, a heterodimer of the integrin family, as a receptor that not only mediates Tau fibril entry into astrocytes, but also activates integrin signaling upon Tau binding. We show that distinct Tau species differentially activates integrin signaling, with filamentous Tau being a more robust stimulator. This leads to NFκB activation and differential upregulation of pro-inflammatory cytokines and chemokines. Additionally, a sub-group of neurotoxic astrocyte markers are also induced in an integrin-dependent manner preferentially by filamentous Tau, causing astrocytes to release a neurotoxic factor(s). Together, these findings establish a paradigm that astrocytes can be directly converted into a neurotoxic state by filamentous Tau via an integrin receptor, which as a sensor transducing unfolded protein pathology, may provide a new therapeutic target for Tauopathies.

MULTI-OMICS APPROACHES REVEAL A LINK BETWEEN THE MS4A GENE LOCI, TREM2, AND MICROGLIA FUNCTION

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Background: Soluble triggering receptor expressed on myeloid cells 2 (sTREM2) in cerebrospinal fluid (CSF) has been associated with Alzheimer's disease (AD). TREM2 plays a critical role in microglial activation, survival, and phagocytosis; however, the pathophysiological role of sTREM2 in AD is not well understood. Understanding the role of sTREM2 in AD may reveal new pathological mechanisms and lead to the identification of therapeutic targets. We recently identified common variants in the membrane-spanning 4-domains subfamily A (MS4A) gene region that were associated with CSF sTREM2 concentrations. One variant (rs1582763) was associated with increased CSF sTREM2 and reduced AD risk, while a second variant (rs6591561) was associated with reduced CSF sTREM2 and increased AD risk. Using human induced pluripotent stem cell-derived microglia, we found that MS4A4A and TREM2 colocalize on lipid rafts at the plasma membrane. Here, we sought to define the molecular mechanism by which variants in the MS4A gene region impact sTREM2, microglia function and AD risk.
Methods: To define the functional effects of MS4A variants, we used genotype and bulk RNAseq data from 579 human brain samples. We then evaluated microglia specific effects using single nuclei RNAseq data obtained from 70 human brains.
Results: Leveraging genotypic and transcriptomic data in human brain tissue, we found that rs1582763 and rs6591561 alter distinct molecular pathways. Rs1582763, which confers AD resilience, impacts pathways associated with cholesterol metabolism, while rs6591561, which confers AD risk, impacts pathways associated with chemokine regulation. Using single nuclei RNAseq data in human brain tissue, we found that variants in the MS4A gene region were sufficient to alter specific microglia populations.
Conclusions: These findings also provide a mechanistic explanation for the original GWAS signal in the MS4A locus for AD risk and indicate that TREM2 may be involved in AD pathogenesis not only in TREM2 risk-variant carriers but also in those with sporadic disease.

DIFFERENTIAL EFFECTS OF APOLIPOPROTEIN E GENOTYPE ON THE MOLECULAR AND CELLULAR PHENOTYPES ASSOCIATED WITH ALZHEIMER'S DISEASE IN AN ISOGENIC HUMAN IPSC-DERIVED NEURON/ASTROCYTE CO-CULTURE SYSTEM

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Apolipoprotein E (APOE) is a major lipoprotein highly expressed in the brain and an established late-onset Alzheimer's Disease (AD) risk gene and consisted with three different variants: APOE2, APOE3 and APOE4. Numerous evidence demonstrate that APOE4 markedly increases AD risk relative to the APOE3, while the APOE2 is considered protective. However, the underlying mechanism of how these APOE variants affect the molecular and cellular phenotypes associated with AD in human cells are still poorly investigated. In this study, we therefore used isogenic human APOE2/2, APOE3/3, APOE4/4 and APOE knock-out (APOE-/-) iPSC lines which were generated by CRISPR-Cas9 system and differentiated into functional neurons and astrocytes to explore the differential effects of APOE isoforms on AD-related pathology.
In the co-culture system where isogenic human neurons were co-cultured with the same APOE genotype of iAstrocytes, we tested the expression of AD and neuroinflammation-related genes, tau phosphorylation and amyloid-β peptide secretion in vitro. APOE2 iAstrocytes exhibited the highest intracellular APOE level than other iAstrocytes, while we observed no APOE production in APOE-/- iAstrocytes and significant reduction of APOE secretion in conditioned media from APOE2 and APOE4 compared to that from APOE3 iAstrocytes. Expression of PPP2CB was significantly increased in APOE2 compared to APOE3 and APOE4 iNeurons, while there were no significant differences in C4A/B expression among the APOE-defined groups of iNeurons. No significant differences of Aβ (Aβ40 and Aβ42) levels among different APOE iNeurons were found. However, we observed the highest level of pTau231/tTau ratio from APOE2 while the pTau181/tTau ratio was the lowest from APOE4 iNeurons, compared that from APOE-/- or other APOE carrying iNeurons. Correlation analysis suggested the positive correlation of PPP2CB expression with pTau231/tTau ratio (P=0.013) but not with pTau181/tTau ratio (P=0.74). C4A/B expression was not correlated with levels of pTau231 or pTau181, while C4A/B expression was inversely correlated with Aβ42 level. Furthermore, we found the significant positive correlation between PPP2CB and C4A/B expression (P=2.0x10-4) in iNeurons co-cultured with isogenic iAstrocytes regardless of the APOE genotype of the iNeurons.
Our findings suggest the role of APOE isoforms on pTau accumulation, PPP2CB and C4A/B expression as a cross-talk between human neurons and astrocytes in vitro and potentially in AD brains.

