14th Annual Les Turner Symposium on ALS

Brought to you by the Les Turner ALS Center at Northwestern Medicine

Monday, Nov. 4, 2024

8 a.m.-5 p.m. Central Time
Feinberg Pavilion
Feinberg Krumlovsky Atrium
251 E. Huron St. 3rd Floor
Chicago, IL 60611

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Welcome Letter

Dear Friends and Colleagues,

It is with great pleasure that we extend our warmest welcome to the 14th Annual Les Turner Symposium on ALS. Our symposium is a day of celebration for our collaborative efforts, providing hope and excitement within the ALS community. Advancements in translational research have been very rapid. We have many reasons to celebrate.

Our Les Turner ALS Center has expanded its research efforts with the addition of new faculty, investigating different aspects of the disease. The Lois Insolia ALS Clinic continues to be one of the most effective hubs for patient care, with the highest number of active clinical trials. The Les Turner ALS Foundation, one of the first ALS foundations in the nation and in the world, continues to support both the clinic and the research efforts. We are blessed and humbled to have their continued support.

Symposiums like this bring us together and remind us of the need to work together as a team. ALS is a horrific disease, attacking patients from many different angles with many different underlying causes. To end ALS, we need to be stronger than ALS. We need to fight the disease with different sets of ammunition, with different sets of expertise, but with a unified determination.

Here, at our 14th Annual Les Turner Symposium on ALS, you will meet the most passionate patients and caregivers, and you will meet scientists, doctors, students, nurses, representatives from drug companies, and members of the Les Turner ALS Foundation, all united towards one goal: End ALS.

This year, we prepared a one-of-a-kind program. Dr. Angela Genge, the Director of Montreal Neurological Institute, will be giving the keynote address. Dr. Genge will be giving an update on the current and emerging clinical trials for ALS, and the importance of outcome measures. We are thrilled to have Dr. Genge with us.

Members of our Les Turner ALS Center, namely Dr. Robert Kalb, Dr. David Gate, Dr. Marco Martina and I will be giving seminars to share our most recent research efforts. Our friends and colleagues Dr. Lindsey Hayes from Johns Hopkins and Dr. Pietro Fratta from University College London will be giving seminars about the importance of RNA-based regulation for TDP-43 function and how that relates to pathology in ALS. We will hear from Laura Freveletti, the CEO of the Les Turner ALS Foundation, about the recent and most exciting achievements the foundation has made. Lauren Webb, the Chief Advocacy and Outreach Officer at the Les Turner ALS Foundation, will moderate the Clinical Conversations panel, which will host Dr. Senda Ajroud-Driss, the Director of the Lois Insolia ALS Clinic; Dr. Angela Genge, our keynote speaker; and Kelly Goodman and Melissa Diaz- Viera, members of the Support Services Committee at the Les Turner ALS Foundation. Dr. Robert Kalb, the Director of the Les Turner ALS Center and the Chief of Neuromuscular Disease will share recent developments at the Les Turner ALS Center and will make the closing remarks. This year, we have 30 poster abstracts from different laboratories and institutions, all sharing exciting research developments in the field.

As we work all our might to end ALS, we are happy to have you here with us.

Welcome, welcome and welcome.

P. Hande Ozdinler, PhD

P. Hande Ozdinler, PhD
Associate Professor, Department of Neurology, Northwestern University Feinberg School of Medicine

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Agenda

8:00 a.m. Registration and Breakfast

8:45 a.m. Welcome and Opening

Hande Ozdinler, PhD, Associate Professor of Neurology, Northwestern University Feinberg School of Medicine

Robert Kalb, MD, Joan and Paul Rubschlager Professor of Neurology, Chief of Neuromuscular Disease; Director, Les Turner ALS Center at Northwestern Medicine

9:00 a.m.- Noon Research Presentations

9:00-9:15 a.m. **Targeting the Ubiquitin-Proteasome System in Neurodegenerative Diseases** by Robert Kalb, MD

9:20-9:35 a.m. **Immune System Alteration in ALS** by David Gate, PhD, Director, Abrams Research Center on Neurogenomics Assistant Professor of Neurology, Northwestern University Feinberg School of Medicine

9:40-9:55 a.m. **Cell Type-Specific Alterations of Cortical Excitability in a Model of Familial ALS** by Marco Martina, MD, MSc, PhD, Professor of Neuroscience and Psychiatry and Behavioral Science, Northwestern University Feinberg School of Medicine

10:00-10:15 a.m. **TDP-43 Pathology in the ALS Motor Cortex** by Hande Ozdinler, PhD

10:20-10:45 a.m. Morning Break

10:50-11:20 a.m. **RNA-Based Regulation of TDP-43 Nuclear Localization** by Lindsey Hayes, MD, PhD, Associate Professor of Neurology, Johns Hopkins Medicine

11:25-11:55 a.m. **Cryptic Splicing: From Foe to Friend in Tackling Amyotrophic Lateral Sclerosis** by Pietro Fratta, MD, PhD, Professor of Cellular and Molecular Neuroscience at the UCL Queen Square Institute of Neurology and the Francis Crick Institute

Noon-2:00 p.m. Lunch Break/Poster Session

2:00-2:10 p.m. Opening Remarks for Afternoon Session

Laura Freveletti, CEO, Les Turner ALS Foundation

2:15-3:10 p.m. Keynote Address

Introduction: Senda Ajroud-Driss, MD, Les Turner ALS Foundation/Herbert C. Wenske Professor of Neurology; Director, Lois Insolia ALS Clinic; Les Turner ALS Center at Northwestern Medicine

Keynote: **Advances in ALS Clinical Trial Outcome Measures** by Angela Genge, MD, FRCP(C), Professor of Neurology, Director, Montreal Neurological Institute and Hospital's ALS, Global Center of Excellence, ALS Clinic

3:20 p.m. Afternoon Break

3:30 p.m. Clinical Conversations Panel

Moderator: Anne Marie Doyle, M.A., CCC-SLP, Community Education Manager, Les Turner ALS Foundation

Panelists:

Senda Ajroud-Driss, MD

Melissa Diaz-Viera, LCSW, Support Service Committee, Les Turner ALS Foundation and ALS Research Ambassador for the Northeast ALS Consortium, Operations Coordinator for Project ALS

Angela Genge, MD

Kelly Goodman, Support Services Committee, Les Turner ALS Foundation

4:35 p.m. Closing Remarks

Robert Kalb, MD

*All Northwestern faculty members presenting at the symposium are affiliated with the Les Turner ALS Center at Northwestern Medicine unless otherwise noted.

Angela Genge, MD, FRCP(C)

Director, ALS Centre of Excellence for Research and Patient Care at Montreal Neurological Institute-Hospital, McGill University

Advances in ALS Clinical Trial Outcome Measures

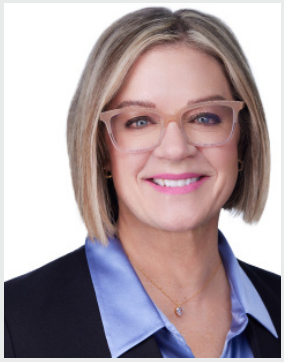
Angela Genge, MD, is internationally recognized for her work in clinical trial design and development for rare neurological conditions, with an emphasis on ALS/MND. She has served as director of the ALS Global Center of Excellence and ALS Clinic at the Montreal Neurological Institute and Hospital since 1998.

In 2023 she stepped down from her twenty-year tenure as executive director of the Institute's clinical research unit, where, under her direction, it evolved into the most active neuroscience unit in the country, conducting over 100 clinical trials from Phase 1 to Phase 4. She developed a Phase 1 unit dedicated to neurological diseases, the ALS Global Center of Excellence, and ACCESS ALS, all of which fuel the drug discovery pipeline and accelerate the development of new therapies for rare and terminal neurological diseases. Angela serves as the global principal investigator for AL-S Pharma, as well as sits on numerous advisory boards and data and safety monitoring boards. She previously served as a distinguished clinical investigator for Novartis Global.

Angela advocates tirelessly for therapeutic innovation in neuroscience and access to therapeutics for the rare disease community. Throughout her career, she has received awards in recognition of her exceptional care and management of ALS studies and patients including being named the first international recipient of the 2023 Wings Over Wallstreet Diamond Award, the 2018 Forbes Norris Award, the DIVA of Distinction Award, the YMCA Woman of the Year Award, and the Governor General Diamond Jubilee Award. She completed her medical degree at Memorial University of Newfoundland and earned a B.Sc. from Dalhousie University. A fellow of the Royal College of Physicians and Surgeons (Canada), Angela completed her Canadian and American certifications in internal medicine and neurology at McGill University prior to completing a fellowship in neuromuscular diseases at the Montreal Neurological Institute.

Remarks

Laura Freveletti, CEO of the Les Turner ALS Foundation



Laura Freveletti is Chief Executive Officer of the Les Turner ALS Foundation. She comes to the ALS field with 30 years of experience in executive leadership, most recently as senior program officer at The Allstate Foundation. She has deep connections to the Chicago non-profit community, with experience as a senior fundraising executive at the YMCA of Metropolitan Chicago and Lyric Opera of Chicago, as well as service as board president of the Sudden Infant Death Services (SIDS) of Illinois. Laura also has led corporate marketing and community involvement at companies including Kraft Heinz, LaSalle Investment Management, and Chicago Title & Trust Company, and earned a degree in business administration from the University of Louisville. She brings a personal understanding of the impact of ALS to the position, having lost her brother-in-law to the disease.

