LBSL: Defects in energy metabolism and in-vitro response to treatment with aminolaevulinic acid

J. Abdenur, M.D.
Chief, Division of Metabolic Disorders
Director, Metabolic Laboratory
CHOC Children's, Orange CA, USA

2022 LBSL International Patient Conference
Kennedy Krieger Institute
July 30 - August 01, 2022

CHOC Research
GO BEYOND

No conflicts of interest related to this presentation

CHOC Research
GO BEYOND

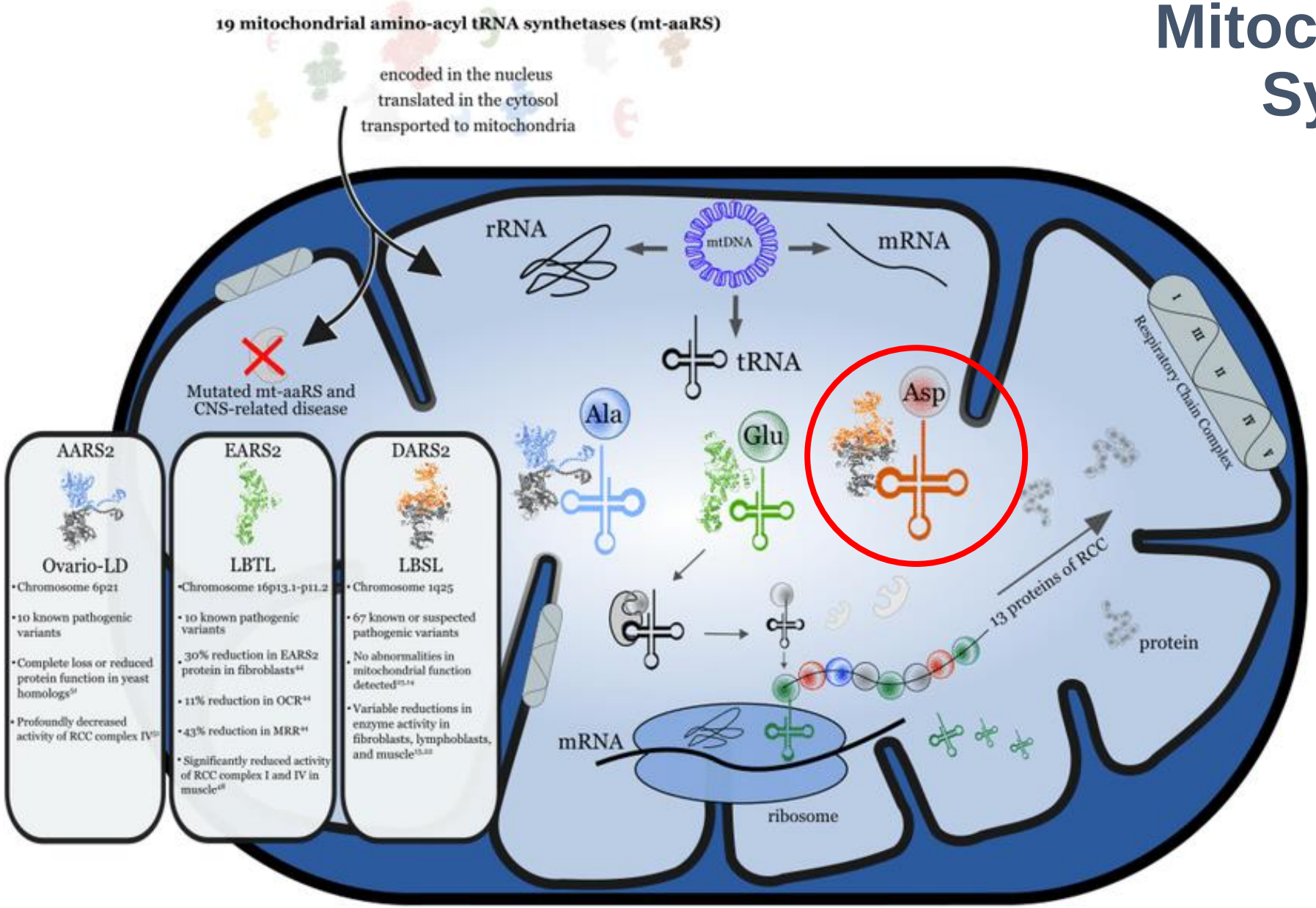
CHOC Mitochondrial Disease Patients (April 2022)

Mitochondrial Disease due to mtDNA defects	Number of patients
mtDNA mutation known to be deleterious	18
mtDNA mutation of unknown significance	5
mtDNA deletion	7
Mitochondrial Disease due to nuclear DNA defects	
ETC Complex / Assembly Defects:	
NDUFAF5 (3), UQCRC2 (2), SURF1 (2), NUPBL (2), BCS1L, SCO2	11
mtDNA Synthesis / Stability:	
SUCLG1, POLG1- Alpers (3), *RRM2B (2), TFAM, MRPS22,*	8
Protein folding, import, export and turnover. Iron cluster biogenesis	
LONP1 (2), FBXL4 (4), SPATA5 (2), SFX4, FDXR	*10*
Mitochondrial Protein Synthesis	
GFM1, MTO1, NARS2 (2), EARS2 (2), KARS, VARS2, DARS2 (2), RARS2 (2), PDE12	13
Other	
TAZ (Barth syndrome), *HIBCH (2), ECHS1 (6), ETHE1 (2), PC (1), PDH (9)*	21
Total	**93**

CHOC Mitochondrial Disease Patients (April 2022)

Mitochondrial Disease due to mtDNA defects	Number of patients
mtDNA mutation known to be deleterious	18
mtDNA mutation of unknown significance	5
mtDNA deletion	7
Mitochondrial Disease due to nuclear DNA defects	
ETC Complex / Assembly Defects:	
NDUFAF5 (3), UQCRC2 (2), SURF1 (2), NUPBL (2), BCS1L, SCO2	11
mtDNA Synthesis / Stability:	
SUCLG1, POLG1- Alpers (3), *RRM2B (2), TFAM, MRPS22,*	8
Protein folding, import, export and turnover. Iron cluster biogenesis	
LONP1 (2), FBXL4 (4), SPATA5 (2), SFX4, FDXR	*10*
Mitochondrial Protein Synthesis	
GFM1, MTO1, NARS2 (2), EARS2 (2), KARS, VARS2, ***DARS2*** *(2), RARS2 (2), PDE12*	13
Other	
TAZ (Barth syndrome), *HIBCH (2), ECHS1 (6), ETHE1 (2), PC (1), PDH (9)*	21
Total	**93**

Mitochondrial tRNA Synthetases



Smith Fine et al., Journal of Neurodevelopmental Disorders 11 (2019) 29

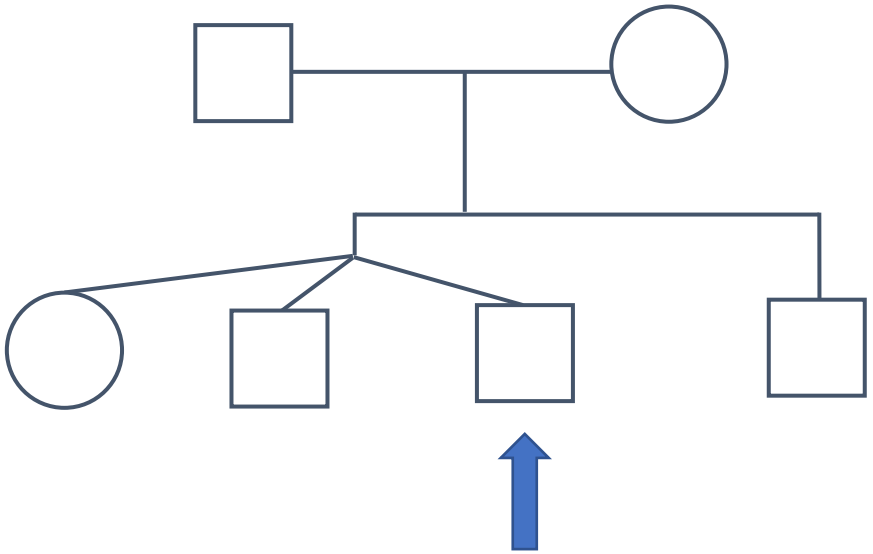
Aspartyl tRNA synthetase *(DARS2)* deficiency

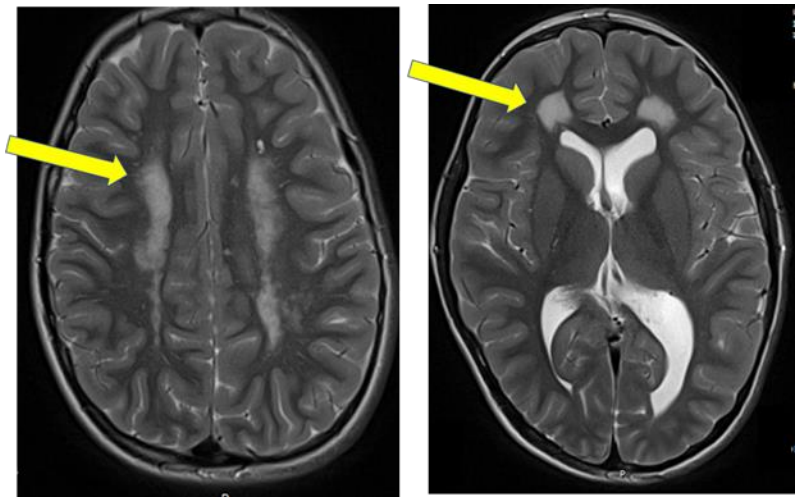
- **Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevations (LBSL)**
- Heterogeneous clinical presentation
  - Onset in infancy, severe
  - Adult onset, mild and slowly progressive
  - Exercise Induced Paroxysmal Ataxia (rare)
- Characteristic MRI pattern
- Genotype appears to determine the phenotype

Scheper GC, et al., Nature Genetics 39 (2007) 534-539
Berge LV, et al., Brain 137 (2014) 1019–1029
Synofzik M, et al., J Med Genet 48 (2011) 713e715

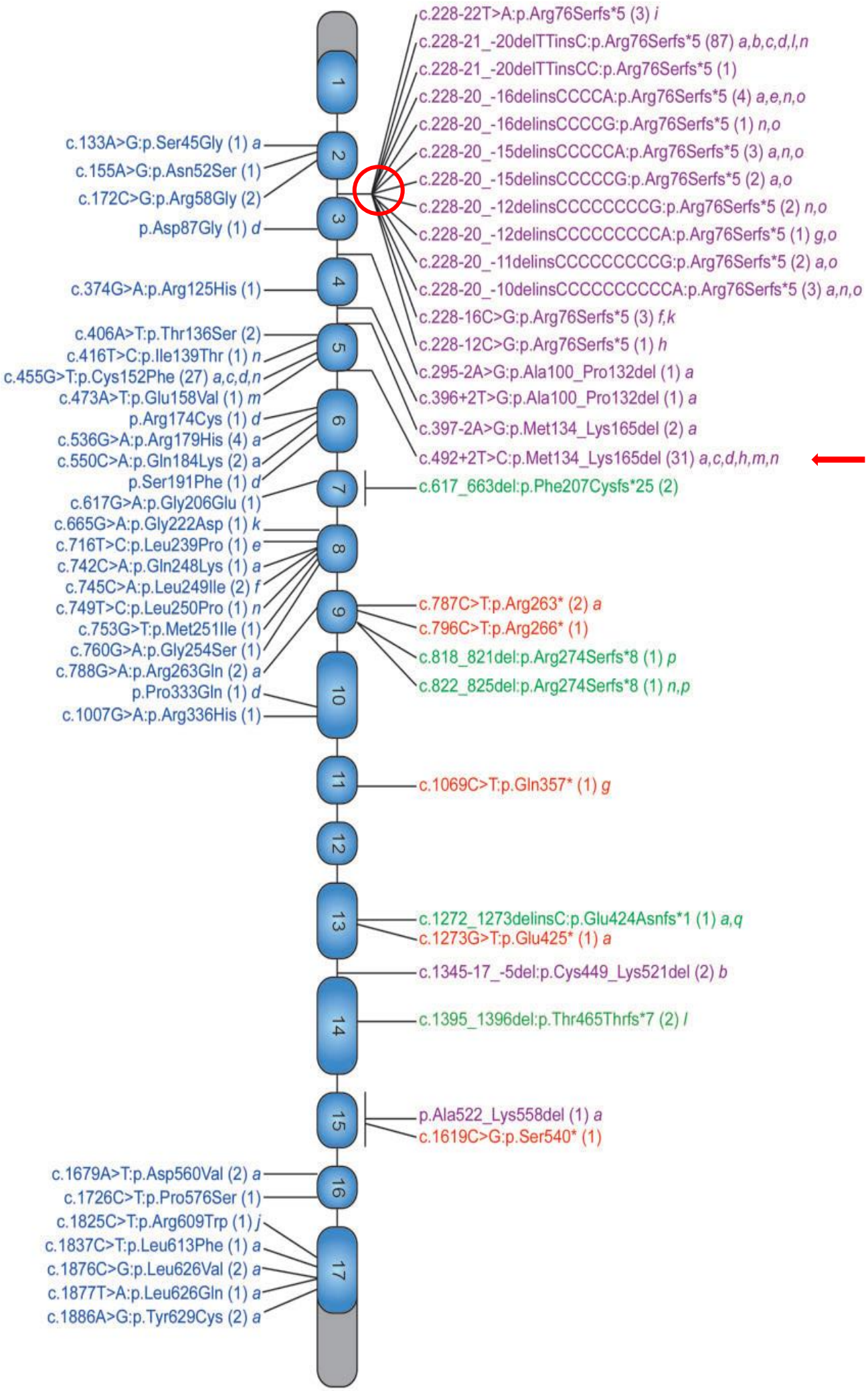
Patient 1: Initial evaluation

- Triplet pregnancy, 28-week preemie, 3 m NICU stay
- History of intermittent headaches since age 4.9 y
- Referred at age 8 y due to worsening headaches and abnormal MRI