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HSP70 CHAPERONES RNA-FREE TDP-43 INTO ANISOTROPIC INTRANUCLEAR LIQUID SPHERICAL SHELLS

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The RNA binding protein TDP-43 naturally phase separates within cell nuclei and forms intranuclear or cytoplasmic aggregates in multiple age-related neurodegenerative diseases, including ALS and frontal temporal dementia (FTD). Here we show that RNA-binding deficient TDP-43 (produced by ALS/FTD-causing mutations or post-translational acetylation in either of its two RNA recognition motifs) drives TDP-43 de-mixing into symmetrical, intranuclear liquid spherical shells with anisotropy (iLSA). Shells exhibit birefringence, evidence of liquid crystal formation by macromolecules within a living cell. Guided by modeling that predicts iLSA to be driven by components with strong self-interactions but weak interaction with TDP-43, we identify the major components of cores to be HSP70 family chaperones, whose activity is required to maintain liquidity of shells and cores. In vivo proteasome inhibition within neurons - to mimic reduction in proteasome activity during aging – is sufficient to induce iLSA with TDP-43-containing shells that convert into aggregates when ATP levels are reduced. Thus, acetylation, HSP70, and proteasome activities regulate TDP-43 phase separation and conversion to a solid phase.

EFFECTS OF CARDIOTONIC STEROID MARINOBUFAGENIN ON NEURODEGENERATION, NEUROINFLAMMATION AND COGNITION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Background Cardiotonic steroids, including marinobufagenin (MBG), participate in cardiac function, intracellular signaling transduction, and anti-neuroinflammatory response. The 15-mo old double-mutant mice (APPswe/PS1dE9; 2xTg-AD; AD), a model of Alzheimer's disease, have lower level of MBG than wild type (WT) mice. This study examined whether treatment of AD mice with MBG affects cardiovascular function, neuroinflammation, neurodegeneration, and cognitive function.
Methods Male AD and WT mice (15-mo old) were given MBG (100 μg/day/kg body weight; AD-MBG, n=8; WT-MBG, n=4) or control (AD-C, n=8; WT-C, n=4) via subcutaneous ALZET minipumps for 3-mo. Systolic blood pressure (SBP; by tail cuff plethysmography), cognitive flexibility (reversal learning in a water T-maze), plasma (for MBG measurement), and brain samples (for qPRC analysis) were collected at 18-mo. Data was analyzed by 2-way ANOVA and presented as mean±SE.
Results SBP did not differ between WT-C and AD-C (98±4 vs. 92±1 mmHg), MBG did not affect SBP. Heart rate was higher in WT-MBG vs. WT-C (594±4 vs. 457±11 beats/min; $p<0.05$) and was the same in AD-C vs. AD-MBG (513±29 vs. 557±19 beats/min). Endogenous plasma MBG was lower in AD-C vs. WT-C (125±21 vs. 223±41 pmol/L; $p<0.05$). Treatment with MBG increased plasma MBG in both WT-MBG and AD-MBG (483±116 vs. 657±176 pmol/L; $p<0.05$). Hippocampal mRNA expression of inflammatory marker IL6 was upregulated 1.5-fold in AD-C vs. WT-C ($p<0.05$); MBG reduced this expression in AD-MBG vs. AD-C ($p<0.01$). Hippocampal mRNA expression of mouse amyloid precursor protein (APP) was upregulated 2.6-fold in AD-C vs. WT-C ($p<0.01$); MBG reduced this expression in AD-MBG vs. AD-C ($p<0.01$). AD mice showed normal learning for a turn-based discrimination rule but were impaired during the reversal phase vs. WT mice. MBG did not affect this behavior.
Conclusion Old AD mice had lower endogenous plasma MBG, impaired cognitive flexibility, and higher hippocampal IL6 and APP mRNAs vs. WT mice. MBG treatment significantly reduced levels of hippocampal IL6 and APP mRNA. The absence of the effect of MBG on cognitive behavior in AD-MBG vs. AD-C mice is likely because MBG may prevent the formation of new amyloids plaques from APP but does not affect existing accumulation. The association of MBG with neuroinflammation, neurodegeneration, and cognitive function will be further explored in this AD mouse model.
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RIPK1 ACTIVATION MEDIATES NEUROINFLAMMATION AND DISEASE PROGRESSION IN MULTIPLE SCLEROSIS