Presenters at the 2023 Les Turner Symposium on ALS

Pietro Fratta, MD, PhD

Professor of cellular and molecular neuroscience at the UCL Queen Square Institute of Neurology and the Francis Crick Institute

Cryptic Splicing: From Foe to Friend in Tackling Amyotrophic Lateral Sclerosis

Dr. Fratta's lab studies the cellular and molecular mechanisms that regulate TDP-43, a major pathological hallmark of ALS and an important therapeutic target. Using in vitro model systems, he identified a major role for RNA in regulating TDP-43 localization, and now seeks to apply that knowledge to better understand TDP-43 mislocalization in ALS and test candidate therapeutic approaches.

TDP-43 is an important protein in ALS as it is found to aggregate and form clumps in patient neurons. This happens in virtually all cases of ALS, other than some specific genetic forms of disease. When TDP-43 aggregates, the processing of RNA within the cell is altered and mistakes – called "cryptic exons" – are introduced. This talk will discuss how to target cryptic exons for therapeutic purposes and how to use these to our advantage to create safer therapies for ALS.

Pietro Fratta, MD, is a professor of cellular and molecular neuroscience at the UCL Queen Square Institute of Neurology and the Francis Crick Institute. His research centers on motor neuron diseases and RNA biology. His laboratory uses sequencing and visualization tools to understand disease mechanisms in patient derived tissue, iPS cells, and mouse models.

David Gate, PhD

Assistant professor of neurology, Director, Abrams Research Center on Neurogenomics at Northwestern University Feinberg School of Medicine

Immune System Alteration in ALS

This presentation will discuss the relationship between the immune system and ALS. Dr. Gate will detail findings on the peripheral immune system of the blood of ALS patients. He will also describe immune cells in the spinal cord of ALS patients.

David Gate, PhD, is a genomicist with a background in neuroimmunology. Dr. Gate's work focuses on the intersection of the brain and immune system in ALS. His laboratory employs multi-omics strategies to interpret immune system changes related to ALS. His group is particularly interested in the interplay between neurons and inflammatory immune cells.

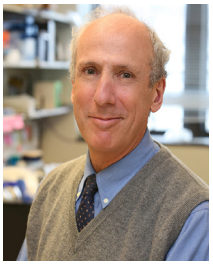
Lindsey Hayes, MD, PhD

Associate professor of neurology at the Johns Hopkins University School of Medicine and Brain Science Institute

RNA-Based Regulation of TDP-43 Nuclear Localization

Dr. Hayes studies the cellular and molecular mechanisms that regulate TDP-43, a major pathological hallmark of ALS and an important therapeutic target. Using in vitro model systems, she identified a major role for RNA in regulating TDP-43 localization, and now seeks to apply that knowledge to better understand TDP-43 mislocalization in ALS and test candidate therapeutic approaches.

Lindsey Hayes, MD, PhD, is an associate professor of neurology at the Johns Hopkins University School of Medicine and Brain Science Institute. She is a physician-scientist who specializes in neuromuscular disorders including ALS. Her laboratory studies the disruption of RNA and protein trafficking in neurodegeneration with a focus on TDP-43. Her laboratory employs cellular and molecular techniques to analyze mechanisms that regulate TDP-43 localization and function, to inform understanding of disease mechanisms and as a platform for preclinical therapy development.



Robert G. Kalb, MD

Joan and Paul Rubschlager Professor, Chief of the Division of Neuromuscular Medicine, and Director of the Les Turner ALS Center at Northwestern Medicine

Targeting the Ubiquitin-Proteasome System in Neurodegenerative Disease

Most neurodegenerative diseases exhibit the accumulation of ubiquitinated protein aggregates at autopsy and this reflects an imbalance in the protein homeostasis capacity. Reducing the burden of misfolded proteins in cells can be healthful. Through forward genetic screens he has identified components of the ubiquitin proteasomal system that control the abundance of misfolded proteins and represent new potential therapeutic targets.

Robert G. Kalb, MD, is the Joan and Paul Rubschlager Professor, Chief of the Division of Neuromuscular Medicine, and Director of the Les Turner ALS Center at Northwestern Medicine. His laboratory studies the basic mechanisms underpinning ALS using genetically engineered mice, primary neuron culture, c.elegans, and yeast disease models. His work has focused on the fundamental molecular processes that go awry during disease. Dr. Kalb's group discovered that derangement of energy metabolism is a key contributor to neuronal death in models of ALS. The Kalb Lab is passionately committed to bending the arc of disease and finding a cure for ALS through innovative and collaborative research.

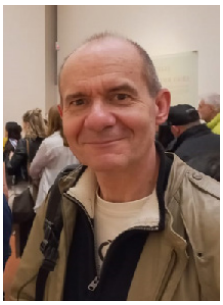
Hande Özdinler, PhD

Associate professor of neurology (neuromuscular disease) at the Northwestern University Feinberg School of Medicine

TDP-43 Pathology in the ALS Motor Cortex

TDP-43 pathology is one of the most common causes of neurodegeneration and it is also observed in the motor cortex of ALS and ALS/FTD patients. Dr. Özdinler developed two different mouse models of TDP-43 pathology, one for recapitulating the familial and the other for sporadic cases of the ALS disease. The goal is to understand the cellular and the molecular basis of upper motor neuron degeneration with respect to TDP-43 pathology.

Hande Özdinler, PhD, is an associate professor of neurology (neuromuscular disease) at the Northwestern University Feinberg School of Medicine. She is a faculty member at the Les Turner ALS Center, Mesulam Center for Cognitive Neurology and Alzheimer's Disease, Robert H. Lurie Comprehensive Cancer Research Center, and Chemistry of Life Processes Institute at Northwestern University. Dr. Özdinler's research aims to understand the cellular and molecular mechanisms responsible for selective vulnerability and progressive degeneration, with special interest in upper motor neurons. The Özdinler lab is currently working towards developing drug discovery platforms that incorporate upper motor neuron health as a read-out, and towards the identification of target engagement and pharmacokinetic biomarkers that can be utilized in the upcoming clinical trials, including patients with upper motor neuron loss.



Marco Martina, MD, PhD

Professor of neuroscience at the Northwestern University Feinberg School of Medicine

Cell Type-Specific Alterations of Cortical Excitability in a Model of Familial ALS

In both inherited and non-inherited forms of ALS, motor cortex hyperexcitability has been detected. This indicates its key role in the disease, although the underlying mechanisms are still unclear. He studied Alsin-KO mice, a well-known model for juvenile cases of ALS, to explore how different neurons in the motor cortex behave electrically at an early stage, before symptoms appear.

Marco Martina, MD, PhD, is a professor of neuroscience at the Northwestern University Feinberg School of Medicine and has investigated the functional and pharmacological properties of ion channels for nearly 25 years. In particular, his expertise concerns the impact of individual ion channel types on action potential generation and propagation, the role of dendrites in synaptic integration, and the properties and modulation of GABAA currents.

Dr. Martina has also contributed to the first characterization of the cellular pathology of corticospinal motor neuron in an alsin knockout mouse, a rodent model of genetic ALS, and to the characterization of the electrophysiological dysfunction of upper motor neurons of SODG93A mice.

Clinical Conversations Panel

Join us for an engaging afternoon discussion on the latest in ALS care and clinical research, from the perspectives of a clinician and people with lived experiences of ALS. By uniting the expertise of specialists in fields like pulmonology, nutrition, and social work, multidisciplinary care is making a difference in the quality of life and health of people living with ALS and their caregivers.

The Lois Insolia ALS Clinic at the Les Turner ALS Center at Northwestern Medicine has a comprehensive multidisciplinary team and is actively involved in multi-center drug trials and other clinical research. In this panel, you'll learn how clinicians and people with lived experience of ALS are shaping the future of multidisciplinary care, research, and community support. There will also be opportunities to ask questions about the latest advancements in these fields.

Senda Ajroud-Driss, MD

Les Turner ALS Foundation / Herbert C. Wenske Professor of Neurology Professor of Neurology; Director, Lois Insolisa ALS Clinic, Les Turner ALS Center at Northwestern Medicine; Director, MDA

Dr. Driss received her medical degree from The Medical School of Tunis, Tunisia, then completed her neurology residency at the University of Illinois at Chicago, and a neuromuscular fellowship at Northwestern. She is board-certified in neurology and in neuromuscular medicine and has been treating patients with ALS in the Lois Insolia ALS Clinic for nearly 20 years. Dr. Driss also leads the Les Turner ALS Center's clinical trial program and Scientific Advisory Board Member for Northeast Amyotrophic Lateral Sclerosis Consortium.

Melissa Diaz-Viera, LCSW

Support Service Committee, Les Turner ALS Foundation and ALS Research Ambassador for the Northeast ALS Consortium, Operations Coordinator for Project ALS

Melissa Diaz-Viera joined the Les Turner ALS Foundation's Support Services Committee in 2024. As a Licensed Clinical Social Worker, she brings years of experience in helping others navigate difficult life circumstances within complex systems, as well as a strong passion for advocacy, community support and collaborative care. Since her ALS diagnosis in 2023, Melissa has been involved in multiple groups within the ALS Community including Her ALS Story. She has contributed to research through participation in multiple observational studies and clinical trials. Melissa currently serves as a NEALS Research Ambassador and is the Research Operations Coordinator for Project ALS.

