LES TURNER ALS RESEARCH AND PATIENT CENTER AT NORTHWESTERN MEDICINE

5th Annual Les Turner Symposium on ALS and NeuroRepair

Celebrating Research, Patient Care and Education

Monday, November 16, 2015
Conference Room L, 3rd Floor
Prentice Women's Hospital
250 E. Superior Street, Chicago, IL
8am - 4:30pm

Keynote speaker

Kevin Eggan, PhD

Professor of Stem Cell and Regenerative Biology at Harvard University

LES TURNER ALS FOUNDATION

Northwestern Medicine®

5th Annual Les Turner Symposium Organizing Committee

Andrea P. Backman
Tara Y. Davis
Shari Diamond
P. Hande Ozdinler, PhD
Suzanne R. Pressler
Judy Richman

* Biosketches are provided by the speakers.

Dear Friends and Colleagues,

Welcome to the 5th Les Turner Symposium on ALS and NeuroRepair. This year is very special for us, as we celebrate the first year of Les Turner ALS Research and Patient Center at Northwestern Medicine. Les Turner ALS Foundation is one of the first ALS foundations in the world; started in 1977 by the friends and family members of Les Turner, who was diagnosed with ALS as a young father of three boys. What a few good people started at the day turned into a world-renowned foundation that supports both research and patient care.

We initiated this Symposium 5 years ago with the hopes of creating a momentum for collaborative efforts among scientists who work on different aspects of the big puzzle. We all focus on our individual research, but sometimes we fail to see the forest for the trees. Meetings like this broaden our perspective, enhance our will to work together. Given the complexity of the diseases, we need to be as collaborative as the neurons in our brain, which makes millions of connections to be functional.

As you know, each year we focus on a different topic and this year we will be celebrating the establishment of the new Les Turner ALS Research and Patient Center at Northwestern Medicine, and thus the title of our Symposium is "*Celebrating Research, Patient Care and Education*". We are thrilled to have Dr. Kevin Eggan join us from Harvard Medical School as the Keynote speaker. We have a very full program with poster presentations, Data Blitz talks given by seven renowned scientists from three different institutions in the Chicago area, followed by clinical and research sessions that include presentations from Les Turner Center faculty senior faculty members and clinicians.

Today, as we celebrate our will and determination to join our forces toward one common goal under the umbrella of one Center, we also salute our patients, caregivers, families, and friends. Without their courage, their unyielding will to overcome ALS, and their generous support, we would not be here today. As we set the stage for a better future for patients, scientists, researchers, doctors, care givers and friends, we are very pleased to have you with us. Welcome! Welcome to the 5th Annual Les Turner Symposium on ALS and NeuroRepair. Let's celebrate the birth of a new ALS Center at the heart of the windy city, the city that is reborn from its ashes, the city of change and hope.

Sincerely,

Pembe Hande Ozdinler

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

Participating Vendors

Brightstar Care – Chicagoland Region

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Les Turner ALS Foundation would like to thank

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CMIDD
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Program

8: 00 am -4: 30 pm: **Poster Session** (Abstracts are in alphabetical order)
9: 00 am- 4: 30 pm: **Vendor and Health Professional Session**
9: 00am – 11: 00 am: **Data Blitz presentations**

- 9: 00 pm: **Dr. Raymond Roos;** Marjorie and Robert E. Strauss Professor of Neurological Science, University of Chicago.
- 9: 20 am: **Dr. Jeffrey Loeb**; Department Chair, Neurology Department, University of Illinois at Chicago
- 9: 40 am: **Dr. Jane Wu;** Charles Louis Mix Professor of Neurology, Northwestern University
- 10: 00 am: **Dr. CJ Heckman**; Professor of Physiology, Northwestern University
- 10: 20 am: **Dr. Christine DiDonato;** Associate Professor, Pediatrics-Human Molecular Genetics, Northwestern University
- 10: 40 am: **Dr. Trevor T. Nicholson;** Instructor, Pulmonary Medicine, Northwestern University
- 10: 50 am: **Dr. Javier Jara;** Research Assistant Professor, Department of Neurology, Northwestern University

11: 00am-11: 15 am: **Break/Refreshments**

11:15-am- 12:15am: **Keynote Speaker**
Dr. Kevin Eggan; Professor of Stem Cell and Regenerative Biology, Harvard University

“ A Novel Therapeuitic Candidate for ALS from Studies of Stem Cell Derived Motor Neurons”

12:15am-1:20 pm: **Lunch Reception**
1:20pm-1:30 pm: **Freeze ALS Video Presentation**

1:30pm- 2:30 pm: **Clinical Session**

- 1: 30pm: **Dr. Robert Sufit;** Director, Lois Insolia ALS Clinic; Professor, Neurology
- 1: 50 pm: **Dr. Senda Ajroud-Driss;** Associate Professor, Neurology
- 2: 10 pm: **Dr. Lisa Wolfe**; Associate Professor, Neurology

2: 30pm: 3: 00pm: **Break/Refreshments**

3:00pm- 4:30 pm: **Research Session**

- 3: 00 pm: **Dr. Hande Ozdinler;** Assistant Professor, Neurology
- 3: 30 pm: **Dr. Evangelos Kiskinis;** Assistant Professor, Neurology
- 4: 00 pm: **Dr. Teepu Siddique;** Director of Neuromuscular Medicine, Professor, Neurology

Keynote Speaker

Kevin Eggan, PhD.

Professor in the Department of Stem Cell and Regenerative Biology, Harvard University.

As a young investigator in the burgeoning field of stem cell biology, Dr. Eggan has garnered international recognition for his seminal work and a number of high profile awards for his creativity and productivity, including the MacArthur Foundation "Genius Grant" in 2006. He has made fundamental contributions to the fields of stem cell biology and cellular reprogramming, which in turn led his group to pioneer an entirely new strategy for studying human disease.

