2022 LBSL International Patient Conference

Riboflavin stabilzes motor degradation in early onset LBSL

Observation of 2 LBSL early onset patients with extended follow-up

1:30 – 2:00: TBD, Enrico Bertini, MD, Pediatric Neurologist, Bambino Gesù Children's Hospital, Rome, Italy

Pt. 1

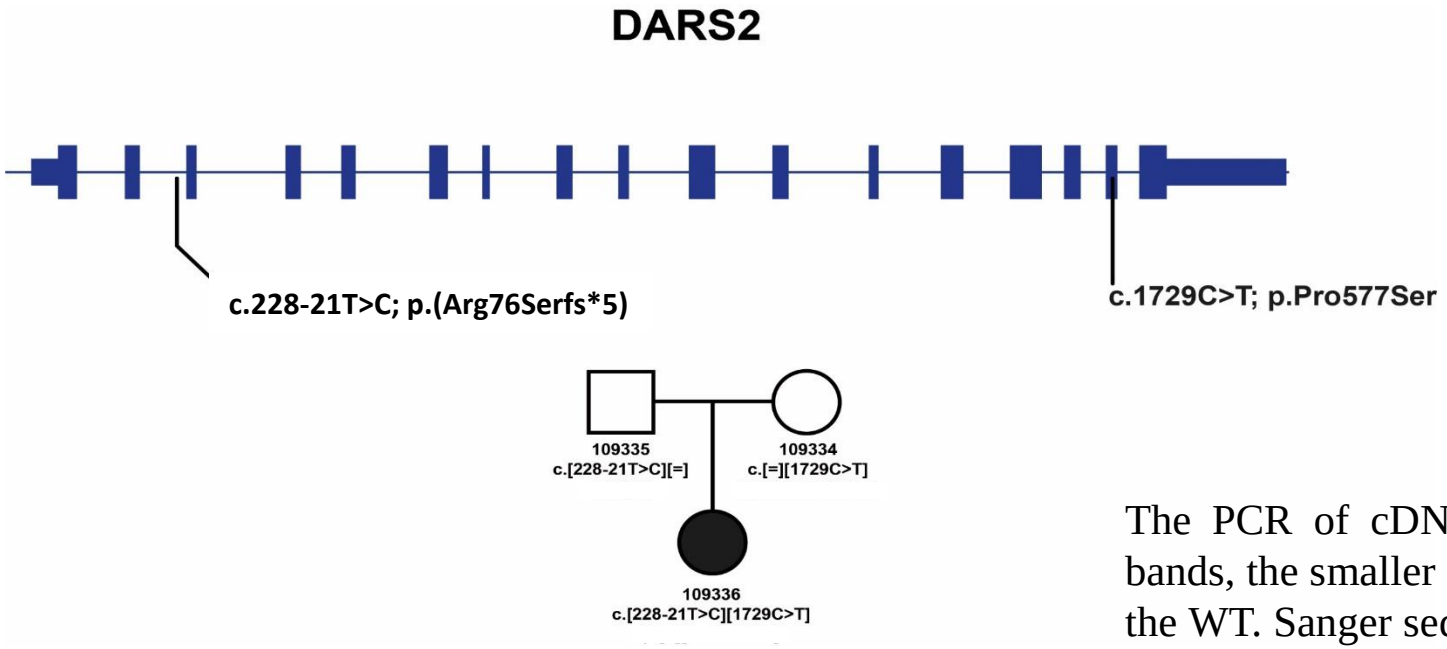
- Female (b. 2009); unrelated healthy parents.
- Normal early milestones (crawling at 8-9 months, standing at age 11 months, walking at age 13 months)
- At age 3 years, after an accidental traumatic fall she started ever since with motor difficulties showing ataxic gait, slight right hemiparesis and lost wallking for 2-3months.
- Two months later MRI was performed showing the pattern of a mitochondrial leukodystrophy (Increased lactate at spectroscopy), and diagnosis workup excluded a lysosomal leukodystrophy. The girl started to walk spontaneously again after 3-4 months with increased unsteadiness for ataxia and right hemiparesis.
- At age 3 years and 6 months the girl started with Riboflavin 10 mg/kg/day as antioxidant treatment suspecting a mitochondrial leukodystrophy, and parents said that after a few weeks the girl improved consistently with reduction of fatigue and ataxia.
- We have a follow up of 10 years, and during this period she has remained stable, with persistence of right hemiparesis.
- At age 10 years she manifested a scoliosis that has 50° Cobb angle this year (age 13 years).

