Meet the LBSL Team

Christina Nemeth Mertz, PhD
Postdoctoral Fellow
2016 - present

Philippe Hubo
Graduate Student
2017 - present

Sophia Tomlinson, BS
Research Technician
2017 - present

Melissa Rosen, BS
Research Technician
2018 - present
Lab Undergraduate
2015-2017

Ali Fatemi, MD, MBA

Oscar Larraza, JHU ‘19
Lab Undergraduate
2017 - present

Connor Murray, BA
Research Technician
2016-2017

Joel Marx, MS
Research Technician
2011-2016
Outline

**Mouse Models**
- how we generate mice
- what we know about DARS2 in mice
  - behavior and histology so far

**Induced Pluripotent Stem Cells**
- what they are and how they’re made
  - utility
  - our data so far

**Potential for Therapeutic Testing**
- Understanding numbers
  - Animals and cells as platforms

**Collaborations**
- Cerebral Organoids
- RNAseq
Outline

**Mouse Models**
- how we generate mice
- what we know about DARS2 in mice
- behavior and histology so far
Why mice?

Comparable genetic makeup to humans

Genetically easy to modify
Genetically identical!

Similar reproductive and nervous systems to humans

Relatively short life span (can study their *entire* life span)

Excellent model for studying changes in motor function, behavior

Useful for testing therapies

Not perfect!
LBSL and *DARS2*

- Mitochondrial aspartyl-tRNA synthetase
  - Aspartic acid in mitochondrial protein translation (mtAspRS)
- Nuclear encoded
- Decreased activity; “leaky”
van Berge et al., 2014; *Brain*

DARS2 gene

frameshift
missense
deletion
nonsense
Mitochondria

Nucleus

Cytosol

transcription

mRNAs

genomic-DNA

19 mt-aaRSs

Ribosome

EF-Tu

aaRS

mt-DNA

mitochondrial translation

13 mRNAs

22 tRNAs

2 rRNAs

transcription

NADH + H+

FAD

$\text{F}_{1}$

$\text{F}_{0}$

$\text{NAD}^{+}$

$\text{ADP} + \text{P}^{i}$

$\text{ATP}$

Succinate

Fumarate

$1/2\text{O}_{2} + 2\text{H}^{+}$

$\text{H}_{2}\text{O}$

$4\text{H}^{+}$

$4\text{F}^{+}$

$2\text{H}^{+}$

$\text{NADH} + \text{H}^{+}$

$\text{ADP} + \text{P}^{i}$
Succinate dehydrogenase
Cytochrome b/c complex
Cytochrome b oxidase
F0/F1 ATP synthase
O2 + e− → O2− → SOD → H2O2 → CAT → H2O

NADH dehydrogenase
NAD → H+ + NADH → O2 → H2O

α-Ketoglutarate → Malate → Acetyl-CoA → Glucose
Modeling LBSL

Full knockout of DARS2 is embryonic lethal and selective knockout results in mitochondrial dysfunction

Dogan et al, 2014; Cell Metab
How does Cre-Lox work?

Allows us to specify which cells are affected.
Our mice

Received from Dr. Aleksandra Trifunovic at CECAD, Cologne, Germany

Purchased from Jackson Labs:

**Nestin**: neuronal precursor cells (all)

**CamKIIα**: neurons in forebrain

**PDGFRα**: oligodendrocyte precursors (myelin)
Brain-specific cre transgene

Homozygous loxP “floxed” mouse

2-3 months

CamKII

Dars2

Dars2

Dars2

Dars2

CamKII

cre

loxE

loxE

loxE

loxE

FOUNDER

1st GENERATION

50%

50%
Brain-specific cre transgene

homozygous loxP “floxed” mouse

25% 
25% 
25% 
25%
50% Dars2 fl/fl Cre - (Control)
50% Dars2 fl/fl Cre + (Mutant)
Day 0                  28                    42
-56

**Behavior, Imaging, Histology, Therapies**

determine end date based on behavioral observations

- brain/spinal cord histology

maintain colonies

Activity
Gait
Cognition
Reflexes
Affect
Weight

Oscar at work
Full knockout of DARS2 is embryonic lethal and selective knockout results in mitochondrial dysfunction
Impaired mitochondrial translation

Disrupted mitochondrial proteostasis

ATF4

↑ FGF21

PGC-1α stability

Systemic change in metabolism

Mitochondrial biogenesis

Autophagy

UPRmt

increased mito production

reduced clean-up

increased stress
Our mice
Cre-Lox Recombination

Nestin

Neuronal-restricted progenitors

Glial-restricted progenitors

- Multiple neuron types
- Motor neurons
- CamKII expressing neurons
- Oligodendrocyte precursor cells
- Astrocytes
- Mature oligodendrocytes
Cell type specific deletion

