Recent developments on disease mechanisms in Friedreich ataxia; *Implication for therapy*

Where are we, dealing with Frataxin function?

For some, Frataxin binds iron… *not true*, others claim!

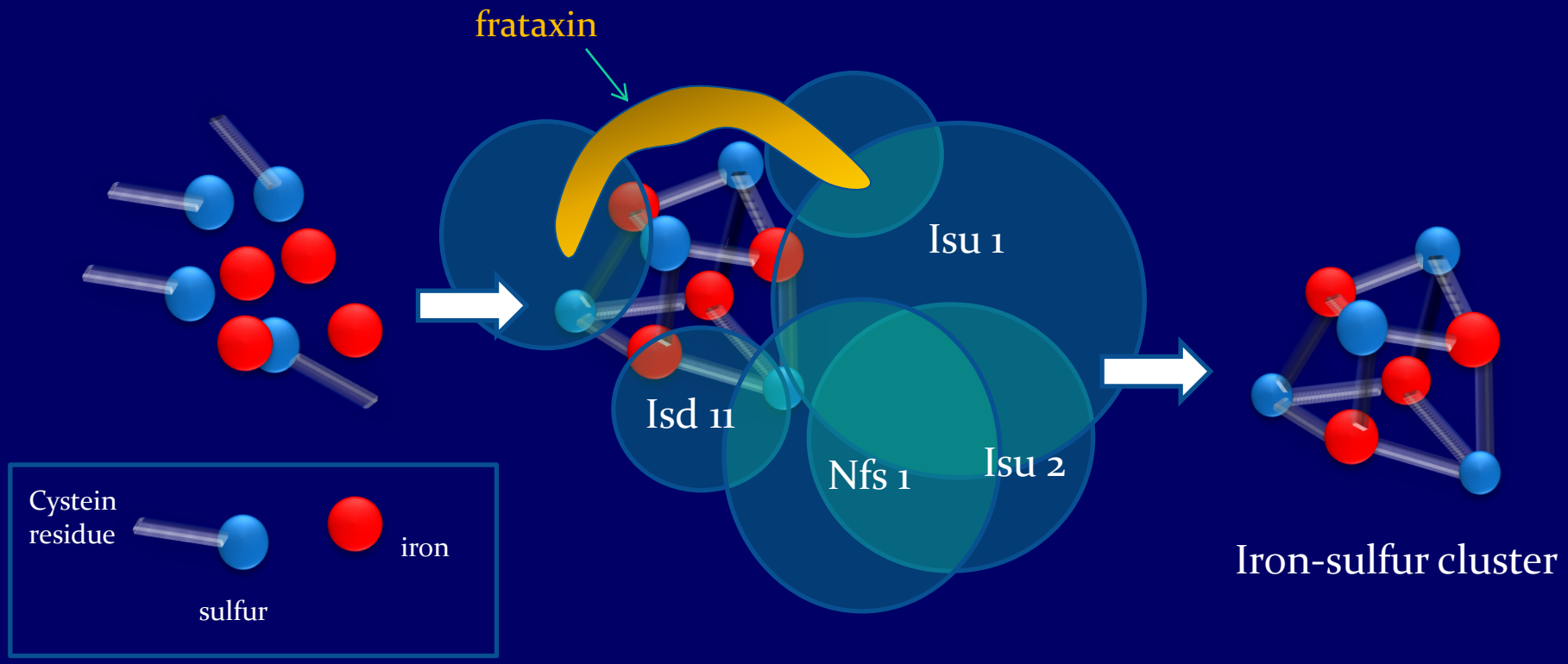
Frataxin provides iron for ISC synthesis… *not true*, it limits iron providing for synthesis!

Lack of Frataxin results in oxidative stress… *not true* claim others

etc…

Where are we, dealing with Frataxin function?