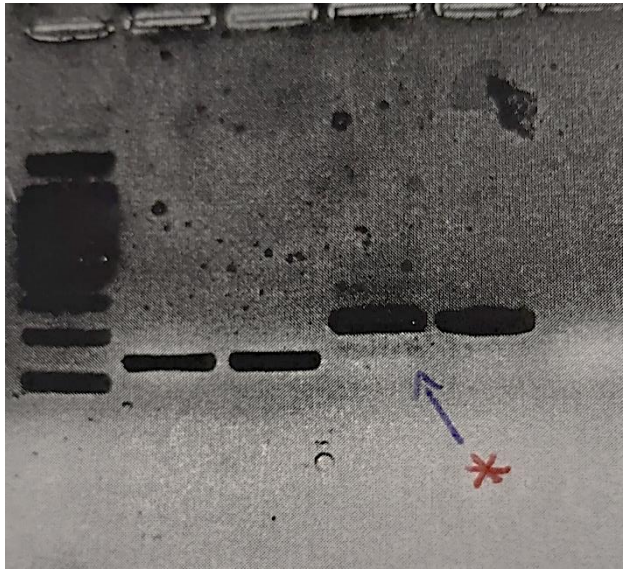
Genetics

WES

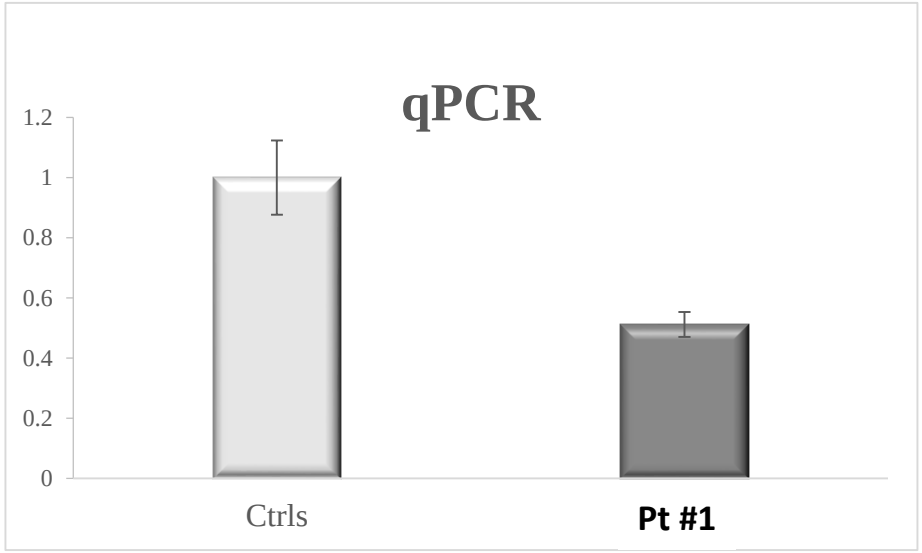
DARS2

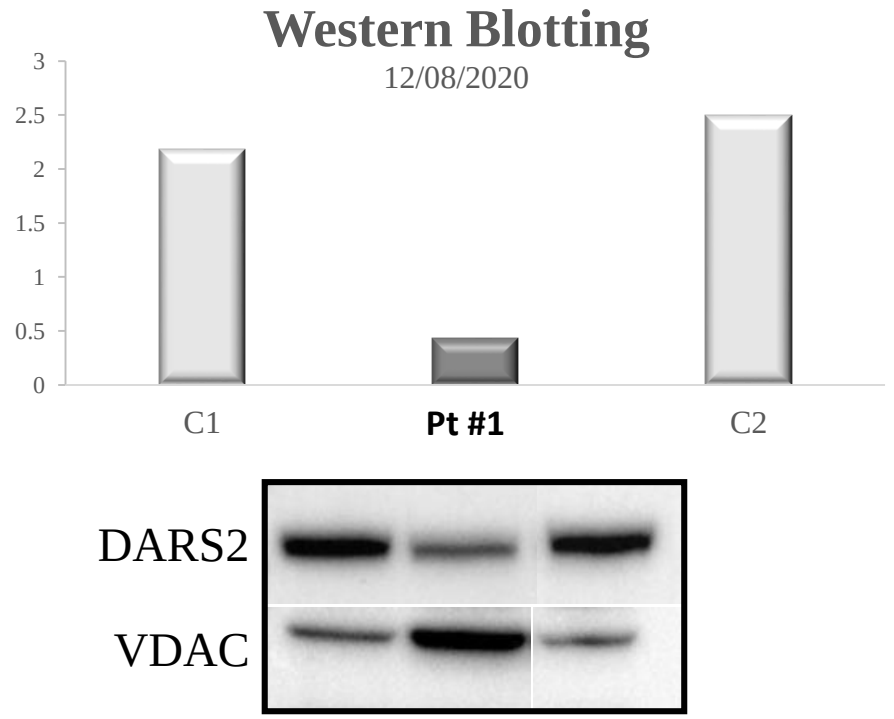
Re-reading the Whole Exome Sequencing file, in addition to the first variant identified as heterozygous in the gene ***DARS2*** (NM_018122.5) [**c.1729C> T; p.Pro577Ser**] a second intronic variant was found always in heterozygosity **(c.228-21T> C)**. Amplifying by PCR the region around this latter mutation, on cDNA obtained from fibroblasts, 2 bands were identified, one of equal size to that of the wild-type and a second smaller, the latter is present in a much lower percentage than that of wild-type (10-20%). From Sanger sequencing of the small band the absence of exon 3 and the consequent production of truncated protein was highlighted [p.Arg76Serfs * 5].]