Angela Genge, MD

Professor of Neurology, Director, Montreal Neurological Institute and Hospital's ALS, Global Center of Excellence, ALS Clinic

Angela Genge, MD, is internationally recognized for her work in clinical trial design and development for rare neurological conditions, with an emphasis on ALS/MND. She has served as director of the ALS Global Center of Excellence and ALS Clinic at the Montreal Neurological Institute and Hospital since 1998. In 2023 she stepped down from her twenty-year tenure as executive director of the Institute's clinical research unit, where, under her direction, it evolved into the most active neuroscience unit in the country, conducting over 100 clinical trials from Phase 1 to Phase 4. She developed a Phase 1 unit dedicated to neurological diseases, the ALS Global Center of Excellence, and ACCESS ALS, all of which fuel the drug discovery pipeline and accelerate the development of new therapies for rare and terminal neurological diseases. Angela serves as the global principal investigator for AL-S Pharma, as well as sits on numerous advisory boards and data and safety monitoring boards. She previously served as a distinguished clinical investigator for Novartis Global.

Kelly Goodman

Support Services Committee, Les Turner ALS Foundation

Kelly Goodman joined the Les Turner ALS Foundation's Support Services Committee in 2024. She was first introduced to the Les Turner ALS Foundation when her husband Jason was diagnosed with ALS in 2017. Kelly cared for Jason throughout his illness, and she learned many of the needed caregiver skills from the clinic. After 26 years of marriage, and having ALS for 6 years, Jason passed away in March 2023. Kelly is the Director of Treasury and Business Services at Terlato Wine Group in Lake Bluff and has been working there for 25 years. She is also a member of the Foundation's Gratitude Group.

Moderator: Anne Marie Doyle, M.A., CCC-SLP

Community Education Manager, Les Turner ALS Foundation

Anne Marie serves as the Community Education Manager at the Foundation. Anne Marie earned a Bachelor's degree in Communication Sciences & Disorders from Saint Xavier University followed by a Master's Degree in Speech & Hearing Sciences from the University of Illinois Urbana-Champaign. For the last 13 years she worked as a speech-language pathologist at Shirley Ryan AbilityLab serving both the inpatient and outpatient populations with a specialty in adult neurological conditions.

Since 2015, Anne Marie worked directly with people living with ALS initially providing traditional voice and swallowing therapy, and more recently, focusing on Augmentative & Alternative Communication (AAC). She maintains a membership with the American Speech-Language & Hearing Association, as well as participates in their Special Interest Group for AAC. She has lectured at both the state and national level, as well as participated in clinical research. Anne Marie resides in Chicago with her husband and dog. She is humbled and honored to be joining the Foundation to continue to directly serve those with ALS, their families, and their caregivers.

Identification of a highly dynamic pathway regulating mitochondrial RNA and cytosolic RNA crosstalk by super-resolution live microscopy

Abby Woods[1], Yvette C Wong[1], Catherine Molakal[1]

[1]*Ken & Ruth Davee Department of Neurology, Feinberg School of Medicine, Northwestern University*

Mitochondria are highly dynamic organelles, which require proper regulation to maintain neuronal health. ALS is a devastating neurodegenerative disease characterized by the loss of upper and lower motor neurons. Of note, misregulation of both mitochondria and RNA have been proposed to contribute to the molecular mechanisms underlying ALS, suggesting that further understanding their crosstalk may shed light on ALS pathogenesis. Moreover, while mitochondria contain their own mtRNA, the mechanisms regulating mtRNA and its ability to be dynamically co-regulated with cytosolic RNA is still not well understood. Using live super-resolution microscopy over time, we identify a mitochondrial RNA binding protein which undergoes novel trafficking dynamics and phase separation dependent on mitochondrial function. Importantly, these dynamics were directly dependent on its ability to bind RNA. Interestingly, modulation of cytosolic RNA was further able to disrupt its proper trafficking dynamics. Finally, we find that trafficking dynamics of this mitochondrial RNA binding protein may be disrupted in models of ALS, potentially contributing to mitochondrial dysfunction in disease. In summary, our findings highlight a novel highly dynamic pathway for co-regulating mitochondrial RNA and cytosolic RNA, which may have important implications for understanding mitochondrial homeostasis and ALS cellular mechanisms.

The role of chondroitin sulfate proteoglycan 4 in toxic DPR uptake and intercellular transmission

Alexandra Sutter[1], Benito Buksh[2], Jelena Mojsilovic-Petrovic[1], David MacMillan[2], Robert G. Kalb[1]

[1]*Ken & Ruth Davee Department of Neurology, Feinberg School of Medicine, Northwestern University*
[2]*Department of Chemistry, Princeton University*

C9orf72 ALS is the most common monogenic form of ALS, comprising 25-40% of familial ALS cases. This form of ALS results from an intronic GGGGCC hexanucleotide repeat expansion in the chromosome 9 open reading frame 72, C9orf72, gene. One proposed mechanism of disease is a toxic gain of function from dipeptide repeat proteins (DPRs) which are products of repeat-associated non-AUG translation. DPRs have been shown in vitro to participate in a neurodegenerative disease spreading mechanism termed cell-cell transmission. We utilized a culture technique, with donor and receiver neurons, to demonstrate cell-cell transmission of one of the toxic arginine-rich DPRs, poly-PR, in primary rat hippocampal neurons. Bath application of hemagglutinin (HA) tagged PR of twenty repeats (HA-PR20) are internalized and rapidly localized to the nucleolus. We applied several endocytosis inhibitors to demonstrate that poly-PR internalization is accomplished through endocytic processes. Poly-PR endocytosis was potently inhibited by temperature shifts, clathrin-mediated inhibitors, dynamin inhibitors, and macropinocytosis inhibitors.

To understand how poly-PR is internalized into cells, we used a novel proximity labeling procedure and mass spectrometry to generate a candidate list of putative mediators of HA-PR20 uptake. A candidate of particular interest is the chondroitin sulfate proteoglycan 4 (CSPG4). The participation of proteoglycans in cellular uptake of HA-PR20 uptake is supported by three observations – uptake is inhibited by: 1) RNAi knockdown of CSPG4, 2) removal of chondroitin sulfate GAG chains by pre-treatment of cells with Chondroitinase ABC, and 3) RNAi knockdown of XYLT2, an enzyme involved in GAG chain synthesis. Furthermore, uptake of both poly-PR and poly-GR is reduced in CSPG4 null cells. We plan to next explore the role of CSPG4 in cell-cell spreading of toxic DPRs. We hypothesize that its depletion can prevent transmission of toxic DPRs between cells and slow disease progression.

Exploring a Neuroprotective role for BET proteins in C9orf72 pathology

Amber Ruccia[1], Jelena Mojsilovic-Petrovic[1], Robert G. Kalb[1]

[1]*Ken & Ruth Davee Department of Neurology, Feinberg School of Medicine, Northwestern University*

The most common genetic form of amyotrophic lateral sclerosis (ALS) is caused by a GGGGCC hexanucleotide repeat expansion in the C9orf72 locus. C9orf72 expansions lead to the transcription of repeat RNA, which can undergo noncanonical translation to form toxic dipeptide repeat (DPR) proteins. In a modifier screen in C. elegans, knockdown of Speckled type POZ Protein (SPOP) was found to suppresses DPR toxicity. SPOP functions in the ubiquitin proteosome system by presenting clients to cullin-type E3 ligases for ubiquitination and degradation. In a secondary screen targeting SPOP's known substrates, co-knockdown of Bromodomain and Extra-terminal Domain (BET) proteins suppressed the beneficial effects of SPOP knockdown on DPR toxicity, suggesting these proteins play a neuroprotective role. In the current study, we aim to understand the mechanism of this suppression of toxicity through BET proteins.

Nucleolar stress has been associated with DPR toxicity in various studies. We confirm hallmarks of nucleolar stress upon DPR expression in our model system. We also demonstrate BRD4, a BET protein family member, localizes to the nucleolus of neurons. Upon expression of DPRs, we find mislocalization of BRD4 to the periphery of the nucleolus. This mislocalization is rescued by SPOP knockdown, providing a potential mechanism of BET proteins' neuroprotection against DPR toxicity.

Dysregulated excitability caused by impaired homeostasis in mSOD1^{G93A} motoneurons

Amr Mahrous[1], Bradley Heit[1], CJ Heckman[1]

[1]*Department of Physical Therapy & Huan Movement Sciences, Feinberg School of Medicine, Northwestern University*

Multiple studies have reported changes in motoneuron excitability in the SOD1^{G93A} (mSOD1) mouse model of ALS. The observed alterations in motoneuron electrophysiological properties display both increases and decreases throughout the time course of disease progression. This has led us to hypothesize that homeostatic plasticity of spinal motoneurons is dysregulated in ALS. The dysregulation manifests as an increased 'gain' of compensatory mechanisms leading to overcompensation and oscillations that could contribute to motoneuron morbidity. To test this novel hypothesis, we evaluated changes in mouse motoneuron excitability following 10-days treatment with riluzole. This drug is known to decrease motoneuron excitability, and hence can trigger a compensatory homeostatic increase in excitability.

Young adult mice (P30 – P40, no detectable motor deficits at this age) were treated with riluzole (added to drinking water) for 10 days. At the end of treatment, the whole-tissue sacrocaudal spinal cord along with the attached roots was extracted, maintained ex vivo, and used to assess motoneuron excitability. Individual motoneurons were then recorded with sharp glass electrodes and subjected to different patterns of depolarizing currents to evaluate their excitability based on the gain of frequency-current (F-I) relationship. Data from mSOD1 mice was compared to that from age-matched non-transgenic (NT) mice which received similar treatment protocol.

Our data shows that chronic riluzole treatment at this dose caused no significant change in the F-I relationship of NT motoneurons. On the other hand, mSOD1 motoneurons exhibited a significant increase in F-I gain, indicating a hyperactive homeostatic response in this ALS model. These results has important implications for pharmacological treatment and possible early interventions in ALS.