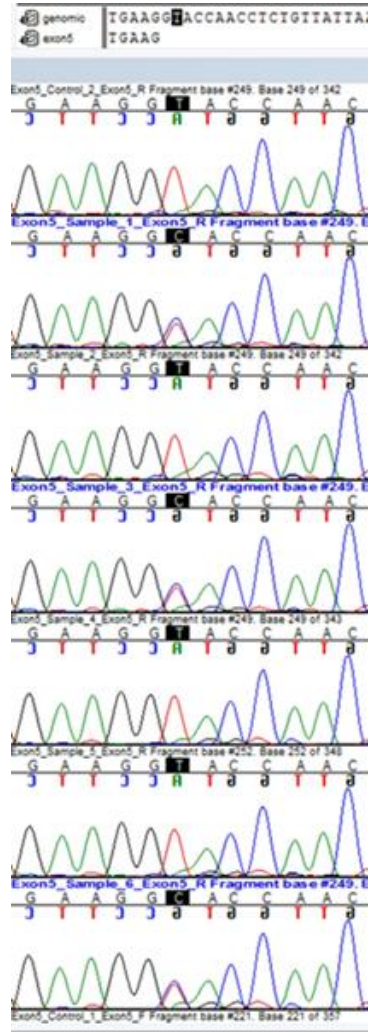
Hyperintensities in white matter, brainstem, and spinal cord.
Increased lactate in MRS


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
c.228-22T>A:p.Arg76Serfs*5 (3) i
c.228-21_-20delTTinsC:p.Arg76Serfs*5 (87) a,b,c,d,l,n
c.228-21_-20delTTinsCC:p.Arg76Serfs*5 (1)
c.228-20_-16delinsCCCCA:p.Arg76Serfs*5 (4) a,e,n,o
c.228-20_-16delinsCCCCG:p.Arg76Serfs*5 (1) n,o
c.228-20_-15delinsCCCCCA:p.Arg76Serfs*5 (3) a,n,o
c.228-20_-15delinsCCCCCG:p.Arg76Serfs*5 (2) a,o
c.228-20_-12delinsCCCCCCCCG:p.Arg76Serfs*5 (2) n,o
c.228-20_-12delinsCCCCCCCCCA:p.Arg76Serfs*5 (1) g,o
c.228-20_-11delinsCCCCCCCCCG:p.Arg76Serfs*5 (2) a,o
c.228-20_-10delinsCCCCCCCCCCA:p.Arg76Serfs*5 (3) a,n,o
c.228-16C>G:p.Arg76Serfs*5 (3) f,k
c.228-12C>G:p.Arg76Serfs*5 (1) h
c.295-2A>G:p.Ala100_Pro132del (1) a
c.396+2T>G:p.Ala100_Pro132del (1) a
c.397-2A>G:p.Met134_Lys165del (2) a
c.492+2T>C:p.Met134_Lys165del (31) a,c,d,h,m,n
c.133A>G:p.Ser45Gly (1) a
c.155A>G:p.Asn52Ser (1)
c.172C>G:p.Arg58Gly (2)
p.Asp87Gly (1) d
c.374G>A:p.Arg125His (1)
c.406A>T:p.Thr136Ser (2)
c.416T>C:p.Ile139Thr (1) n
c.455G>T:p.Cys152Phe (27) a,c,d,n
c.473A>T:p.Glu158Val (1) m
p.Arg174Cys (1) d
c.536G>A:p.Arg179His (4) a
c.550C>A:p.Gln184Lys (2) a
p.Ser191Phe (1) d
c.617G>A:p.Gly206Glu (1)
c.617_663del:p.Phe207Cysfs*25 (2)
c.665G>A:p.Gly222Asp (1) k
c.716T>C:p.Leu239Pro (1) e
c.742C>A:p.Gln248Lys (1) a
c.745C>A:p.Leu249Ile (2) f
c.749T>C:p.Leu250Pro (1) n
c.753G>T:p.Met251Ile (1)
c.760G>A:p.Gly254Ser (1)
c.788G>A:p.Arg263Gln (2) a
p.Pro333Gln (1) d
c.1007G>A:p.Arg336His (1)
c.787C>T:p.Arg263* (2) a
c.796C>T:p.Arg266* (1)
c.818_821del:p.Arg274Serfs*8 (1) p
c.822_825del:p.Arg274Serfs*8 (1) n,p
c.1069C>T:p.Gln357* (1) g
c.1272_1273delinsC:p.Glu424Asnfs*1 (1) a,q
c.1273G>T:p.Glu425* (1) a
c.1345-17_-5del:p.Cys449_Lys521del (2) b
c.1395_1396del:p.Thr465Thrfs*7 (2) l
p.Ala522_Lys558del (1) a
c.1619C>G:p.Ser540* (1)
c.1679A>T:p.Asp560Val (2) a
c.1726C>T:p.Pro576Ser (1)
c.1825C>T:p.Arg609Trp (1) j
c.1837C>T:p.Leu613Phe (1) a
c.1876C>G:p.Leu626Val (2) a
c.1877T>A:p.Leu626Gln (1) a
c.1886A>G:p.Tyr629Cys (2) a

Targeted mutation analysis for DARS2

Sanger sequencing uncovered a novel mutation in Intron 2 and established phase

	Intron 2	Intron 5
Mother	Normal Normal	c.492+2T>C Normal
Father	c.228-17C>G Normal	Normal Normal
Patient 1	c.228-17C>G Normal	c.492+2T>C Normal



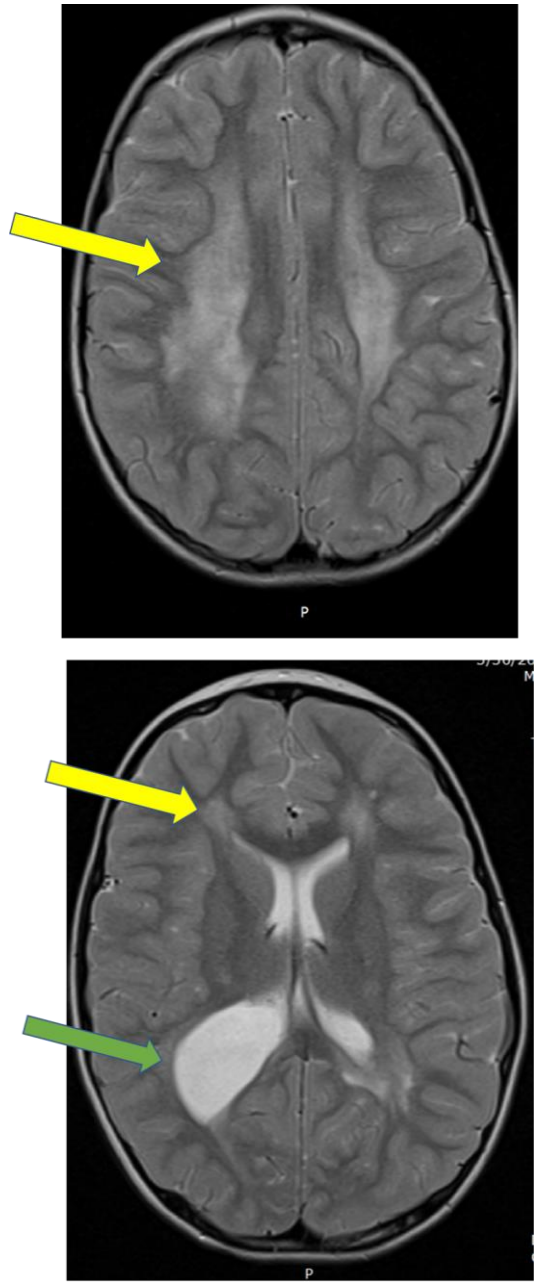
Family Studies

Identification of a 2nd affected family member

	Intron 2	Intron 5
Mother	Normal Normal	c.492+2T>C Normal
Father	c.228-17C>G Normal	Normal Normal
Patient 1	c.228-17C>G Normal	c.492+2T>C Normal
Sibling 1	Normal Normal	Normal Normal
Sibling 2	c.228-17C>G Normal	Normal Normal
Patient 2	c.228-17C>G Normal	c.492+2T>C Normal

Patient 2: Initial evaluation (age 4)

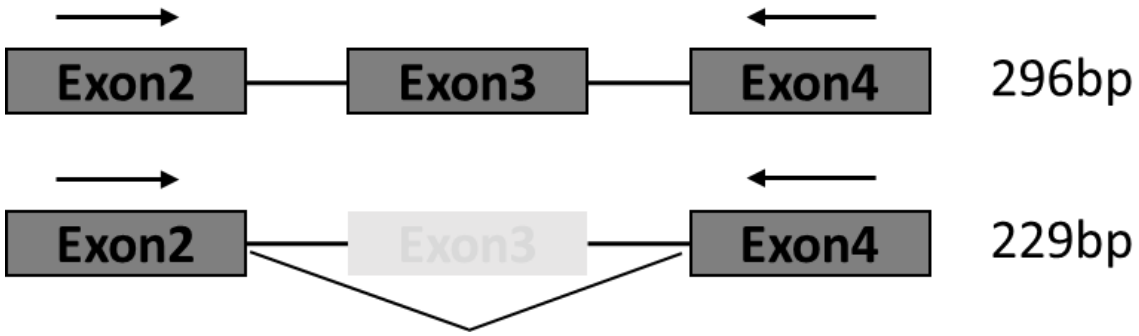
- Normal pregnancy and delivery
- History of intermittent leg pain
- MRI at age 5 y: Bilateral and symmetric hyperintense T2 signal abnormalities in the supraventricular and periventricular white matter (yellow arrows). Abnormal signal intensities in the posterior limbs of the internal capsule, pyramids at the level of the medulla, and dorsal columns of the spinal cord. A right intraventricular cyst (green arrow) was also uncovered.