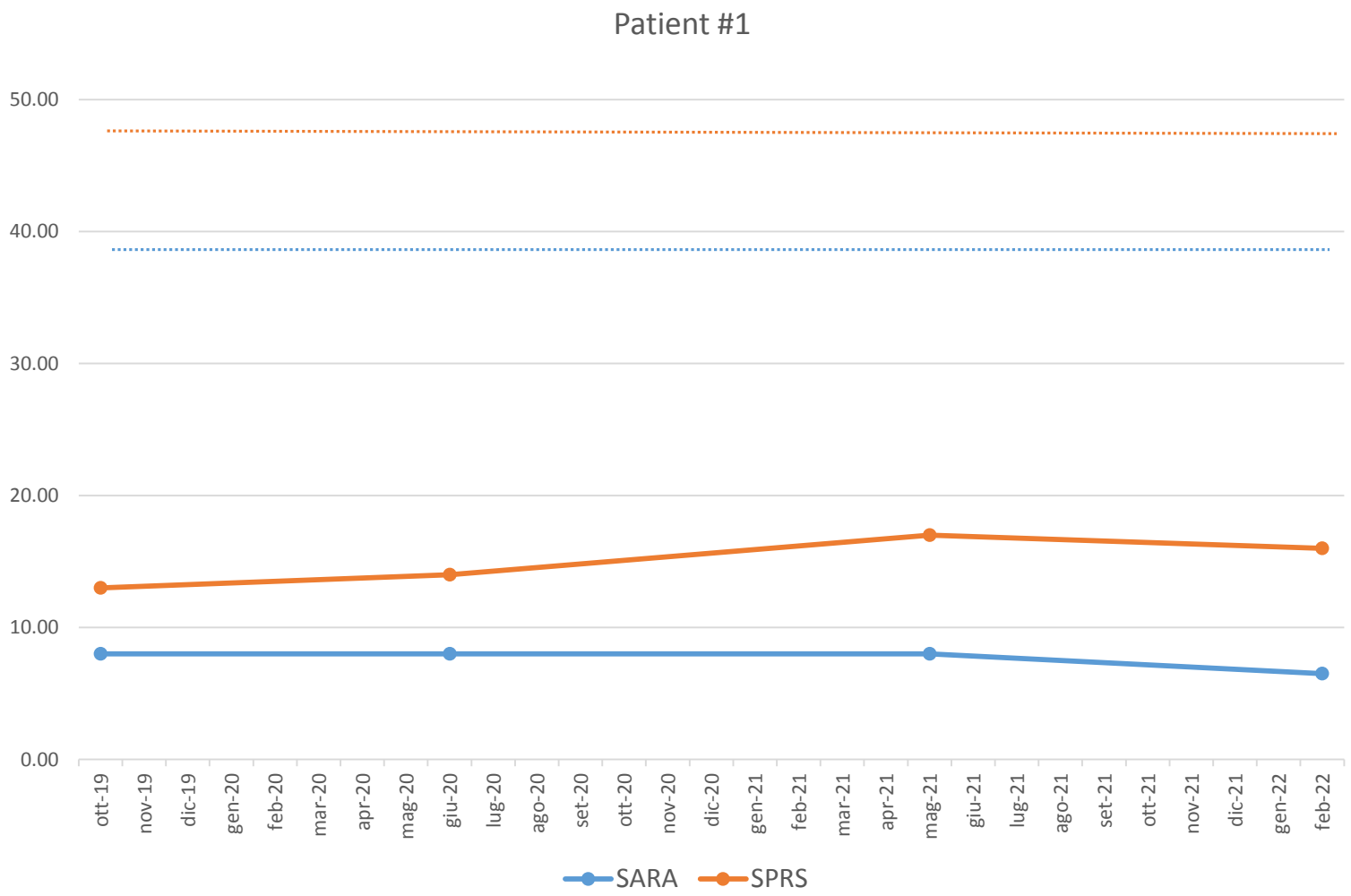




The PCR of cDNA in the restricted region of mutation showed two bands, the smaller one was present in much lower percentage than that of the WT. Sanger sequencing of the lower band showed skipping of exon 3 that produce a truncated protein [**p.Arg76Serfs*5**].