The ALS-associated co-chaperone DNAJC7 mediates neuroprotection against proteotoxic stress by regulating HSF1 activation

Andrew Fleming[1], Nalini R. Rao[1], Matthew Wright[1], Jeffrey N. Savas[1], Evangelos Kiskinis[1]

[1]*Ken & Ruth Davee Department of Neurology, Feinberg School of Medicine, Northwestern University*

The degeneration of neurons in patients with amyotrophic lateral sclerosis (ALS) is commonly associated with accumulation of insoluble and misfolded proteins. Heat shock proteins (HSPs) are central regulators of protein homeostasis as they fold newly synthesized proteins and refold damaged proteins. Heterozygous loss-of-function mutations in the HSP DNAJC7 were recently identified as a cause for rare forms of ALS, yet the precise mechanisms underlying pathogenesis remain unclear. Using mass spectrometry, we found that the DNAJC7 interactome in human motor neurons (MNs) is enriched for RNA binding proteins (RBPs) and stress response chaperones. MNs generated from iPSCs with the ALS-associated mutation R156X in DNAJC7 exhibit increased insolubility of its client RBP HNRNPU and associated RNA metabolism alterations. Additionally, DNAJC7 haploinsufficiency renders MNs susceptible to proteotoxic stress and cell death as a result of an ablated HSF1 stress response pathway. This defect is mediated by an interference of the HSF1/HSP70 transactivation complex. Critically, expression of HSF1 is sufficient to rescue neurotoxic sensitivity to stress. Taken together, our work identifies DNAJC7 as a crucial mediator of both RBP function and proteotoxic stress sensitivity in human MNs and highlights HSF1 as a therapeutic target in ALS.

Les Turner ALS Foundation & NEALS' Collaborative Development of Tools for Increasing Awareness of and Participation in ALS Clinical Research

Anne Marie Doyle[1], Lauren Webb[1], Allison Bulat[2], Judith Carey[2]

[1]Les Turner ALS Foundation
[2]Sean M Healey & AMG Center for ALS & Northeast ALS Consortium

As the field of ALS research evolves, opportunities arise to participate in clinical research that could lead to new treatments and insights into the disease. Given the critical need for rapid enrollment of research studies, the Les Turner ALS Foundation collaborated with the Northeast Amyotrophic Lateral Sclerosis Consortium (NEALS) to develop educational tools to support participation in ALS clinical research; a user-friendly guide and decision support tool for people living with ALS. Both tools can be used in tandem and are currently available in English and Spanish.

The Foundation worked with members of the NEALS research and clinical community, along with people with lived experience, and health literacy professionals to develop resources that use plain language to introduce key concepts related to clinical research, how to participate in clinical research, and the rights of those that choose to participate. The resources lead users through the benefits of participation in clinical research and provides important perspectives of people with lived experience. Critical information to allow people living with ALS to make informed decisions.

We relied on a four-step process involving interviews of people with lived experience and research experts, drafting content, building and testing the prototypes, and launching the final products. The aim is to empower people living with ALS, increasing their confidence in their decisions about clinical research participation. These tools fill a gap in existing resources with convenient, understandable educational support. Since launching in May of 2024 the materials have had 2,000+ unique sessions and 1,800 viewers from 49 of 50 states in America and five out of seven continents. We plan to continually assess the resources' usability through web analytics and user feedback.

The utilization of these resources may enhance enrollment and diversity in clinical research, as well as break down barriers to enable early and informed decisions regarding participation. Additionally, these materials may reduce the burden of staff time by proactively providing materials prior to discussion, thus fostering more productive conversations. We hope that professionals caring for people living with ALS will consider sharing these unique resources, freely available on the Les Turner ALS Foundation website: lesturnerals.org.

Genetic Counseling and Testing Educational Resources: A Call to Action from the Genetic Summit Hosted by the International Alliance of ALS/MND Associations

Anne Marie Doyle[1], Lauren Webb[1], Laynie Dratch[2], Lisa Kinsley[3], Jennifer Roggenbuck[4], Ashley Crook[5]

[1]*Les Turner ALS Foundation*
[2]*University of Pennsylvania*
[3]*Feinberg School of Medicine, Northwestern University*
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[5]*Macquarie University*

In 2022, the International Alliance of ALS/MND Associations researched the genetic landscape of ALS. In 2023, evidence-based, consensus guidelines asserted that everyone with ALS should be offered comprehensive genetic testing, followed by, the first-ever approved gene-targeted therapy for SOD1-ALS. In 2024, The Miami Framework, was published offering more insights for people with a genetic connection to ALS and other related conditions. There is a critical need for resources that provide the most accurate information on genetic counseling and testing for people living with ALS and their family members because navigating genetic counseling and testing is daunting, especially with limited access to genetic counselors, thus supplemental education methods are critical to the community.

The Les Turner ALS Foundation worked with genetic counselors, clinicians, and those with lived experience to develop resources that uses plain language to introduce key concepts related to genetic counseling and testing. The guides and decision support tools explain how genetic counseling and testing works, leads users through the benefits and downsides of genetic counseling and testing, explores the emotional challenges that may come with genetic counseling and testing, and describes what people living with ALS or their family members can learn from genetic counseling and testing, and why this information matters.

We relied on a four-step process involving interviews of people with lived experience and genetic counselors, drafting content, building/testing prototypes, and launching the final products. Our first genetic decision tool launched last year followed by a family member tool this year. The tools have had a combined 4,000 unique sessions and 10,000+ views. The tools have been used in all fifty states in America and six out of seven continents. We are committed to learning more about the effectiveness of the tools and are collaborating with genetic counselors to study the tool using pre- and post-use outcome measures which is currently under IRB approval.

It is our goal that after using these educational materials that people living with ALS and their family members feel more confident in making an informed decision around genetic counseling and testing. To learn more please visit lesturnerals.org/resources.

Ambient glutamate and motoneuronal excitability: the role of system xc- in ALS pathogenesis

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Glutamate excitotoxicity is canonically viewed as an important mediator in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS). In both human and animal studies, neural networks exhibit elevated extracellular glutamate concentrations coupled with hyperexcitability which ultimately begets denervation. Not surprisingly, the role of synaptic glutamate has been heavily scrutinized in ALS etiology; ambient, extrasynaptic glutamate, however, has not been appraised in this regard. In the central nervous system, ambient glutamate is regulated by the cystine/glutamate antiporter, system xc-, which imports cystine from the extracellular compartment and exports glutamate in exchange. Importantly, system xc-, with protein subunit xCT, is markedly upregulated in both animal ALS models and human patients. The current study therefore explored the role of system xc- in ALS pathophysiology by assessing the antiporter's effect on lifespan, extrasynaptic glutamate concentrations, and motoneuronal excitability. Three mouse genotypes were examined: 1) xCT KO (xCT-/-) mice, a novel transgenic model lacking a functional system xc-, 2) SOD1G93A mice, a transgenic model of ALS pathology, and 3) wild-type (WT) controls. Remarkably, our initial studies found that xCT-/- mice exhibited extended lifespan when compared to WT controls, while, as expected, SOD1G93A mice displayed truncated lifespan. We next employed an in vitro spinal cord preparation to electrophysiologically measure motoneuronal excitability, as well as mass spectrometry to quantify ambient glutamate concentrations in the cerebral spinal fluid (CSF). Our experiments revealed decreased motoneuronal excitability in xCT-/- mice, as evidenced by enhanced paired-pulse depression (PPD), when compared to WT and SOD1G93A counterparts. Contrarily, SOD1G93A mice exhibited attenuated PPD as compared to xCT-/- and WT mice, thus revealing increased motoneuronal excitability. Moreover, the decreased excitability in xCT-/- mice was concomitant to reduced CSF glutamate concentrations, whereas the increased excitability in SOD1G93A mice was attendant to elevated glutamate concentrations. Importantly, treatment with system xc- inhibitor, CPG, mitigated hyperexcitability in SOD1G93A mice by increasing PPD. These data suggest that ALS-induced system xc- upregulation, and the obligate release of ambient glutamate, drive the motoneuronal hyperexcitability which elicits excitotoxicity and degeneration. Future studies will probe whether genetic deletion and/or in vivo pharmacological inhibition of system xc- can protract lifespan and rescue motoneuronal function in SOD1G93A mice. Collectively, these data portend a novel therapeutic intervention as several FDA-approved drugs, which function as system xc- antagonists, are currently available.

Modifiers of mTDP43 aggregation in cellular models

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TAR DNA-binding protein 43 (TDP-43) is a major pathological protein in Amyotrophic Lateral Sclerosis (ALS). At autopsy, aggregated TDP-43 inclusions are found in the cytoplasm of >95% of ALS patients. TDP-43 likely contributes to pathophysiological events by both loss-of-function (i.e., nuclear depletion) and gain-of-function (i.e., cytoplasmic aggregation) mechanisms. Many missense mutations in TDP-43 have been determined to be causative of familial ALS (fALS). These mutations increase aggregation propensity, aggravate cytoplasmic mislocalization, disrupt endogenous interactions and influence stability leading to resistance to degradation. Due to its vital endogenous functions and auto-regulatory activities, direct depletion of TDP-43 is unlikely to lead to a promising therapy. Therefore, we have explored the utility of targeting modifier genes.