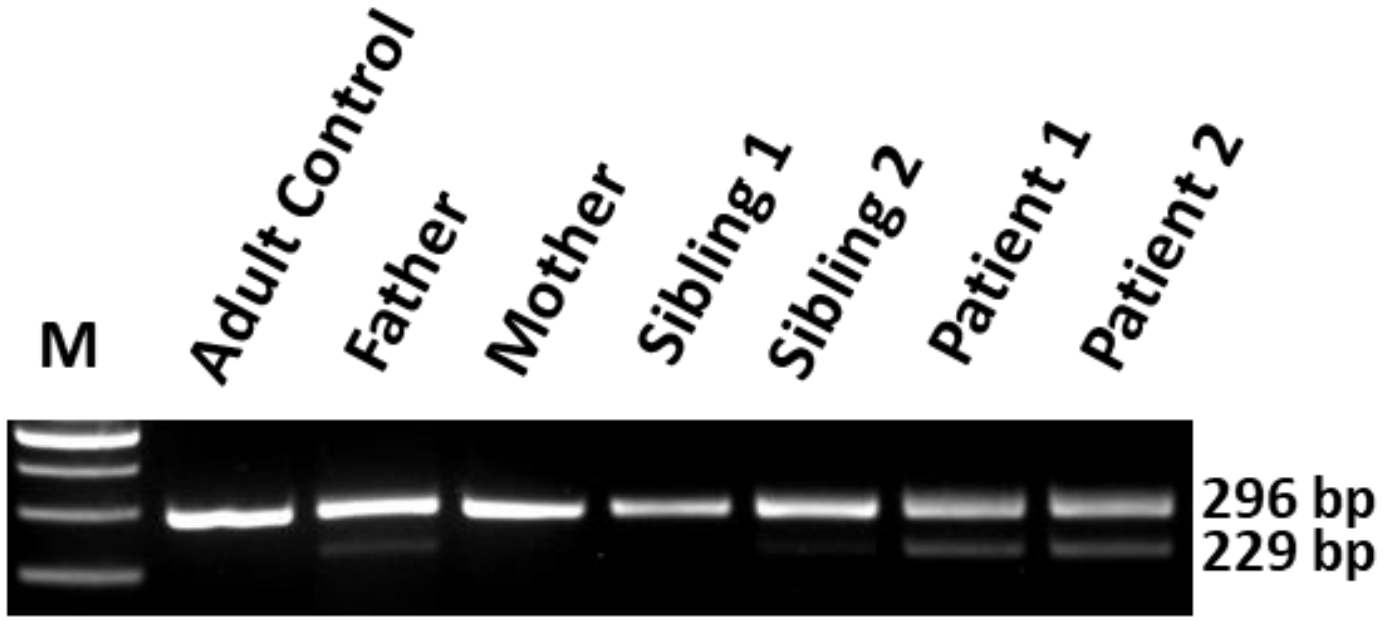
E-Lab
Fibroblasts studies

CHOC Research
GO BEYOND

Skipping of exon 3 in patients



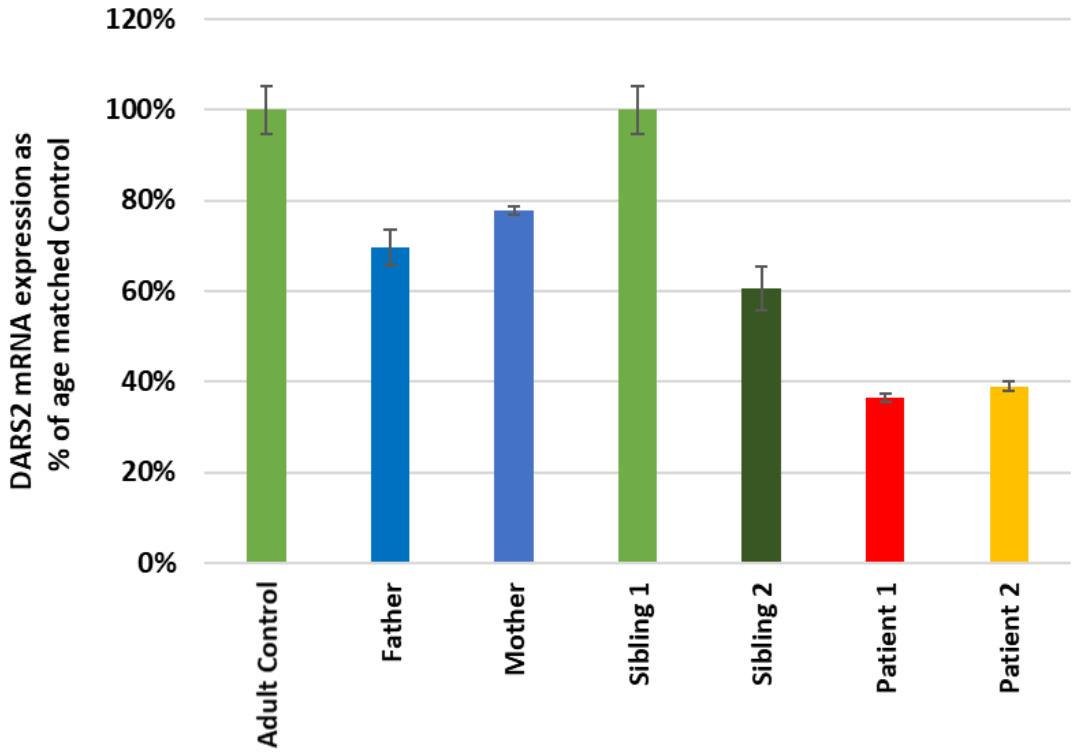
RT-PCR results suggested skipping of exon 3 in the father and the two patients harboring the intron 2 mutation



DARS2 mRNA levels – qPCR

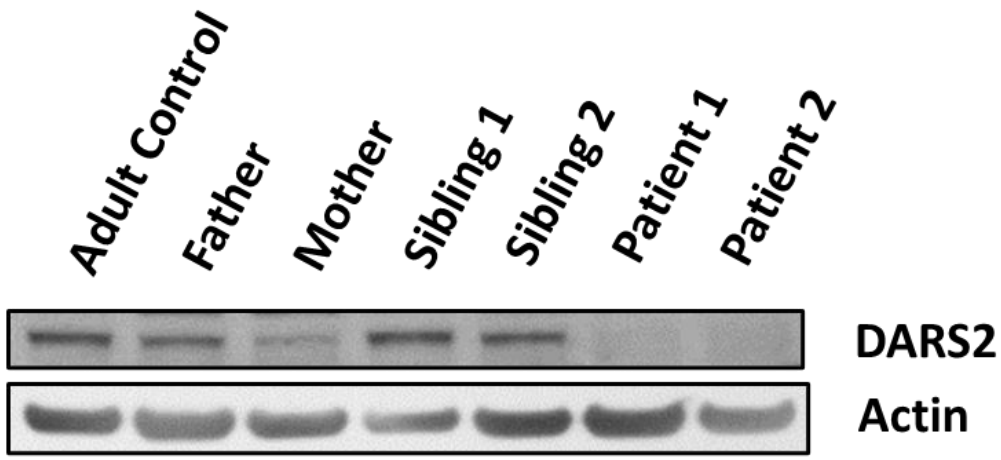
Carriers and patients showed lower expression of mRNA levels

qPCR Method: Taqman Assay
Primer set: Thermo Fisher Hs01016220_m1

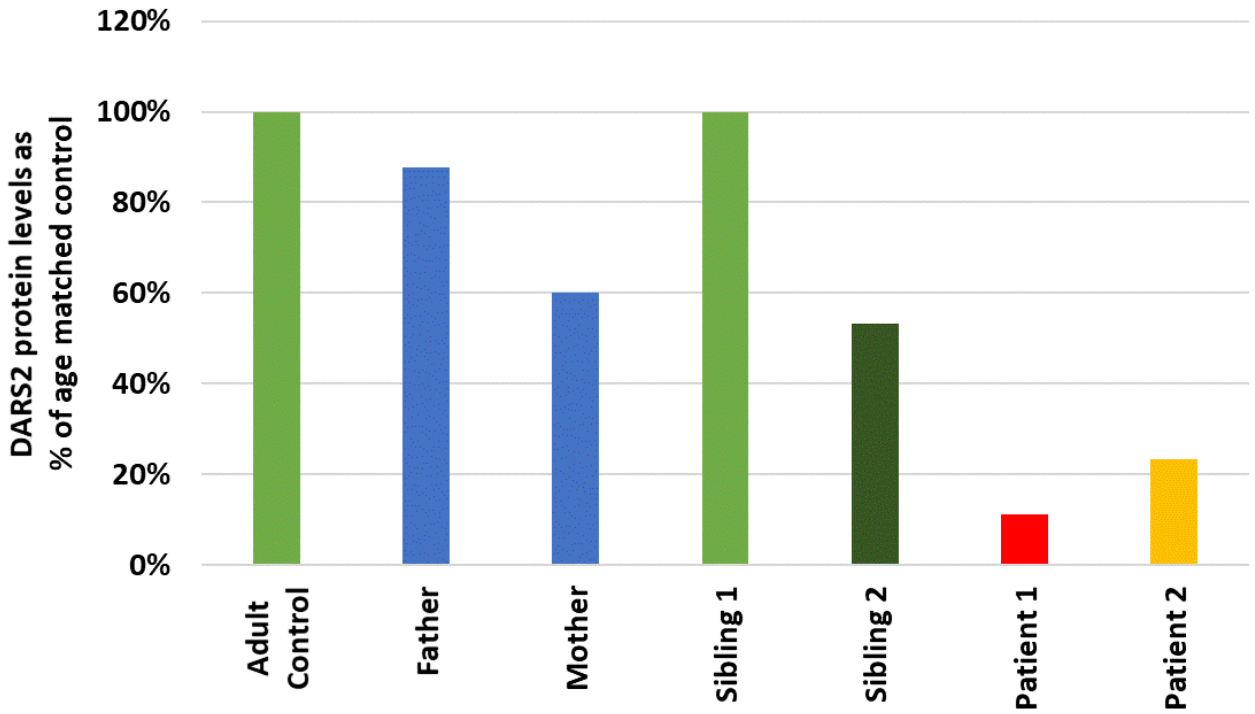


DARS2 Protein levels – Western Blot

Patients showed reduced levels of DARS2 protein

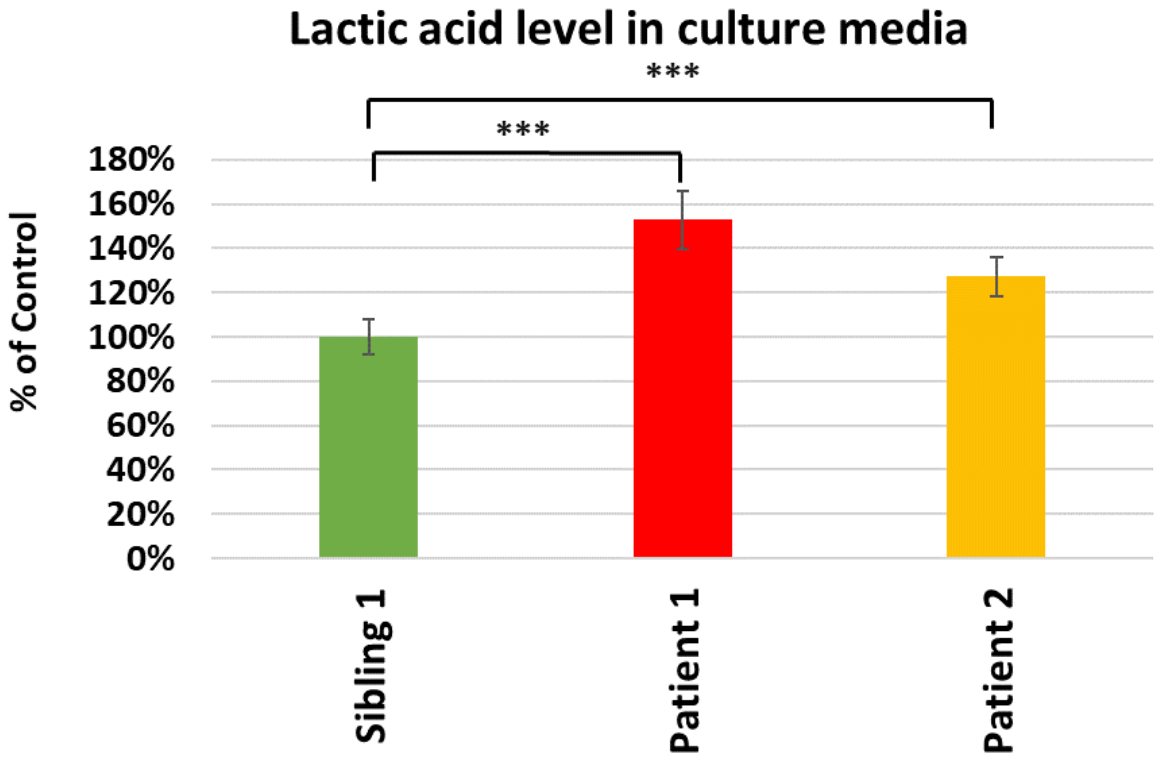


Primary Antibody : DARS2 (ab154606)
Secondary Antibody: Goat anti-Rabbit HPR (ab6721)



Lactic acid levels in cultured media

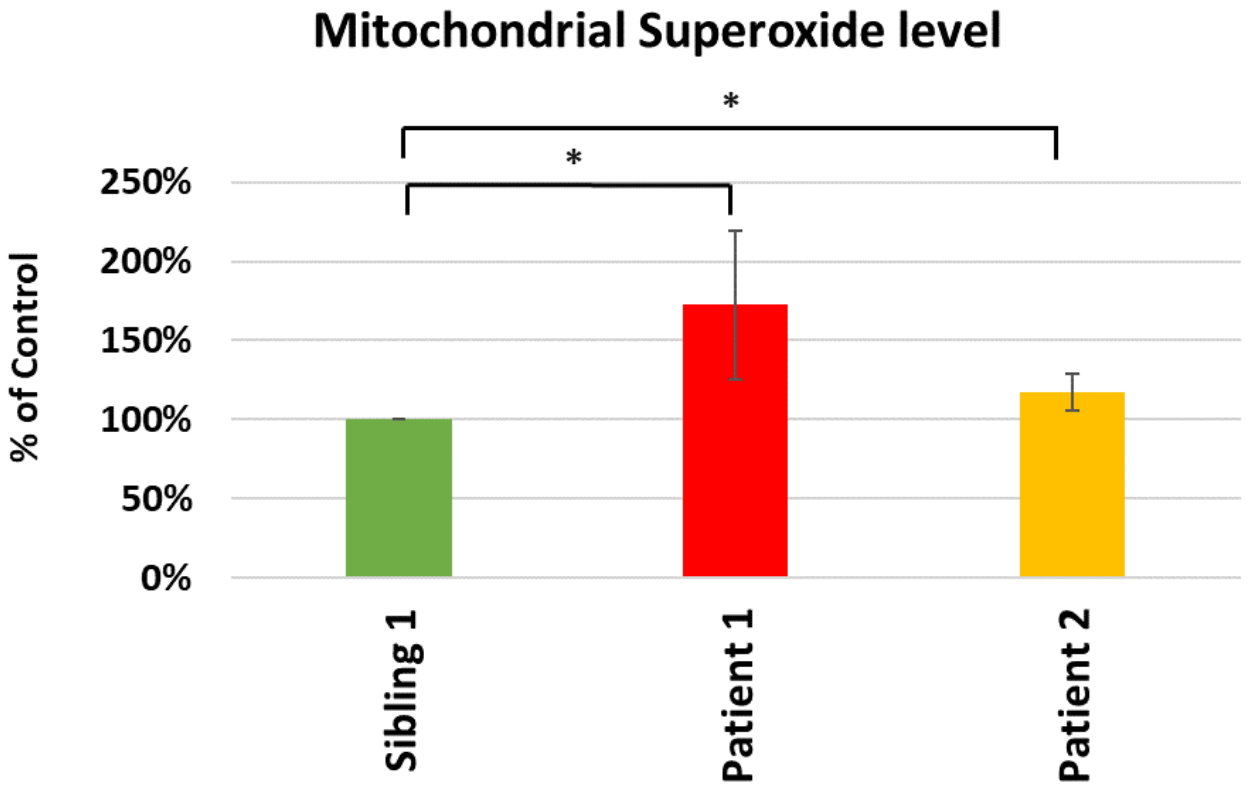
Patients showed elevated lactic acid levels in the culture media



Instrument: Infinite M Plex
Kit : POINTE™ Lactate Reagent Set (L7596-50)