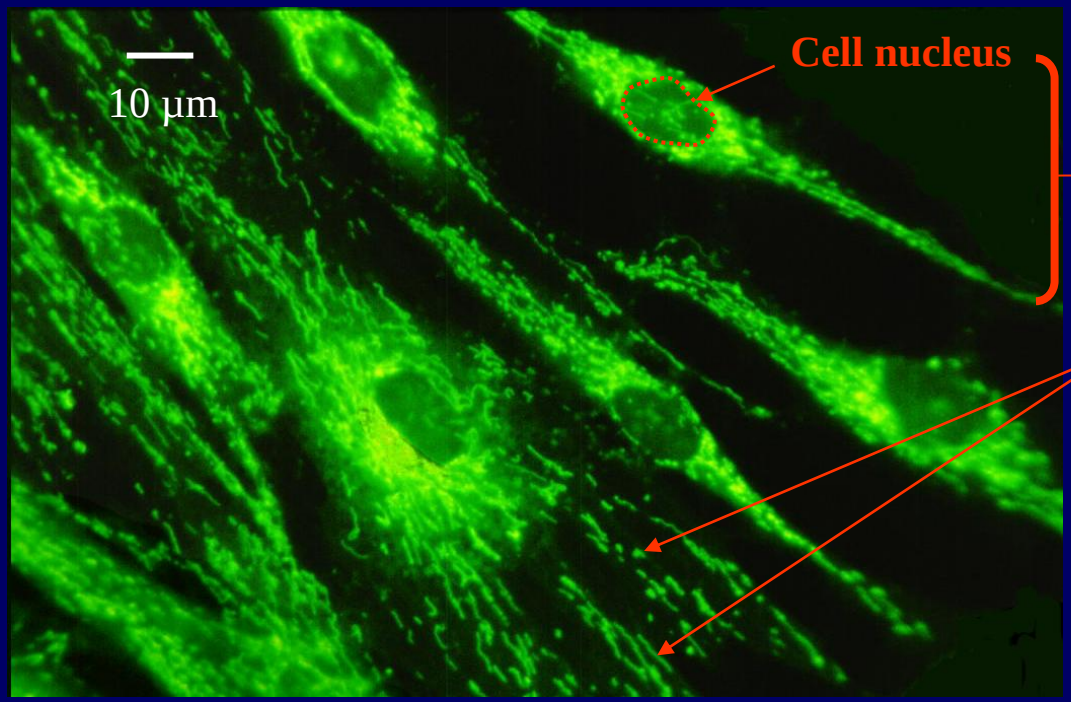
. Frataxin has the capacity to interact with the machinery responsible for iron-sulfur cluster (ISC) synthesis



Where are we, dealing with Frataxin function?

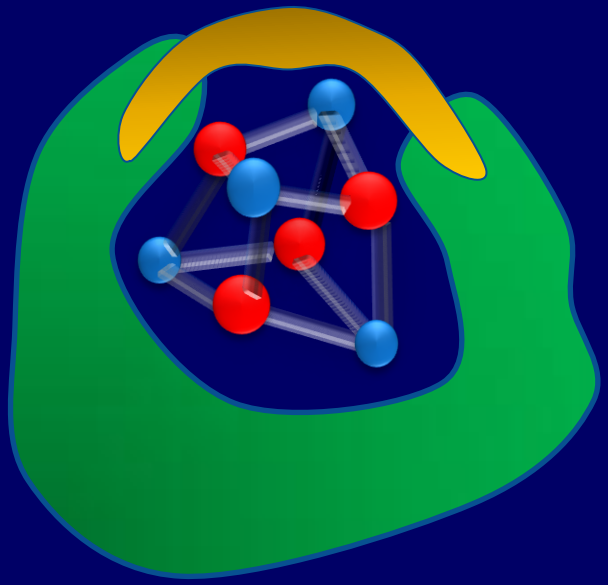
. This synthesis mostly takes place in the mitochondria where frataxin is mainly (only?) found

➡ When frataxin is not in sufficient amount, it results in human in a typical mitochondrial disease



Where are we, dealing with Frataxin function?

