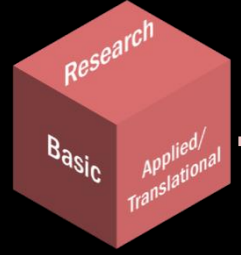
Characterizing Patient Specific Cells for Understanding and Treating Mitochondrial Diseases

Shilpa Iyer, PhD

2017 MIP Conference Hradec Kralove

Nov 16th 2017



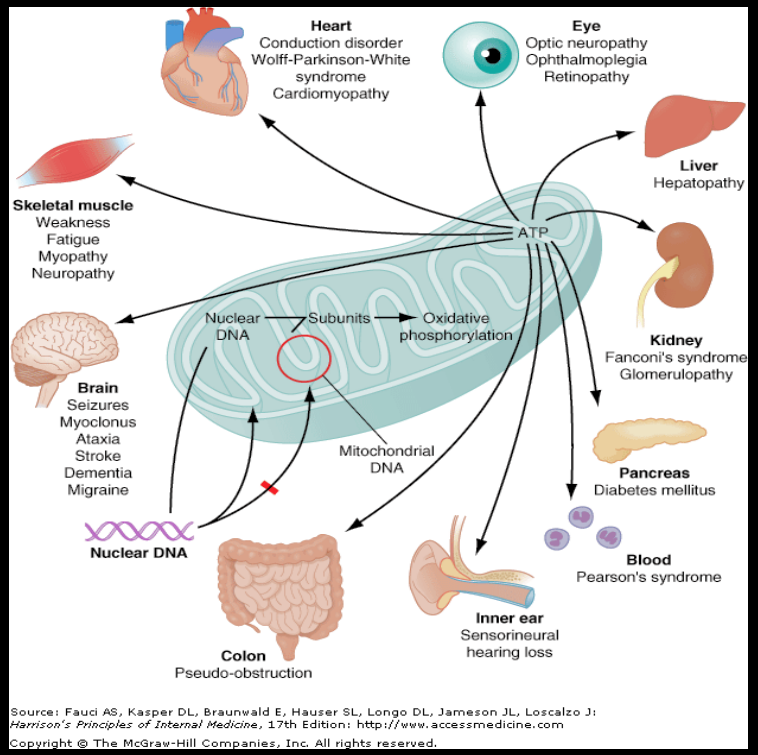
Symptoms faced by patients with mitochondrial disorders…

Heart

Dizziness
Low blood pressure
Poor circulation

Brain

Seizures
Memory loss
Cognitive delay
Migraines
Blindness
Speech impairment



Gut

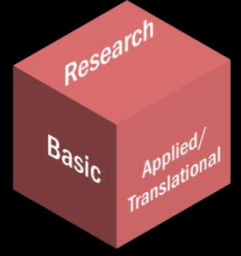
Nausea
Lack of appetite
Difficulty gaining weight
Digestive issues

Muscles

Fatigue
Muscle cramps
Weakness
Exercise intolerance

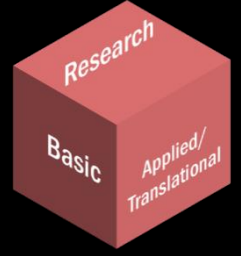
*Look for three or more systems to be involved

(*In prep*)