Reactive oxygen species (ROS) levels

Patients showed elevated ROS production

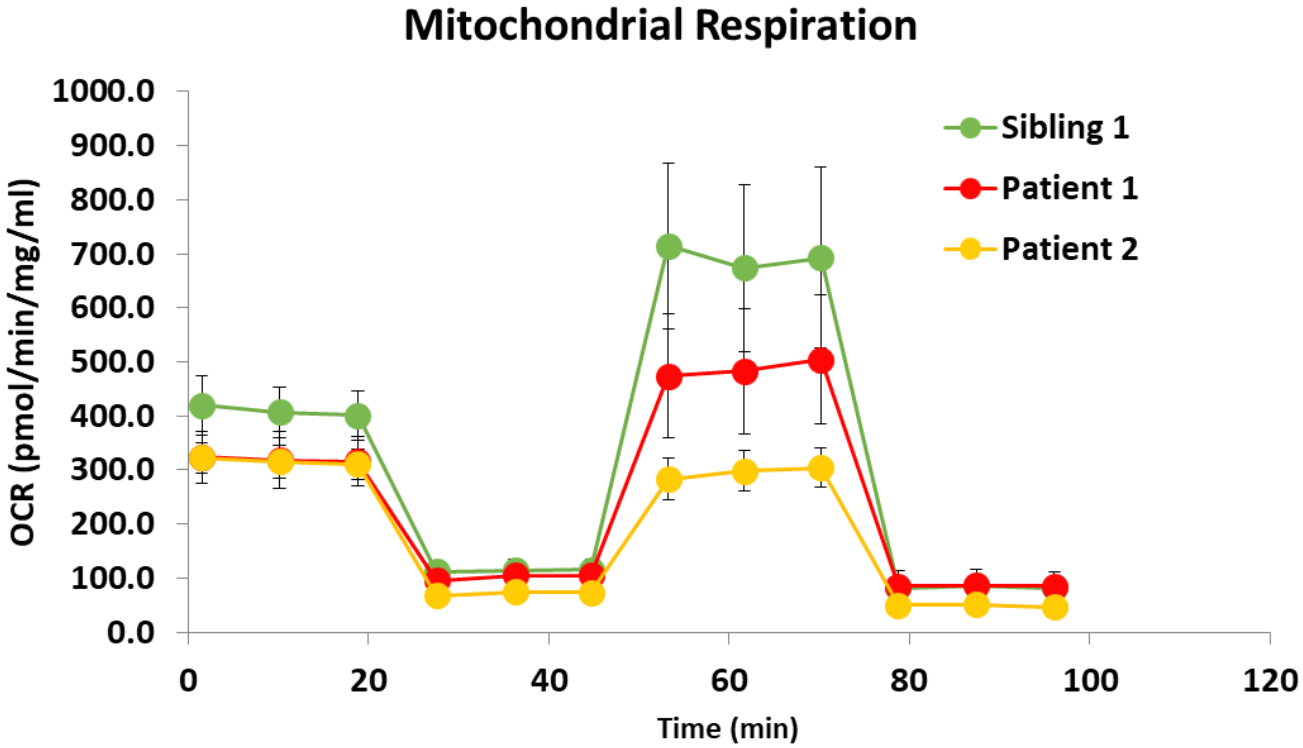


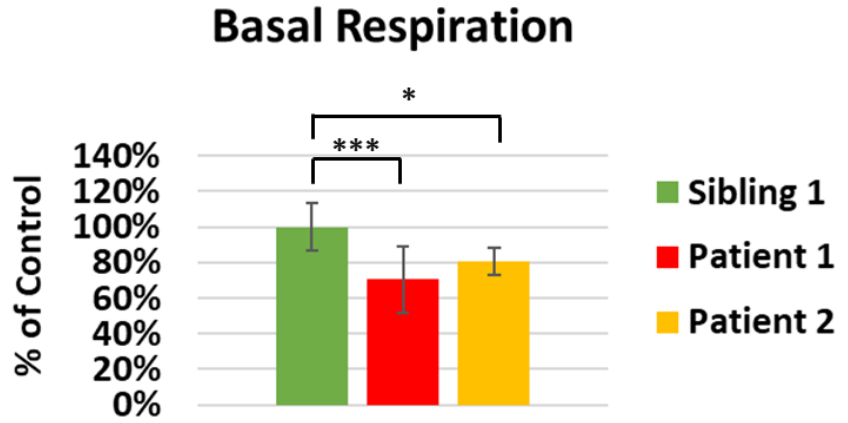
Flow cytometry Assay (BD Melody)
Leipnitz G et al. Sci Rep. 2018; 8: 1165.

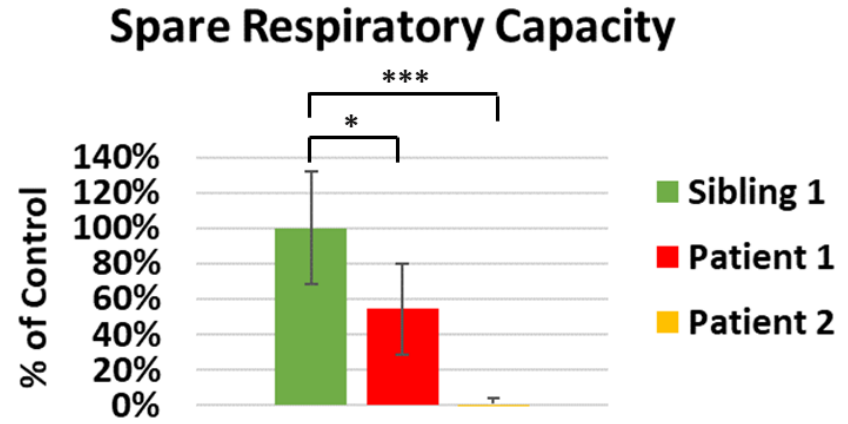
- MitoSOX™ Red: Mitochondrial Superoxide
- MitoTracker™ Green: mt content (Normalization)

Oxygen consumption rate

Patients showed decreased basal respiration and spare respiratory capacity

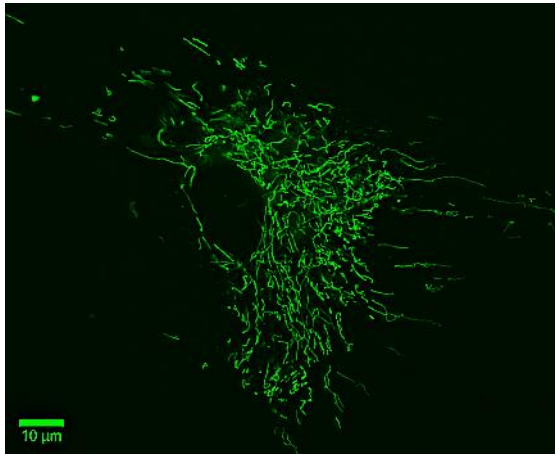
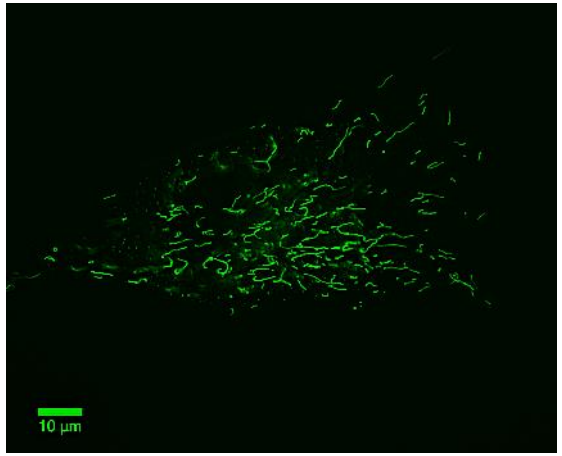
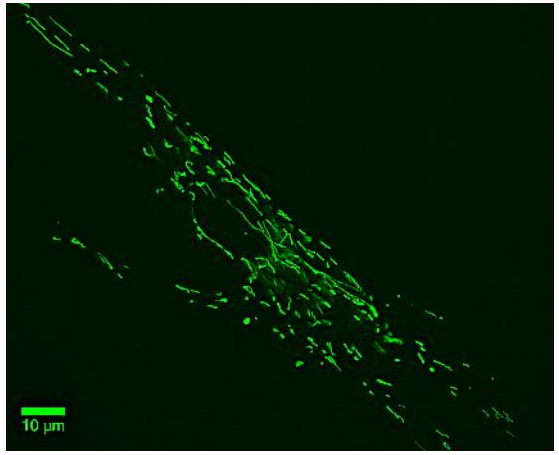
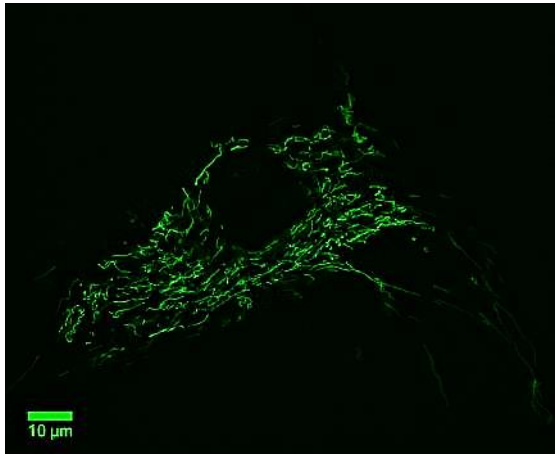
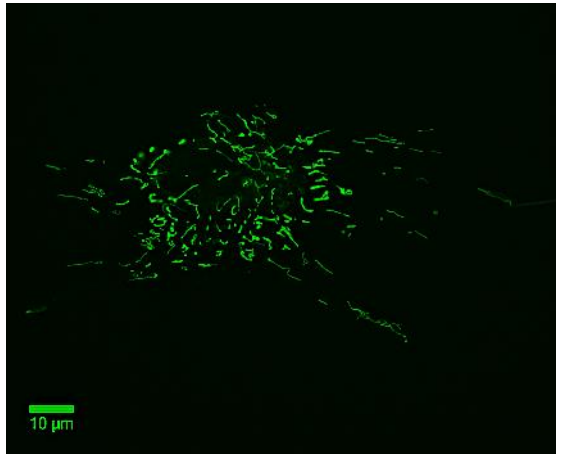
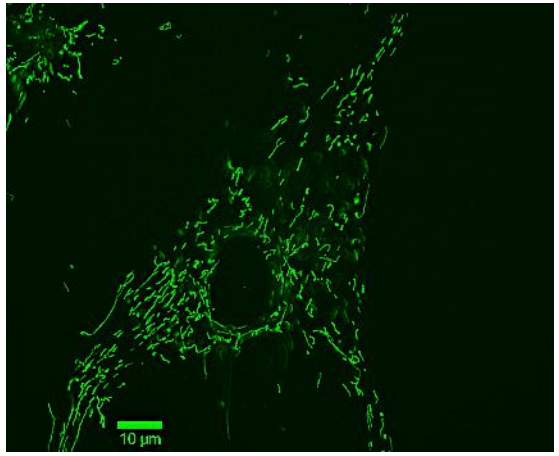






Mitochondria morphology - Microscopy

Patients showed fragmented mitochondrial network

Sibling 1	Patient 1	Patient 2
		
		

Fluorescent Microscope:
Live-cell staining
MitoTracker™ Green

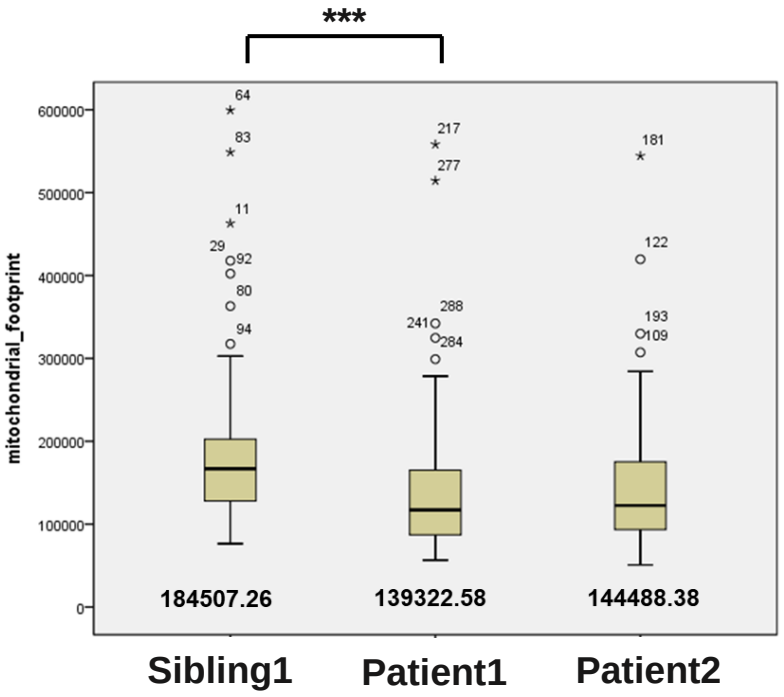
Deconvolution: Image J plug-in

Mitochondrial Network Analysis (MiNA)

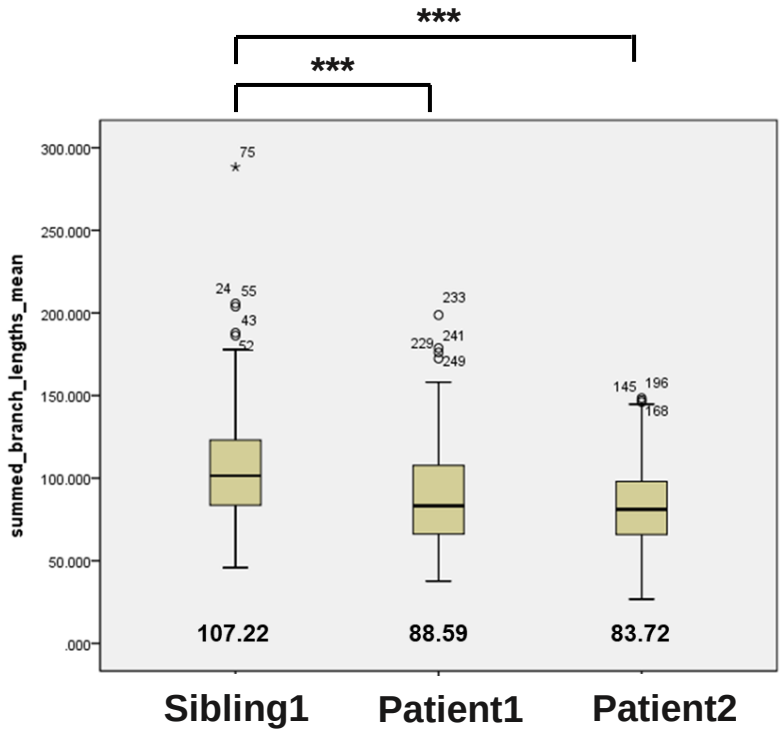
Patients showed decreased mitochondrial intensity, length, and number of branches