qPCR
1.2
1
0.8
0.6
0.4
0.2
0
Ctrls
Pt #1

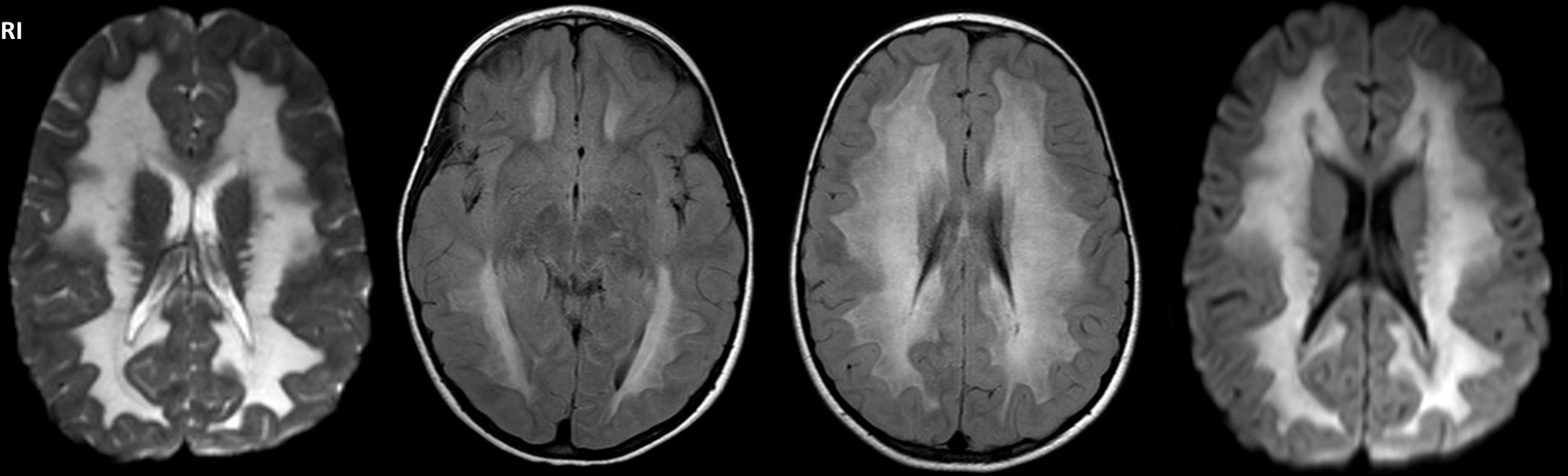

Western Blotting
12/08/2020
3
2.5
2
1.5
1
0.5
0
C1
Pt #1
C2
DARS2
VDAC



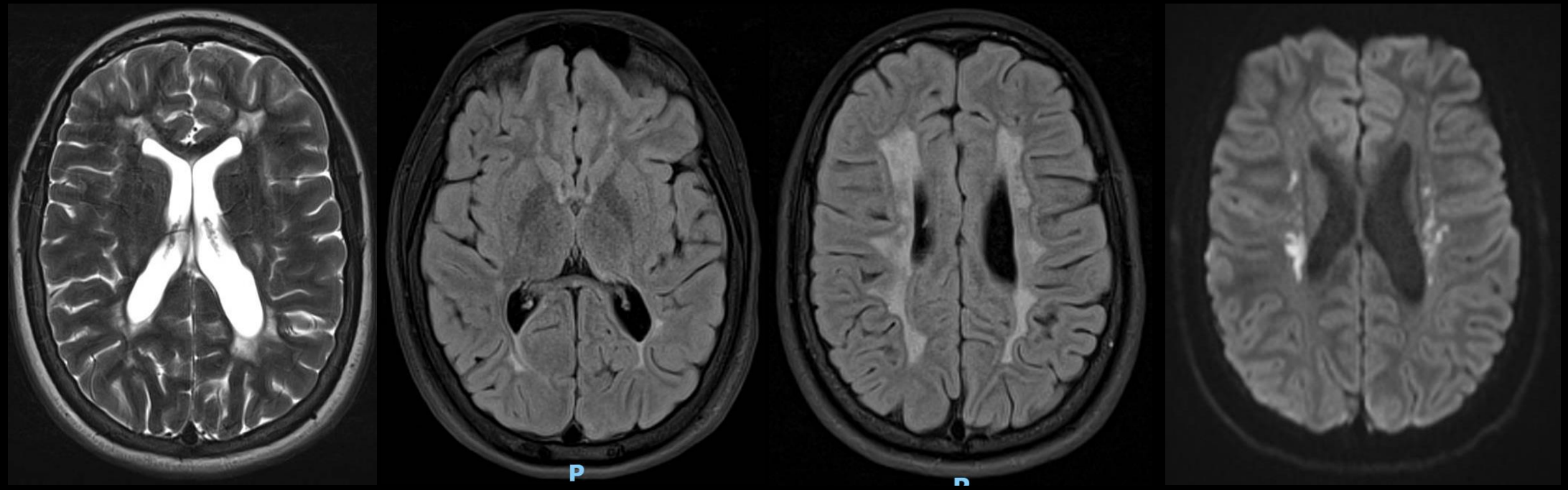
10 y 11 y 12y 13y

Brain MRI

4 y

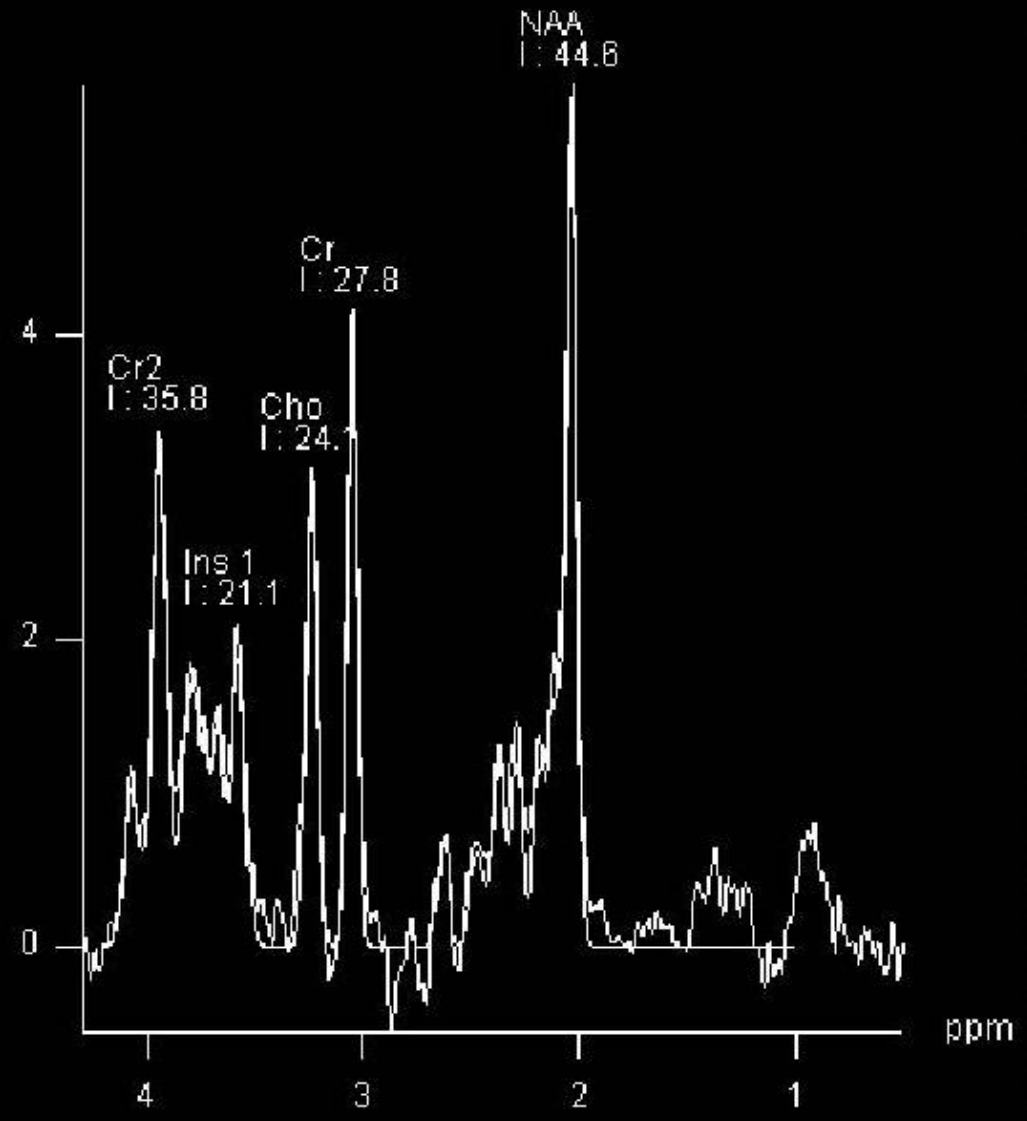


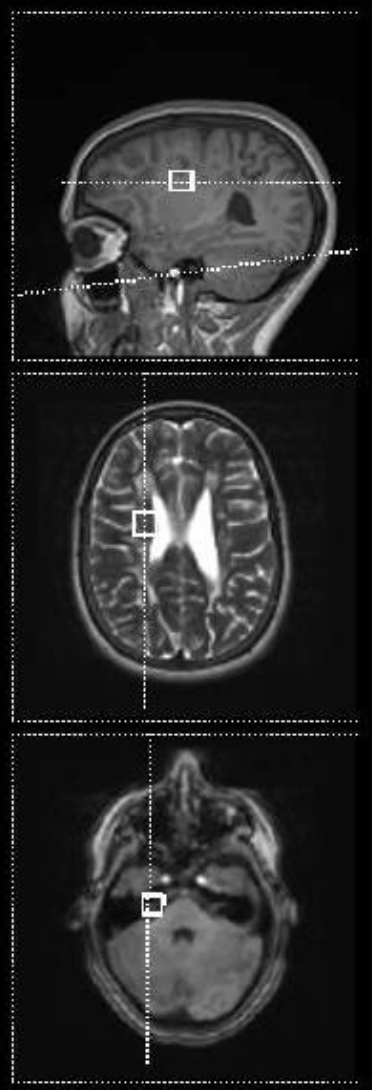
13 y



MRS

13 y





Pt. 2

- Female (b. 2019). Born from unrelated healthy parents by assisted reproductive technology (ART).
- Normal early milestones (crawling at 8-9 months, standing at age 10 months)

- At age 12 months, after a mandatory vaccination with fever, the girl loss the ability to stand and had difficulty in crawling.

- At age 13 months she was admitted; MRI: leukodystrophy and lactic aciduria; a LBSL was confirmed a few months later