While training, Dr. Eggan performed nuclear transfer studies that challenged preconceived notions concerning the limits of cellular plasticity (Eggan *et al*., 2000, 2001, 2004). His own lab then became the first to demonstrate that human somatic cells could be reprogrammed to an embryonic stem (ES) cell state (Cowan *et al*., 2005). This demonstration that human ES cells harbored reprogramming activities has been cited as an inspiration for the discovery of factors used to generate induced pluripotent stem cells (iPSCs). Through persistent reprogramming attempts, his lab became the first to generate patient-specific iPSCs and use them to produce the cell type that degenerated in that individual (Dimos *et al*., 2008).

As these patients suffered from ALS, he was inspired to explore stem cells as a renewable source of motor neurons for studying mechanisms leading to neural degeneration. These experiments were the first "stem cell models" of disease and enabled the discovery that astrocytes are important non-cell autonomous contributors to motor neuron degeneration in ALS (DiGiorgio *et al*., 2007 and 2008). Subsequently, Dr. Eggan's group used this novel approach to study disorders that were intractable in rodents (Mekhoubad et *al.*, 2012), discover new mechanisms that lead to motor neuron degeneration (Suzuki et *al*., 2013 Kiskinis *et al*., 2014), and finally to identify a candidate ALS therapeutic (Wainger *et al*., 2014).

Dr. Eggan completed his B.S. in microbiology at the University of Illinois in 1996. A two-year Pre-doctoral internship at Amgen at the National Institutes of Health in Bethesda solidified his desire to pursue a career in academic research. He enrolled at the graduate school of Massachusetts Institute of Technology in 1998 shortly after the cloning of Dolly the Sheep was reported. During his Ph.D. training, he

actively pursued projects focused on cloning, stem cells and reprogramming after nuclear transfer under the guidance of genetics pioneer, Dr. Rudolf Jaenisch. He stayed in the Dr. Jaenisch's lab after his graduation for a one-year postdoc training in 2002. During that time, he conducted a collaborative study with Dr. Richard Axel, a Nobel Prize winner at the Howard Hughes Medical Institute. In 2003, he moved to Harvard University as a junior fellow and then became an assistant professor of Molecular & Cellular Biology at the Stem Cell Institute in 2005. In 2009, he was selected as one of 50 Howard Hughes Medical Institute Early Career Scientists receiving six years of dedicated support to conduct transformative research. Dr. Eggan was promoted to Professor in the Department of Stem Cell and Regenerative Biology in 2012. The success of Dr. Eggan's laboratory in the study of motor neuron disease has led to his appointment as the Director of the Stem Cell Program at the Stanley Center for Psychiatric Research at the Broad Institute. In this role he is leading a group of scientists to expand the platform to increase reproducibility of stem cell and reprogramming technologies with the ultimate goal of improving understanding and treatment of psychiatric diseases.

DATA BLITZ Speakers

Raymond Roos, M.D. Marjorie and Robert E. Strauss Professor of Neurological Science, University of Chicago.

Raymond P. Roos, MD, is an established expert on neurodegenerative diseases (such as amyotrophic lateral sclerosis (ALS) and prion diseases (e.g., Creutzfeldt-Jakob disease), multiple sclerosis (MS), inflammatory central nervous system diseases, and neuropathy. He directs the ALS/Motor Neuron Disease clinic.

A basic goal of Dr. Roos' research studies is to use molecular techniques to better understand the pathogenesis of MS and ALS. For instance, in one investigation, he and his colleagues sought to identify genes and gene products critical to the death and survival of motor neurons--especially as related to neurodegenerative diseases such as ALS. The lab uses viruses and transgenic mouse models in order to clarify the mechanisms by which mutant genes kill the target neurons.

Jeffrey Loeb, M.D., Ph.D. Department Chair, Neurology Department, University of Illinois at Chicago.

Dr. Loeb currently serves as the department head of the UIC Department of Neurology & Rehabilitation and previously was a professor in the department of neurology and the associate director of The Center for Molecular Medicine and Genetics at Wayne State University School of Medicine in Detroit, Michigan. He received his MD and PhD from the University of Chicago and went on to complete a residency in neurology at the Massachusetts General Hospital in Boston then followed by fellowship training in epilepsy and clinical neurophysiology at Harvard's Beth Israel Deaconess Hospital. At the same time, Dr. Loeb held a faculty appointment at the Harvard Medical School where he performed research on nervous system development with Gerald Fischbach in the Department of Neurobiology.

During his 20 year career, Dr. Loeb has authored numerous publications and has been the recipient of research and teaching awards. Among these awards, Dr. Loeb has been listed as one of the Best Doctors in America 2007-2013, and honored by Wayne State University with the Outstanding Graduate mentor Award and numerous Faculty Research Awards. He is a member of the American Academy of Neurology, the American Epilepsy Society, the Society for Neuroscience, and the American Neurological Association. He is on the professional advisory board of the

Epilepsy Foundation of America and is a regular member of the developmental neuroscience NICHD NIH study section.