Quantification method
Image J, Plug-in / MiNA

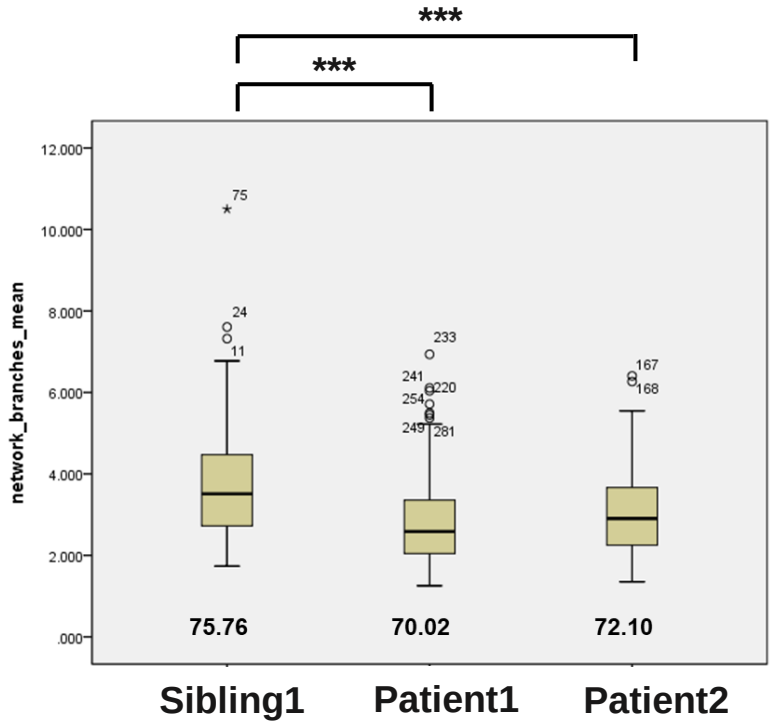
Footprint (Intensity)



Length

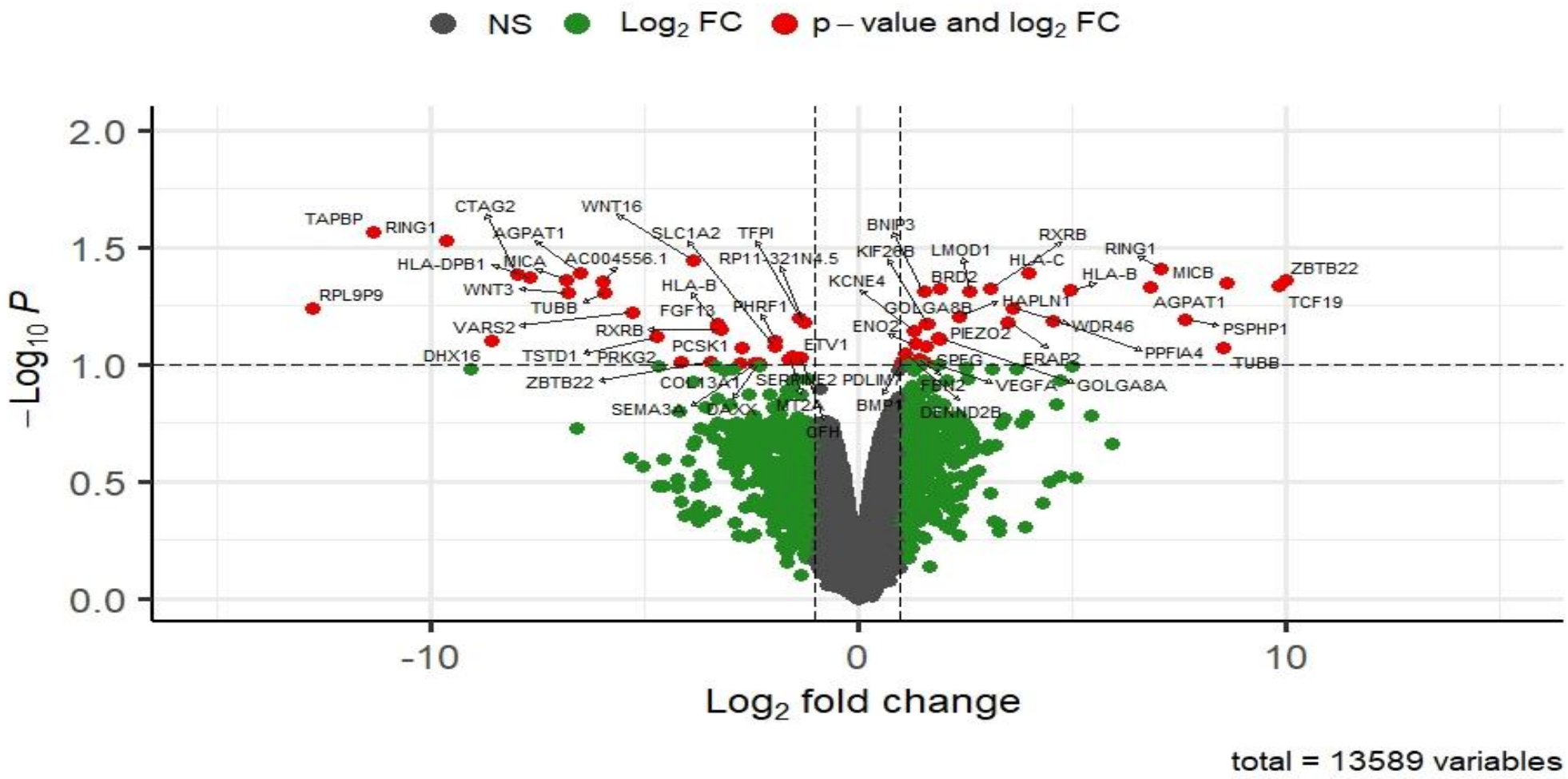


Number of branches



Transcriptome Analysis

Method: RNAseq
Software: Kallisto and limma (R & R Studio)



MitoCarta3.0 collection

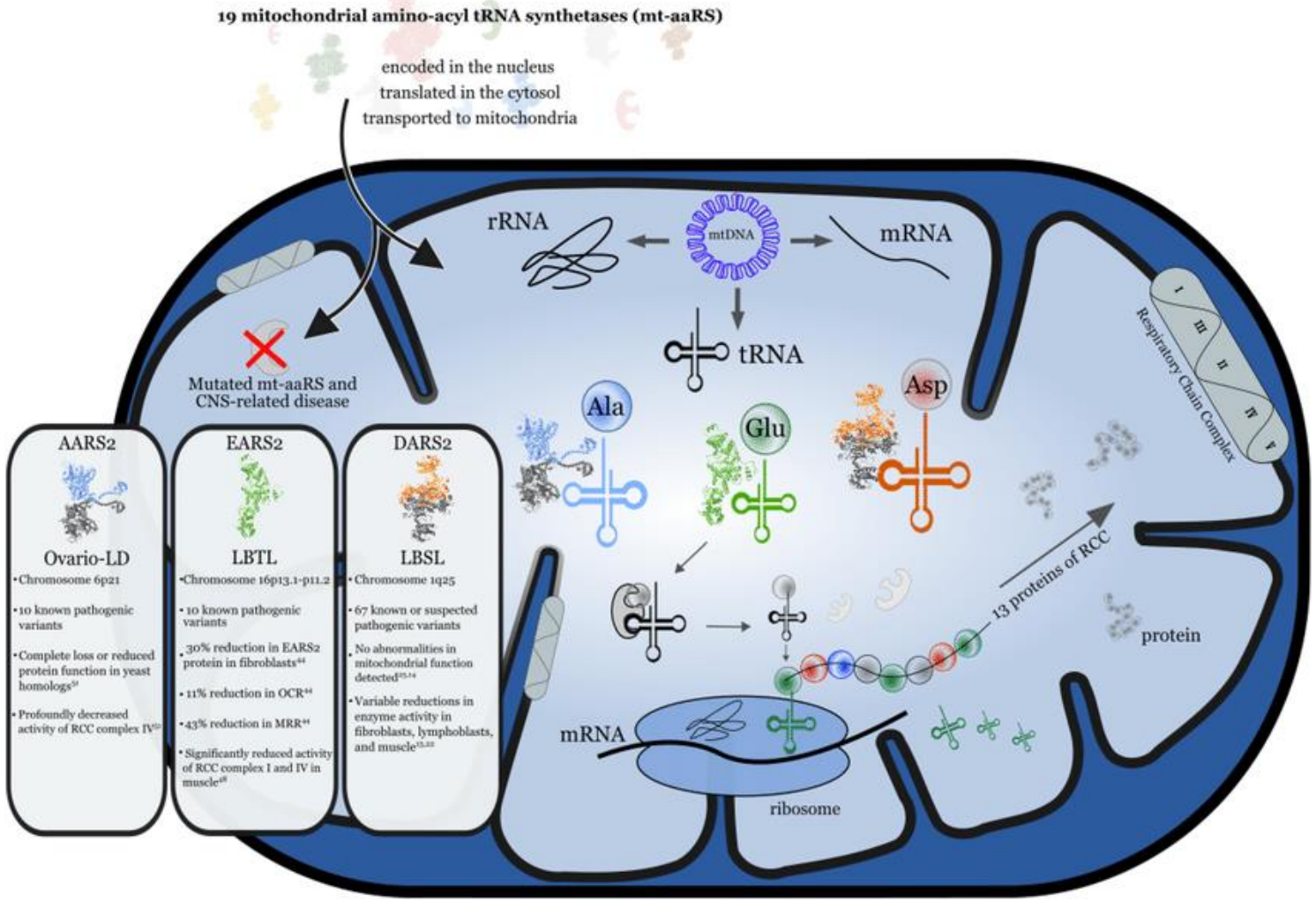
Downregulated genes

	Gene Symbol	Fold change (log_2)	adj.P.value
	VARS2	-5.29	0.06
	TSTD1	-4.70	0.08
	PDK4	-2.68	0.24
C V →	MT-ATP6	-1.99	0.18
C V →	MT-ATP8	-1.91	0.15
C I →	MT-ND3	-1.78	0.23
C IV →	COX7A1	-1.66	0.14
C IV →	MT-CO2	-1.62	0.20
C I →	MT-ND4L	-1.45	0.18
	OXCT2	-1.42	0.24
C I →	MT-ND6	-1.40	0.21
C I →	MT-ND4	-1.37	0.22
C III →	MT-CYB	-1.26	0.25
C I →	MT-ND1	-1.25	0.18
C I →	MT-ND5	-1.18	0.21
	ACSS3	-1.17	0.18
	MTHFS	-1.11	0.30
C V →	ATP23	-1.09	0.34
C IV →	MT-CO1	-1.01	0.28
	ACOT11	-1.00	0.19

Upregulated genes

Gene Symbol	Fold change (log_2)	adj.P.value
C6orf136	1.76	0.25
PDK1	1.74	0.13
AMT	1.72	0.18
C17orf47	1.71	0.28
HSD17B8	1.67	0.73
BNIP3	1.55	0.05
MGARP	1.26	0.32
MTFP1	1.20	0.33
MRPL23	1.12	0.16

DARS2 Pathway



Smith Fine et al., Journal of Neurodevelopmental Disorders 11 (2019) 29

Treatment ?