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Receptor interacting protein kinase 1 (RIPK1) mediates inflammatory and cell death signaling and is increased in multiple sclerosis (MS) brain samples. Here, we investigate the role of RIPK1 kinase activity in glial cells in mediating MS pathogenesis. We demonstrate that RIPK1 levels correlate with MS disease progression. We find microglia are susceptible to RIPK1-mediated cell death and identify a pro-inflammatory gene signature that may contribute to the neuroinflammatory milieu in MS patients. However, we uncover a distinct role for RIPK1 in astrocytes in regulating inflammatory signaling in the absence of cell death. Using a murine MS model we show therapeutic RIPK1 inhibition attenuates disease progression and suppresses deleterious signaling in astrocytes and microglia. Finally, we demonstrate robust RIPK1 kinase activity in human microglia and astrocytes. Our results suggest RIPK1 kinase activation in microglia and astrocytes can induce a detrimental neuroinflammatory program that contributes to the neurodegenerative environment in progressive MS.

TARGETING THE NF-kB PATHWAY BY SENOLYTIC DRUGS ALLEVIATES AB-ASSOCIATED OLIGODENDROCYTE PROGENITOR CELL SENESCENCE IN AN ALZHEIMER'S DISEASE MODEL

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Alzheimer's disease (AD) is the most common neurodegenerative disorder to date, with no curative or preventive therapy. Recent animal studies suggest that senolytic agents kill senescent cells to alleviate pathological phenotypes in age-related diseases in part via the inhibition of anti-apoptotic Bcl-2 family proteins. However, the target of true senolytics is not only senescent cells but also their senescence-associated secretory phenotype (SASP), a "root cause contributor" to local inflammatory environment. It is critical to investigate whether targeting NF-kB signaling, a master transcriptional regulator of inflammation is necessary for the suppression of SASP in senescent brain cells during the early stage of AD. Previously, we documented the presence of oligodendrocyte progenitor cells (OPCs) exhibiting the senescence phenotype and SASP in the Aβ plaque of an AD mouse model. Intermittent administration of senolytic compounds, dasatinib plus quercetin (D+Q) selectively removed senescent cells from the plaque environment, reduced neuroinflammation, lessened Aβ load, and ameliorated cognitive deficits1. To further investigate the action of NF-kB pathway in senescent OPCs, we conducted an unbiased transcriptomic profiling analysis using γ-radiation induced senescent OPC cultures. We found that ~26% of significantly upregulated transcripts were NF-kB putative targets along with 2.87% of the AD-network associated genes, implicating a correlation between AD network and NF-kB activation. Moreover, immunostaining brain sections of a mouse AD model, we found that the nuclear accumulation of total p65 and phospho-p65, a marker for NF-kB activation, were significantly increased in Aβ-associated senescent OPCs. So far, our preliminary data indicated that the NF-kB signaling may contribute to the proinflammatory milieu surrounding Aβ plaques, which hampers the differentiation of OPCs for regeneration of myelin and axons, and thus likely serves as one of key targets for developing new senolytic strategy of AD treatments.

Reference:
1. Zhang P, et al. (2019) Senolytic therapy alleviates Abeta-associated oligodendrocyte progenitor cell senescence and cognitive deficits in an Alzheimer's disease model. Nature neuroscience. doi: 10.1038/s41593-019-0372-9. PubMed PMID: 30936558.