RAD23 functions as a shuttle factor that binds ubiquitinated substrates and presents them to the proteasome for degradation. We have previously shown that loss of rad23 improves motor phenotypes in C. elegans models of fALS. In order to better understand the mechanism through which these protective effects are occurring, we generated doxycycline-inducible hemagglutinin-tagged Q331K human TDP-43 (mTDP-43) expressing HEK293 cells. Upon doxycycline treatment and "SarkoSpin" subcellular fractionation, knockdown of rad23a reduces the abundance of mTDP-43 that resides in the insoluble fraction. We replicated these studies in primary rat cortical neurons infected with A315T human mutant TDP-43 and similarly find that knockdown of rad23a reduces the percentage of mTDP-43 in the insoluble fraction.

We then conducted tandem mass tag proteomics in our doxycycline-inducible HA-tagged mTDP-43 expressing HEK293 cells to gain further insight into how loss of rad23a remodels the proteome. Proteins of interest were then identified based on total fold change, pathway analysis, and known interplay with neurodegenerative disease. After initial screening, we found that knockdown of usp13, a deubiquitinase, reduces the abundance of mTDP-43 that resides in the insoluble fraction in our doxycycline-inducible HEK293 cells and in primary rat cortical neurons. Additionally, a viability assay in primary rat motor neurons infected with A315T human mutant TDP-43 showed a blunting of neuronal cell death after usp13 knockdown. We next plan to explore if the deubiquitinase activity of USP13 is critical for the observed reductions in insoluble TDP-43 and if USP13 is a deubiquitinase of TDP-43.

Generation of a Novel Reporter Mouse Model for Investigating Neuroinflammatory Changes of Upper Motor Neurons in TDP-43 Pathology

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Protein aggregation and neuroinflammation are two common features in numerous neurodegenerative diseases. Transgenic mice expressing the human disease-causing TAR DNA binding protein-43 mutation (TDP-43A315T) are invaluable tool for studying frontotemporal degeneration (FTD) and amyotrophic lateral sclerosis (ALS). However, the role of peripheral or innate immune cells in cortical degeneration, especially the degeneration of the upper motor neurons (UMNs), remains unknown. In this study, we aimed to bridge this gap by developing a novel transgenic mouse model, in which the UMNs as well as the cells that are important for the initiation of the innate immune system are fluorescently labeled. We took advantage of UCHL1-eGFP reporter mice to specifically label corticospinal motor neurons (CSMN, a.k.a UMNs in mice) and crossed them with monocyte chemoattractant protein-1 (MCP1)-monomeric red fluorescent protein-1 (mRFP) reporter line, thereby creating a double transgenic reporter mouse model. Subsequently, we introduced prpTDP-43A315T transgenic mice into this double transgenic to generate a triple transgenic mice, in which MCP1-positive cells and CSMN are fluorescently labeled within the context of TDP-43 pathology. This transgenic line allows cellular visualization of both the key cells of the innate immune system as well as the motor neurons that degenerate in ALS and ALS/FTD. This significant shift from mice to neuron/cell enables more direct and translational analyses of the cellular defects observed in ALS and ALS/FTD patients with TDP-43 pathology. We conducted immunocytochemical analyses on paraformaldehyde fixed brain sections of both healthy and diseased animals at postnatal days 30, 60, and 100. Our results begin to reveal the intricate encounters of MCP1-positive cells with CSMN that become vulnerable and display progressive degeneration over time. We can, now, visualize how, when and where they interact at a cellular level. Flow cytometry analyses are also beginning to reveal dynamic changes on the identity of cells that express MCP1 and how they differ between periphery and the CNS. Our study provides compelling evidence of enhanced MCP1 expression and microglia-CSMN interactions in with respect to disease, emphasizing the significance of early neuroinflammatory influences in TDP-43 pathology. This novel triple transgenic mouse model offers cellular clarity on the complex cellular dynamics of neuroimmune modulation in TDP-43 pathology, providing insights directly relevant to human ALS and ALS/FTD patients.

Accelerated Centers of Enrollment for ALS Clinical Trials

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Published rates of enrollment in ALS clinical trials across the country range from 0.5 to 1 participant per site per month. With an ever-growing pipeline of ALS trials and research studies, these rates limit access to research for people living with ALS and slow down progress in drug development. Site bandwidth is one of the main limiting factors.

To pilot a new funding model to explore whether better resourced site teams can make an impact on the rates of enrollment at the respective site.

The Sean M. Healey & AMG Center for ALS at Massachusetts General Hospital and the Les Turner ALS Center at Northwestern Memorial Hospital are launching an innovative partnership to increase the rate of enrollment of study participants in ALS clinical research.

Funding was awarded to two pilot centers ("Accelerated Centers of Enrollment") to increase site capacity and infrastructure. Support for this program will initially fund training and salaries for an ALS enrollment team at each site. This team includes effort of a medical doctor or nurse practitioner, clinical research nurses, clinical research coordinators and project managers who will review the available clinical trials, expanded access protocols, and non-interventional studies with the goal of boosting enrollment across programs.

This program will explore whether upfront funding to build research infrastructure and hire dedicated clinical research teams will support higher enrollment rates across research programs.

We would like to thank an Anonymous donor for launching this effort, as well as Les Turner ALS Foundation and ALS ONE for their support.

Utilizing Physiologic Media to Model ALS Motor Neuron Metabolism

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Amyotrophic Lateral Sclerosis (ALS) is a debilitating neurodegenerative disease which selectively targets upper and lower motor neurons (MNs) of the brain and spinal cord. Mislocalization of RNA-binding protein TDP-43 and mitochondrial dysfunction are two common pathologic hallmarks of degenerating ALS MNs. However limited emphasis has been placed on accurately modeling human MN metabolism in vitro, and thus investigating the causal relationship between metabolic dysfunction, the aberrant action of TDP-43, and neurodegeneration. Traditional cell culture media utilized to maintain human neurons is specifically formulated to reduce metabolic stress and is not physiologic. Little is known about how a physiologic metabolic milieu affects MN metabolism, and what metabolic mechanisms in this context contribute to disease. Here, we aim to address this fundamental limitation by utilizing Human Plasma-Like Media (HPLM), which closely approximates the extracellular glucose and small metabolite composition of human plasma & cerebrospinal fluid. In preliminary experiments, we find HPLM can support iPSC-derived MNs and patterns of electrophysiologic activity. Using mass spectrometry analysis, we show HPLM remodels the intracellular polar metabolome of healthy MNs, and severely depletes intracellular glucose and downstream glycolytic metabolites. Additionally, we show that Phosphofructokinase-P (PFKP), a rate-limiting glycolytic enzyme, is pathologically mis-spliced in the context of TDP-43 knock down. Together, these results provide a promising platform for further investigation into novel metabolic mechanisms of MN vulnerability in ALS.

Developing A Semi-High Throughput Platform to Advance Drug Discovery Efforts for Upper Motor Neuron Diseases

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There are no preclinical drug discovery platforms for upper motor neurons (UMNs), which degenerate in ALS patients. Our overall goal is to identify compounds that improve the health of diseased UMNs. Therefore, we utilize reporter lines of UMNs that degenerate due to different underlying causes of ALS and that are labelled by eGFP expression. This approach allows development of a cell-based and mechanism-focused drug discovery approach that utilizes improved health of diseased UMNs as a readout.

Motor cortex is isolated from postnatal-day 3 (P3) UCHL1-eGFP (control) as well as SpastC448Y-UeGFP, hSOD1G93A-UeGFP, PFNG118V-UeGFP, and TDP43A315T-UeGFP (diseased) mice, dissociated and plated on 96-well plates with a density of 20,000 cells/well. They are cultured 3 DIV either in serum free medium or with compounds of interest (i.e. riluzole, edaravone, NU-9) and in combination (i.e NU9+Riluzole, NU9+Edaravone). They are fixed and subjected to immunocytochemistry to visualize UMNs (GFP), astrocytes (GFAP), and oligodendrocytes (Olig2). Images are taken on Molecular Devices ImageXpress Micro Confocal System at 10X to assess neuronal survival and the extent of gliosis and at 20X for detailed cellular analyses of UMN.

UMNs are distinguished from other cells/neurons by their eGFP expression. High-throughput imaging and analyses help determine and quantitate the changes in the numbers of cells (neurons, astrocytes and microglia), and changes in total neurite outgrowth, max process outgrowth, mean outgrowth intensity, total branch points, for each UMN in each well, for each genotype and treatment condition. These outcome measures reveal UMNs' response to treatment. Our initial results indicate that diseased UMNs have limited axon outgrowth and that they respond to treatment by extending their axon and neurite outgrowth. There are differences between UMNs that become diseased due to different underlying causes, and they respond differently to treatment.

We are developing a novel platform that can detect responses of diseased UMNs to different compound treatments. Our ongoing studies will help improve preclinical selection criteria and will also include UMN health in drug discovery efforts.

Mechanistic insights into mitochondrial oxidative phosphorylation regulation of TDP-43

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease affecting upper and lower motor neurons which currently has no cure. Patients suffer from muscle weakness leading to paralysis and eventual death within 2-4 years of diagnosis. TAR DNA Binding Protein (TDP-43) is an RNA binding protein associated with pathological aggregates in 97% of ALS patients. TDP-43 pathological aggregates accumulate in the cytoplasm and have been proposed to be the result of TDP-43's misregulated shuttling between the nucleus and cytoplasm. However, the mechanisms regulating TDP-43's dynamics over time are still not well understood. In addition, while mitochondrial dysfunction has also been previously linked to ALS pathophysiology, how this pathway modulates TDP-43 shuttling dynamics remains to be further elucidated. Through the utilization of super-resolution live microscopy, we identified a novel mechanistic pathway for mitochondrial regulation of TDP-43. We conducted live imaging to investigate the dynamics of TDP-43 shuttling from the nucleus to the cytoplasm upon modulation of mitochondrial oxidative phosphorylation. Interestingly, we found that this pathway may directly modulate post-translational modifications on TDP-43 to further regulate its shuttling dynamics, with important downstream consequences for additional key cellular proteins. Furthermore, modulating this pathway was sufficient to alter TDP-43's cytoplasmic aggregation, and may inform future therapeutic strategies targeting TDP-43 dynamics and its homeostatic role in both health and disease.