CHOC Research
GO BEYOND

DARS2 Treatment

SCIENTIFIC REPORTS

OPEN

Effects of 5-aminolevulinic acid and sodium ferrous citrate on fibroblasts from individuals with mitochondrial diseases

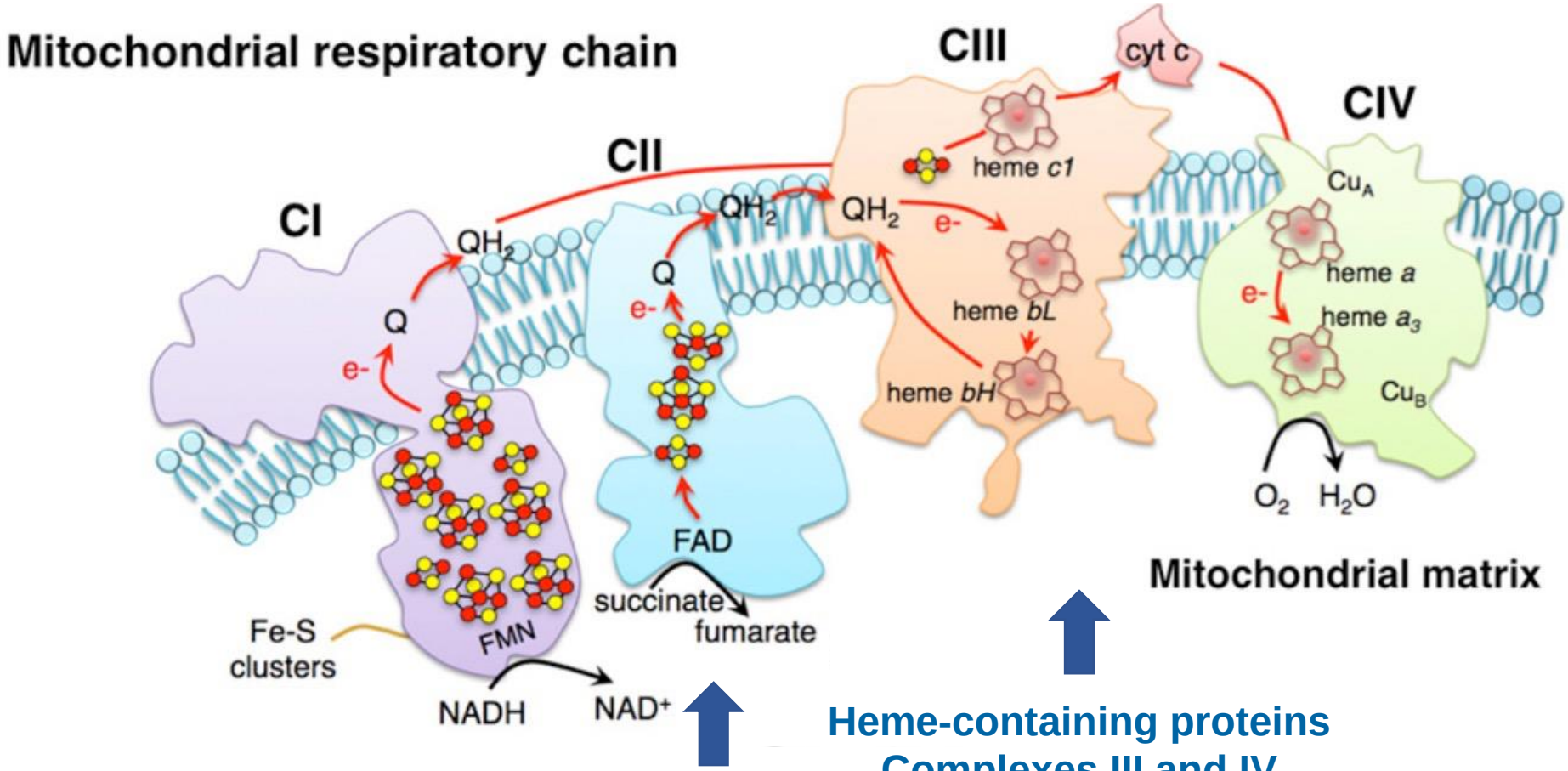
Masaru Shimura[1], Naoko Nozawa[2], Minako Ogawa-Tominaga[1], Takuya Fushimi[1], Makiko Tajika[1], Keiko Ichimoto[1], Ayako Matsunaga[1], Tomoko Tsuruoka[1], Yoshihito Kishita[3], Takuya Ishii[2], Kiwamu Takahashi[2], Tohru Tanaka[2], Motowo Nakajima[2], Yasushi Okazaki[3], Akira Ohtake[4,5] & Kei Murayama[1]

No.	ID	Sex	Age of onset	Clinical diagnosis	Affected complex	Gene	Mutations
1	Pt25	F	5 m	IMD	CI	*ACAD9*	c.811T > G:p.C271G/ c.1766-2A > G
2	Pt27	M	1 y	LS	CIV	*SURF1*	c.743C > A:p.A248D/ c.743C > A:p.A248D
3	Pt67	M	0 d	IMD	CI	*NDUFB11*	c.391G > A:p.E131K (hemizygous)
4	Pt100	M	8 m	ND	CI	*NDUFV2*	c.580G > A:p.E194K/ unknown
5	Pt101	M	11 m	LS	CI	*NDUFAF6*	c.371T > C:p.I124T/ c.805C > G:p.H269D
6	Pt276	M	1 y 11 m	MH	CI + IV	*MRPS23*	c.119C > G:p.P40R/ c.119C > G:p.P40R
7	Pt346	F	0 d	IMD	CI	*ECHS1*	c.176A > G:p.N59S/ c.476A > G:p.Q159R
8	Pt1177	F	9 m	LS	CI	*NDUFV2*	c.427C > T:p.R143X/ c.580G > A:p.E194K

- 5-Aminolevulinic acid(ALA)/sodium ferrous citrate (SFC) treatment in patients with different mitochondrial diseases
- Increased oxygen consumption rate, ATP levels and mitochondrial copy number after 5-ALA/SFC treatment

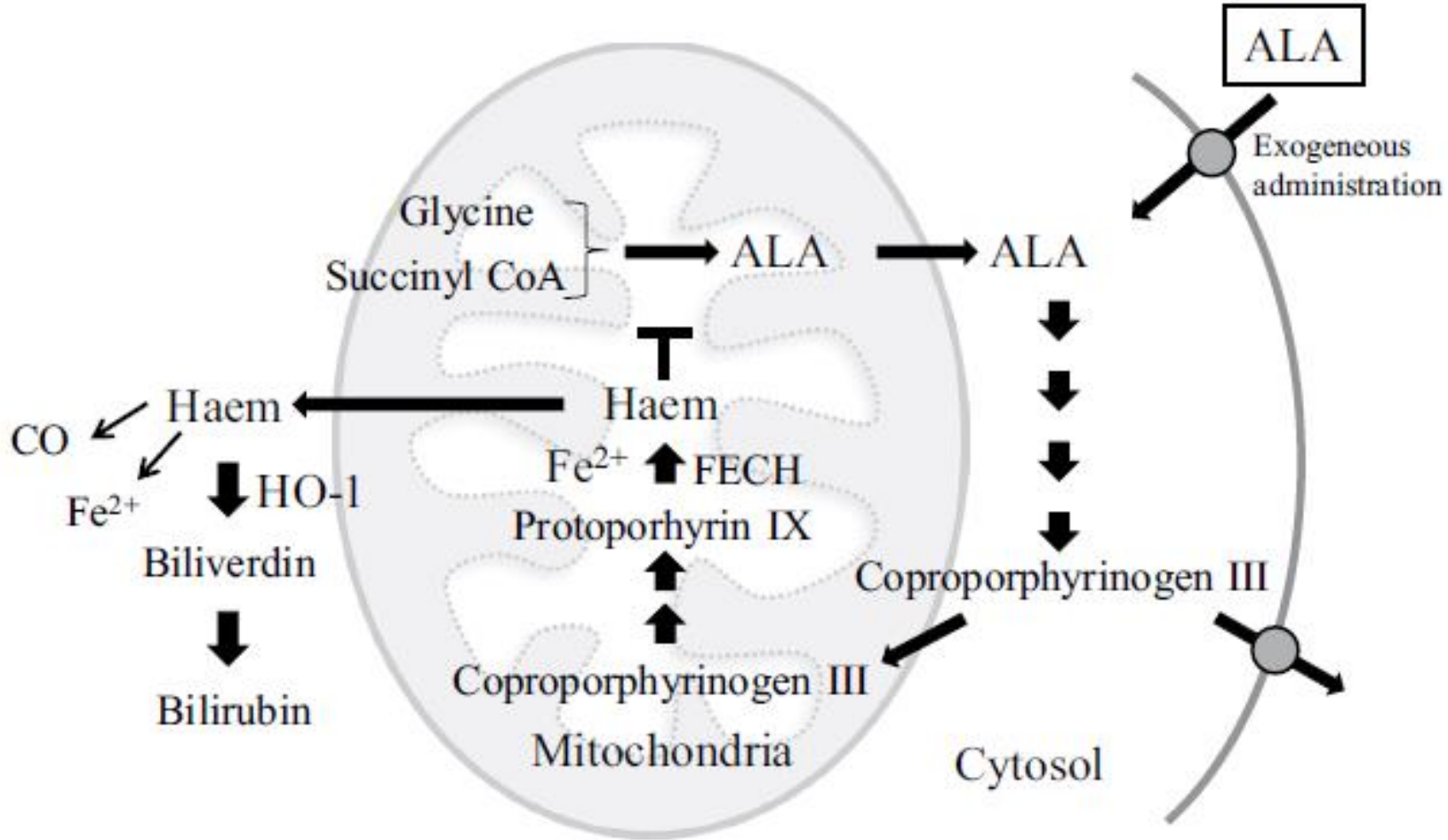
Shimura et al., Scientific Reports 9 (2019) Article #10549

Mitochondrial iron-containing proteins



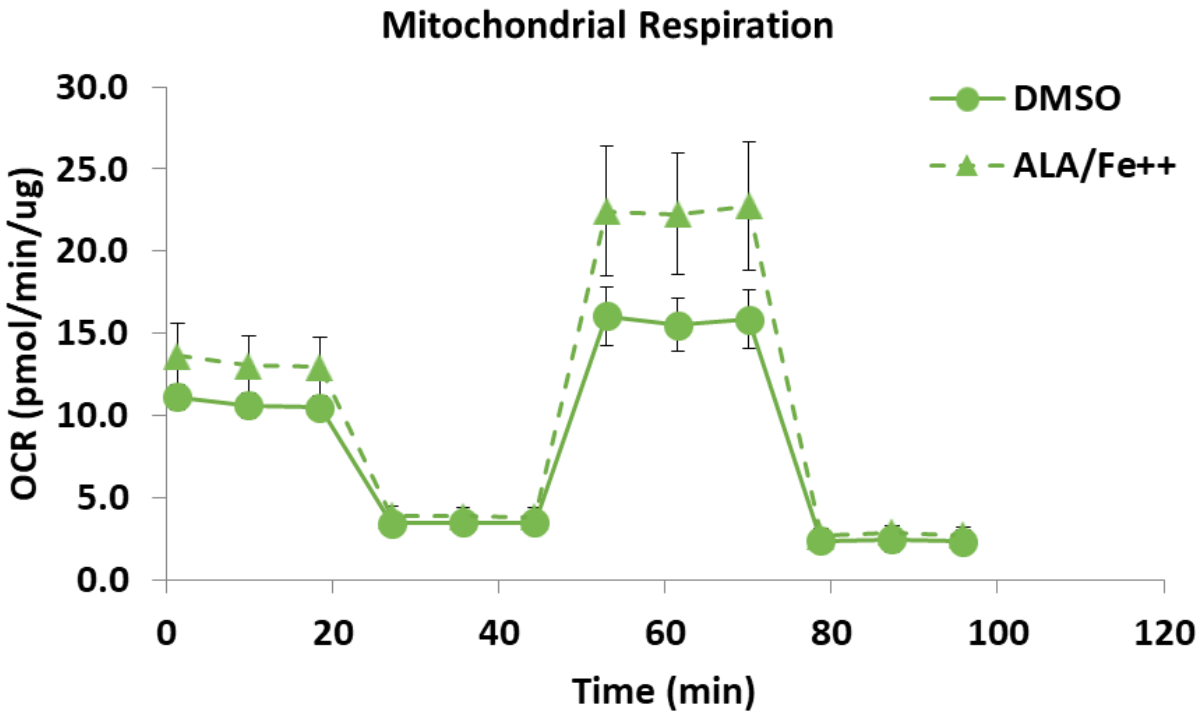
Adapted from *Maio et al, Cell Metabolism 25 (2017) 945–953*