A MYOSIN-7B–DEPENDENT ENDOCYTOSIS PATHWAY MEDIATES CELLULAR ENTRY OF α-SYNUCLEIN FIBRILS AND POLYCATION-BEARING CARGOS

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Cell-to-cell transmission of misfolding-prone α-synuclein (α-Syn) has emerged as a key pathological event in Parkinson's disease. This process is initiated when α-Syn–bearing fibrils enter cells via clathrin-mediated endocytosis, but the underlying mechanisms are unclear. Using a CRISPR-mediated knockout screen, we identify SLC35B2 and myosin-7B (MYO7B) as critical endocytosis regulators for α-Syn preformed fibrils (PFFs). We show that SLC35B2, as a key regulator of heparan sulfate proteoglycan (HSPG) biosynthesis, is essential for recruiting α-Syn PFFs to the cell surface because this process is mediated by interactions between negatively charged sugar moieties of HSPGs and clustered K-T-K motifs in α-Syn PFFs. By contrast, MYO7B regulates α-Syn PFF cell entry by maintaining a plasma membrane-associated actin network that controls membrane dynamics. Without MYO7B or actin filaments, many clathrin-coated pits fail to be severed from the membrane, causing accumulation of large clathrin-containing "scars" on the cell surface. Intriguingly, the requirement for MYO7B in endocytosis is restricted to α-Syn PFFs and other polycation-bearing cargos that enter cells via HSPGs. Thus, our study not only defines regulatory factors for α-Syn PFF endocytosis, but also reveals a previously unknown endocytosis mechanism for HSPG-binding cargos in general, which requires forces generated by MYO7B and actin filaments.

GENOME-WIDE IDENTIFICATION OF THE GENETIC BASIS OF AMYOTROPHIC LATERAL SCLEROSIS

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Genetic association studies suffer from poor statistical power which limits discovery. To address this we have integrated functional genomics with disease genetics in a hierarchical Bayesian model called RefMap. Amyotrophic lateral sclerosis (ALS) is an archetypal complex disease centred on progressive death of motor neurons. Functional and epigenetic profiling of iPSC-derived motor neurons enabled RefMap to fine-map causal genes and pathways in ALS. We identified 690 candidate ALS genes; our list is enriched with known ALS genes and ALS-associated biological functions, and is up-regulated in undiseased motor neurons but progressively down-regulated in patient tissue and ALS disease models. The most significant new ALS gene is KANK1, which is enriched with coding and non-coding, common and rare, ALS-associated genetic variation; CRISPR/Cas9 perturbation proximate to patient mutations reduces KANK1 expression and produces neuronal toxicity. RefMap can be applied broadly to increase power in genetic association studies of complex human traits.

HAP40 IS A CONSERVED CENTRAL REGULATOR OF HUNTINGTIN AND A POTENTIAL MODULATOR OF MUTANT HUNTINGTIN TOXICITY

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Perturbation of huntingtin (HTT)'s physiological function is one postulated pathogenic factor in Huntington's disease (HD). However, little is known how HTT is regulated in vivo. In a proteomic study, we isolated a novel ~40kDa protein as a strong binding partner of Drosophila HTT and demonstrated it was the functional ortholog of HAP40, an HTT associated protein shown recently to modulate HTT's conformation but with unclear physiological and pathologic roles. We showed that in both flies and human cells, HAP40 maintained conserved physical and functional interactions with HTT, loss of HAP40 resulted in similar phenotypes as HTT knockout, including animal viability and autophagy, and more strikingly, HAP40 depletion significantly reduced the levels of endogenous HTT, while HAP40 was mostly degraded via the proteasome in the absence of HTT. Interestingly, polyglutamine expansion in HTT did not affect its affinity for HAP40. However, HAP40 modulated HD pathogenesis in Drosophila model by regulating the overall protein levels and the toxicity of full-length mutant HTT. Together, our study uncovers a conserved mechanism governing the stability and in vivo functions of HTT, and demonstrates that HAP40 is a central and positive regulator of HTT and a potential modulator of HD pathogenesis and a promising candidate for an HTT-lowering strategy against HD.