- At age 15 months she started with Riboflavin 20 mg/kg/day and 6 months later at age 21 months she was able to walk with support with mild ataxic unsteadiness

- One months later she had a fever, and motor difficulties worsened with dysmetric tremor

- Two months later at age 2 years the girl was able to walk independently, and slowly, unsteadiness improved persisting till now at the age of 3 years, 3 months

Genetics

Clinical Exome

DARS2 c.788G>A [p.Arg263Gln]/ c.932C>T [p.Pro311Leu]

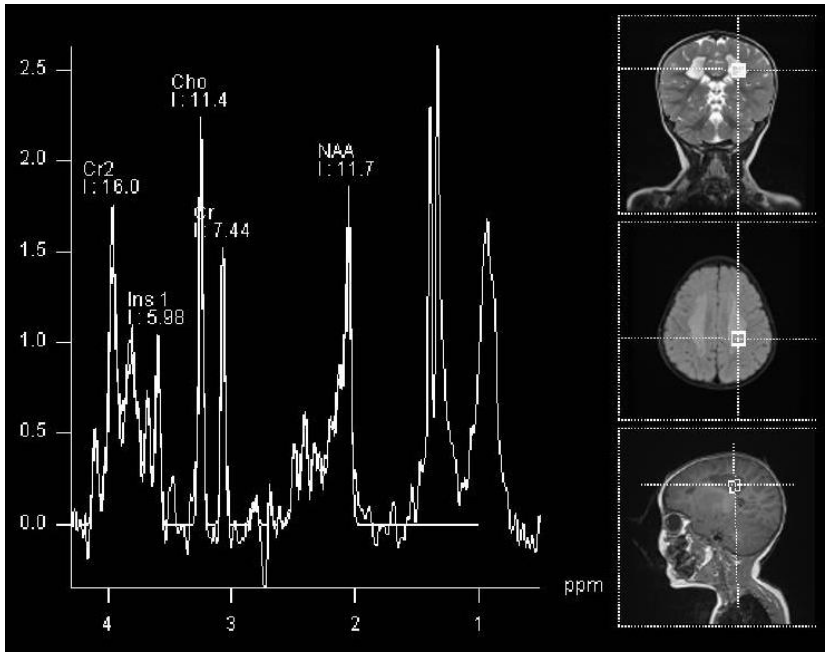
mother c.932C>T [p.Pro311Leu]

father c.788G>A [p.Arg263Gln]

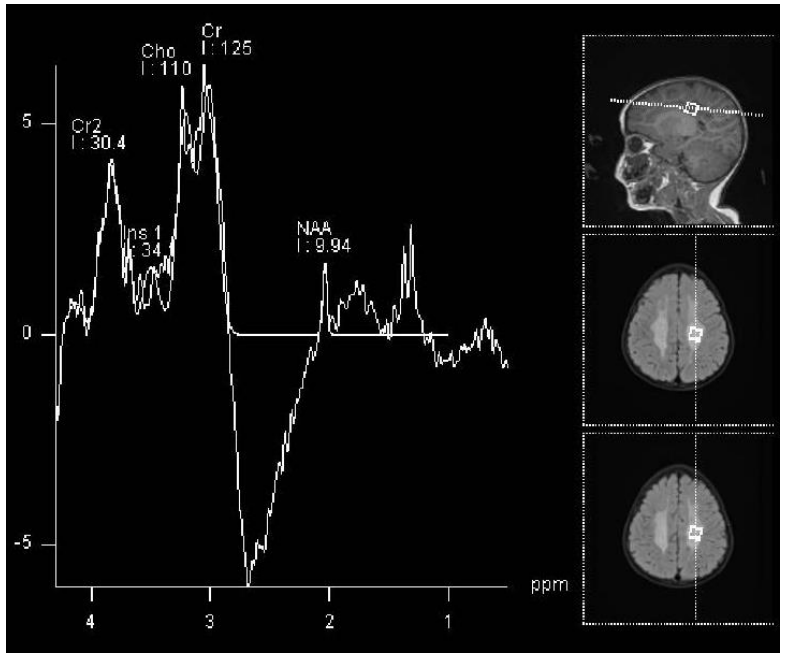
Laboratory examinations

No organic aciduria, mild lactic acidosis (+ spectroscopy)