The ALS-associated NEK1 kinase regulates microtubule stability and axonal transport in human iPSC-derived motor neurons

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by the loss of motor neurons (MNs) in the brain and spinal cord. Breakthrough genetic studies of familial ALS patients have enabled the identification of causative mutations in genes that may play a role in disease pathogenesis. Recently, loss-of-function variants in a novel identified gene NEK1, which encodes the NIMA-related kinase 1 (NEK1) protein, have been demonstrated to confer susceptibility for up to 3% of all ALS patients and can thus be considered a major genetic cause of ALS. While little is known about the role of NEK1 in normal MN physiology, preliminary evidence from our lab has implicated a dysregulation of microtubule (MT) homeostasis in NEK1 loss-of-function iPSC-derived MN models.

Using unbiased mass spectrometry-based proteomics analyses, we discovered that NEK1 interactors and protein expression changes in response to NEK1 loss-of-function converge on proteins enriched for function in the microtubule (MT) cytoskeleton. We have additionally identified alpha-tubulin (TUBA1B), a major structural component of MTs, as a NEK1-interacting protein that undergoes phosphorylation by NEK1 in vitro. Follow-up unbiased phosphoproteomic analysis in conditional NEK1-knockout cells revealed a reduction in phosphorylation of TUBA1B at the T56 residue, which we then generated an antibody against and confirmed diminished phosphorylation in NEK1 loss-of-function iPSC-derived MN models. We then performed a series of functional assays to assess the effects of NEK1 loss-of-function on the MT network and discovered a reduction in MT stability associated with NEK1 reduction (via siRNA) or ALS-linked NEK1 mutations in iPSC-derived MNs. This phenotype is accompanied by decreases in MT detyrosination and acetylation, two post-translational tubulin modifications associated with long-lived and/or stable MTs. NEK1 loss-of-function also resulted in compromised neurite regeneration following axotomy and alterations in axonal transport in iPSC-derived MNs. Together, these data suggest that NEK1 may function in the regulation of MTs in human MNs, and that ALS-associated NEK1 mutations may contribute to degeneration of MNs through disruptions in MT stability.

TDP-43 Dependent Mis-Splicing of KCNQ2 Triggers Neuronal Hyperexcitability in ALS/FTD

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Cortical hyperexcitability is a broadly observed, yet poorly understood clinical feature of familial and sporadic amyotrophic lateral sclerosis (ALS). Nuclear depletion and cytoplasmic aggregation of the RNA splicing modulator TDP-43 is a unifying neuropathological feature identified in most ALS patients. Here, we sought to examine a potential association between TDP-43 dysfunction and neurophysiology. By integrating gene expression datasets from human iPSC-derived neurons depleted of TDP-43 and postmortem ALS tissue we identify spurious skipping of exon 5 of the voltage-gated potassium channel KCNQ2 (Kv7.2). KCNQ2 forms heterotetrameric channels with other Kv7 subunits to conduct M-current and regulate repetitive firing and excitability in neurons. We show that KCNQ2 is sensitive to TDP-43 levels and aberrant pre-mRNA processing yields a non-functional protein that disrupts neuronal excitability, accumulates within the ER in iPSC-derived neurons and forms abundantly present ubiquitinated aggregates in postmortem spinal cord tissue from ALS patients. This event strongly correlates with phosphorylated TDP-43 levels and age of disease onset in patients and can be leveraged as a novel biomarker for TDP-43 pathology and ALS diagnosis. Collectively, our work reveals that nuclear TDP-43 maintains the fidelity of KCNQ2 expression and function in human neurons and provides a mechanistic link between established excitability disturbances in ALS and TDP-43 dysfunction.

Addressing contributions of aberrant p38alpha MAPK signaling to axonal degeneration in the SOD1^{G93A} mouse model of amyotrophic lateral sclerosis

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Axonal pathology represents an early pathogenic event affecting motor neurons in both familiar and sporadic forms of amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disorder. The mechanism(s) by which axons degenerate in ALS are largely unknown. Numerous independent studies documented alterations in fast axonal transport (FAT), a major cellular process sustaining axonal heath, in a wide variety of familial ALS (fALS) models, including transgenic mice expressing mutant forms of superoxide dismutase 1 (mSOD1). The potential disease relevance of these observations was highlighted by genetic evidence showing that mutations in motor proteins powering FAT suffice to cause axonopathy and degeneration of motor neurons, but a mechanistic basis linking mSOD1 to such deficits remained elusive. Filling this gap in our knowledge, our studies in isolated squid axons showed that the toxic effect of mSOD1 proteins on FAT involves abnormal activation of the protein kinase p38alpha (p38α) and aberrant phosphorylation of axonal proteins, including the motor protein kinesin-1 and neurofilaments. Although numerous reports spanning decades documented enhanced phosphorylation (and hence activation) of p38 kinases, in ALS-affected tissues and mSOD1-based fALS mouse models, their potential contribution to axonal pathology elicited by mutant SOD1 in vivo remained unknown. We addressed this gap in our knowledge by examining specific contributions of p38α, the most abundant p38 isoform expressed in neurons. Towards this, we used a genetic approach to promote ubiquitous attenuation of p38α signaling in transgenic SOD1^{G93A} mice, an animal model where the axonal pathology phenotype of ALS is faithfully recapitulated. Remarkably, we found that genetically based attenuation of p38α signaling in SOD1^{G93A} mice prevented degeneration of spinal cord axons and loss of spinal motor neurons at an age when motor deficits are already manifested in this model. Interestingly, this effect was not associated with changes in transgenic mSOD1 expression and glial activation. Collectively, our findings reveal a significant contribution of p38-alpha to mutant SOD1-induced axonal pathology and neuronal preservation, suggesting this kinase might represent a potential therapeutic target to treat ALS.

Cell type changes and immune involvement in the central nervous system of ALS patients

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Amyotrophic lateral sclerosis (ALS) is a devastating progressive neurodegenerative disease which causes loss of motor neurons, leading to eventual paralysis and death. Despite being the most common form of motor neuron disease, with approximately 400,000 people affected worldwide, few effective treatments exist, and there is currently no known cure. As such, many collaborative projects have been established in order to gather patients, create large-scale data sets, and accelerate research by providing these to scientists.

By combining RNA-Seq data from two such projects, Target ALS and the New York Genome Center ALS Consortium, we analyzed 1464 samples taken from cortical and spinal tissues of 405 people with ALS and FTD, as well as healthy subjects. Leveraging recent single cell data sets from human spinal cord, motor cortex, and frontal cortex, we performed tissue-specific cell type deconvolution of these bulk tissue samples to determine cell type proportions in each tissue and examine how these populations change in ALS. In the frontal and motor cortices of ALS patients, we discovered modest but significant increases in microglia and inhibitory neuron cell types, as well as decreases in endothelial cell populations.

Deconvolution of spinal tissues, on the other hand, revealed drastic changes in nearly all profiled cell types, particularly in the cervical and lumbar spine. Specifically, we observed decreases in oligodendrocyte, endothelial, and neuronal populations, and increases in the proportion of astrocytes, microglia, and macrophage populations. Notably, we also observed increases in the proportion of lymphocytes, suggesting infiltration of immune cells into the ALS spine. Subclustering of the lymphocyte population revealed it is composed of T cells and NK cells, implicating these cell types in the progression of ALS.

Finally, we validated our findings by performing staining of cervical spine sections. Indeed, we observed a loss of MAP2+ motor neurons and an increase in IBA1+ microglia/macrophages in ALS spine compared to control tissue. Additionally, we were able to detect the presence of CD8+ T cells in ALS spine.

Together, this serves as a large-scale investigation into underlying cell type changes and immune cell infiltration that occurs in the CNS of ALS patients. We hope to link these changes to clinical features in order to determine how cell type changes affect and are affected by disease progression.

Regulation of nucleoporin proteostasis through SPOP and impacts on nucleocytoplasmic transport in cellular ALS models

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A hexanucleotide repeat expansion (HRE) in C9orf72 is the most commonly known mutation in familial frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS), two well studied neurodegenerative diseases. This expansion results in the transcription of G4C2 repeats that can lead to the production of toxic dipeptide repeat proteins (DPR) through repeat-associated non-ATG (RAN) translation. In an unbiased genome-wide RNA interference screen in C. elegans to discover genes that can suppress DPR toxicity, spop emerged as a significant suppressor of PR-50 toxicity.

Our lab has shown that speckle-type non-POZ domain containing protein (SPOP), a CUL3 ubiquitin ligase adaptor, interacts with certain nuclear pore proteins through proximity ligation assay and TURBO-labeling. These nucleoporins include NUP153, NUP214 and NUP98. Knocking down SPOP in cell models also increases protein levels of NUP153 and 214 as seen on a western blot. Coupled with cell and neuronal immunocytochemistry data that shows cytoplasmic Ran GTPase Activating Protein 1 (RANGAP1) mislocalizing into the nucleus in the presence of PR-50, we believe that nucleocytoplasmic transport is dysregulated in our ALS models. Here we propose that SPOP is a major regulator for the proteostasis of nucleoporins and can become a possible target to restore transport across the nuclear pore.