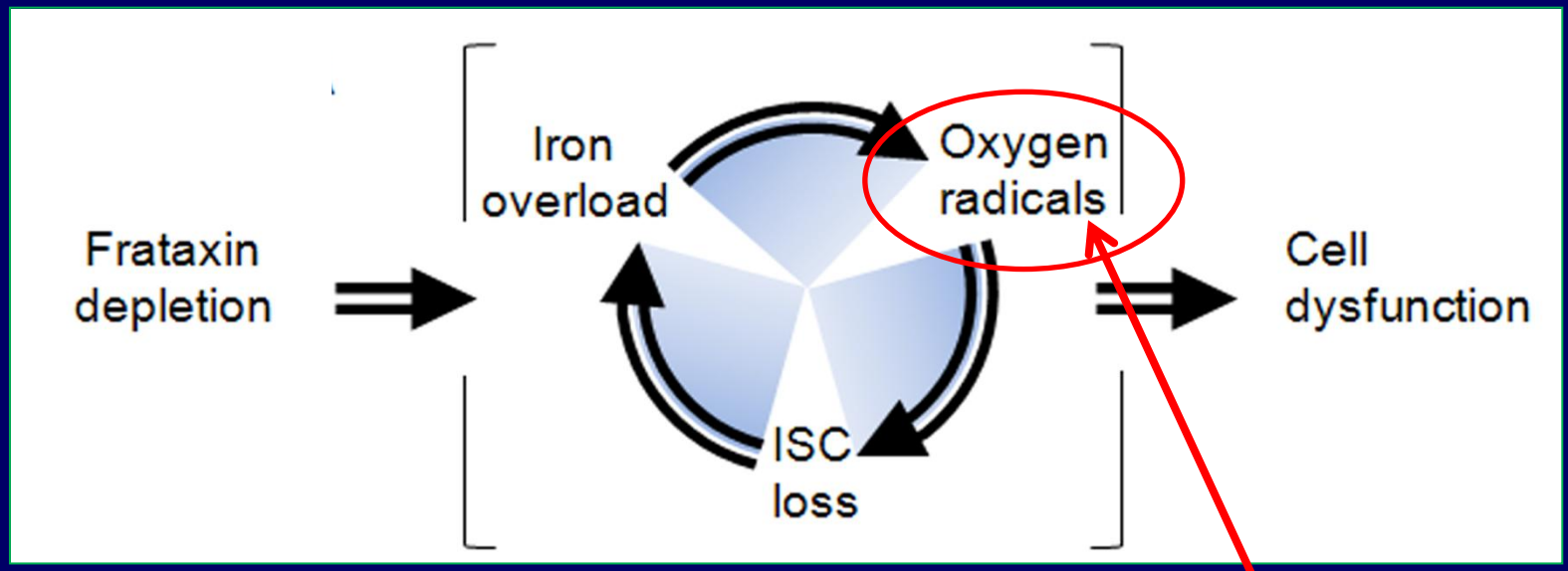
. Frataxin has the capacity to interact with different ISC-containing proteins after their synthesis



➡ Frataxin protects ISC during and after their synthesis

What about the consequences of frataxin depletion?

-In 1998, we proposed the occurrence of a vicious cycle :



From there our idea in 1998 to use idebenone, an antioxidant

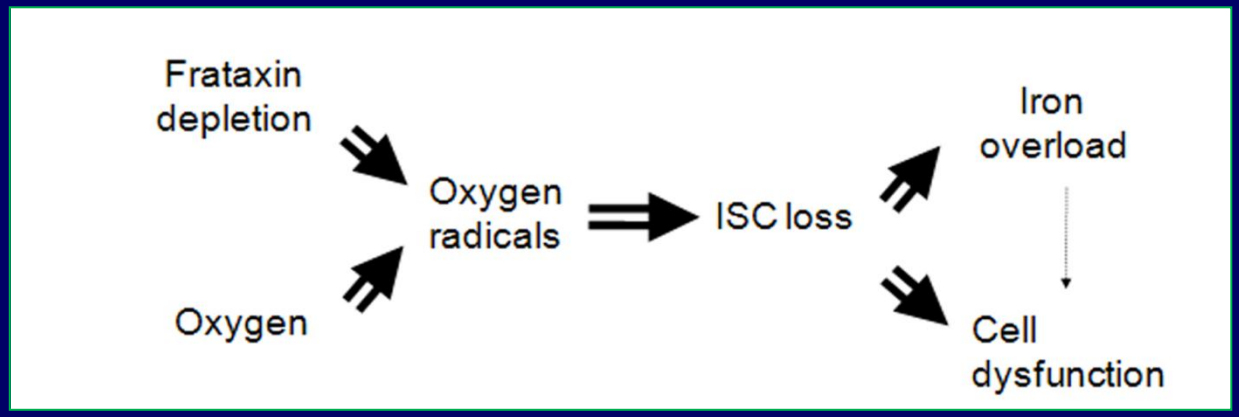
What about the consequences of frataxin depletion?

Now, because :

1. Fxn depletion is not always associated with significant ISC protein deficiency (human skeletal muscle, liver, fibroblasts, lymphocytes);
2. ISC synthesis is only partially reduced in the absence of Fxn as long as oxygen is not there (yeast);
3. Iron accumulation is mostly a late event presumably resulting from ISC destruction
4. No significant increase of oxygen radicals results from frataxin depletion
5. Vitamin E deficiency, rather than either ABC7 (iron), or GLRX5 or ISCU (ISC synthesis) protein deficiency resembles FA disease.
6. **In essentially all situations and models** where this have been looked for, Fxn deficiency consistently results in hypersensitivity to oxidative insults.

What about the consequences of frataxin depletion?

For these six additional reasons, we have to reconsider our initial proposal of a vicious cycle. In frataxin-depleted cells, 1) deficient signalling of antioxidant defences 2) sensitises the frataxin-free iron-sulfur clusters to reactive oxygen species. This antioxidant-sensitisation process results 3) in cell dysfunction and 4) late intra-mitochondrial iron accumulation, mostly as amorphous non-reactive precipitates.