Jane Wu, MD., Ph.D. Charles Louis Mix Professor of Neurology, Northwestern University

Dr. Wu's research focuses on the molecular and cellular mechanisms underlying pathogenesis of age-related diseases, especially neurodegeneration and cancer. 1) Her discovery and characterization of molecular interactions among spliceosomal proteins and splicing regulators are considered milestones in the field. 2) In collaboration with other teams, the Wu lab has identified molecular cues regulating neuronal guidance and cell migration, including the discovery of the first and prototypical repellent neuronal guidance cues-Slit family members, their receptors Roundabout (Robo) family. Slit-Robo signaling plays an important role in modulating chemokine activation and migration of cells of different lineages, including neuronal and non-neuronal cell types. 3) Supported by genetic studies from many other groups, Dr Wu's recent data indicate that Slit-Robo signaling pathways represent a major endogenous tumor suppression mechanism in limiting cancer invasion and metastasis. 4) In the last several years, Dr. Wu's team has established cellular and animal models for studying neurodegenerative diseases including dementia and motor neuron diseases. Their data have provided biochemical and structural evidence for the similarity between TDP-43 proteinopathy and classical amyoid diseases 5). The recent work by Dr. Wu's group has revealed a direct link between the nuclear RNA binding protein FUS and mitochondrial damage, providing new insights into molecular pathogenesis of frontotemporal lobar degeneration and ALS asssociated with FUS. Their studies provide new information that is valuable for future development of new diagnostic and treatment strategies for these devastating diseases.

C.J. Heckman, Ph.D. Professor of Physiology, Northwestern University.

The focus of research in Dr. Heckman's lab is on the role of neural circuits in the mammalian spinal cord for the control of movement. They are interested in both basic and clinical research. The latter is aimed at understanding the impairment of spinal circuits in spasticity, which develops following injuries to the spinal cord, and in the mechanisms of degeneration of motoneurons in ALS. A variety of techniques are utilized, ranging from single electrode voltage clamp

studies of spinal neurons to extracellular recordings of discharge patterns of spinal interneurons to measurements of mechanical behaviors of muscle fibers and whole muscles to studies in human subjects. The Heckman group has also developed computer simulations that use their data measured in individual cells to realistically reconstruct the behavior of entire neural networks controlling motor output. This simulation works provides a direct bridge between cellular and systems data and guides translational studies in human subjects.

Christine DiDonato, Ph.D. Associate Professor, Pediatrics and Human Molecular Genetics, Northwestern University.

Dr. DiDonato's goal is to develop a strong translational research program for SMA and other motor neuron diseases in which spinal motor neurons are affected. The research will be multi-faceted and use biochemistry, cell biology, molecular biology and animal modeling. We will use these approaches to decipher SMN function within nerve and muscle, the two tissues affected in SMA. We will also create a hypomorphic allelic series of Smn mutations in mice. This will provide a classical approach to study Smn function in vivo and determine the biochemical mechanism of motor neuron death in SMA. These animals will be an invaluable resource for testing potential treatment modalities in vivo. Finally, since a treatment for SMA may require both gene and pharmacological therapy, we are investigating the potential of delivering genes encoding either SMN or other neuroprotective agents to motor neurons in animal models of SMA. To this end, we have already demonstrated that cellular deficits in skin cells from severe SMA patients can be rescued through adenoviral delivery of SMN. We are now moving forward to test this strategy in animal models of SMA.

Trevor T Nicholson, MD. Instructor, Medicine-Pulmonary, Northwestern University.

Dr. Trevor Nicholson currently holds an appointment as Clinical Instructor in the Division of Pulmonary and Critical Care at Northwestern University. A native of Ireland, he graduated in 2000 from University College Dublin and is a Member of the Royal College of Physicians of Ireland and the Royal Colleges of Physicians of the United Kingdom. He completed a Fellowship in Pulmonary and Internal Medicine in Ireland and in 2012 moved to Chicago for a Pulmonary Critical Care Fellowship, which he completed in 2015. His research interests currently include mechanisms of muscle dysfunction in critical illness and also non-invasive

ventilation in ALS. Specifically he is examining the role of various modes of non-invasive ventilation, in addition to the use of airway clearance devices, in ALS respiratory disease progression and overall survival. His findings will be presented at the MND Association International Symposium later this year and have been submitted to the American Thoracic Society scientific meeting in 2016.

Javier Jara Ph.D. Research Assistant Professor, Department of Neurology at Northwestern University.

Javier H. Jara received his PhD at University of North Dakota at the Dept. of Pharmacology, Physiology and Therapeutics where he investigated the role of TNF alpha receptors in primary cortical neurons and their relationship to Alzheimer's disease. He is now a Research Assistant Professor at the Dept of Neurology and his main work is focused to understand upper motor neuron biology in ALS. He was the recipient of the ALSA Milton Safenowitz Postdoctoral Fellowship for ALS research in 2010 to investigate the role of innate and adaptive immune responses in ALS. He has published several papers that have been well received in the ALS field. These include two reviews and three research articles which include novel approaches to study corticospinal motor neurons using AAV approaches that revealed selective apical dendrite degeneration in corticospinal motor neurons, and the characterization of the UCHL1 -/- mouse model as an upper motor neuron degeneration mouse model. Dr. Jara plans to continue working in the ALS field and recently received funding from ALS Association for the grant titled “Understanding the role of traumatic brain injury in ALS” to start his work as an independent investigator."

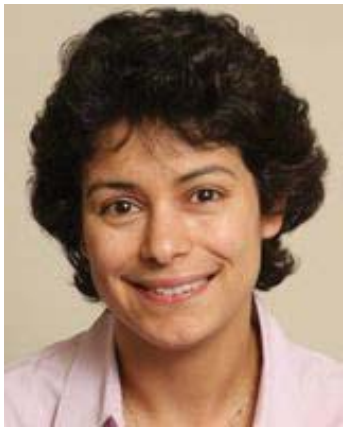
Les Turner ALS Center Clinical and Research Faculty Speakers

Robert L. Sufit, M.D. Professor in Neurology - Ken and Ruth Davee Department and Surgery-Organ Transplantation, Northwestern University.

Dr. Sufit received his medical training at the University of Virginia School of Medicine in 1976, and completed Neurology Residency at the University of Pittsburgh Medical Center in 1980. He is Board Certified in Neurology. Dr. Sufit has clinical expertise in Amyotrophic lateral sclerosis, Muscular dystrophies, Myasthenia gravis, Neurogenetic disorders, Neuromuscular diseases, and Peripheral neuropathies.