NEURAL SPECIFIC *BAK1* ALTERNATIVE SPLICING IN NEURONAL LONGEVITY AND NEURODEGENERATIVE DISEASES

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Due to limited regeneration capacity mammalian brains are equipped with multiple strategies to promote neuronal longevity for mature neurons to live and function throughout the life of organisms (i.e., years). Prevailing thoughts assume the default cell fate of neurons is death and neuronal survival is promoted by target cells and tissues through extrinsic neurotrophic factors and synaptic activity. We have identified an intrinsic genetic control mechanism that enables neuronal longevity at birth. Developmental downregulation of the splicing regulator PTBP1 in neurons allows neural-specific splicing of the evolutionarily-conserved *Bak1* exon 5. Exon 5 inclusion triggers nonsense-mediated mRNA decay and unproductive translation of *Bak1* mRNA, leading to suppression of pro-apoptotic BAK1 proteins and allowing neurons to reduce apoptosis. Ablation of exon 5 increases BAK1 proteins exclusively in the brain, inflates neuronal apoptosis, and leads to animal mortality. Therefore, neural-specific exon 5 splicing and depletion of BAK1 proteins are necessary for uninterrupted neuronal survival. Dysregulation of the PTBP1-Bak1 exon 5 axis causes neuronal death and may underlie the neuronal loss in various neurodegenerative diseases.

SULFORAPHANE AS A POTENTIAL PROTECTIVE AGENT IN THE TREATMENT OF MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a chronic autoimmune, inflammatory neurological disease of the central nervous system (CNS) which attacks the myelinated axons in the CNS, destroying the myelin and the axons to varying degrees. In most patients, the disease is characterized initially by episodes of reversible neurological deficits, followed by progressive neurological deterioration. The cause remains unknown, but it appears to involve a combination of genetic and environmental triggers that together result in a self-sustaining autoimmune disorder that leads to recurrent immune attacks on the CNS. Although progress has been made in the therapy of this disease, effective treatment of progression remains an unmet need because current therapies confer only partial protection against the neurodegenerative component of MS. Therefore, intervention approaches targeting phytochemicals have been recommended as an alternative form of treatment. Sulforaphane (SFN) is a sulfur-rich dietary phytochemical which has drawn considerable attention for the treatment of neurological disorders in recent years due to its neuroprotective, antioxidative, and detoxification actions. The aim of this study was to elucidate the molecular mechanisms of SFN as a potential neuroprotective agent in the therapy of MS by using in silico data mining approach. The STITCH database (version 5.0) (http://stitch.embl.de) was used to obtain the information about chemical–protein interactions, while GeneMania prediction server (https://genemania.org) revealed detailed gene interactions.ToppGene Suite (https://toppgene.cchmc.org) gene ontology analysis revealed biological processes involved in SFN -mediated neuroprotection. According to the STITCH database, human genes that have the strongest interaction with SFN are: NQO1, NFE2L2, CASP3, HSP90AA1, MAPK14, HDAC6, HPGDS, KEAP1, GSTA1, GSTM1. GeneMANIA predictive server identified the tight interaction network comprising the selected genes (Genetic Interactions (29.54%), Physical Interactions (26.69%), Interactions Predicted by the server (17.97%), Shared protein domains (10.23%), Pathway interactions (7.57%), Co-expression (5.74%) and Co-localization (2.25%)). ToppGene Suite (https://toppgene.cchmc.org) gene ontology analysis revealed biological processes noted as a SFN target (glutathione metabolic process, cellular response to toxic substance, response to toxic substance, glutathione derivative metabolic process, glutathione derivative biosynthetic process, cellular detoxification, reactive oxygen species metabolic process). These results confirmed that SFN could be helpful adjuvant phytochemical in the future combined treatment of MS.

LOSS OF TAX1BP1-MEDIATED AUTOPHAGY RESULTS IN PROTEIN AGGREGATE ACCUMULATION IN THE BRAIN

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Misfolded protein aggregates disrupt cellular homeostasis, causing toxicity, a hallmark of numerous neurodegenerative diseases. Selective autophagic elimination of protein aggregates, or aggrephagy, is critical to protein quality control, but how aggregates are selectively recognized and targeted for degradation is not well understood. Therefore, we compared the requirements for 5 autophagy receptor proteins: OPTN, NBR1, p62, NDP52, and TAX1BP1 in proteotoxic stress-induced aggregate clearance. We identify TAX1BP1 as an autophagy receptor protein required for aggregate clearance in response to multiple forms of cytotoxic stress, which is essential in maintaining protein homoeostasis. Endogenous TAX1BP1 is both recruited to and required for the clearance of stress-induced aggregates. Ectopic expression of TAX1BP1 increases clearance through autophagy, promoting viability in human induced pluripotent stem cell-derived neurons. In contrast, TAX1BP1 depletion sensitizes cells to several forms of aggregate-induced proteotoxicity. We further demonstrate that TAX1BP1 is more specifically expressed in the brain compared to other autophagy receptor proteins. In vivo, TAX1BP1 contributes to aggregate clearance as we observed increased accumulation of high molecular weight ubiquitin conjugates in brains of young TAX1BP1 knockout mice accompanied by premature lipofuscin accumulation. We propose a role for TAX1BP1 in the clearance of a broad range of cytotoxic proteins with the potential to be a therapeutic target in clearance of protein inclusions in neurodegenerative diseases.

eCIRP ACTIVATES THE IL-6Rα/STAT3/CDK5 PATHWAY IN NEURONS AND CIRP-DERIVED SHORT PEPTIDE C23 ATTENUATES IT.

