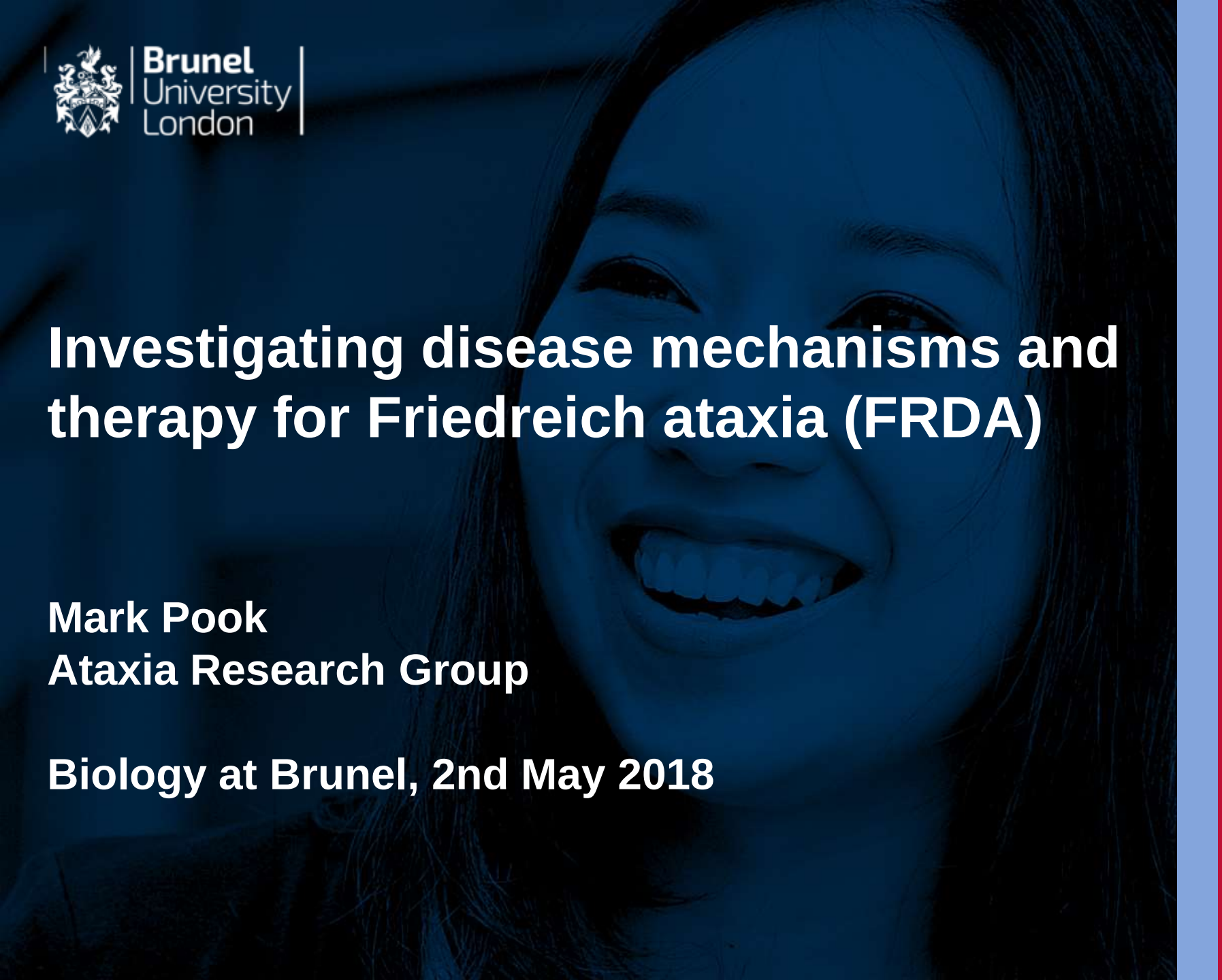


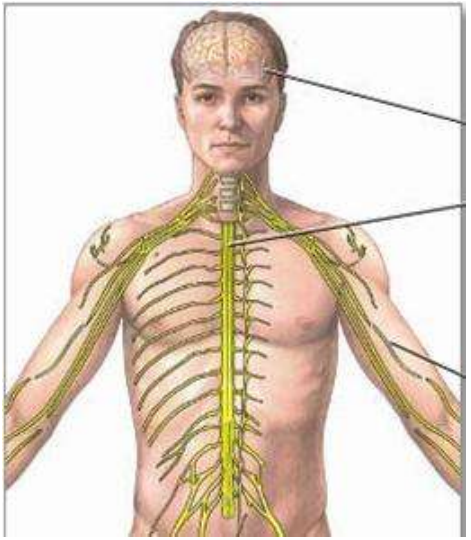
Investigating disease mechanisms and therapy for Friedreich ataxia (FRDA)

Mark Pook
Ataxia Research Group

Biology at Brunel, 2nd May 2018

Friedreich ataxia (FRDA) Disease

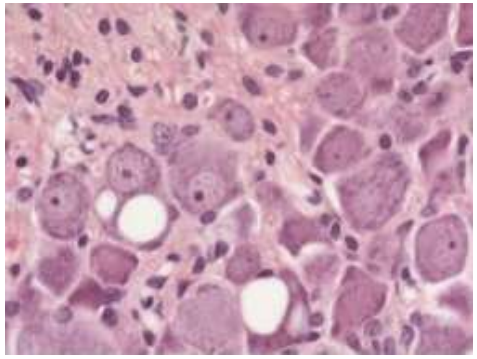
Lethal, inherited, progressive neurodegenerative disorder



Ataxia (uncontrolled movement) onset in childhood

Wheel-chair bound in teens

Premature death in 20-40s

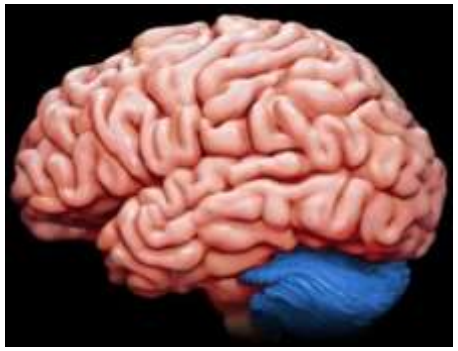


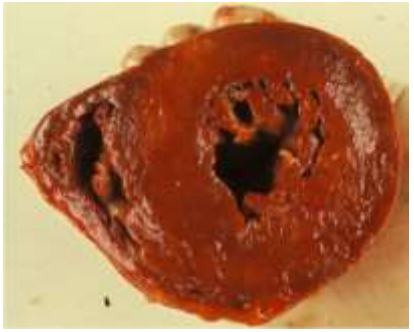
Primary pathology:

> Neurons of dorsal root ganglia (DRG)