Lisa F. Wolfe, M.D. Associate Professor in Medicine-Pulmonary and Neurology - Ken and Ruth Davee Department, Northwestern University.

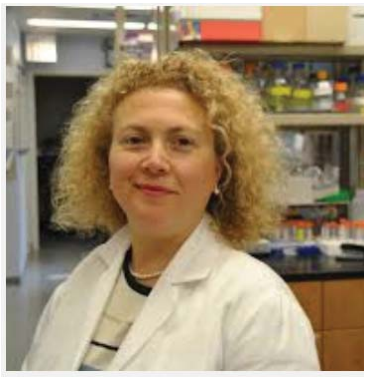
Dr. Wolfe received her medical training at Ohio State University, and completed her Internal Medicine Residency as well as the Sleep Medicine, and Pulmonary and Critical Care fellowships at Northwestern University. She serves as the Pulmonary Consultant at the MDA program at Northwestern University. She has more than 24 peer-reviewed publications and is actively involved in the pulponary care of ALS patients. Dr. Wolfe conducts research to improve breathing capacity of patients and to improve the quality of life.



Senda Ajroud-Driss, M.D. Associate Professor in Neurology - Ken and Ruth Davee Department, Northwestern University.

Dr Senda Ajroud-Driss received her medical degree from The Medical School of Tunis, Tunisia; she completed her Neurology Residency at the University of Illinois at Chicago and her Neuromuscular Fellowship at Northwestern University. Prior to her residency, she worked as a post-doctoral fellow in Dr. Teepu Siddique's Neurogenetic laboratory at Northwestern University, establishing linkage of an autosomal dominant mitochondrial myopathy and later identifying the causative gene. She is Board Certified in Neurology and in Neuromuscular Medicine. She is currently an Associate Professor in the department of Neurology at Northwestern. She is involved in research and the clinical care of patient with familial and sporadic ALS.

Les Turner ALS Center Clinical and Research Faculty Speakers



P. Hande Ozdinler, PhD. Assistant Professor in Neurology-Ken and Ruth Davee Department, Northwestern University.

Dr. Ozdinler's research focuses on the upper motor neurons, the motor neurons that reside in the brain and are an important component of motor neuron circuitry that degenerates in ALS. Dr. Ozdinler received training and education in the fields of molecular biology, genetics, biochemical and genetic engineering during her undergraduate and Masters of Science at the Bogazici University, Turkey. She was the first person to clone genes from *Thermus aquaticus* to *E. coli* in Turkey, and developed ways to purify recombinant proteins of interest with continues production, overcoming batch purification. She was awarded by "Best Thesis" for her work and was invited to United States to continue her research. She received her PhD from LSU Health Sciences Center in the fields of Cell Biology, Anatomy and Neuroscience and moved to Harvard Medical School, Mass General Hospital, Department of Neurosurgery for her postdoctoral training with Profs Jeffrey Macklis and Robert H. Brown. She received Harvard Center for Nervous System Repair fellowship and award, and became a Harvard Fellow and faculty. Upon completion of her training with a stellar record on publication and awards, Dr. Ozdinler joined Department of Neurology at Northwestern and initiated the second Les Turner ALS Laboratory in 2008.

Dr. Ozdinler developed novel approaches to retrogradely label upper motor neurons and to study their cell biology in culture. In addition, Dr. Ozdinler's lab has generated the first reporter line for upper motor neurons, in which the upper motor neurons that die in the disease are labeled with fluorescence. Together with Dr. Bohn, Ozdinler received NUCATS Translational Innovation Award at Northwestern for their ability to selectively transduce upper motor neurons with AAV-mediated gene delivery. In addition, Ozdinler lab characterized a novel mouse model for upper motor neurons and found that ER-stress is an important contributor for selective upper motor neuron vulnerability. Dr. Ozdinler's research on upper motor neurons has been a game changer in the field as she was able to shine light onto these mostly neglected, but very important neuron populations. She is invited to give talks, seminars and Webinars by many associations, such as American Neurology Association, Society for Neuroscience and ALS/MND Association. She also organizes meeting and symposiums herself.

Dr. Ozdinler received grants from NIH, NIA, ALS Association, Brain Research Foundation, NUCATS, and major gifts from the Les Turner ALS Foundation and the Wenske Foundation. Dr. Ozdinler's research will uncover the underlying causes of upper motor neuron degeneration in ALS, and will reveal novel early detection markers and biomarkers for ALS, especially that has prominent upper motor neuron involvement.

Les Turner ALS Center Clinical and Research Faculty Speakers

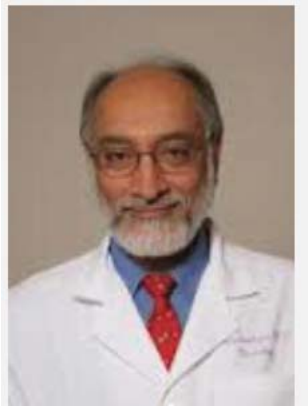
Evangelos Kiskinis, PhD. Assistant Professor in Neurology - Ken and Ruth Davee Department, Northwestern University.

In 2008, Dr. Kiskinis moved to the US to carry out postdoctoral research training in the Lab of Professor Kevin Eggan at the Department of Stem Cell and Regenerative Biology at Harvard University. While at Harvard, Evangelos acquired expertise in human stem cell biology and explored the possibility of using patient-specific stem cells to investigate neurodegenerative diseases with a particular focus on Amyotrophic Lateral Sclerosis (ALS). His research efforts lead to the discovery of molecular pathways that become dysfunctional in the motor neurons of ALS patients as well as to the discovery of a small molecule therapeutic that is currently being tested in clinical trial for ALS patients.