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Extracellular cold-inducible RNA-binding protein (eCIRP) stimulates microglial inflammation causing neuronal damage during ischemic stroke and is a critical mediator of alcohol-induced cognitive impairment. However, the precise role of eCIRP in mediating neuronal damage remains to be addressed. In this study, we hypothesized that eCIRP activates IL-6Rα/STAT3-mediated induction of neuron-specific cyclin-dependent kinase-5 (Cdk5) hyperactivator p25 which is neurotoxic. Conditioned medium (CM) from N2a cells stably transfected with human APP constructs containing extracellular amyloid β (Aβ) was used to measure the effect of neuronal Aβ stress on the release of eCIRP (measured by ELISA) from mouse microglial BV2 cells after 6h and 24h stimulation. The released eCIRP levels from BV2 cells showed 3.2-fold increase on stimulation with N2a CM containing Aβ compared to control N2a CM in a time-dependent manner. Primary neurons were isolated from C57BL/6 pups on postnatal day 1, seeded in poly-L-ornithine and laminin coated 12-well plates and maintained for 10-12 days. N2a cells and DIV 10 primary neurons were treated with eCIRP with or without 30 min. pretreatment with IL-6Rα neutralizing antibody or C23 peptide and cultures continued for additional 1, 16 or 48 h. Total protein was extracted from the cells and p25 levels and phosphorylation status of STAT3 were determined by Western blot analysis. Stimulation of N2a cells and primary neurons with eCIRP upregulated the neuronal Cdk5 activator p25 expression in a time and dose-dependent manner. eCIRP directly induced phosphorylation of STAT3 (pSTAT3) and p25 increase in N2a cells and primary neurons via its novel receptor IL-6Rα. Next, we showed using surface plasmon resonance BIAcore assays that eCIRP-derived peptide C23 inhibited the binding of eCIRP to IL-6α with a 40-fold increase in equilibrium dissociation constant ($K_{d)}$ value at 25 μM (from $8.08x10^{-8}$ M to $3.43x10^{-6}$ M) and complete abrogation of binding at 50 μM. Finally, C23 reversed the eCIRP-induced increase in neuronal pSTAT3 and p25 levels. In conclusion, the current study provides upregulation of neuronal IL-6Rα/STAT3/cdk5 pathway as the mechanism for eCIRP's role in neuronal damage with C23 as the potential inhibitor. Supported by NIH grant 1R01AA028947-01.

MEMBRANE REMODELING IS ASSOCIATED WITH IMPAIRED INSULIN SIGNALING IN AGING BRAIN

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Aging is considered the major risk factor for Alzheimer's disease (AD), though other factors such as diabetes and impaired brain insulin signaling have been linked to AD pathogenesis. In aging, neuronal membrane dysfunction was reported, involving membrane remodeling. Such modifications are also observed in mice fed a high fat diet, whereas a diet containing controlled amounts of n-3 polyunsaturated fatty acids such as docosahexaenoic acid (DHA, C22:6) seems to prevent or limit these alterations, most presumably by promoting optimal lipid environment to membrane protein function.

With the aim to explore how brain insulin signaling is impaired in aged mice, we focused our attention on the β subunit of insulin receptor (IR-β) and its downstream signaling protein AKT. Comparing young (3-months of age) to old (18-month) mice, we used immunohistochemistry analyses to study the localization of these proteins in the cerebral cortex.

Distinct distribution patterns were observed, though sustained by no significant quantitative variation. IR-β subunit tended to scatter, while Akt protein seemed to form large clusters in aged mice. Furthermore, comparison of membrane architecture using membrane fractioning and lipid raft isolation revealed that the IR-β subunit seemed to be excluded from lipid rafts upon aging, while Akt rather appeared sequestered within these microdomains.

These results suggest a possible mechanism that may explain impaired brain insulin signaling observed in aged brain. Ongoing functional experiments will have to confirm this hypothesis.