The role of Aurora B kinase in the development of neuron dysfunction in amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that affects the upper and lower motor neurons. While the cause of ALS is unknown, the most common genetic risk factor for ALS is an expansion of a hexanucleotide repeat in the chromosome 9 open reading frame 72 (C9orf72) gene. This repeat expansion leads to the expression and accumulation of 5 different dipeptide repeat proteins; however, it is unknown how these dipeptide repeats contribute to disease pathogenesis. There is evidence that neuronal nuclear dysfunction, such as abnormal size and shape, occurs in ALS. Our data shows that two of these C9orf72 dipeptide repeats, poly-RP and poly-RG, are cytotoxic when expressed in neuronal cells, producing nuclear and cellular pathological phenotypes. To explore a potential mechanism underlying the development of these pathologies, we focused on a signaling pathway that is critical for regulating nuclear processes. Aurora B kinase is a serine/threonine kinase that localizes to chromosomes and has been reported to play an important role in both mitosis and cytokinesis in differentiating cells, as well as neurite outgrowth in neurons. Based on its predominantly nuclear localization and functions, our hypothesis is that an impairment in Aurora B kinase activation contributes to the development of both nuclear pathologies associated with the poly-RP and poly-RG dipeptides. To address this hypothesis, we transfected rat primary cortical neurons from 18-day embryos with expression plasmids containing the C9orf72 dipeptides. We found that there are alterations in Aurora B kinase levels in cells expressing the poly-RP and poly-RG dipeptides, specifically a decrease in cells expressing the poly-RP dipeptide and an increase in cells expressing the poly-RG dipeptide. Treating primary cortical neurons that were transfected with the dipeptides with a selective Aurora B kinase inhibitor, AZD1152, resulted in a decrease in neurite length in cells expressing the poly-RP dipeptide and an increase in neurite length in cells expressing the poly-RG dipeptide, suggesting that Aurora B kinase plays a role in axonal processes. Our findings indicate that alterations in Aurora B kinase may be involved in the development of pathological phenotypes in C9orf72-associated ALS, perhaps establishing an overall mechanism of neurodegeneration.

Cholinergic neuron-specific molecular clock ablation drives ALS disease phenotypes

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Sleep disruption and poor sleep quality are extremely common in patients with ALS and may arise from various etiologies. These disturbances substantially add to disease burden for both patients and caregivers, underscoring the importance of research into potential methods of amelioration. Here, we investigate a connection between ALS disease phenotypes and disruption of the molecular circadian clock, which regulates the physiological sleep/wake cycle. ALS is a disease that originates in motor neurons, which are cells that have long axons and high energetic demands, making them uniquely susceptible to metabolic stress and degeneration. To elucidate the effect of motor neuron circadian clock disruption on ALS disease phenotypes, we generated a mouse model with cholinergic neuron-specific molecular clock ablation and monitored activity using a metabolic chamber. Our analysis revealed normal locomotor activity compared to controls, with increased activity in both groups during the dark period, as expected. Interestingly, we also observed an increase in whole-body energy expenditure in the experimental mice, along with an increase in the rate of oxygen consumption and the rate of carbon dioxide emission in both the light and dark periods. This hypermetabolic state has been similarly reported in mouse models of ALS as well as in patients with ALS. Lower motor neurons innervate hindlimb skeletal muscle, and our preliminary qPCR analysis also revealed changes in skeletal muscle oxidative and glycolytic fiber mRNA expression levels in the experimental animals. Finally, our functional DigiGait analysis demonstrated a decrease in hindlimb swing time, or the amount of time the limb is in the air while walking, supporting that lower motor neuron clock ablation alters downstream muscle function. Together, this suggests a role for the cholinergic lower motor neuron molecular clock in ALS disease outcomes.

Ataxin-2 Regulates Microtubule Dynamics in *Drosophila* Neurons

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Ataxin-2 (Atx2), is a highly conserved RNA-binding protein, and the expansion of its polyglutamine (polyQ) repeats is strongly associated with neurodegenerative diseases, including Amyotrophic Lateral Sclerosis (ALS). Notably, studies in ALS models of *Drosophila* and mice have shown that either upregulation or downregulation of Atx2 can modulate cytoplasmic aggregation and lifespan, suggesting that Atx2 is a potent modifier in ALS. However, the biological functions of Atx2 in the nervous system remain largely unknown, leaving unanswered questions about how varying levels of Atx2 modify cellular aggregation. Our lab previously identified Atx2 as a regulator of microtubules (MTs) during neuronal development. We demonstrated that Atx2 knockdown (KD) decreases MT dynamics, leading to severe defects in neurite outgrowth, and MT-dependent organelle transport in *Drosophila* motor neurons. Additionally, we observed impaired larval locomotion and early lethality. Recently, we found that Atx2 overexpression enhances MT dynamics. These findings suggest that Atx2 levels fine-tune MT dynamics, which may explain how its up- or downregulation affects cellular aggregation. We also identified that Atx2 regulates MT dynamics through its RNA-binding domains, known as Lsm (Like-Sm) domains. Remarkably, the Lsm domains of human ATXN2 (hsATXN2) can reverse the MT phenotype caused by Atx2-KD in *Drosophila*, indicating an evolutionarily conserved function of Atx2 in MT regulation. By analyzing the mRNA targets of Atx2 in recent studies and conducting RNAi screening, we identified Uncoordinated-76 (UNC-76), the ortholog of human FEZ1, as a potential downstream factor in Atx2's function during neuronal development. Depletion of UNC-76 caused defects in neurite outgrowth and locomotion, mirroring the observations seen in Atx2 KD. Collectively, our findings demonstrate that Atx2 regulates MT dynamics through its conserved RNA-binding domain and propose that UNC-76 functions downstream of Atx2 during neuronal development.

The Burden of Delayed ALS Diagnosis: Unnecessary Procedural Intervention & Healthcare Costs

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Patients with ALS often experience long diagnosis delays, characterized by multiple visits with both neurologist and non-neurologist specialists. Along this diagnostic journey, patients may be misdiagnosed and undergo inappropriate and unnecessary procedures. Further, ALS is often a diagnosis of exclusion, thus requiring a comprehensive work-up that may be costly. More research and understanding of the diagnostic path to ALS are necessary for better care and avoidance of economic burden.

The objective of this research is to provide an estimate of ALS costs accrued from symptom onset to diagnosis, thus including costs associated with misdiagnosis (unnecessary intervention) and early-stage diagnosis (exclusionary testing).

The sample comprised 150 patients with ALS referred to the Les Turner ALS Center for suspected ALS diagnosis, second-opinion, or transfer of care. Data was collected via retrospective chart review of a cohort of patients participating in the ALS/MND Natural History Consortium Study. For each patient, we noted the occurrence of incorrect procedural intervention prior to ALS diagnosis and the assessments included within the diagnostic workup. Cost estimations were calculated using Medicare Claims Database / Procedure Price Lookup Tool and Northwestern Hospital Price Transparency documents.

Among the 150 patients within our sample, 47 patients (31%) underwent procedural intervention prior to ALS diagnosis. The most common category of surgery reported was spinal surgery (n=17/42, 40%), followed by tunnel release (n=16/47, 34%). Non-surgical interventions reported most frequently included steroid injections of the back, knee and shoulder (n=10, 24%) and endoscopy with or without biopsy (n=5, 12%). Of the subgroup that underwent intervention, the average procedural cost per patient was $12,285 US Dollars. The average diagnostic cost per patient, including laboratory tests, MRI brain, MRI spine, and CSF analysis was $16,967 US Dollars. Lastly, the cost including all diagnostic assessments, as well as procedural intervention if present, was $19,915 US Dollars per patient.

The path to ALS diagnosis is long and costly, requiring extensive diagnostic workup and potentially resulting in unnecessary invasive procedural intervention. Further, delayed diagnosis carries an emotional burden and delays access to both multidisciplinary care and clinical trials. By providing further data on the ALS diagnostic workup, we hope to shed light on a topic that if confronted can alleviate healthcare costs and increase patient quality of life.

Co-targeting TDP-43 loss-of-function-induced mis-splicing using multiplexed antisense oligonucleotides

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TDP-43 is a DNA/RNA binding protein primarily residing in the nucleus to regulate RNA metabolism. TDP-43 mislocalization has been found in >97% of amyotrophic lateral sclerosis (ALS) cases, where it aggregates in the cytoplasm and is depleted in the nucleus. The TDP-43 nuclear depletion leads to the mis-splicing of several dozen genes, including UNC13A, KCNQ2, and STMN2. In this ongoing study, we aim to target these three genes simultaneously using multiplexed antisense oligonucleotides (ASOs) encapsulated in spherical nucleic acids (SNAs), to test the hypothesis that co-targeting mis-spliced genes can effectively improve neuronal function and survival. We designed gene-specific splice-modulating ASOs for UNC13A and KCNQ2 and completed primary screens in human induced pluripotent stem cell (iPSC)-derived motor neurons identifying lead candidate molecules for each gene. We further tested the uptake efficiency of two forms of functionalized SNAs. We are currently optimizing the encapsulation of three ASOs (for UNC13A, KCNQ2, and STMN2, respectively) into SNAs. After optimization, functionalized ASO-SNAs will be delivered to TDP-43 loss-of-function motor neurons to restore the splicing of UNC13A, KCNQ2, and STMN2. Together, this study will develop multiplexed ASO-SNAs to target three mis-splicing events upon TDP-43 nuclear depletion as a proof-of-concept experiment of co-targeting TDP-43 loss-of-function-induced mis-splicing in ALS.