Dr. Kiskinis earned a degree in Molecular Biology from the University of Surrey and a Masters and PhD in Molecular Endocrinology from Imperial College, London, England. During his graduate work he established a mechanism by which transcriptional co-repressors define cellular function by regulating the metabolic profile of muscle and neurons. Dr. Kiskinis also spend a year in Basel, Switzerland working as a research associate at the headquarters of Novartis Pharmaceuticals, setting up novel high-throughput screening assays for evaluating cytotoxicity of candidate therapeutic compounds.

In January 2015, Evangelos was appointed as an Assistant Professor of Neurology and Physiology at the Feinberg School of Medicine, Northwestern University. Dr. Kiskinis' lab is a core member of the newly founded Les Turner ALS Research and Patient Centre. Evangelos is focusing his research efforts on the fight against neurological diseases and is developing novel stem cell based models of ALS. He has been the recipient of prestigious fellowships from the European Molecular Biology Organization as well as from the New York Stem Cell Foundation and the Charles A. King Trust Medical Foundation. His research has also been supported by the Project ALS and Target ALS initiatives. At Northwestern University, Dr. Kiskinis serves as the Scientific Director of the Stem Cell Core Facility.

Les Turner ALS Center Clinical and Research Faculty Speakers



Teepu Siddique, MD. Professor in Neurology - Ken and Ruth Davee Department, Northwestern University.

Teepu Siddique earned his MBBS from Dow Medical College of the University of Karachi and completed his internship and neurology residency at the University of Medicine and Dentistry of New Jersey. He was awarded fellowships in neuromuscular medicine at both the Hospital for Special Surgery-Cornell Medical Center and the National Institutes of Health. He held faculty appointments at the University of Southern California and Duke University before coming to Northwestern University, where he has joint appointments in the departments of Neurology and Cell and Molecular Biology. He was the first recipient of the Abbott Laboratories Duane and Susan Burnham Research Professorship at Northwestern and currently holds the Les Turner ALS Foundation-Herbert C. Wenske Chair in Neurology, where he is the director of the Division of Neuromuscular Medicine and a practicing neurologist.

Dr. Siddique's research has focused on the molecular genetics of several neuromuscular diseases, most specifically ALS (amyotrophic lateral sclerosis), commonly called Lou Gehrig disease, and ALS with dementia. ALS is a progressive disorder in which people lose their ability to use their muscles, resulting in the inability to move and take care of themselves. Patients die when the muscles that control breathing no longer work, 90% within 5 years of onset. Dr. Siddique has employed a range of strategies to identify several genes that cause inherited ALS, including SOD1, ALSIN, FUS, UBQLN2 and SQSTM1, as well as additional genetic loci. His research team developed the first animal model for ALS, the SOD1 transgenic mouse and the first model for ALS/FTD, the ubiquilin2 mouse. Additional models have since been developed for genes more recently identified in his laboratory to study disease mechanism and test potential therapies. His recent work with UBQLN2 and SQSTMI demonstrated that a defect in protein degradation pathways is a common disease mechanism in all types of ALS, sporadic as well as inherited, and the ALS-dementias. This has paved the way for targeted, and therefore effective, treatments of ALS.

A recent editorial in the journal Neurology, the most widely read and quoted journal in the field, cited both Dr. Siddique's identification of the first causative gene in ALS in 1993 and his team's 2010 report of malfunction in the ubiquitin-proteasome and autophagosome-lysosome system as paradigm shifts in understanding ALS.

Professor Siddique has received numerous national and international awards, including the Rattigan Gold Medal from the University of the Punjab, the first Shelia Essey Award in ALS from the American Academy of Neurology, the Hope through Caring Award from the Les Turner ALS Foundation and the Forbes Norris Award from the International Alliance of ALS-MND Associations.

Nuclear-cytoplasmic protein dysregulation in *C9orf72* ALS-FTD

Aaron Earley, J. Alberto Ortega, Jeff Savas and Evangelos Kiskinis

Department of Neurology & Physiology, Feinberg School of Medicine, Northwestern University

The most common cause of familial and sporadic amyotrophic lateral sclerosis (ALS)-frontotemporal dementia (FTD) is a hexanucleotide, $(G_4C_2)_n$, intronic repeat expansion (HRE) in the *C9orf72* gene. Accumulating evidence suggest several pathomechanisms related to nuclear and cytoplasmic protein inclusions as well as nuclear RNA foci in ALS-FTD patients. Recent studies demonstrate that ALS-FTD neurotoxicity might be due to the anomalous high repeats of *C9orf72* HREs (C9-HREs). Pathways mediating C9-HRE toxicity include: 1) RNA gain of function mechanism perturbing nuclear protein import and RNA export; and 2) repeat length associated non-ATG (RAN) translation of toxic polydipeptides. The downstream effects of disrupted nucleocytoplasmic transport machinery on protein homeostasis, however, are largely unknown. In this study, we propose that C9-HREs result in mislocalized cellular proteins and alter nuclear-cytoplasmic protein homeostasis. Previous studies have showed high repeat C9-HRE $(G_4C_2)_{58}$ but not $(G_4C_2)_8$ result in defective nucleocytoplasmic RNA and protein transport. To elucidate global protein dysregulation by C9-HREs, we plan to use a heterologous HEK293 system to study nuclear and cytoplasmic proteomic changes induced by high repeat C9-HRE expression. We have successfully developed a system to express RAN translation dependent C9-HRE reporter plasmids, $(G_4C_2)_{8,58}$::GFP, via transient HEK293 transfection. GFP^+HEK293 cells expressing $(G_4C_2)_8$ and $(G_4C_2)_{58}$ plasmids will be sorted by Flourescently Activated Cell Sorting (FACS) and then harvested for nuclear-cytoplasmic protein fractionation and mass spectrometry. We expect that $(G_4C_2)_{58}$ overexpression compared to $(G_4C_2)_8$ results in a repeat-dependent dysregulation of nuclear-cytoplasmic protein localization. Our proteomic analysis will hopefully uncover novel dysregulated proteins underlying ALS-FTD neurotoxicity and potentially direct new avenues of research in ALS-FTD neurodegeneration. The results from our initial experiments in HEK cells will be validated in patient-specific iPSC derived human motor neurons.