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Pathophysiologie et Thérapie des Maladies Mitochondriales

A 3 years fellowship to Dr Aurelien Bayot

A long-term project (5 years): 1) identify the components of the peri-mitochondrial complex associating Nrf2/Keap1/XXX/actin (defective in FRDA) and 2) screen for molecules able to restore the function of this structure

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Pathophysiologie et Thérapie des Maladies Mitochondriales

Year 1

(...familiarize himself with the delicate biochemistry of mitochondrial enzymes)

Immunohistochemical studies of co-localisation of various candidates proteins (Nrf2/Pgam5/Keap1/actin/ park7:dj1 plus mitochondrial markers) in the mitochondria vicinty (cultured skin fibroblasts, HEK)

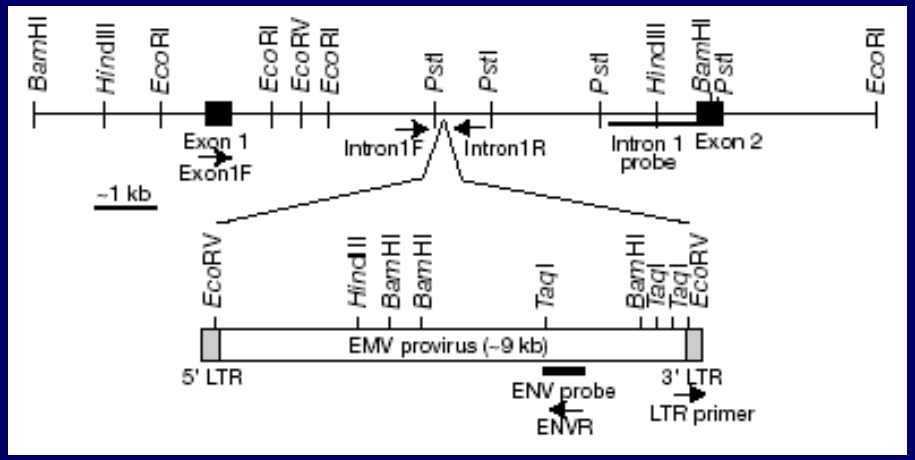
Obtention and characterizaton of mitochondrial properties of differentiated neuron-like cells from 3 patient's fibroblasts_derived Induced Pluripotent Stem Cell Lines IPS (Collab with Laetitia Aubry I-STEM)

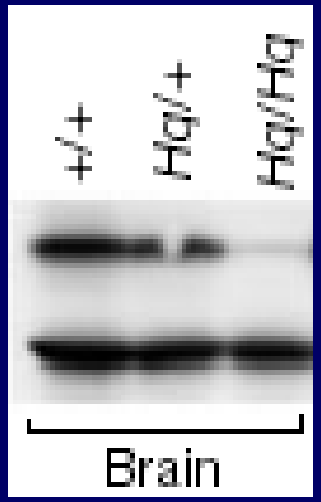
The strange and long story of the *Harlequin* mouse…

- **1971**: The Harlequin mouse is detected because of anomalous fur (Barber BR, Mouse News Lett)

- **1990**: twenty years later… the Hq mouse is also ataxic .
- (Bronson et al. Mouse Genome)

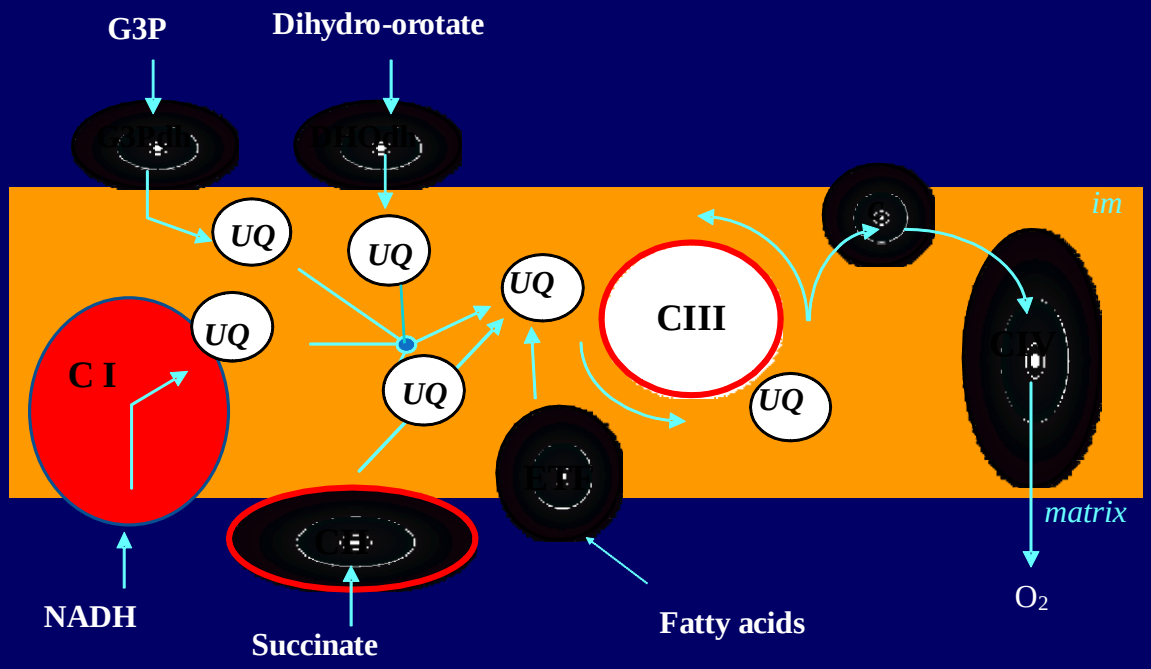
- **2002**: … the mouse is also blind. Identification of AIF as the mutant gene (Klein JA et al. Nature)