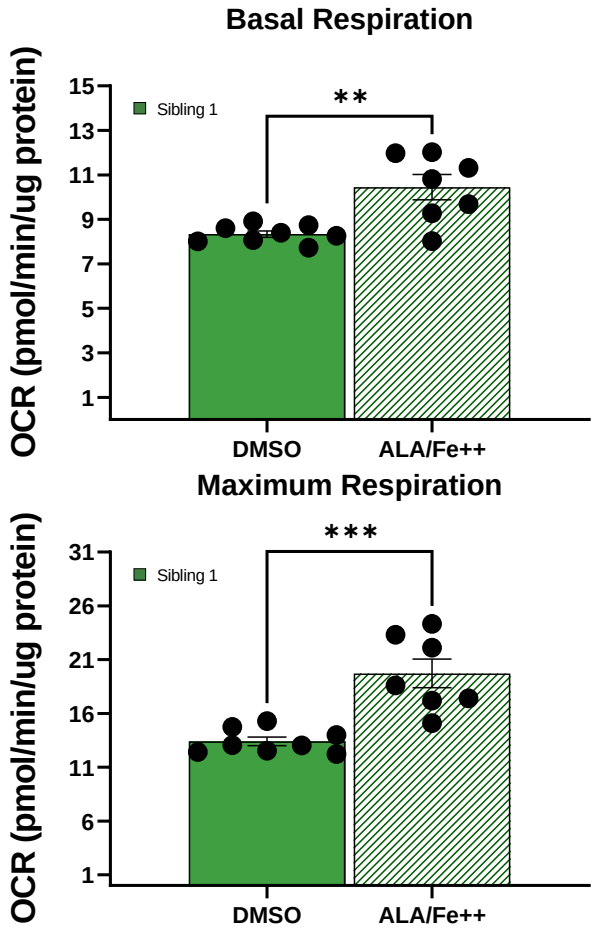
Haeme biosynthesis



Oxygen consumption rate Seahorse

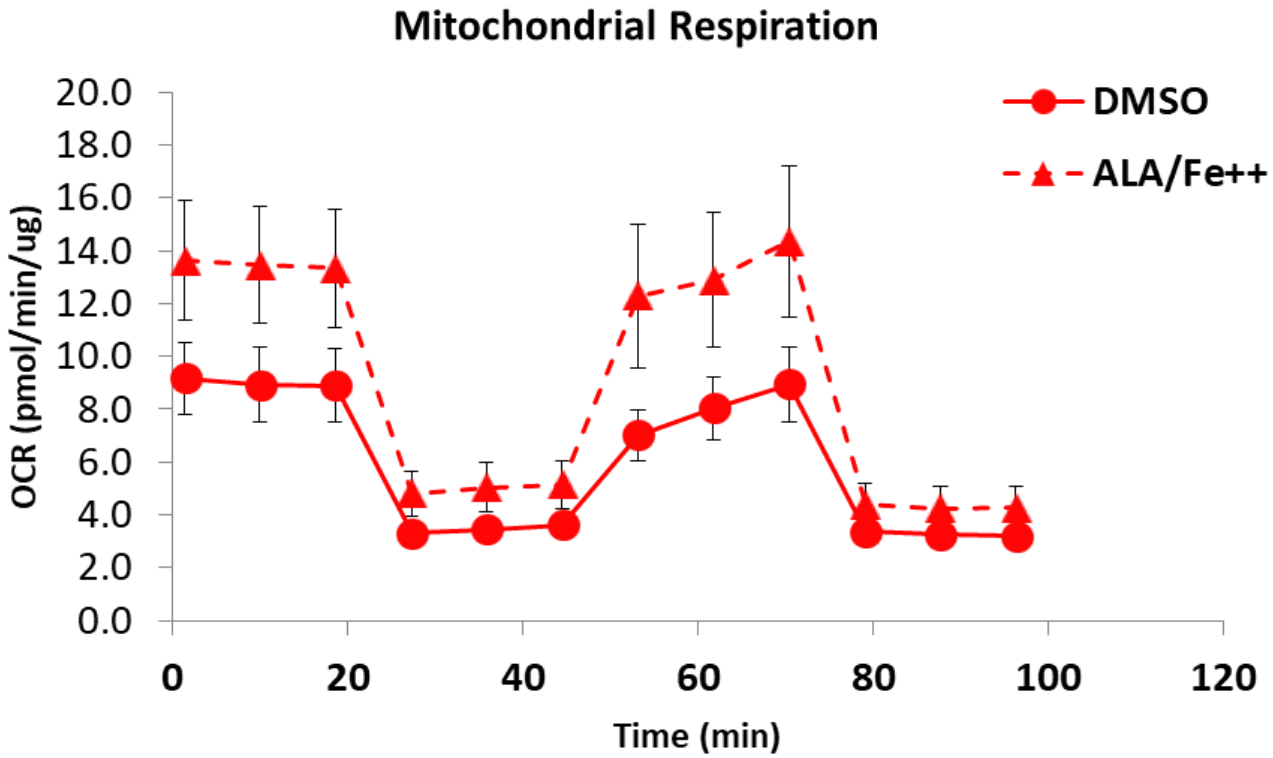
Treatment for 5 weeks on Sibling1

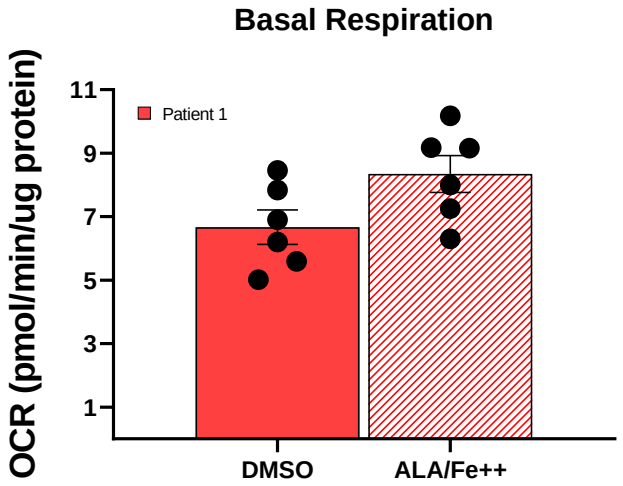


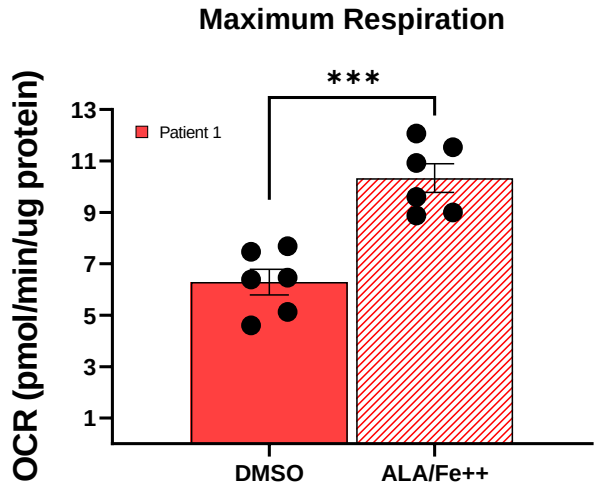


Oxygen consumption rate Seahorse

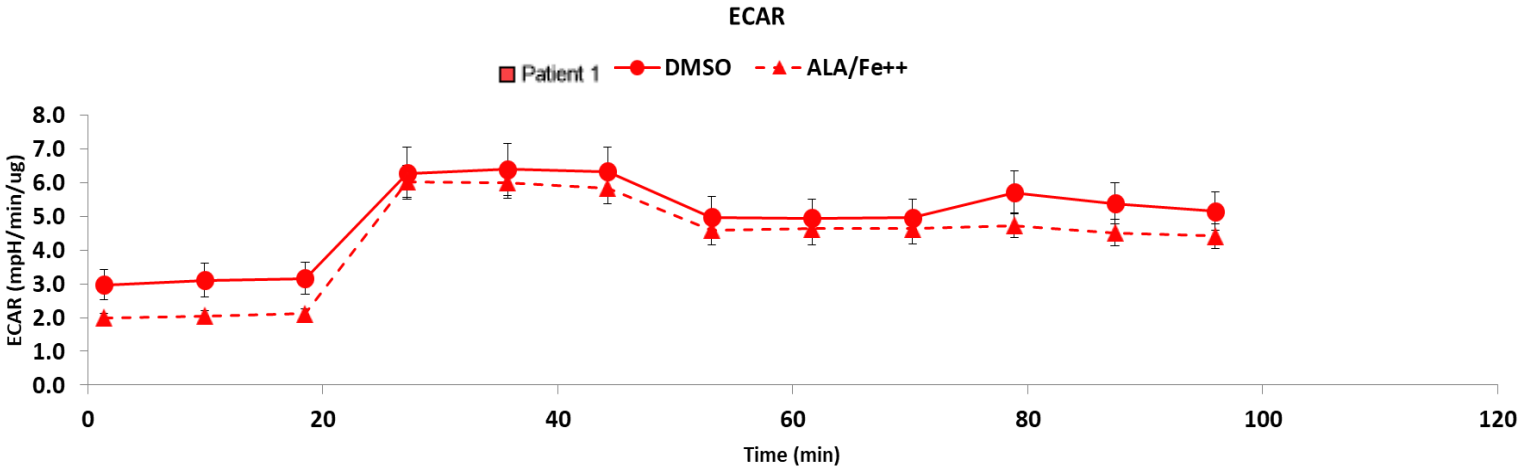
Treatment for 5 weeks on Patient1

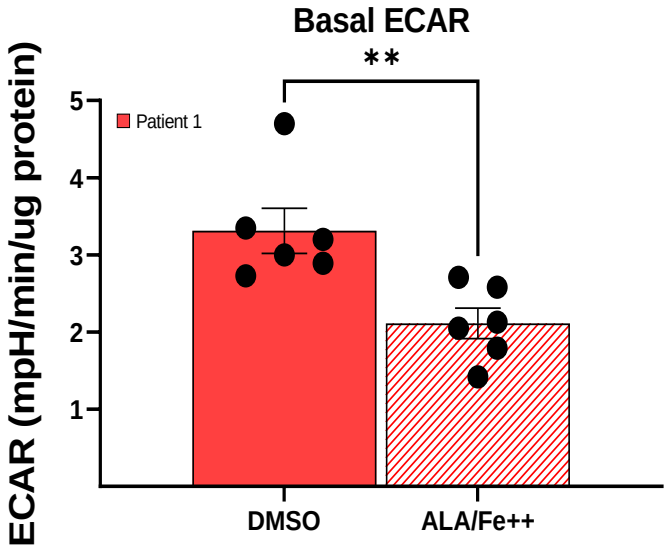


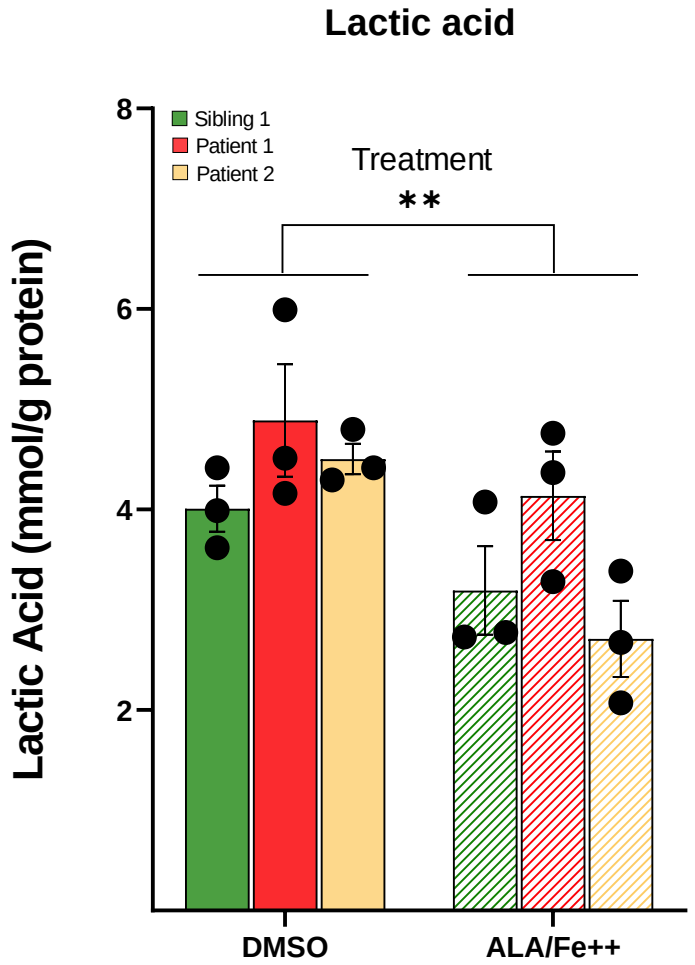




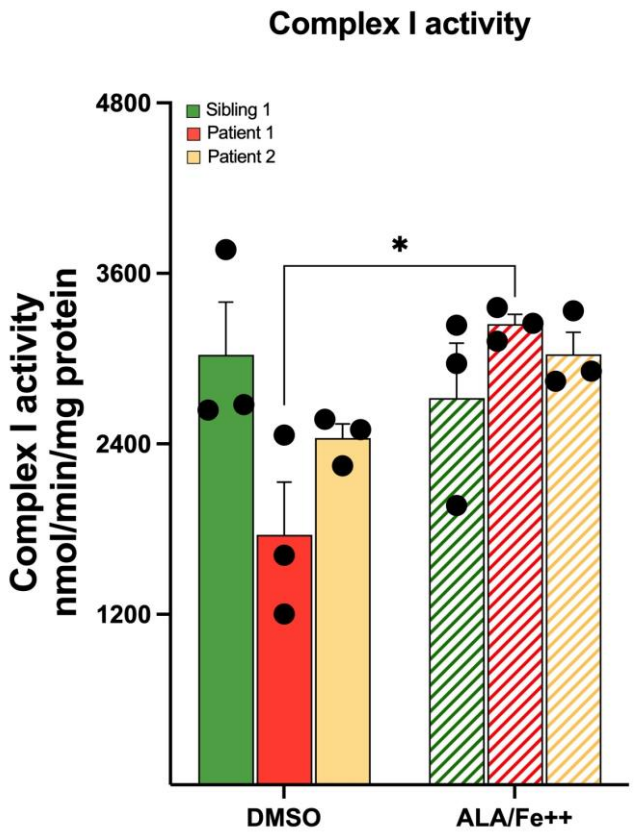
Extracellular acidification rate (ECAR) Seahorse



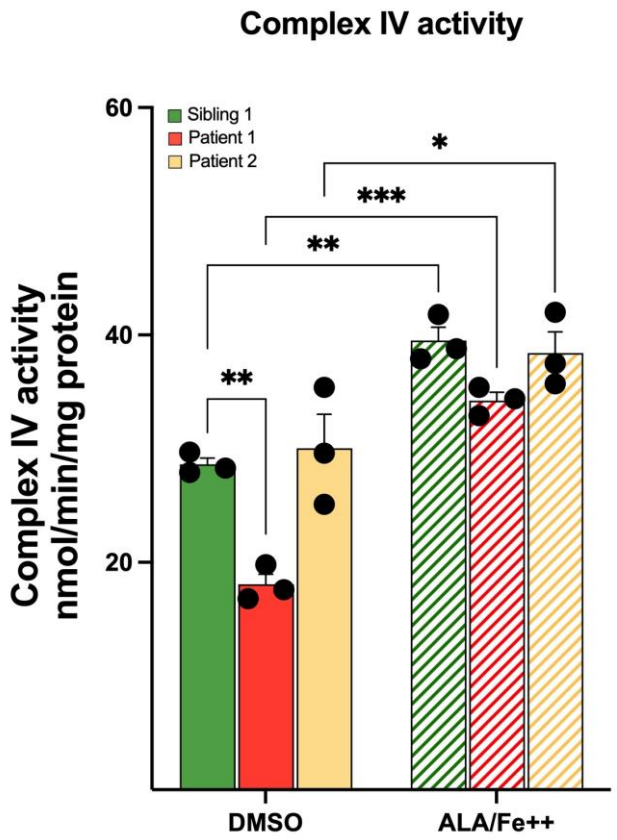




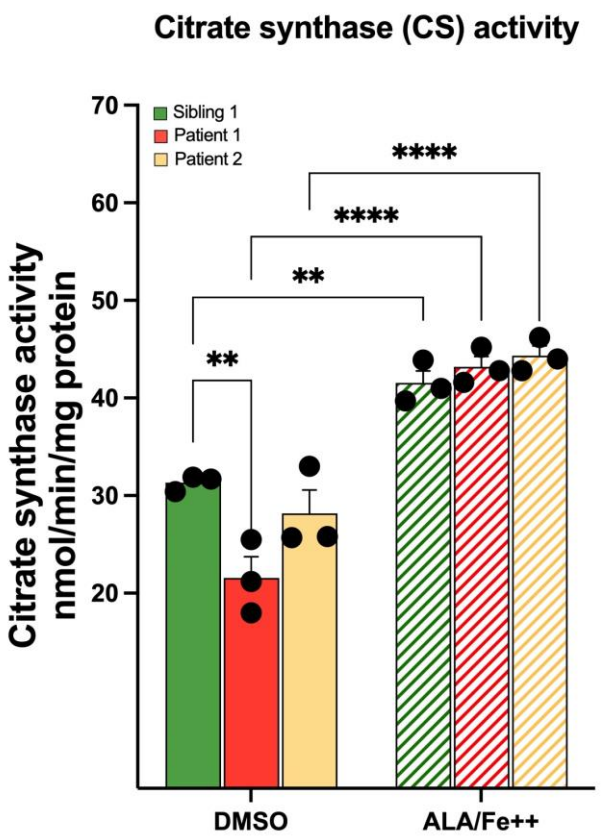
Respiratory chain complex enzyme activities