Alsin^{KO}-UeGFP mice, the CSMN reporter line for Alsin, display CSMN-specific cellular defects without major cell loss.

Mukesh Gautam[1], Gabriella Sekerkova[2], Javier H Jara[1], Marina V. Yasvoina[1], Han-Xiang Deng[1], Marco Martina[2] and P. Hande Özdinler[1,3,4]

[1]Davee Department of Neurology and Clinical Neurological Sciences, Northwestern University, Chicago IL, 60611; [2]Department of Physiology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, [3]Robert H. Lurie Comprehensive Cancer Center; and [4]Cognitive Neurology and Alzheimer's Disease Center, Northwestern University, Chicago IL, 60611

Corticospinal motor neurons (CSMN) are unique in their ability to collect, integrate, translate and transmit cerebral cortex's input towards spinal cord targets. Their degeneration is the key in numerous neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS). Mutations in the *Alsin 2* (*ALS2)* gene are reported to be responsible for juvenile primary lateral sclerosis, infantile onset ascending hereditary spastic paraplegia, and are the most common cause for autosomal recessive juvenile ALS. In addition, upper motor neuron signs and bulbar symptoms are often prevalent in patients with juvenile ALS. However, cellular and molecular aspects of CSMN degeneration has not been studied in detail due to lack of selective markers to visualize these neuron populations *in vivo*. By crossing UCHL1-eGFP with Alsin^{KO}, we generated Alsin^{KO}-UeGFP mice, a CSMN reporter line to investigate upper motor neuron defects in the absence of Alsin. This novel reporter line helped us visualize and study CSMN at different stages of disease progression. Different from the hSOD1^{G93A} mice or the TDP-43 mouse models, the numbers of CSMN do not show dramatic reduction in the absence of Alsin. However, detailed cellular analysis using immunocytochemistry coupled with electronmicroscopy (EM) revealed very precise aspects of cellular defects that are restricted to CSMN. We find that even though CSMN do not undergo massive cell loss, the neurons are not healthy. The apical dendrites of CSMN become vacuolated, and this cellular defect is observed only in CSMN in the motor cortex. In addition, there are defects in the mitochondria, and there are signs of defective autophagy, with enlarged lysosomes that contain defective mitochondria, in addition to other proteins. The integrity of the cell membrane is impaired and becomes leaky especially towards end-stage. These findings suggest that Alsin is an important protein for proper CSMN function, and in its absence CSMN display precise neuronal defects, but such defects do not initiate their clearance. Therefore, even though the neurons are still present at layer V of the motor cortex, they are unhealthy and potentially nonfunctional.

Progressive and selective degeneration of genetically labeled corticospinal motor neurons and related subcerebral projection neurons in hSOD1^{G93A}-UeGFP mice

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Understanding mechanisms that control motor neuron vulnerability and degeneration require visualization, identification, isolation and detailed cellular analysis of affected neuron populations at different stages of disease initiation and progression. hSOD1^{G93A}-UeGFP mouse lines are generated by crossbreeding hSOD1^{G93A} transgenic ALS mice with UCHL1-eGFP reporter mice. Corticospinal motor neurons (CSMN) in layer 5 of motor cortex and subcerebral projection neurons (SCPN) located outside of motor cortex are genetically labeled by eGFP expression between P0 and P365. CSMN identity of layer 5 eGFP+ neurons in the motor cortex is confirmed by anatomy, axon projection path, retrograde labeling, molecular marker expression, electrophysiological analysis and cortical circuit mapping. CSMN degeneration in the motor cortex and SCPN loss in the somatosensory cortex in the hSOD1^{G93A}-UeGFP mouse closely correlate with the well-established neuron loss in the hSOD1^{G93A} mouse. This further confirmed motor neuron identity of eGFP+ neurons in the motor cortex and revealed synchronous and progressive CSMN/SCPN degeneration in hSOD1^{G93A}-UeGFP mouse. This novel reporter line will now allow cell type-specific visualization, purification and analysis of CSMN with respect to motor neuron circuitry degeneration in models of ALS and other motor neuron disorders.

EARLY CONNECTIVITY DEFECTS OF CORTICOSPINAL MOTOR NEURONS IN ALS

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Corticospinal motor neurons (CSMN) degenerate in various motor neuron disorders such as amyotrophic lateral sclerosis (ALS), primary lateral sclerosis, and hereditary spastic paraplegia. CSMN are long-distance projection neurons located in the layer V of the motor cortex that collect, integrate and transmit cerebral cortex' inputs toward spinal cord targets. Therefore, their proper modulation is essential for the initiation and modulation of voluntary movement. CSMN receive both excitatory and inhibitory inputs from many different neuron types in the cerebral cortex, and the fine balance between these excitatory and inhibitory inputs both from local circuitries and long-distance projection neurons modulate CSMN activity. We previously revealed a selective pattern of apical dendrite degeneration with spine loss especially in layer II/III of the hSOD1^{G93A} ALS mouse motor cortex. This could account for early cellular CSMN vulnerability due their inability to process cerebral input. We developed an anatomical and electrophysiology approach to investigate mechanisms that could potentially be involved in CSMN vulnerability and progressive degeneration. Using electrophysiological recordings, coupled with retrograde labeling and analysis of distinct neurons types in different layers of the motor cortex, we begin to reveal the details of early connectivity defects in ALS. Our ongoing studies suggest that CSMN degeneration could be due to a complex imbalance between excitation and inhibition signals, in addition to their intrinsic vulnerability.