In 2004 we established that AIF deficiency causes a mitochondrial disease in the mouse

Vahsen N et al **EMBO J. 2004**



A similar conclusion was reached for human in 2010 by italians colleagues

Ghezzi D et al **Am J Hum Genet**. 2010

Harlequin mouse

- Optic atrophy +++
- Cerebellar ataxia +++
- Retinitis pigmentosa+++
- Growth retardation +++
- Inconsistent hypertrophic cardiomyopathy +/-

CI deficiency

CI deficient patients

- Optic atrophy
- Cerebellar ataxia
- Retinitis pigmentosa +++
- Growth retardation ++
- Inconsistent hypertrophic cardiomyopathy +/-

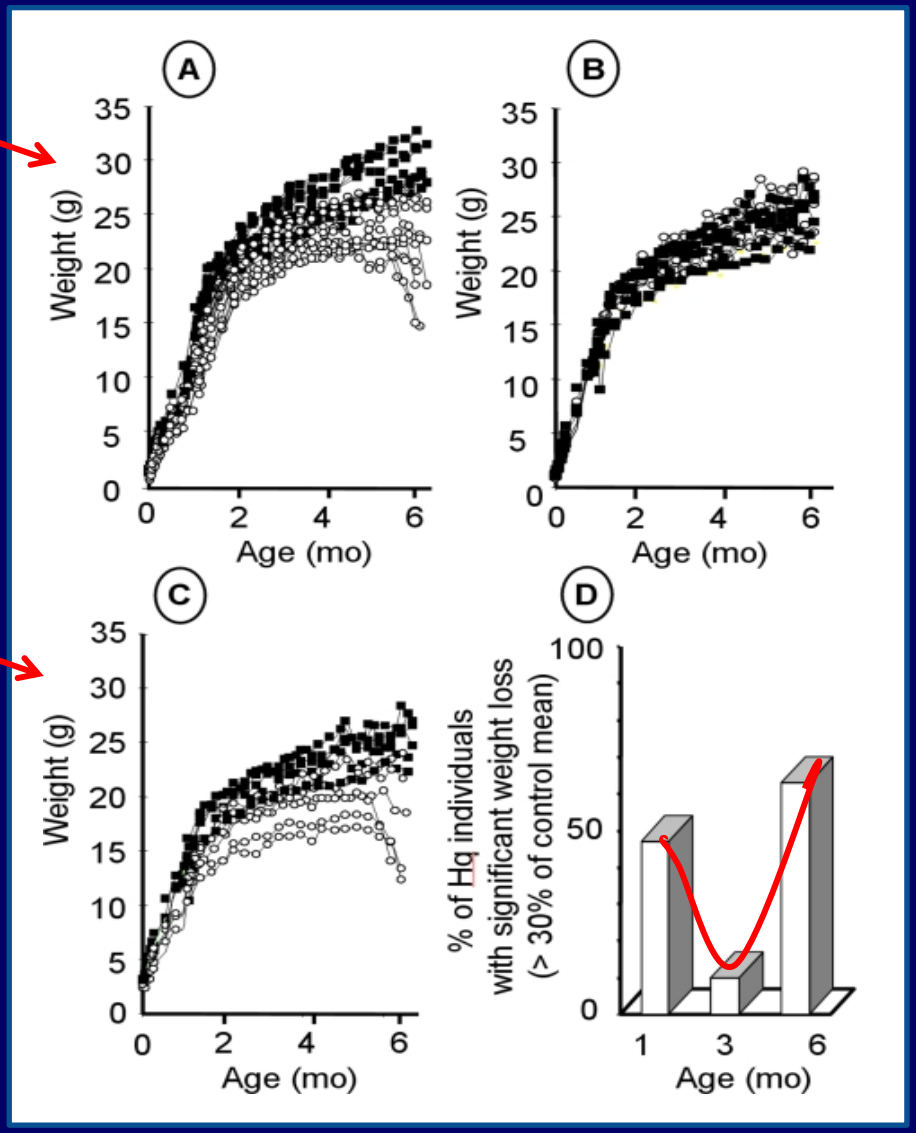
As in Friedreich ataxia, partial and tissus specific deficiency, progressive and heterogeneous phenotype