PIAS1 MODULATES STRIATAL TRANSCRIPTION, DNA DAMAGE REPAIR, AND SUMOYLATION WITH RELEVANCE TO HUNTINGTON'S DISEASE

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DNA damage repair genes are modifiers of disease onset in Huntington's disease (HD), but how this process intersects with associated disease pathways remain unclear. Here we evaluated the mechanistic contributions of protein inhibitor of activated STAT-1 (PIAS1) in HD mice and HD patient-derived iPSCs and find a link between PIAS1 and DNA damage repair pathways. We show that PIAS1 is a component of the transcription-coupled repair complex, that includes the DNA damage end processing enzyme polynucleotide kinase-phosphatase (PNKP), and that PIAS1 is a SUMO E3 ligase for PNKP. Pias1 knock-down in HD mice had a normalizing effect on HD transcriptional dysregulation associated with synaptic function and disease-associated transcriptional co-expression modules enriched for DNA damage repair mechanisms as did reduction of PIAS1 in HD iPSC-derived neurons. Knock-down also restored mutant HTT-perturbed enzymatic activity of PNKP and modulated genomic integrity of several transcriptionally normalized genes. The findings here now link SUMO modifying machinery to DNA damage repair responses and transcriptional modulation in neurodegenerative disease.
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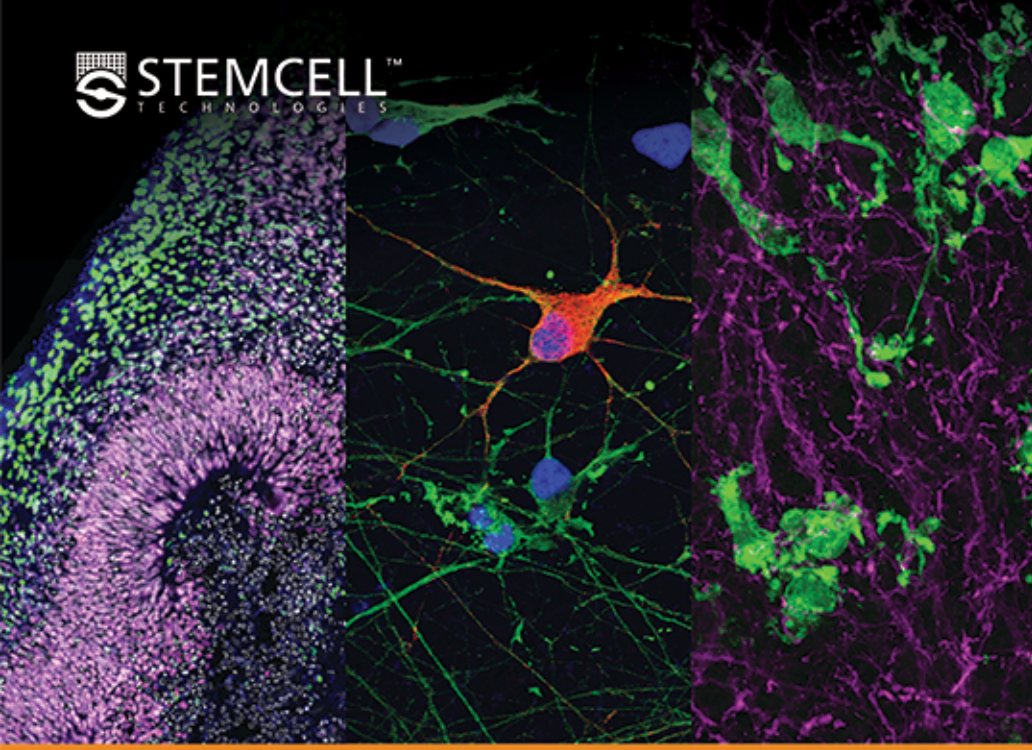
TECHNICAL ABSTRACTS
FOR WORKSHOPS



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The study of neurodegenerative disease requires systems to model human behavior, aging, anatomy, and cellular and molecular function. The complexity of data can't be fully captured with just one system; a combination of approaches is needed. In this workshop, we will discuss modeling cellular and molecular dysfunction in disease using cultured cells from humans and mice as well as from pluripotent stem cell (PSC)-derived cellular models. We will begin with recent research examining neural activity defects in neurodegenerative disease. We will discuss how using physiological culture media (BrainPhys™) increases neural activity and improves study sensitivity. Going back to recent publications, we'll show the advantages of combining PSC-derived models with animal work to model disease. We will cover the basics of how to set up high-quality PSC cultures and present some considerations for designing PSC-based studies. Finally, we will give examples of how to use the 2D and 3D PSC-derived culture systems available from STEMCELL Technologies to ask questions about the cellular and molecular mechanisms of disease. This workshop will interest those who use either rodent- or human-derived cell culture models. Participants will come away with ideas on how to combine animal and culture approaches to uncover new insights into neurodegenerative disease.