Age 1 year

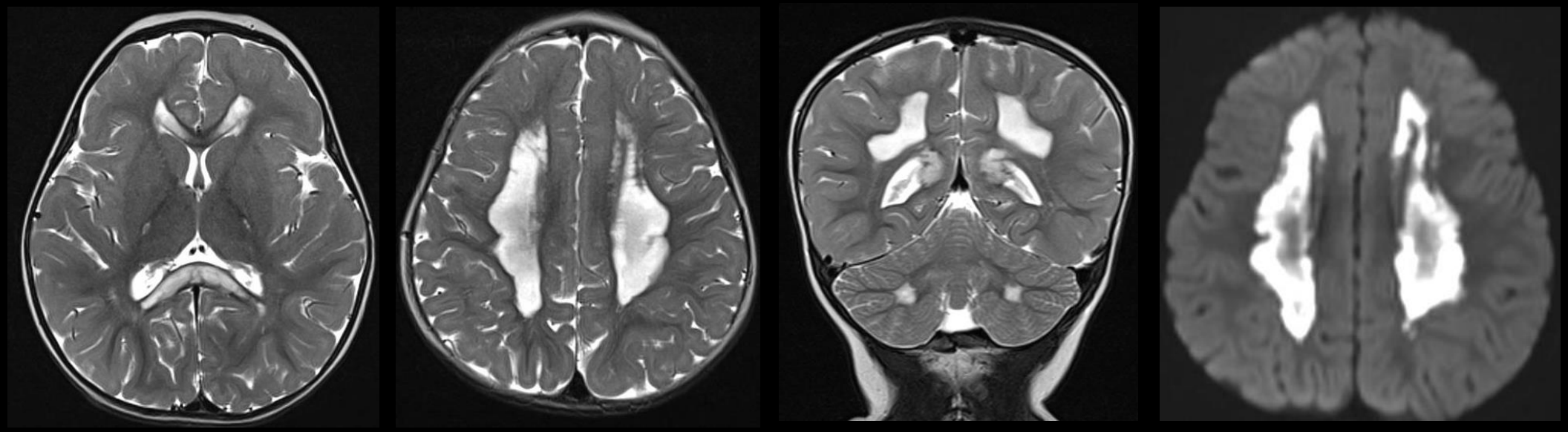


Age 2 years

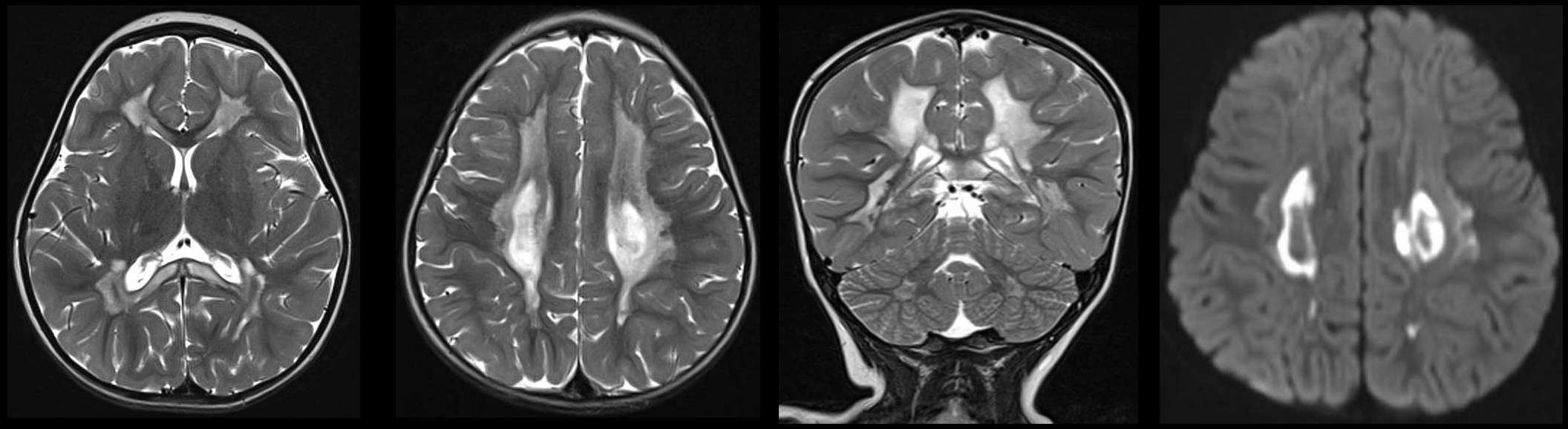


Brain MRI

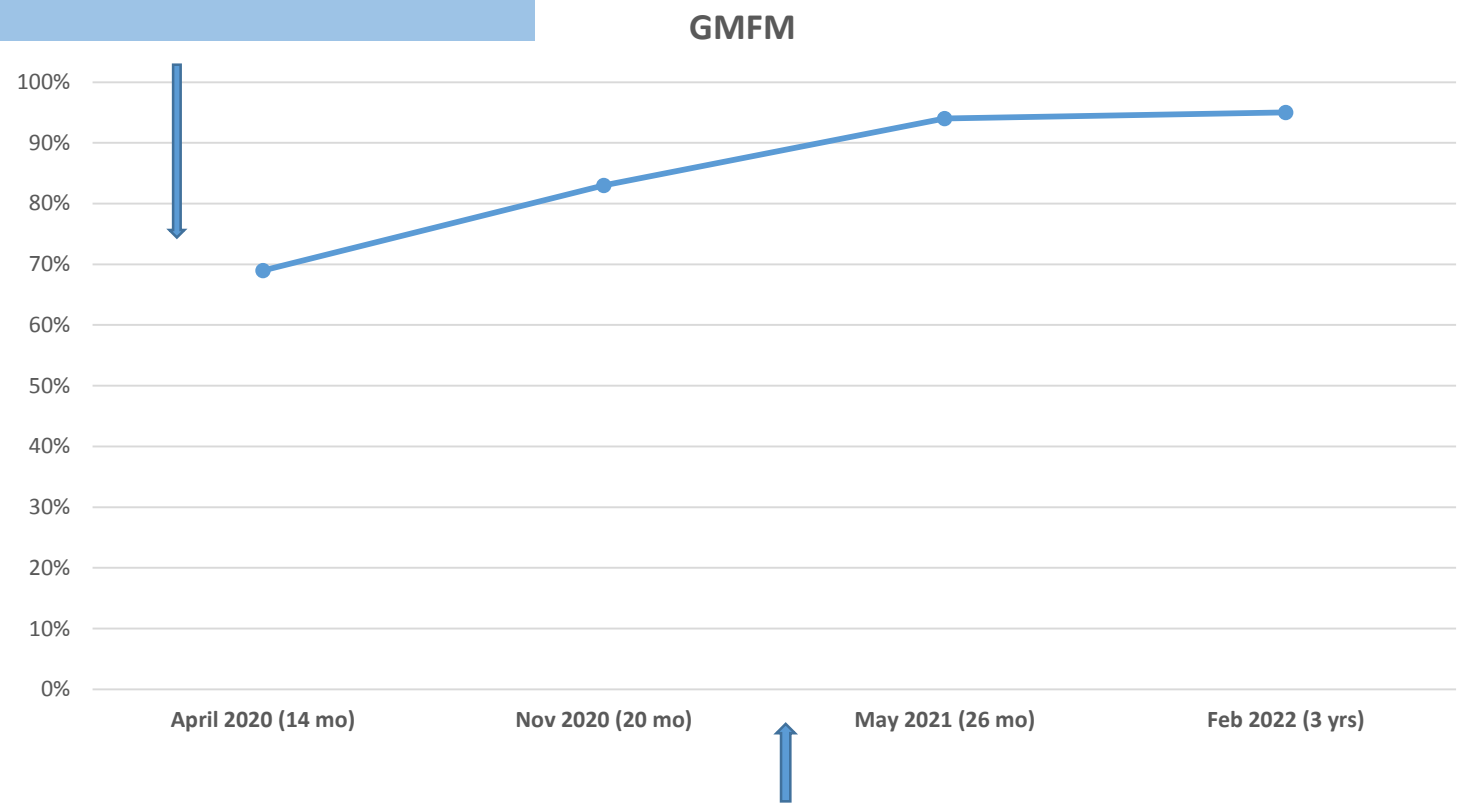
Age 1 year