The highly variable phenotype of the *Hq* mouse

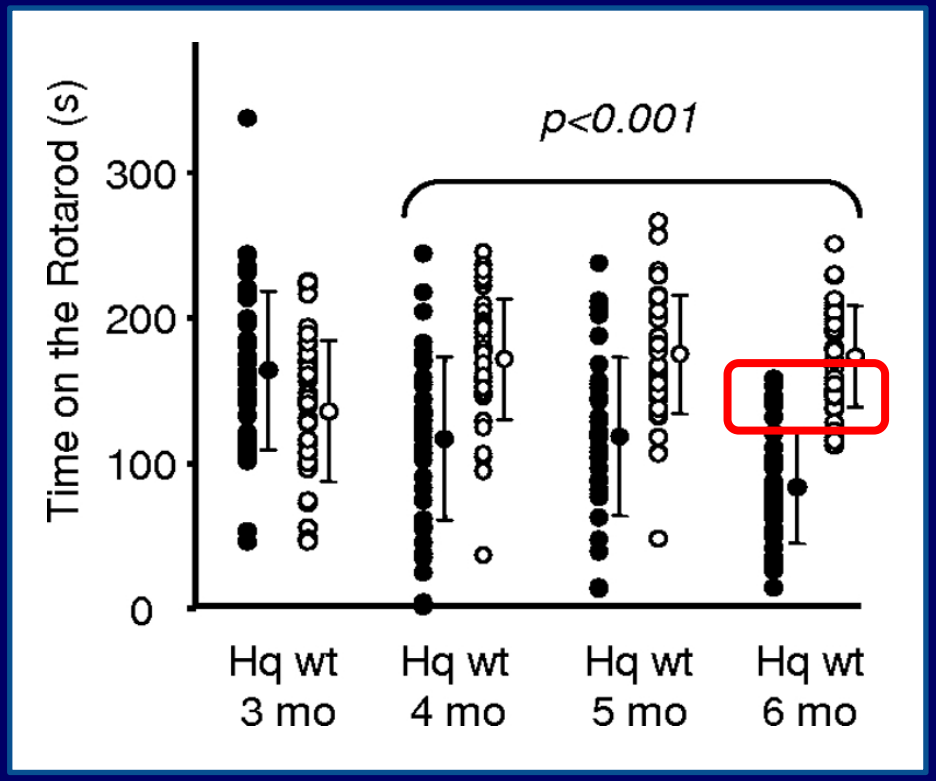
Males

Heterozygous females (AIF on X chr)

Homozygous females (AIF on X chr)