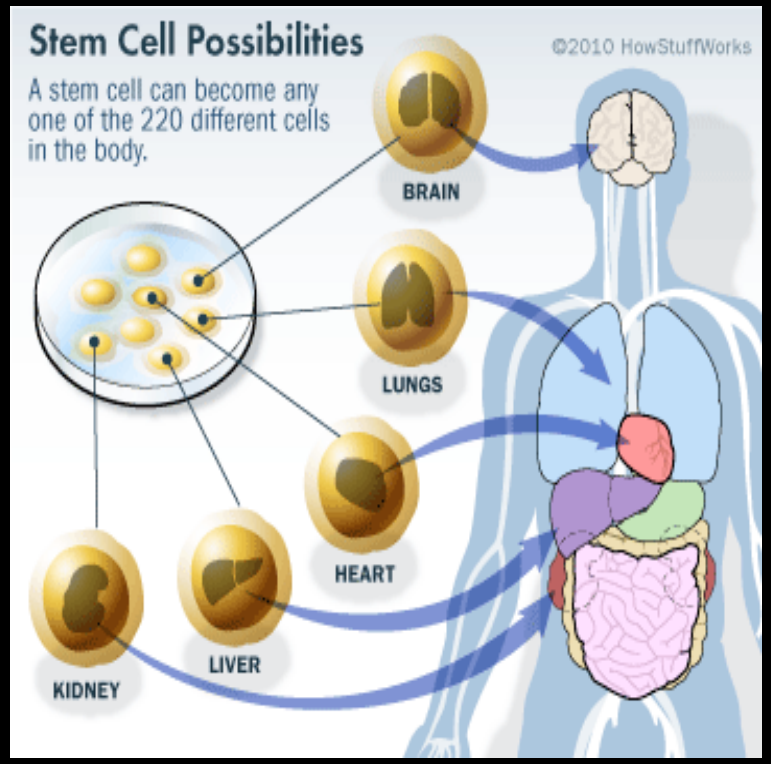


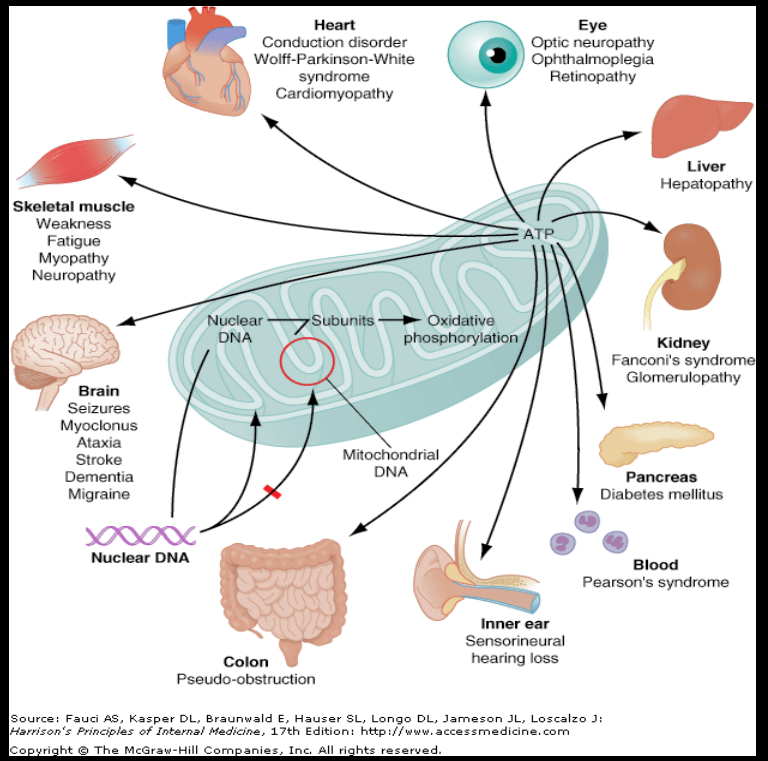
How can one study the perplexing aspects of clinical variability due to mitochondrial defects in different diseases?

(*In prep)*



Stem cells are good model systems for understanding and treating mitochondrial disorders