> Sensory nerves of the spinal cord

> Cerebellum

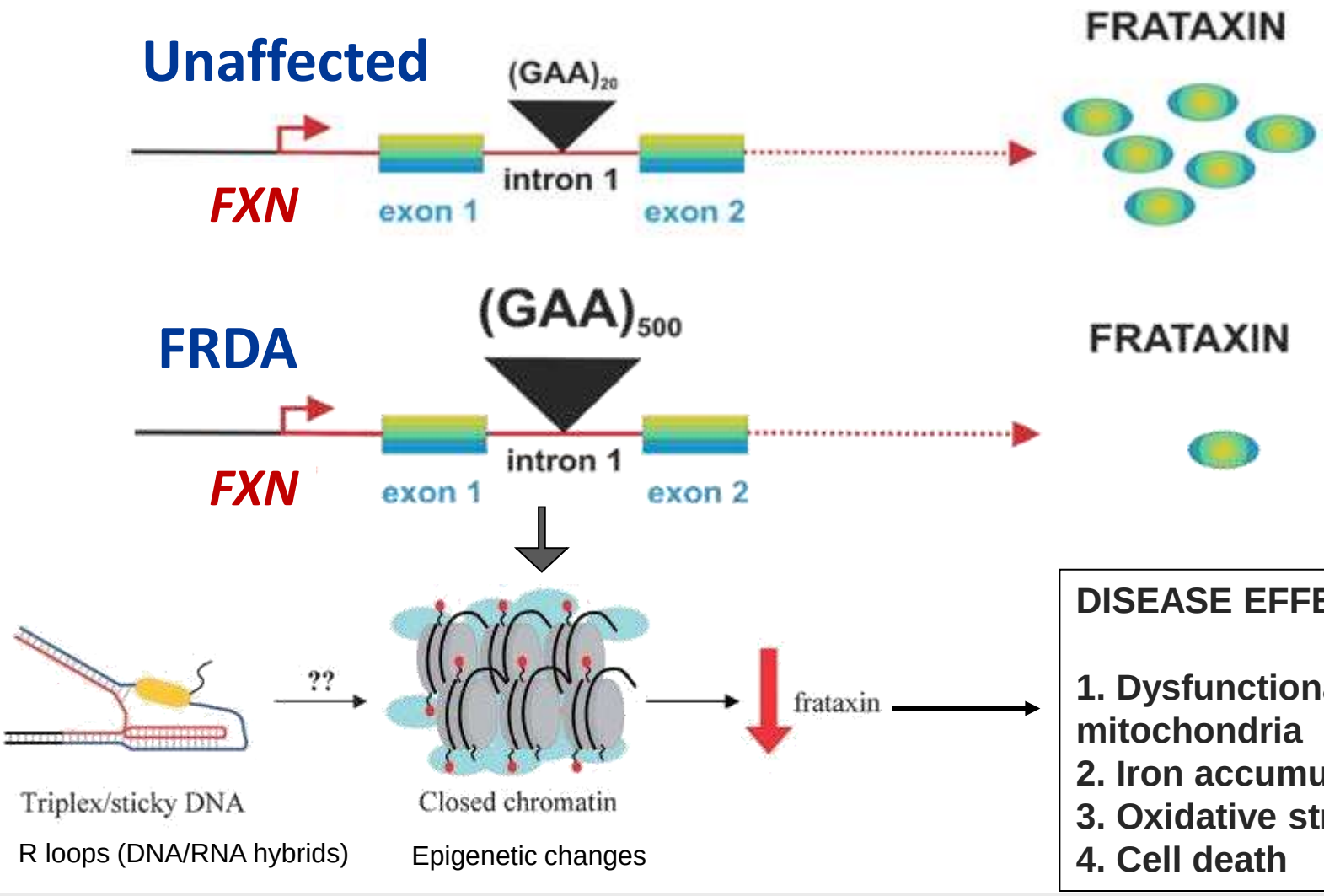