Age 2 years



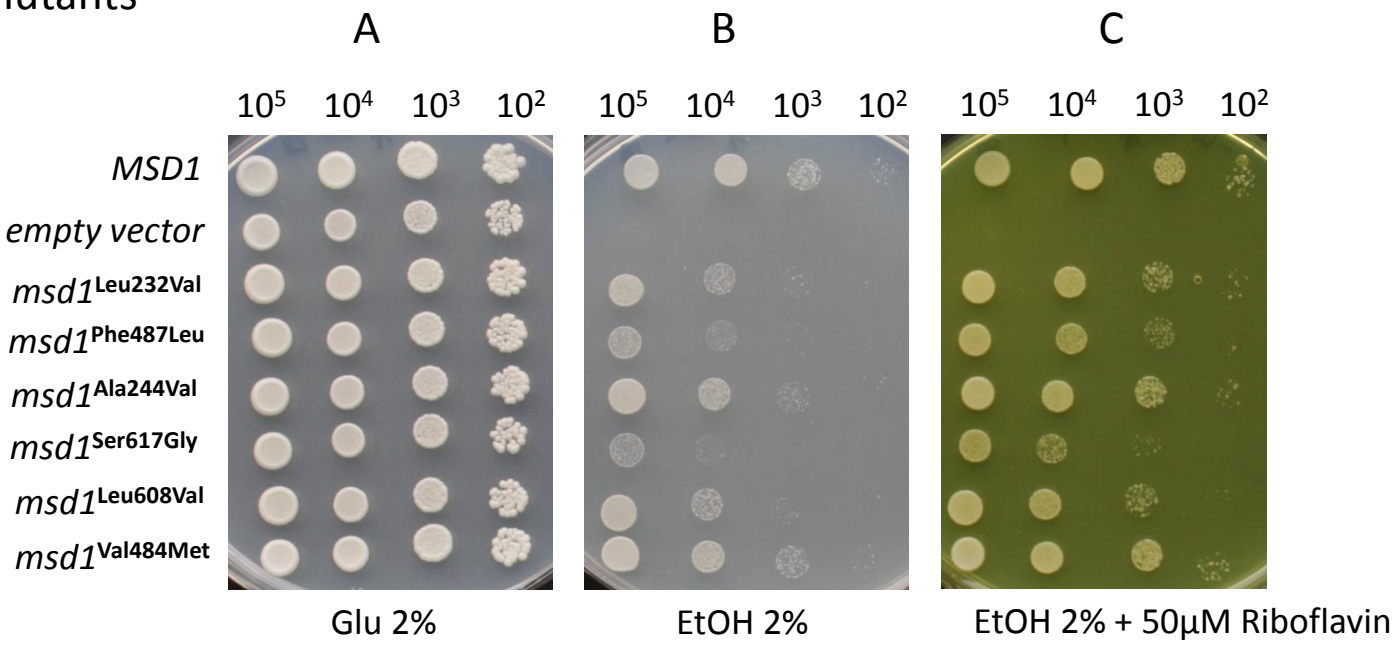
Early March 2020
Onset with psychomotor regression
after normal development
Mid March: start of Riboflavin treatment