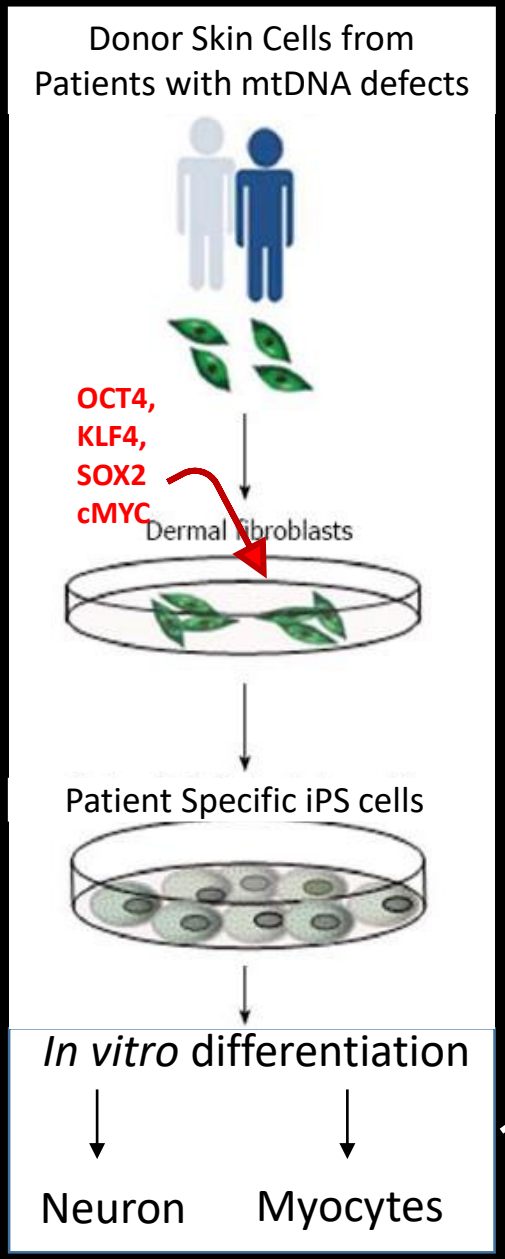




(*In prep*)