The highly variable phenotype of the *Hq* mouse



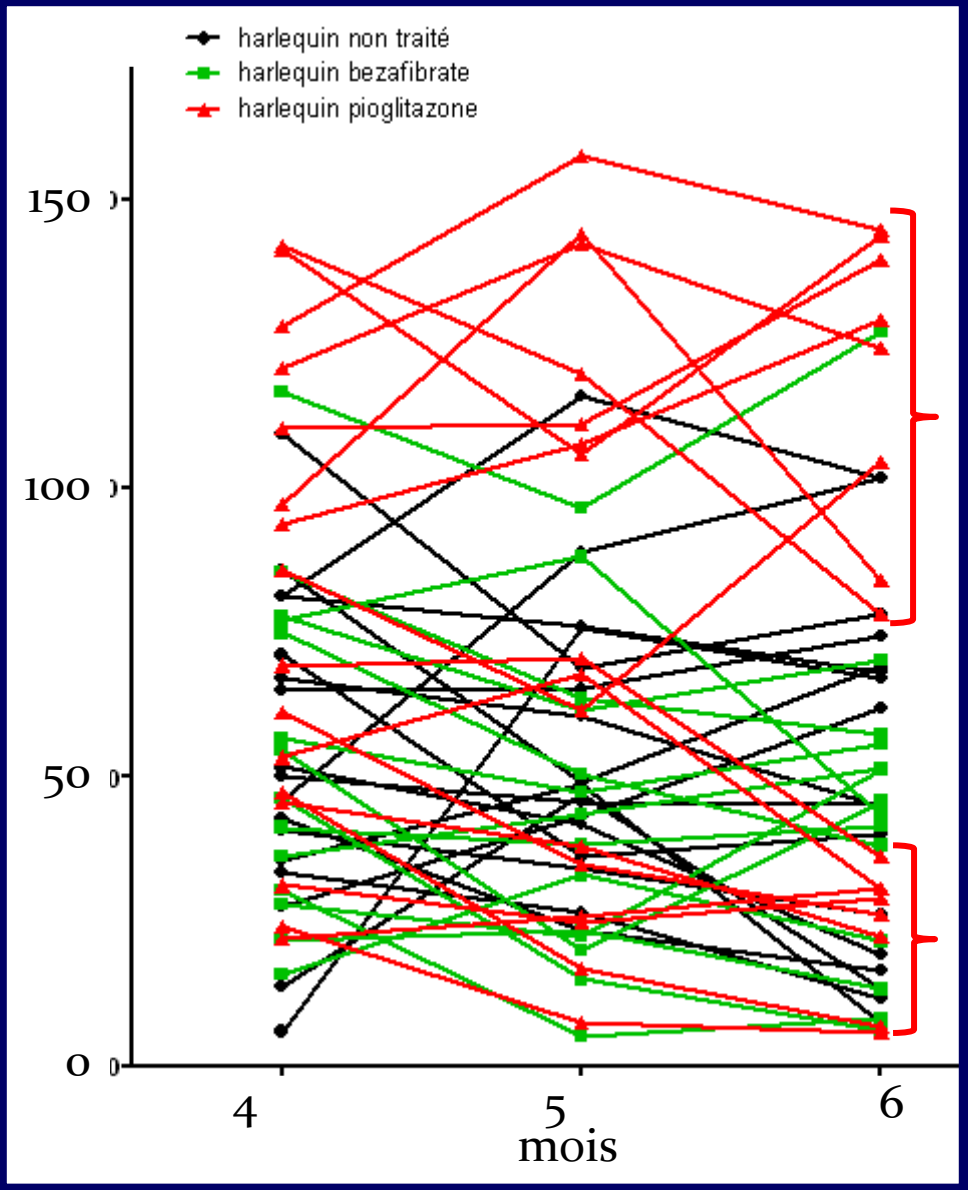
→ is most probably the result of the varying individual genetic background

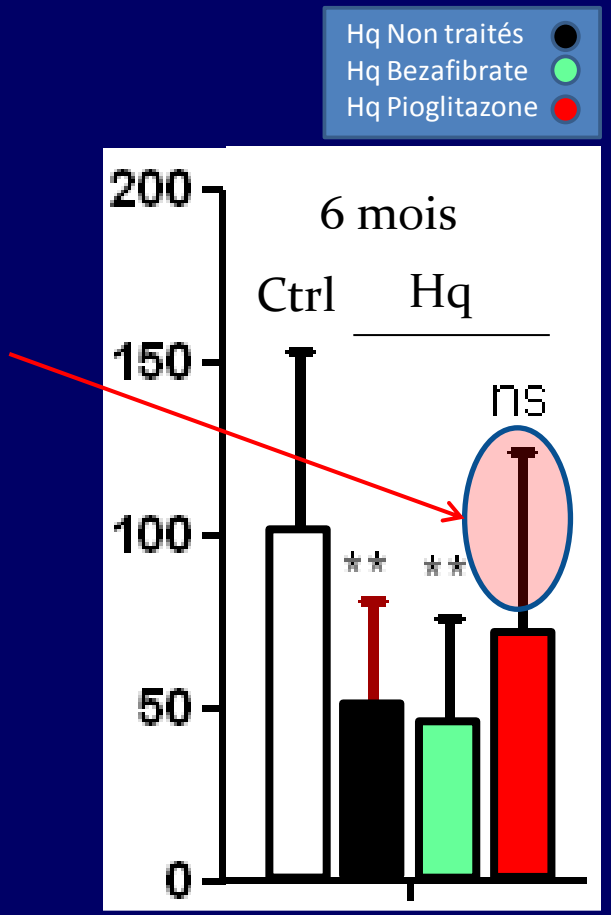
Bénit et al. Trends Mol Med 2010

While such a spectacular effect of the genetic background on mitochondrial diseases such as Friedreich ataxia

- Mitochondria are multifunctional organelles involved in almost all aspects of cell life
- They are quite responsive to environmental conditions
- Their synthesis and functions required more than 2000 genes…

Varying individual genetic background also strongly affects the response to treatments