Specific transduction of corticospinal motor neurons by AAV2 upon direct motor cortex injection

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The application of adeno-associated virus (AAV) in gene therapy has multiple advantages due to its long-term expression in the central nervous system (CNS) and low immunoreactivity in humans. Gene therapy strategies in CNS include Canavan's disease, Alzheimer's disease and motor neuron diseases such as amyotrophic lateral sclerosis (ALS). Targeting only the vulnerable neuron populations without affecting other neuron types within the cerebral cortex is a major obstacle for translational neuroscience. This applies to ALS, in which the corticospinal motors neurons (CSMN; a.k.a upper motor neurons) show selective vulnerability and progressive degeneration. In this study, seven different AAV serotypes that harbor the eGFP gene were tested for their ability to transduce CSMN upon direct injection into the layer V of the motor cortex. CSMN transduction was confirmed by Ctip2 immunocytochemistry and by presence of red fluorescent microsphere in the CSMN after retrograde labeling by injection into the corticospinal tract (CST). Large pyramidal neurons located in layer V showed higher tropism for AAV2-2. In an effort to increase the selective transduction of CSMN by AAV, we used capsid proteins that are engineered, and different promoters to drive the eGFP expression. Our results suggest that the choice of the promoter is critically important to enhance selectivity of gene expression in CSMN. Furthermore, specific transduction of CSMN was feasible in mouse models with progressive CSMN degeneration. Identification of AAV serotypes that transduce only a select set of neuron populations at symptomatic stage of disease is critically important to develop effective and long-term gene therapy approaches in the cerebral cortex.

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Understanding the basis of CSMN vulnerability and degeneration using a proteomics approach

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Ubiquitin carboxy-terminal hydrolase L1 (UCHL1) is a deubiquitinase that plays a critical role in maintaining free ubiquitin levels in neurons. Patients with mutations in their *UCHL1* gene develop motor function defects, paralysis, and upper motor neuron defects. Mice that lack all UCHL1 function (UCHL1^{nm3419}, UCHL1-/-) display motor neuron defects and profound corticospinal motor neuron (CSMN) degeneration characterized by increased ER-stress, vacuolated apical dendrites, and spine loss. These findings show the importance of UCHL1 for CSMN health. Here, we used a bottom-up proteomics approach coupled with UCHL1-immunoprecipitation to reveal the proteins that interact with UCHL1. We then investigated if these interactions were specific to neurons in the motor cortex by including neurons in the spinal cord and the trigeminal ganglia (TG). Our initial findings suggest that UCHL1 binds to and interacts with different proteins in the cortex, spinal cord, and the TG, and this may in part explain why in its absence CSMN display the most prominent cell loss and neuronal vulnerability. CSMN vulnerability is a key component of disease pathology in a number of motor neuron diseases, such as primary lateral sclerosis, hereditary spastic paraplegia, and amyotrophic lateral sclerosis. Understanding the underlying causes of their vulnerability using a proteomics approach will have significant impact on revealing key cellular and molecular pathways responsible for their degeneration.

Moving forward in clinical trials for ALS: Motor Neurons Lead the Way Please

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Amyotrophic lateral sclerosis (ALS) is one of the most complex motor neuron diseases. Even though scientific discoveries are accelerating with an unprecedented pace, to date more than 30 clinical trials have ended with failure and staggering frustration. There are too many compounds that increase life span in mice, but too little evidence that they will improve human condition. Increasing the chances of success for future clinical trials requires advancement of preclinical tests. Recent developments, which enable the visualization of diseased motor neurons, have the potential to bring novel insight. As we change our focus from mice to motor neurons, it is possible to foster a new vision that translates into effective and long-term treatment strategies in ALS and related motor neuron disorders (MND).

Lifespan extension of ALS mice was accepted as a prerequisite for clinical trials, but they all failed. Improvement of the motor neuron health is more relevant to success of clinical trials. Now there is a paradigm shift, which focuses on the health of the motor neuron. Thus, we generated the UCHL1-eGFP reporter line, in which the motor neurons are visible and their health can be easily monitored in the presences of compounds.

Numerous developments and improvements in critical thinking, analysis and understanding suggest that the near future will witness unprecedented achievements in the field of ALS. First, it has been realized that it is not only the mice but the motor neurons in the mouse models that are important for translating biological finding toward clinical trials. This has been a major paradigm shift in thinking. Improvement of preclinical test by focusing on the survival needs of both cortical and spinal motor neurons, determining early detection as well as biomarkers for ALS and improving the design and outcome measures for clinical trials will cumulatively contribute to the identification of compounds that will improve patient health and quality of life.

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ALS Patient Motor Neurons Exhibit Excitability Deficits That Compromise Their Survival

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Cell reprogramming technologies have created unprecedented opportunities for studying patient-specific human cell types that were previously inaccessible. Many studies have used iPSC-based and direct conversion methods to model neurological and neurodegenerative diseases, effectively describing disease-related phenotypes in multiple neuronal subtypes. However, investigating functional electrophysiological deficits in now widely available patient neurons has been limited by currently available techniques. Patch clamp is laborious and has not been amenable to a high-throughput format, while multi-electrode array (MEA) systems only gather population-level data.