> Hypertrophic cardiomyopathy

> Diabetes mellitus

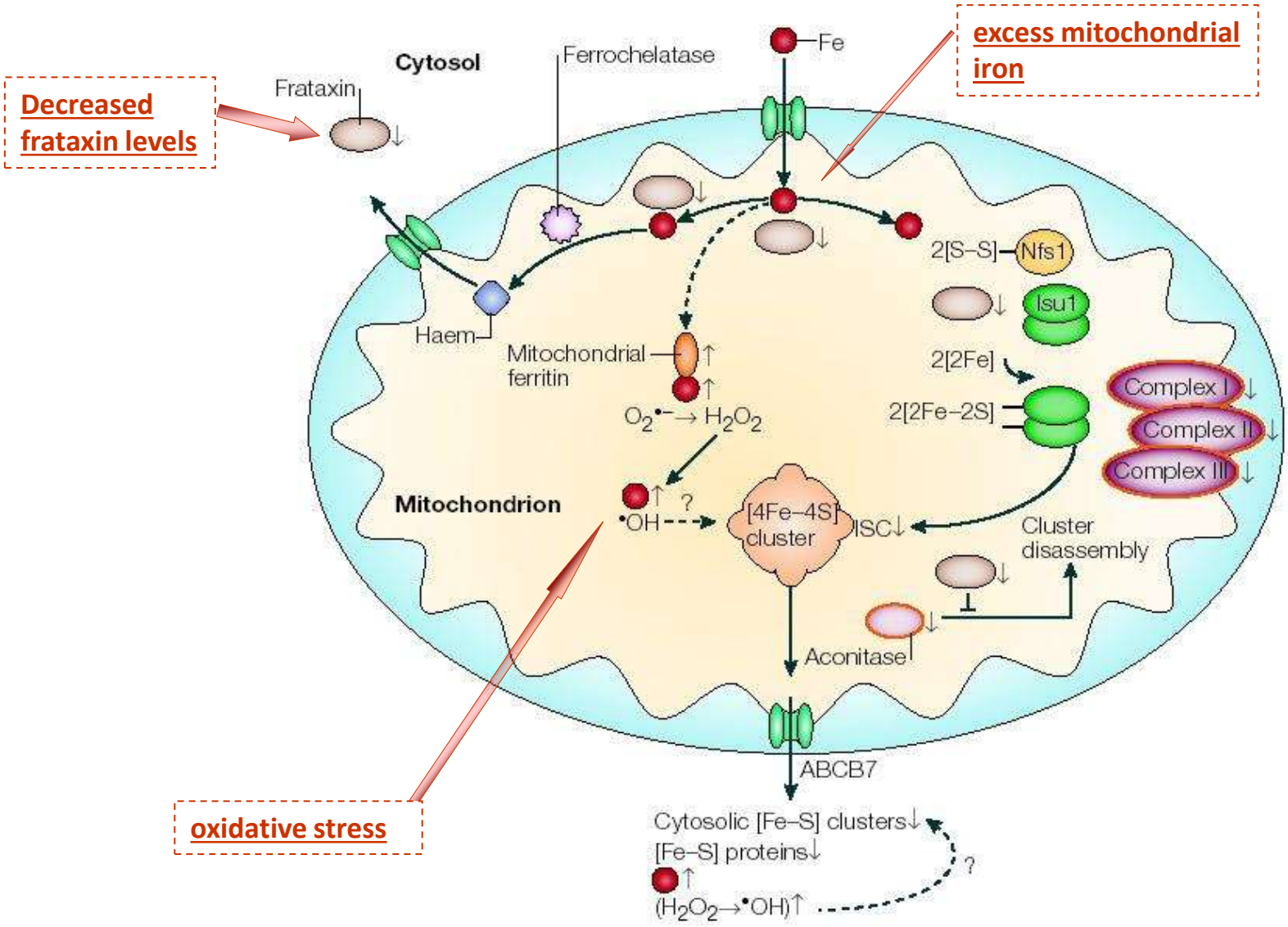
FRDA Molecular Disease Mechanism

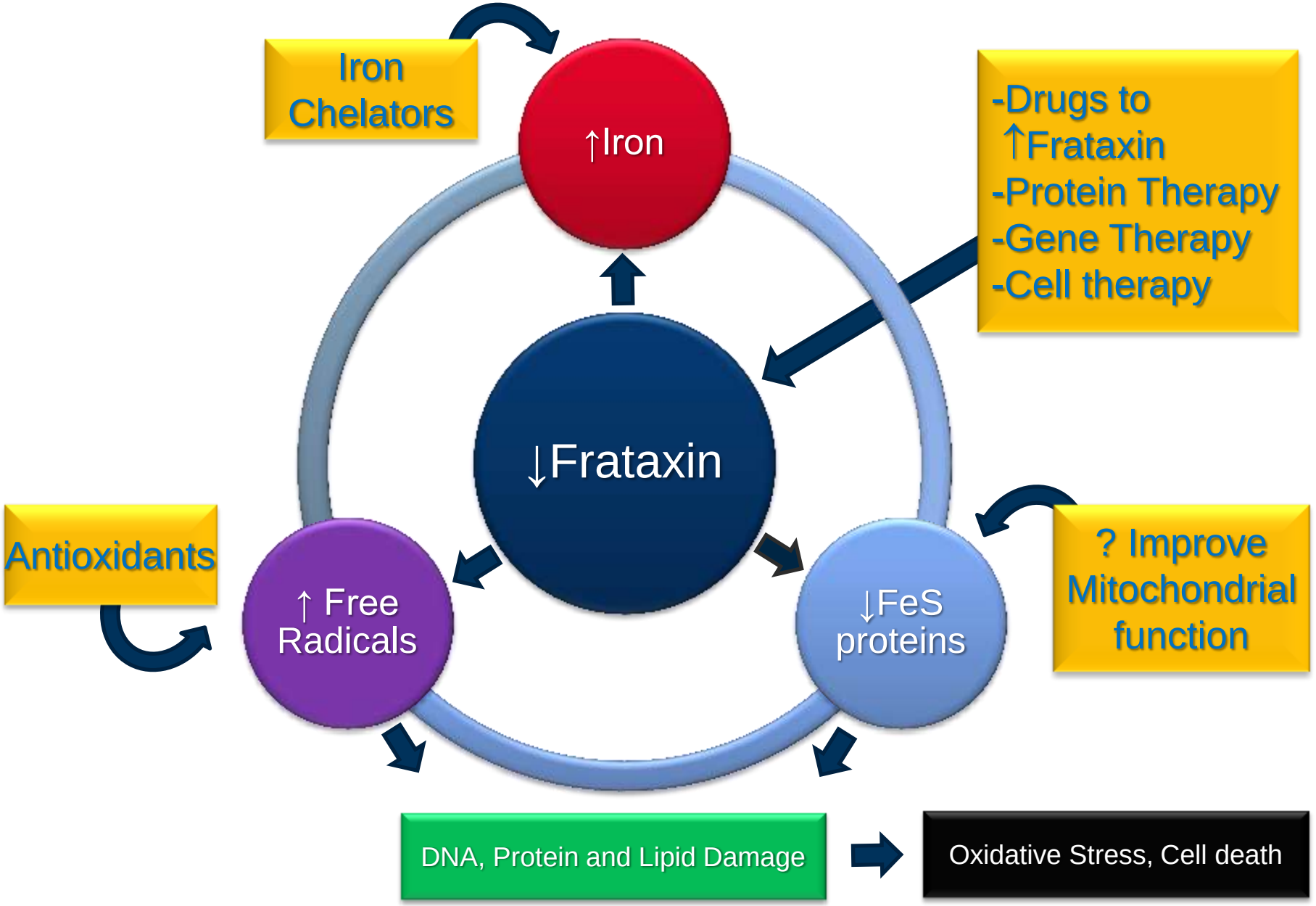