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CODE OF CONDUCT FOR ALL PARTICIPANTS IN CSHL MEETINGS

Cold Spring Harbor Laboratory is dedicated to pursuing its twin missions of research and education in the biological sciences. The Laboratory is committed to fostering a working environment that encourages and supports unfettered scientific inquiry and the free and open exchange of ideas that are the hallmarks of academic freedom. To this end, the Laboratory aims to maintain a safe and respectful environment that is free from harassment and discrimination for all attendees of our meetings and courses as well as associated support staff, in accordance with federal, state and local laws.

Consistent with the Laboratory's missions, commitments and policies, the purpose of this Code is to set forth expectations for the professional conduct of all individuals participating in the Laboratory's meetings program, both in person and virtually, including organizers, session chairs, invited speakers, presenters, attendees and sponsors. This Code is consistent with the Laboratory's internal policies governing conduct by its own faculty, trainees, students and employees.

By registering for and attending a CSHL meeting, either in person or virtually, participants agree to:

1. Treat fellow meeting participants and CSHL staff with respect, civility and fairness, without bias based on sex, gender, gender identity or expression, sexual orientation, race, ethnicity, color, religion, nationality or national origin, citizenship status, disability status, veteran status, marital or partnership status, age, genetic information, or any other criteria prohibited under applicable federal, state or local law.

2. Use all CSHL facilities, equipment, computers, supplies and resources responsibly and appropriately if attending in person, as you would at your home institution.

3. Abide by the CSHL Meeting Alcohol Policy if attending in person (see below).

Similarly, meeting participants agree to refrain from:

1. Harassment and discrimination, either in person or online, in violation of Laboratory policy based on sex, gender, gender identity or expression, sexual orientation, race, ethnicity, color, religion, nationality or national origin, citizenship status, disability status, veteran status, marital or partnership status, age, genetic information, or any other criteria prohibited under applicable federal, state or local law.

2. Sexual harassment or misconduct.

3. Disrespectful, uncivil and/or unprofessional interpersonal behavior, either in person or online, that interferes with the working and learning environment.

4. Misappropriation of Laboratory property or excessive personal use of resources, if attending in person.

Breaches or Violations of the Code of Conduct

Cold Spring Harbor Laboratory aims to maintain in-person and virtual conference environments that accord with the principles and expectations outlined in this Code of Conduct. Meeting organizers are tasked with providing leadership during each meeting, and may be approached informally about any breach or violation. Breaches or violations should also be reported to program leadership in person or by email:

- Dr. David Stewart, Grace Auditorium Room 204, 516-367-8801 or x8801 from a campus phone, stewart@cshl.edu
- Dr. Charla Lambert, Hershey Laboratory Room 214, 516-367-5058 or x5058 from a campus phone, clambert@cshl.edu

Reports can be submitted by those who experience harassment or discrimination as well as by those who witness violations of the behavior laid out in this Code. Reports may also be submitted online via the following form: http://bit.ly/CSHLCoCForm. The Laboratory will take action as needed to resolve the matter, up to and including immediate expulsion of the offending participant(s) from the meeting, dismissal from the Laboratory, and exclusion from future academic events offered by CSHL.

Definitions and Examples

Uncivil/disrespectful behavior is not limited to but may take the following forms: Shouting, personal attacks or insults, throwing objects, and sustained disruption of talks or other meeting-related events.

Harassment/discrimination is not limited to but may take the following forms:

- Threatening, stalking, bullying, demeaning, coercive, or hostile acts that may have real or implied threats of physical, professional, or financial harm.
- Signs, graphics, photographs, videos, gestures, jokes, pranks, epithets, slurs, or stereotypes that comment on a person's sex, gender, gender identity or expression, sexual orientation, race, ethnicity, color, religion, nationality or national origin, citizenship status, disability status, veteran status, marital or partnership status, age, genetic information, or physical appearance.

Sexual misconduct is not limited to but may take the following forms:

- Unwelcome and uninvited attention, physical contact, or inappropriate touching.
- Groping or sexual assault.
- Use of sexual imagery, objects, gestures, or jokes in public spaces or presentations.
- Any other verbal or physical contact of a sexual nature when such conduct creates a hostile environment, prevents an individual from fulfilling their professional responsibilities at the meeting, or is made a condition of employment or compensation either implicitly or explicitly.