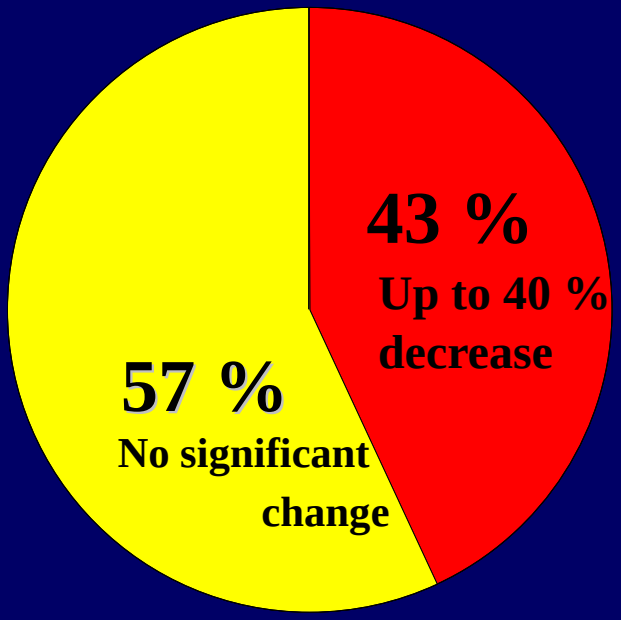


Bénit et al. Trends Mol Med 2010

Human: the effect of idebenone oral supplementation On left-ventricular heart mass index in Friedreich ataxia

Results in 2000

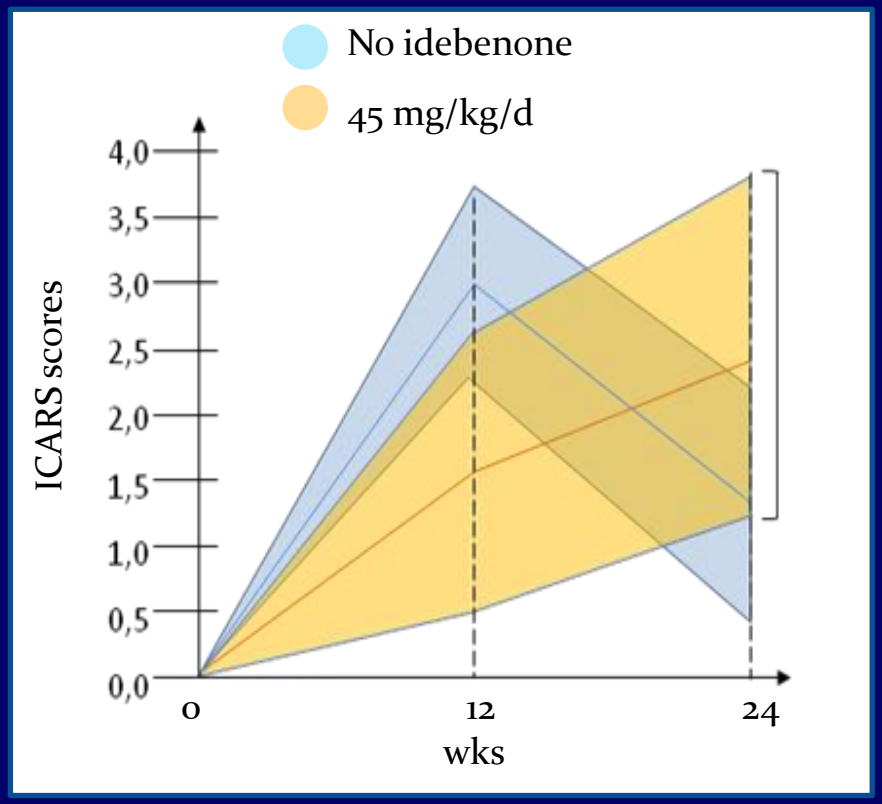
Left-ventricular mass index in 40 FRDA patients treated with 5 mg/kg/d



Rustin P. The use of antioxidants in Friedreich's ataxia treatment. *Expert Opin Investig Drugs.* ***2003*** *12:569-75*

(6 months)

Human: ICARS score in 60 FRDA patients treated with idebenone (10 to 30 mg/kg/d)



Lynch DR, Perlman SL, Meier T. A Phase 3, Double-blind, Placebo-Controlled Trial of Idebenone in Friedreich Ataxia. *Arch Neurol.* ***2010*** *67:941-7*

Individual genetic background ***similarly and strongly*** affects mitochondrial disease expression and course, and the response to treatment

As a direct consequence, any trial which does not take into account the ***individual response*** to treatment has a high probability to result in misleading conclusion on the effect of the tested drug

Noticeably, the statistical tools to handle this question in small cohorts (less than hundred) are clearly insufficient…