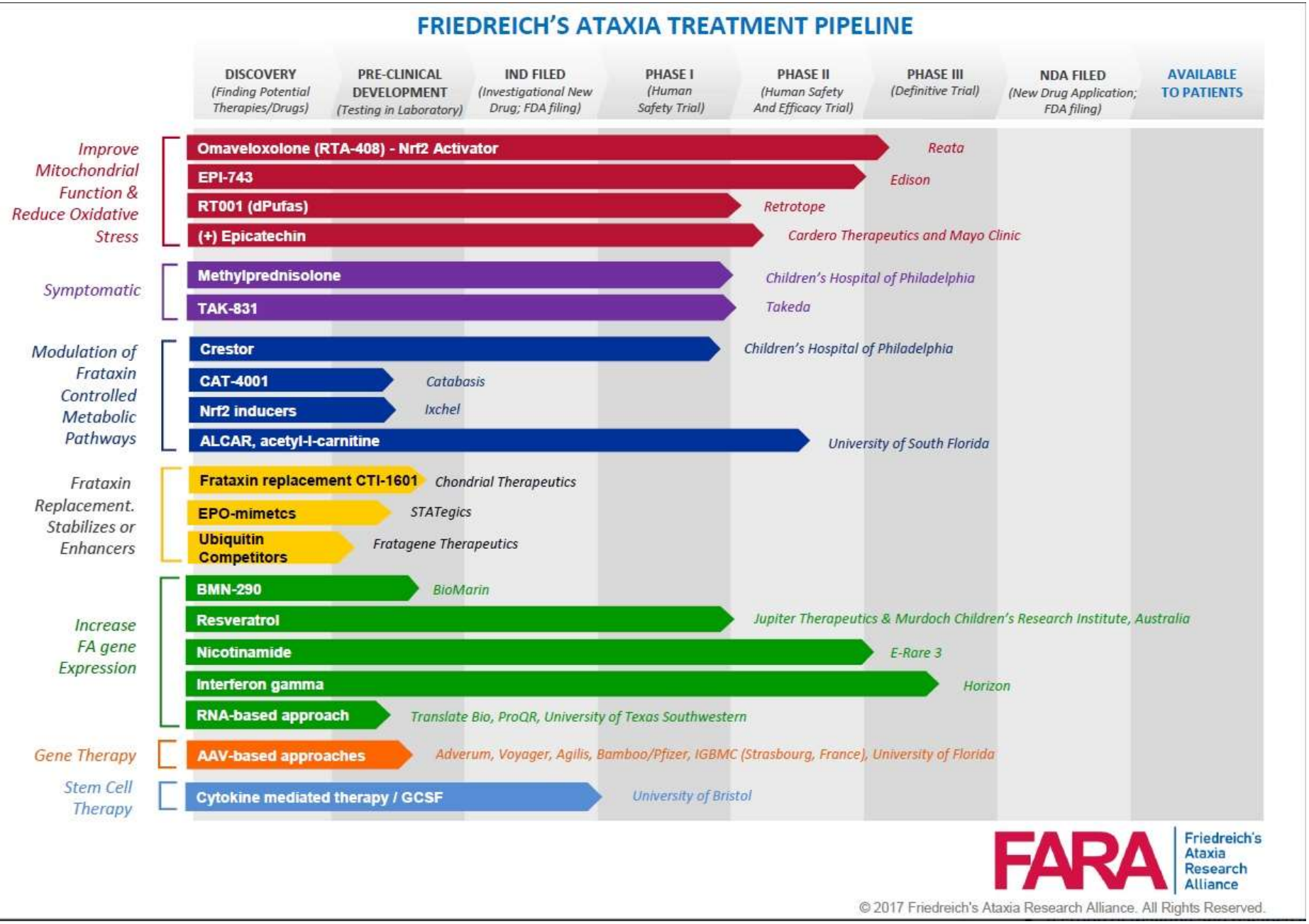
DISEASE EFFECTS:

1. **Dysfunctional mitochondria**
2. **Iron accumulation**
3. **Oxidative stress**
4. **Cell death**

FRDA pathogenesis




Iron Chelators
↑Iron
-Drugs to ↑Frataxin
-Protein Therapy
-Gene Therapy
-Cell therapy
↓Frataxin
Antioxidants
↑ Free Radicals
↓FeS proteins
? Improve Mitochondrial function
DNA, Protein and Lipid Damage
Oxidative Stress, Cell death


FRIEDREICH'S ATAXIA TREATMENT PIPELINE
DISCOVERY (Finding Potential Therapies/Drugs)
PRE-CLINICAL DEVELOPMENT (Testing in Laboratory)
IND FILED (Investigational New Drug; FDA filing)
PHASE I (Human Safety Trial)
PHASE II (Human Safety And Efficacy Trial)
PHASE III (Definitive Trial)
NDA FILED (New Drug Application; FDA filing)
AVAILABLE TO PATIENTS
Improve Mitochondrial Function & Reduce Oxidative Stress
Omaveloxolone (RTA-408) - Nrf2 Activator
Reata
EPI-743
Edison
RT001 (dPufas)
Retrotope
(+) Epicatechin
Cardero Therapeutics and Mayo Clinic
Symptomatic
Methylprednisolone
Children's Hospital of Philadelphia
TAK-831
Takeda
Modulation of Frataxin Controlled Metabolic Pathways
Crestor
Children's Hospital of Philadelphia
CAT-4001
Catabasis
Nrf2 inducers
Ixchel
ALCAR, acetyl-l-carnitine
University of South Florida
Frataxin Replacement. Stabilizes or Enhancers
Frataxin replacement CTI-1601
Chondrial Therapeutics
EPO-mimetcs
STATegics
Ubiquitin Competitors
Fratagene Therapeutics
Increase FA gene Expression
BMN-290
BioMarin
Resveratrol
Jupiter Therapeutics & Murdoch Children's Research Institute, Australia
Nicotinamide
E-Rare 3
Interferon gamma
Horizon
RNA-based approach
Translate Bio, ProQR, University of Texas Southwestern
Gene Therapy
AAV-based approaches
Adverum, Voyager, Agilis, Bamboo/Pfizer, IGBMC (Strasbourg, France), University of Florida
Stem Cell Therapy
Cytokine mediated therapy / GCSF
University of Bristol
FARA
Friedreich's Ataxia Research Alliance
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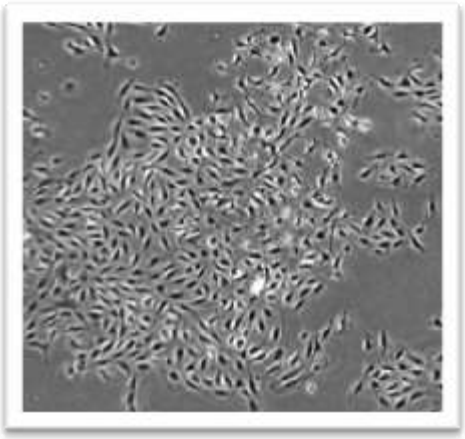
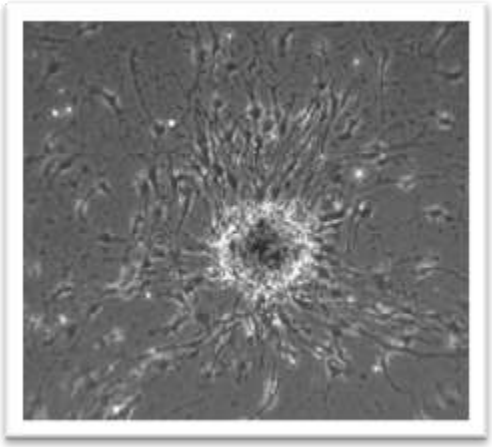
Research into potential new FRDA drug therapies