December 2020:
Tremor and more gait instability following infection.

YEAST Mutagenesis (Paola Goffrini, Parma , <paola.goffrini@unipr.it>

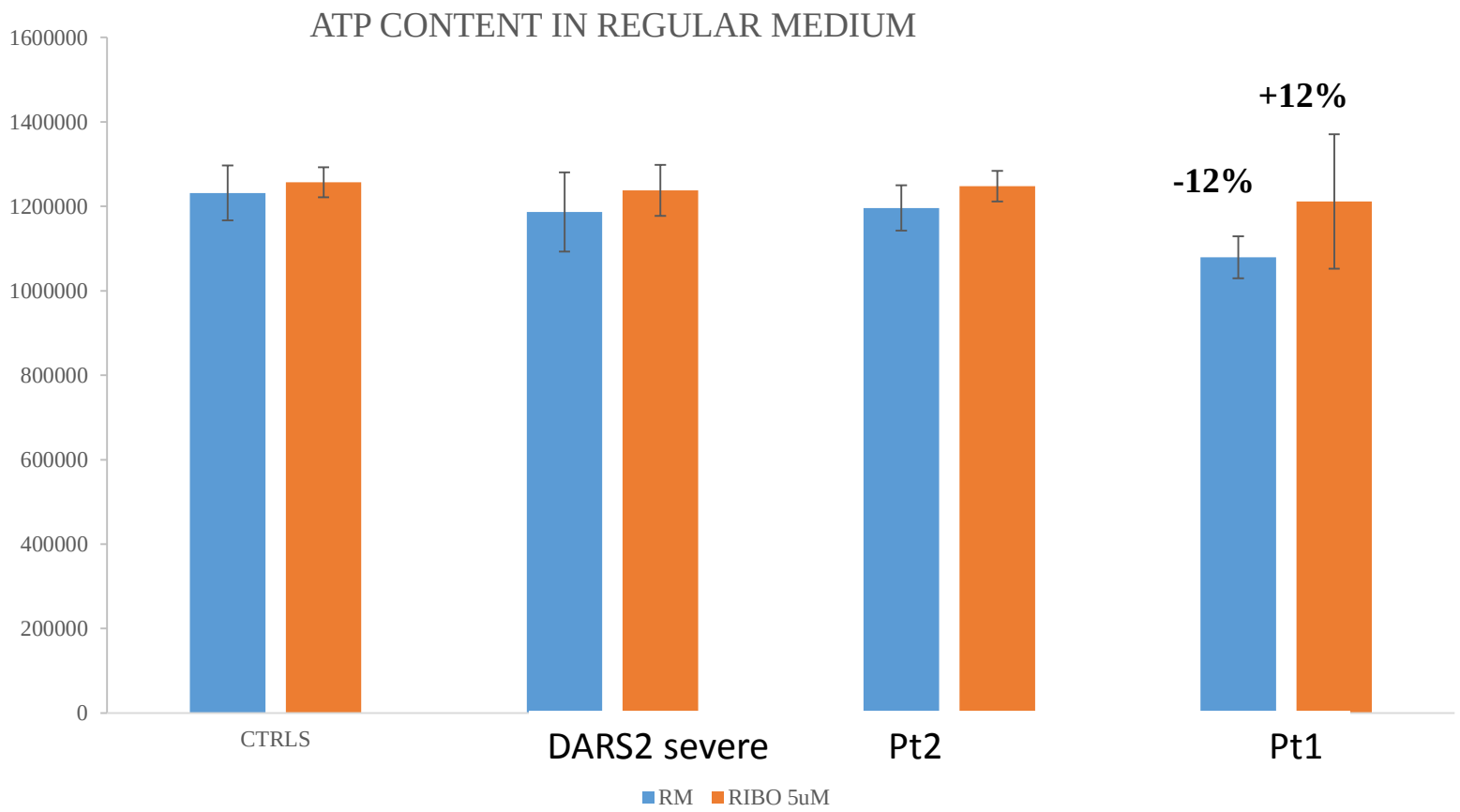
yeast mutagenesis; riboflavin supplementation, protein level of DARS2 (MSD1 in yeast) is not reduced (by WB) in these mutants



Results of the effect of riboflavin on some DARS mutants.

A. Yeast cultures in Glu 2% medium at different concentrations

The rescue on oxidative growth is seen very well in everyone! We carried out the experiments in solid form by trying different concentrations of riboflavin (from 1, 10, 50, 100 micromolar) and the effect begins to be seen at 10 even if 50 is the best concentration (se figure C). At 100 it begins to inhibit growth.


ATP CONTENT IN REGULAR MEDIUM
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
+12%
-12%
CTRLS
DARS2 severe
Pt2
Pt1
RM
RIBO 5uM