Interaction: $F_{(2,12)}$= 5.193; P= 0.02
Treatment: $F_{(1,12)}$= 6.770; P= 0.02
Genotype: $F_{(2,12)}$= 0.9220; P= 0.4

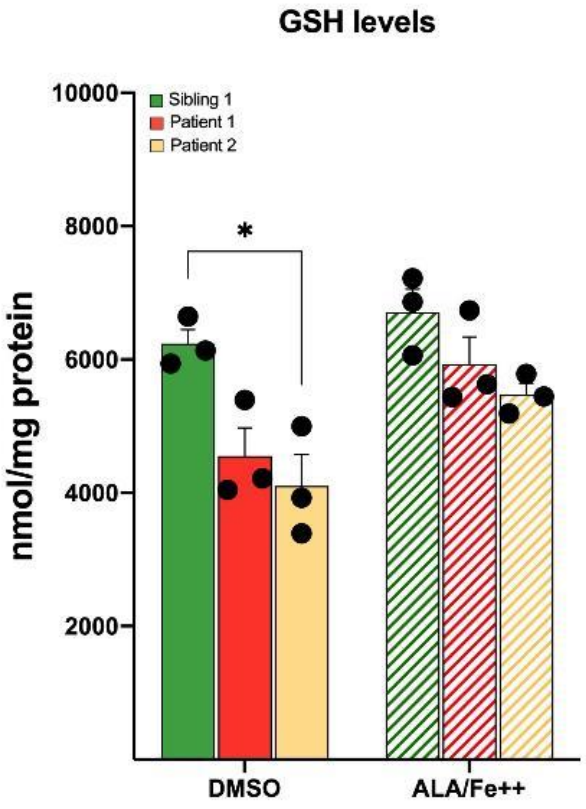


Interaction: $F_{(2,12)}$= 3.087; P= 0.08
Treatment: $F_{(1,12)}$= 81.28; P= <0.0001
Genotype: $F_{(2,12)}$= 16.57; P= 0.0004

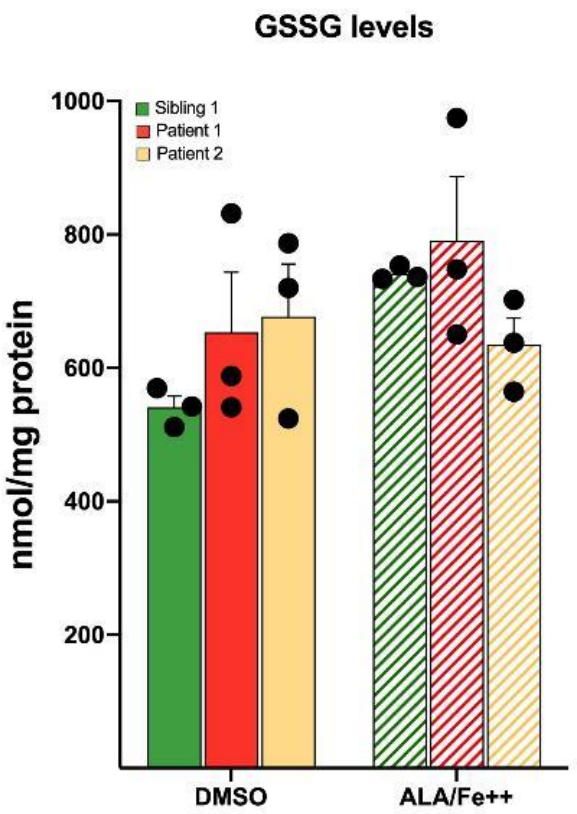


Interaction: $F_{(2,12)}$= 6.796; P= 0.01
Treatment: $F_{(1,12)}$= 159.6; P= <0.0001
Genotype: $F_{(2,12)}$= 4.349; P= 0.03

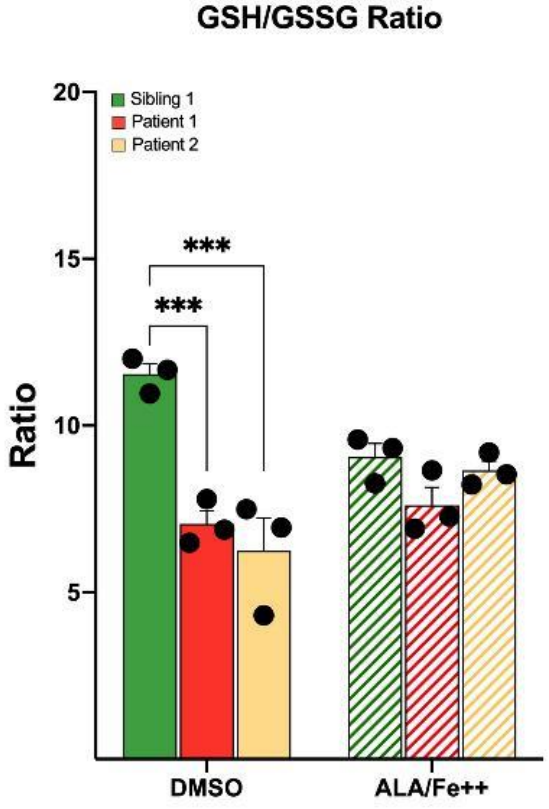
GSH & GSSG levels in cell homogenates



Interaction: $F_{(2,12)}$= 1.07; p= 0.374
Genotype: $F_{(1,12)}$= 13.71; p= 0.003
Treatment: $F_{(2,12)}$= 12.09; p= 0.001



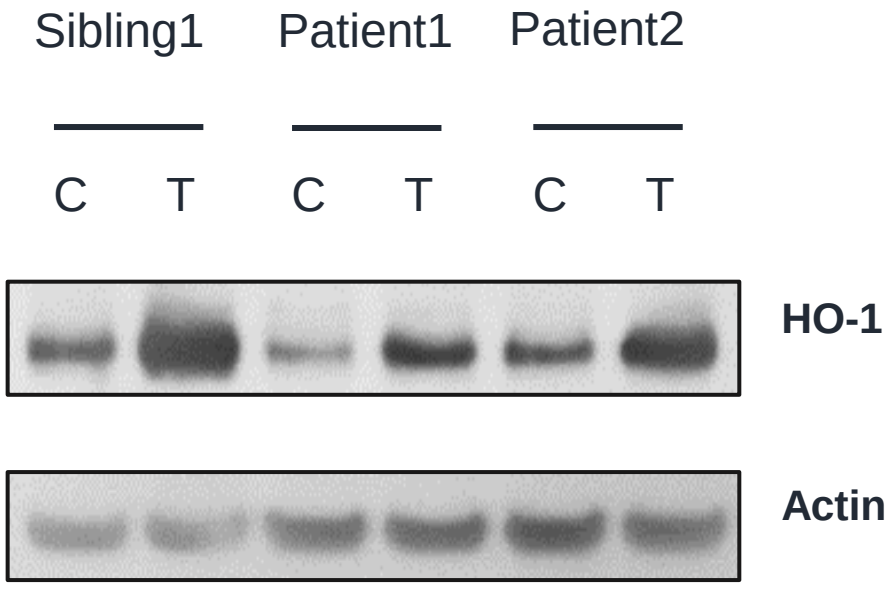
Interaction: $F_{(2,12)}$= 1.86; p= 0.198
Genotype: $F_{(1,12)}$= 3.43; p= 0.090
Treatment: $F_{(2,12)}$= 0.88; p= 0.440

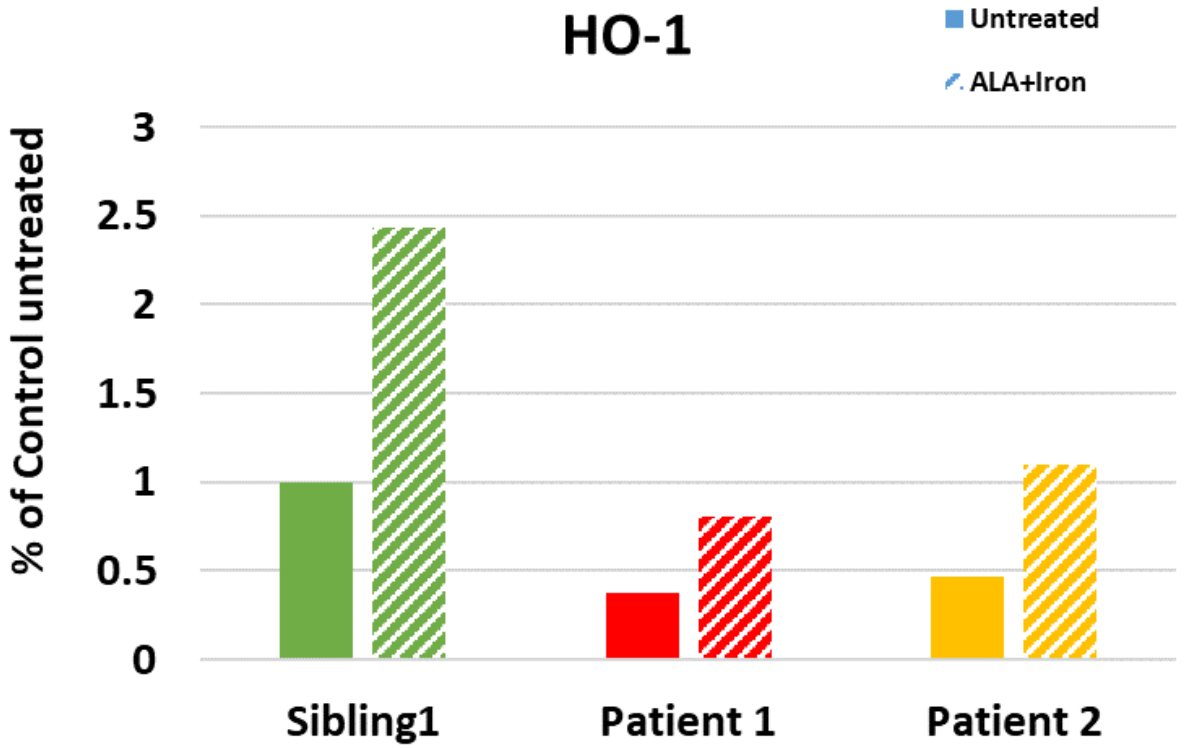


Interaction: $F_{(2,12)}$= 10.56; p= 0.002
Genotype: $F_{(1,12)}$= 0.132; p= 0.723
Treatment: $F_{(2,12)}$= 19.52; p< 0.001

Haemoxygenase 1 (HO-1) content after treatment

C: Control
T: Treatment (ALA + iron)





Summary & next steps

Fibroblast

1. Demonstrate the expression of the disease in fibroblasts

2. Potential treatments can rescue the phenotype

Reprogramming

iPSC

3. Generate iPSC and neuronal cells

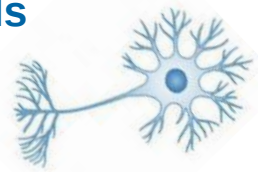
- mRNA and protein expression ↓
- Oxygen consumption rate ↓
- Lactic Acid ↑
- ROS ↑
- Fragmented mitochondria

Treatment with ALA/Fe++

- Oxygen consumption rate ↑
- Lactic Acid / ECAR ↓
- Antioxidant status: Improved
- HO-1 expression ↑

Differentiation

Neuronal cells



4. Demonstrate the expression of the disease in neuronal cells

5. Potential treatments can rescue the phenotype

Acknowledgements

Energy-Lab Team

Jose Abdenur, MD
Alexandra Latini, PhD
Wei-Lin Huang, MS
Alexander Stover MS
Tuany Eichwald, MS
Sarah Ibrahim, BS

Philip Schwartz, PhD
Mariella Simon, PhD
Daniel Nguyen, BS

CHOC Clinical Team

R.Chang MD, R.Wang MD
M.Boyer PNP, R.Sponberg PNP
R.Barrick CGC, K.Schwan CGC
M.Sowa RD, J.Skaar RD, K.Chen RD
C.Daum RN, J.Hager RN
E.Esparza MA
L.Esqueda Admin

CHOC Metabolic Laboratory

D. Butoi CLS
B Evans, S. Xu
M. Aguirre

Thank You!