Here, we describe a novel all-optical electrophysiology method, called Optopatch, based on simultaneous optical voltage imaging and optogenetic actuation. Voltage imaging occurs through a novel genetically encoded fluorescent voltage indicator, called QuasAr2. Optogenetic actuation occurs through a novel blue light-activated channelrhodopsin, called CheRiff. Custom optics and software allow simultaneous stimulation and recording from large numbers of single cells embedded in a complex network. Optopatch enables rapid electrophysiology measurements from hundreds of cells per day with a high signal-to-noise ratio. We developed Optopatch protocols to measure spontaneous and induced activity to characterize cell autonomous excitability and action potential waveforms.

We applied Optopatch assays to stem cell-derived motor neurons. We tested the technology with a previously validated model of ALS with a mutation in the SOD1 gene and an isogenic control cell line generated by genetically correcting the disease-causing mutation. More than 300 individual neurons were measured across both disease and control cells, in multiple differentiations. Each trial showed an increased excitability of the ALS motor neurons at low levels of stimulation, consistent with published MEA and patch clamp data. Interestingly, under strong stimulation, the ALS motor neurons fired fewer action potentials. Tests of several drugs, including retigabine, showed a reversal the ALS excitability phenotype, demonstrating that the Optopatch platform can be used in pharmacological screens. The ease of acquiring Optopatch data creates an opportunity to measure electrophysiology of neuronal subtypes from many models

DNMT3A Plays a Functional Role in the Development of Mature Motor Neurons

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DNA methylation is an epigenetic mark preset in all cell types of the human body that is essential for the regulation of gene expression. There are three main enzymes that regulate the methylation of DNA; *DNMT1*, *DNMT3A* and *DNMT3B*. *DNMT1* is necessary for maintenance DNA methylation, the methylation of a hemi-methylated (half-methylated) strand of DNA. This process occurs most frequently in actively dividing cells. In contrast, *DNMT3A* and *DNMT3B* are important for *de novo* DNA methylation, the methylation of unmethylated DNA.

Recently, a large amyotrophic lateral sclerosis (ALS) exome-sequencing study identified *DNMT3A* as being highly mutated in cohort of ALS patients. Our early work indicated that, of the two *de novo* DNA methyltransferases, *DNMT3A* is expressed significantly higher in motor neurons (MNs) than *DNMT3B.* As a result of these two findings, we hypothesized that *DNMT3A* may be playing a previously unappreciated role in either the development, maturation or both of MNs.

Here, we asses the ability of *DNMT3A*$^{-/-}$ human embryonic stem cell (hESC) lines to generate mature MNs. To answer this question we directed WT and *DNMT3A*$^{-/-}$ hESCs down a differentiation pathway that results in the generation of MNs. We found that in the absence of *DNMT3A* cells are less effective at generating MNs as quantified by *ISL*$^{+}$ cell staining.

We next wanted to address when this issue arises. Is it due to a defect in early neurogenesis, during the transition of neural progenitors to MN progenitors or later in development when MN progenitors transition to fully mature *ISL*$^{+}$ MNs? Again, by directing the cells down a MN lineage and looking at known markers of neurogenesis (*PAX6, OLIG2*) we determined that this issue arises during early neurogenesis.

Our long-term aim is to determine whether mutations in *DNMT3A* can, in fact, cause ALS. We will accomplish this by a combination of gene targeting and biochemical approaches. Gene targeting will allow us to introduce potential ALS causing *DNMT3A* mutations into a healthy control line and asses the phenotypic changes caused by these mutations while biochemical approaches will allow us to asses the effect these *DNMT3A* mutations have on the normal function of the gene.

Importance of UCHL1 function for the motor neuron circuitry and the health of the corticospinal motor neurons.

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Corticospinal motor neurons (CSMN) receive, integrate and relay cerebral cortex's input towards spinal targets to initiate and modulate voluntary movement. CSMN degeneration is central for numerous motor neuron disorders and neurodegenerative diseases, but the cellular and molecular basis of CSMN vulnerability remains unknown. Mutations in the ubiquitin C-terminal hydrolase-L1 (*UCHL1*) gene have been detected in patients with neurodegenerative disease that affect motor function, and recently three siblings displayed early neurodegeneration, including upper motor neuron dysfunction. There is a need for the generation and characterization of novel mouse models that recapitulate upper motor neuron loss in patients. Here we report a unique function of UCHL1 in maintaining CSMN viability and cellular integrity. *Uchl1*^{nm3419} (UCHL1 -/-) mice, which lack all UCHL1 function, display motor neuron circuitry defects. Even though spinal motor neurons remain intact with subtle dysfunction, CSMN show early, selective, progressive and profound cell loss. CSMN degeneration is mediated via increased ER stress and becomes evident at pre-symptomatic stages by cytoarchitectural defects primarily involving the apical dendrites. To reveal the importance of UCHL1 function for the motor neuron circuitry, we now generated novel conditional mutant mice in which UCHL1 function is removed either from the corticospinal or the spinal motor neurons, respectively.

Developing Pairs of ALS Patient-Specific Stem Cells and Isogenic Controls Using the CRISPR/Cas9 System

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Amyotrophic Lateral Sclerosis (ALS) is a progressive neuromuscular disease characterized by the degeneration of motor neurons in both the brain and spinal cord. When motor neuron degeneration occurs the muscles no longer receive impulses from the nerve cells, leading to atrophy, weakness, and eventually fatal paralysis. We reprogram skin fibroblasts or blood cells donated from ALS patients into patient-specific induced pluripotent stem cells (PS-iPSC). We then use the PS-iPS cells to create motor neurons to study the intrinsic molecular mechanisms of the disease. Furthermore, we can also perform genome engineering experiments to repair disease-causing mutations and compare the original, mutated cells to their repaired counterparts. By utilizing the bacterial CRISPR/Cas9 dual nickase system we precisely modify the genome to either repair or introduce mutations. Presently, we are in the process of developing isogenic pairs for the ALS-causing genes FUS, TDP43, and ALS4.

Notes