Enhancing Gut-Brain Axis Integrity: Riluzole and Butyrate as a Combined Therapy in SOD1^{G93A} Mice

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Amyotrophic lateral sclerosis (ALS) is a fatal neuromuscular disease characterized by progressive motor neuron death and paralysis. Emerging evidence has shown that gut-brain barrier dysfunction occurs at the early stages of ALS. Our previous studies demonstrated that feeding the SOD1^{G93A} mice with butyrate significantly decreased aggregation of the human-SOD1^{G93A} (h-SOD1) mutated protein, improved intestinal barriers, delayed the disease onset, and prolonged the life span of ALS mice. Riluzole is the first FDA-approved drug for ALS treatment.

We hypothesize that Riluzole and butyrate combined treatment decreases aggregation of the h-SOD1 mutated protein, restores the gut-brain barrier function and delays the ALS disease onset compared to using Riluzole or butyrate alone.

SOD1^{G93A} mice (Age: 9-10 weeks, male and female) treated with Riluzole (10 mg/kg, I.P. daily) alone, butyrate (2% in drinking water) alone, Riluzole and butyrate combination, with the treatment duration of 6 weeks. Rotarod test, grip strength, intestinal permeability, aggregation of the h-SOD1 mutated protein, and gut-brain axis barrier function markers (ZO-1 and Claudin-5) have been examined.

Butyrate alone, Riluzole alone or Riluzole and butyrate combination treated SOD1^{G93A} mice all had a significantly increased rotarod time, increased grip strength and decreased intestinal permeability compared to control mice. More importantly, Riluzole and butyrate combination showed a significantly longer rotarod time, more increased grip strength, enhanced intestinal barrier, compared with Riluzole or butyrate alone treatment. The aggregation of the h-SOD1 mutated protein was tested as an indicator of ALS progression. More reduced SOD1^{G93A} aggregation was observed in intestinal, spinal cord and brain with Riluzole and Butyrate combination treatment compared with Riluzole or butyrate alone treatment. The expression of tight junction proteins (ZO-1 and Claudin-5) significantly increased in intestinal, spinal cord and brain with Riluzole and butyrate combination treatment, compared with Riluzole or butyrate alone. Claudin-1 expression showed no change in intestinal and brain. Our data suggestion that ALS mice treatment with Riluzole and butyrate combination is more efficient than butyrate or Riluzole alone treatment in delay ALS progress. Restoring barrier function through the gut-brain axis provides a potential therapeutic strategy for ALS.

Development of small and potent promoters for ALS gene therapy

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Gene therapy is presumably the most effective strategy for treating genetic diseases, including ALS that is linked to mutations in over 20 genes, such as SOD1, C9orf72, TDP43 and FUS. Depending on the mechanisms of the diseases, therapeutic strategy can be designed either to supply the functionally lost genes or to reduce the expression levels of the toxic disease-causing genes. Tofersen, an antisense oligonucleotide drug to reduce SOD1 expression, has been approved by FDA for the treatment of SOD1-linked ALS. This approval was primarily based on 35% reduction in CSF SOD1 and 55% reduction in plasma neurofilament light chain observed in treated patients. However, Tofersen was unable to show significant clinical benefit, raising the concerns about the limitations of such an antisense oligonucleotide approach, and suggesting that further reduction of the disease-causing gene expression may be required for sufficient clinical benefits. To further reduce SOD1 expression, we tested a CRISPR/ Cas9 genome-editing strategy to disrupt the SOD1 gene with a transgenic approach. We found that CRISPR/Cas9- mediated genome-editing is an effective and safe strategy to target hSOD1, leading to a disease-free condition in the ALS mice. These data provide proof-of-principle evidence for CRISPR/Cas9 genome editing as a safe and efficient gene therapy.

Currently gene therapy components are mostly delivered to the patients through AAV vector. There are two major limitations for AAV-mediated gene therapy, i.e., the promoters are large in their size, limiting the size of the genes to be expressed, such as the large CRISPR/Cas9 machinery; and the promoters are weak, limiting the therapeutic efficacy or leading to an increased risk of side effects if higher doses are used. The currently most used promoters in clinical studies are CAG and its derivatives, which range from 0.8kb to 1.7kb. Development of small and stronger promoters represents a great challenge for gene therapy. We engineered and tested more than 100 promoters, ranging from <100 bp to ~200bp. We successfully developed more than 10 small and potent promoters. Although 4~10 times smaller than CAG promoters, these promoters showed much higher activities than the currently used CAG promoters, thus providing great advantages to drive gene expression for gene therapies for ALS and other diseases.

Investigation of Maturation and Network Dynamics of Healthy and Diseased Corticospinal Motor Neurons with TDP-43 Pathology, Using High-Density Microelectrode Array System

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Corticospinal motor neurons (CSMN) are one of the key components of the motor neuron circuitry. Their degeneration is a hallmark of ALS. TDP-43 pathology is broadly observed in ALS and ALS/FTD patients. Cortical dysfunctional, such as hyperexcitation followed by hypoexcitation, has been reported as an early event in ALS, potentially contributing to TDP-43 pathology. However, our understanding of the intrinsic and extrinsic controls over cortical dysfunction and how that relates to disease state and pathology is not complete.

Combining high-density microelectrode array (HD-MEA) system and optical imaging with cultures established from reporter lines of neurons diseased in ALS, enables electro-physiological assessment of healthy and diseased neurons with cellular precision. This approach also provides an understanding of the electrophysiological characteristics of healthy and diseased neurons as they form network connections.

We utilize UCHL1-eGFP (G) and TDP-43A315T-UeGFP (TG) mice, in which CSMN are labeled with eGFP expression. The motor cortex cultures from G (healthy) and TG (diseased) mice are established on HD-MEA system, which can record from 4096 active channels simultaneously. Spontaneous neuronal activity is recorded at 12, 15 and 18 days in vitro (DIV). Individual channels that contain recordings from single CSMN are further assessed to determine the firing rate, burst rate, inter-spike interval, inter-burst interval, peak amplitude, burst duration, and percentage spikes in the burst. These outcome measures are studied in both healthy and diseased CSMN at all time points. Cross-correlation analyses are performed to observe network dynamics and how that differs between healthy and diseased neurons and their environment.

We find that based on changes in firing and burst frequencies, healthy CSMN mature, and their connectivity patterns also transition from "random" network to "scale-free" network between 12-18 DIV. Our ongoing studies suggest that CSMN diseased with TDP-43 pathology have a reduced burst duration and inter-spike intervals as early as postnatal day 15 and 18, a very early stage in the disease with no symptoms or neuron loss.

Our study is important for bringing cellular clarity for the investigation and understanding of cortical connectivity, cortical function and dysfunction with respect to disease and TDP-43 pathology.

Peripheral Immune Dysregulation Contributes to Central Nervous System Inflammation in Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease marked by neuroinflammation, characterized by the activation of CNS microglia and macrophages, as well as widespread immune system dysregulation. Recent studies show that genetic risk factors for ALS, such as a repeat expansion in C9orf72 that accounts for 40% of genetic ALS cases, are associated with peripheral immune dysfunction. However, it remains unclear whether peripheral immune cells are differentially affected in genetic versus sporadic forms of the disease. In this study, we employed single-cell RNA sequencing on over 600,000 peripheral blood mononuclear cells (PBMCs) from 22 ALS patients and 18 age-matched healthy controls. Among the ALS patients were four carriers of C9orf72 repeat expansions. We also examined nine patients with fast-progressing and nine with slow-progressing sporadic ALS. Using a novel CRISPR-mediated library preparation method, we enhanced the sensitivity for detecting low-abundance immune gene transcripts. We identified 31 immune cell types through multimodal reference mapping. Specifically, we identified a high number of dysregulated genes in monocytes derived from both C9orf72 carriers and sporadic ALS patients compared to healthy controls. Utilizing the 10X Visium platform, we spatially mapped proteomic and transcriptomic expression in cervical spinal cord sections from 10 healthy controls and 10 ALS patients, including 5 with sporadic ALS and 5 C9orf72 expansion carriers. Our analysis revealed significantly elevated innate immune system-mediated inflammation in both the white matter and anterior horns of ALS patients relative to healthy controls. Altogether, our findings reveal a dysregulated innate immune transcriptome that may contribute to the onset or progression of both sporadic and genetic ALS.

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About The Les Turner ALS Foundation

The Les Turner ALS Foundation was founded in 1977 by the family and friends of Les Turner, who was diagnosed with ALS at the age of 36. Les's wife Ina and their three young boys turned to Les's brother-in-law and best friend, Harvey Gaffen, for help. At a time when information and research on ALS was almost non-existent, they set out to raise funds to provide vital research and resources to people living with ALS and their families.

In 1979, the Foundation established one of the world's first laboratories devoted to ALS research at Northwestern Medicine, followed by the opening of the Lois Insolia ALS Clinic in 1986, which provides world-class multidisciplinary care for people living with ALS. Since then, the Foundation has directly funded over $32 million in ALS research and clinical care, as well as millions more in indirect funding.

Today, the Les Turner ALS Foundation is the Midwest's leading ALS organization. For more than 45 years, it has been our mission to provide the most comprehensive care and support to people living with ALS and their families so they can confidently navigate the disease and have access to the most promising therapies. We treat each person like family, supporting them every step of the way, and provide their loved ones with answers and encouragement.

Under the leadership of Robert G. Kalb, MD, Joan and Paul Rubschlager Professor of Neurology, Chief of Neuromuscular Disease; Director, Les Turner ALS Center, Northwestern University Feinberg School of Medicine, the Les Turner ALS Center comprises more than 70 members working across Northwestern's Chicago and Evanston campuses, uniting expertise across scientific disciplines to generate new insights and significant advances in the fight against ALS.

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