Our overall Goal

Donor Skin Cells from Patients with mtDNA defects

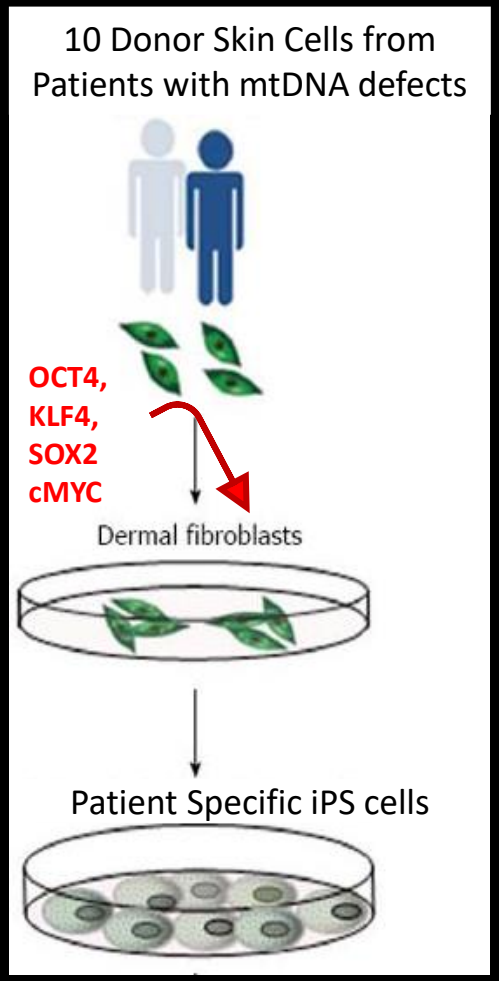


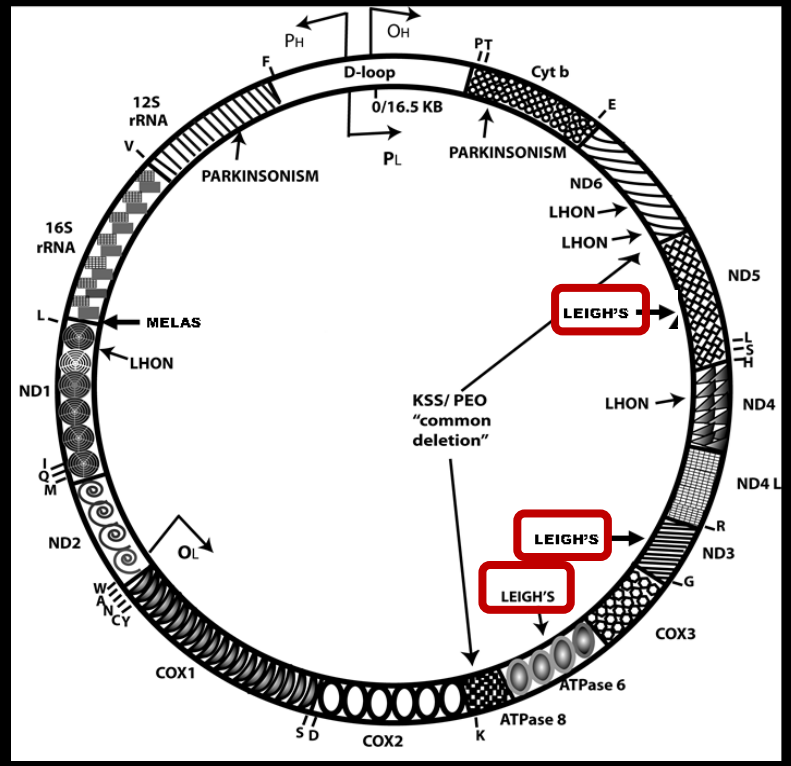
Create patient specific pluripotent stem cell models for understanding bioenergetic defects and tissue-specific variability due to mitochondrial DNA mutations.

OUTCOMES	RELEVANCE
MtDNA next generation Sequencing	Mt DNA heteroplasmy
Mitochondrial Respiration	Energetics (OXPHOS,Glycolysis,FAO)
NADH redox ratio/ETC assay	Measure cellular metabolic activity as well as ETC activity of individual subunits
Specific Drugs to iPS cells	For drug testing

(*In prep*)

Leigh's Syndrome

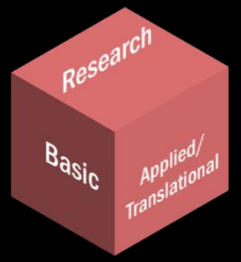




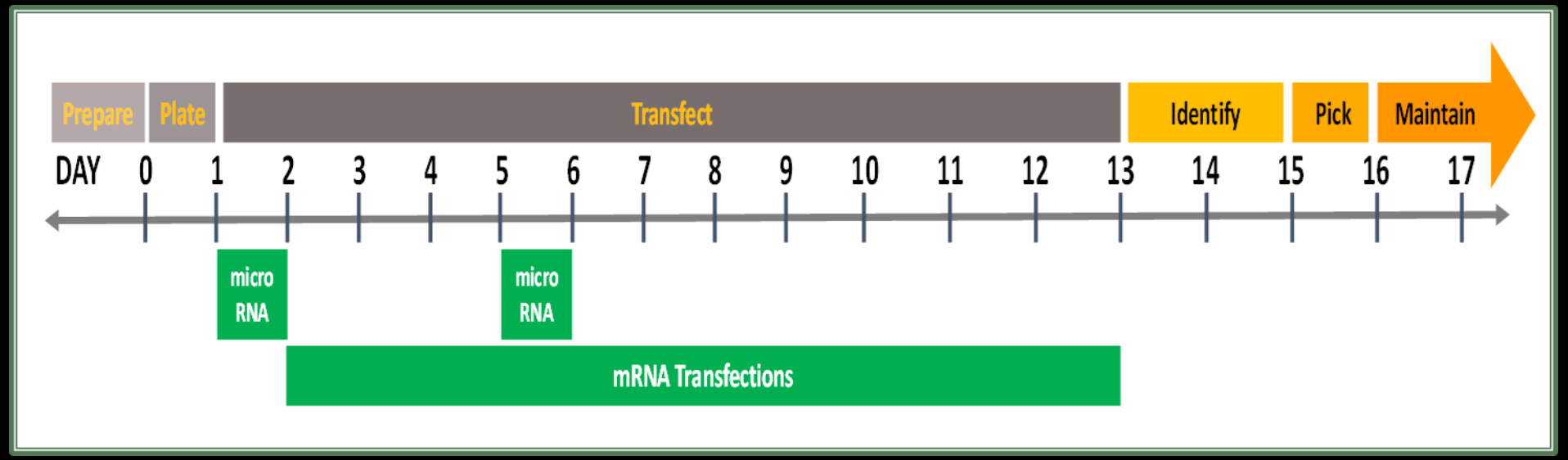
Experimental Design: From ten patient fibroblast samples (already in our laboratory), we will reprogram and differentiate hiPSCs with four specific point mutations present in LS disease, that affect different subunits of the electron transport chain:

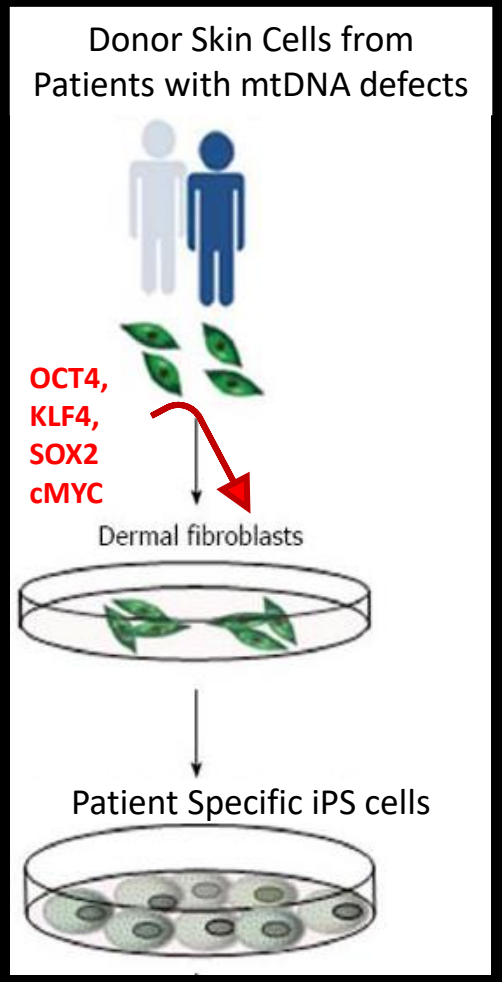
(a) 8993T>G (ATP6) – Defect in the subunit of the F_0 component of the ATP synthase

(b) 10158T>C (ND3) – Defect in the ND3 subunit of the enzyme NADH dehydrogenase (ubiquinone)



Reprogramming Method





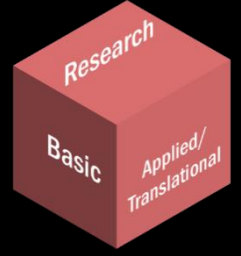
Non-viral induced pluripotent stem cell technology to create clinical-grade patient specific stem cells from patient skin samples.

Reprogramming Timeline.

1. The microRNA-enhanced mRNA Reprogramming System requires a total of 2 microRNA transfections and 11 mRNA transfections.

2. Emerging iPSC colonies are identified by morphology and live staining by Day 13, as shown on the timeline.

(*In prep*)



Summary

We have successfully created a clinical grade induced pluripotent cell line for Leigh's Syndrome carrying m. 8993 T>G mutation which is stably transmitted from dermal fibroblasts to iPS cells .

Our recent preliminary studies indicate patient-derived dermal fibroblasts, have an altered redox ratio depending on whether mutations affect ATP synthase (FB1m 8993 T>G) or NADH dehydrogenase (FB3; m10158T>C)

Our recent preliminary studies indicate patient-derived dermal fibroblasts, have an altered bioenergetics and proton leak depending on the percentage of mutant load and whether they affect ATP synthase (FB1m 8993 T>G) or NADH dehydrogenase (FB3; m10158T>C)

Acknowledgements

University of Georgia
Harrison Grace
Franklin West

University of Arkansas
Raj Rao, Kyle Quinn
Ajibola Bakare, Ahmed Dhamad,Joshua Stabach
Raquel Palmer

Virginia Commomwealth University
Patrick Galdun
Gregory Buck

Va Medical Center
Edward Lesnefsky

Children's Hospital at Innsbruck and Salzburg
Wolfgang Sperl, Daniella Karall and Hans Mayr

Research Support : Iyer (PI)
NIH-1R15NS080157-01A1
DoD-W81XWH-16-1-0181