Nestin
PDGFRα

Neural stem cells

Neuronal-restricted progenitors

Glial-restricted progenitors

Multiple neuron types
Motor neurons
CamKII expressing neurons

Oligodendrocyte precursor cells

Astrocytes

Mature oligodendrocytes
Cell type specific deletion

Neural stem cells

Neuronal-restricted progenitors

Glial-restricted progenitors

Nestin

PDGFRα

CamKIIα

Multiple neuron types

Motor neurons

CamKII expressing neurons

Oligodendrocyte precursor cells

Astrocytes

Mature oligodendrocytes
DARS2 deletion increases overall activity

Genotype, $p < 0.001$
Age, $p < 0.05$

Control
Mutant

males & females
Cre- n = 13
Cre+ n = 12
Body mass of *DARS2* mutant mice decreases at ~6 months of age

Genotype, \( p < 0.001 \)

Age, \( p < 0.001 \)

<table>
<thead>
<tr>
<th>Weight (g)</th>
<th>Control</th>
<th>Mutant</th>
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males & females
Cre-  \( n = 13 \)
Cre+  \( n = 12 \)
DARS2 deletion from neurons leads to severe brain atrophy.
DARS2 deletion leads to neuronal cell loss

Aradjanski et al, 2017; *Hum Molec Genetics*
**DARS2 deficiency increases brain inflammation**

*Microglia* are cells that produce inflammation and keep brain areas free from debris and harmful pathogens.
Microglial cells change shape upon activation

Nemeth et al., 2017
*Microglia* are cells that produce inflammation and keep brain areas free from debris and harmful pathogens.

*DARS2* deficiency increases brain inflammation.
DARS2 deficiency leads to reduced white matter area
Electron Microscopy

- Uses a beam of electrons as illumination source
- Allows for very high magnification and the visualization of tissue ultrastructure

80,000x

myelin
mitochondrion
axon
DARS2 deficiency alters brain axons

**Control**

**Mutant**

JHU MicFac; Hitachi 7600 TEM

Myelin Thickness (nm)

- Control
- Mutant

**Axon Area (μm²)**

- Control
- Mutant

$t$-test; $p = 0.024$

$p = 0.012$
$DARS2$ deficiency increases mitochondrial count in brain axons
DARS2 deficiency in the brain does not alter nerves of the spinal cord.
Outline

Induced Pluripotent Stem Cells
- What are they?
- How do we get them?
- Our data so far
Why iPSCs?

Non-invasive method with lots of flexibility

Allows us to assess the effect of specific mutations

Can be turned in to almost any cell type

They replicate, can be frozen down, and grown again later

No fear of immune rejection if re-introduced to the patient

Can carefully assess cell differentiation

Respond in functional assays

Useful for therapeutic testing
Induced Pluripotent Stem Cells (iPSCs)

- Isolate peripheral blood monocyte cells
- Cells are reprogrammed to induce pluripotency
- Cells are monitored and tested to ensure reprogramming was successful
- Cells are karyotyped, tested for pluripotency, and screened for bacterium
- 3 – 4 months
- Frozen cells are mailed to us

Cells are reprogrammed to induce pluripotency, cells are monitored and tested to ensure reprogramming was successful, cells are karyotyped, tested for pluripotency, and screened for bacterium.
From iPSCs to neurons

Figure 2. Induction of neurogenesis by syn-TFs mRNAs of Neurogenin and NeuroD families
iPSC derived motor neurons

![Graph showing Exon 3 expression relative to Control iPSC](image)

- Control
- LBSL

![Images showing Nuclei/iPSC/Mitochondria](image)

![Images showing Nuclei/Neuron/Mitochondria](image)

iPSC

motor neuron
Outline

Potential for Therapeutic Testing
- Understanding numbers
- Animals and cells as platforms
Sample sizes (# of animals) are calculated based on **statistical POWER**, or the confidence that your groups are truly different.

Animals are randomly assigned to groups and balanced for:
- Sex
- Litter
- Weight
- Housing

*To achieve a less than < 5% chance of incorrect interpretation*
The numbers

Genotype, $p < 0.001$
Age, $p < 0.05$
The Dendrimer Platform

4 – 10 nm in diameter

(a human hair is 50,000 nm thick!)
Outline

Collaborations
- Cerebral Organoids
- RNAseq
From iPSCs to “mini brains”
RNA-Sequencing

Isolate mRNA

Sequence

Align Sequences

Analysis

Sample 1

Sample 2

Gene A

Gene B

Gene A

Gene B
Summary

**Mouse Models**
- Mutant animals show loss of cells
- Increase in brain inflammation
- Increase in overall activity

**Induced Pluripotent Stem Cells**
- Can examine almost any cell type
- Mutation specific

**Potential for Therapeutic Testing**
- Dendrimer platform is in testing
- Cells and animals show deficits that we can attempt to remedy

**Collaborations**
- LBSL patient cells are being grown into cerebral organoids
- Will begin global gene expression studies on mice