Resources:





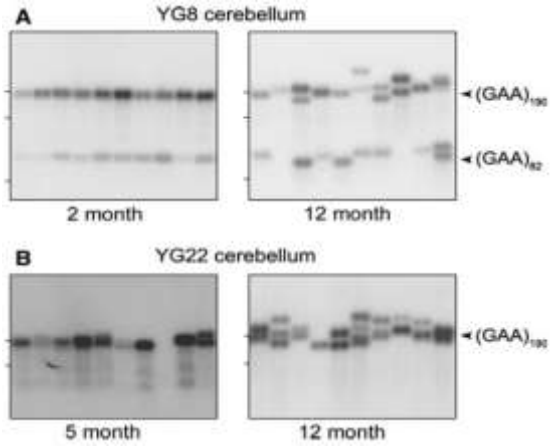
> FRDA Patient cells/autopsy tissues

> Cell cultures

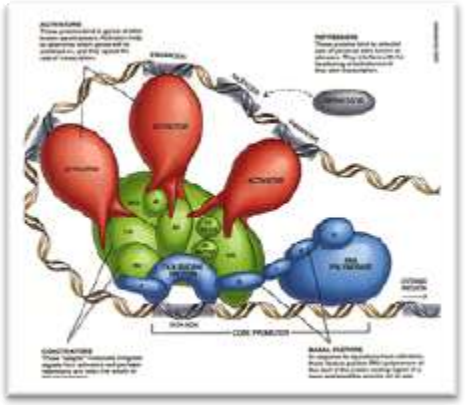
- > Blood cells (lymphoblasts)
- > Skin cells (fibroblasts)
- > Skin cells → (iPS cells) → Cultured neurons and heart cells

> Animal models: Worm, fly, **mouse**

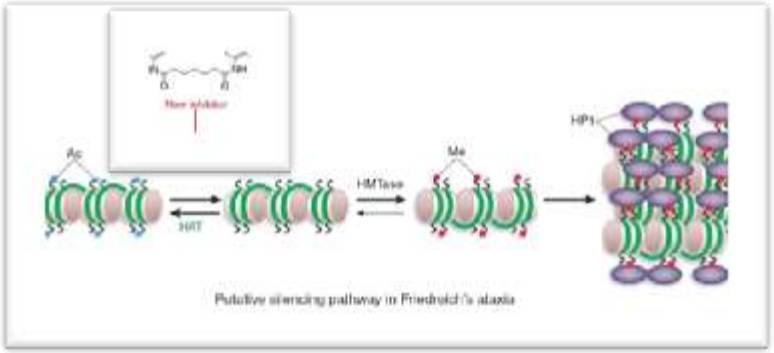
Uses of an FRDA mouse model



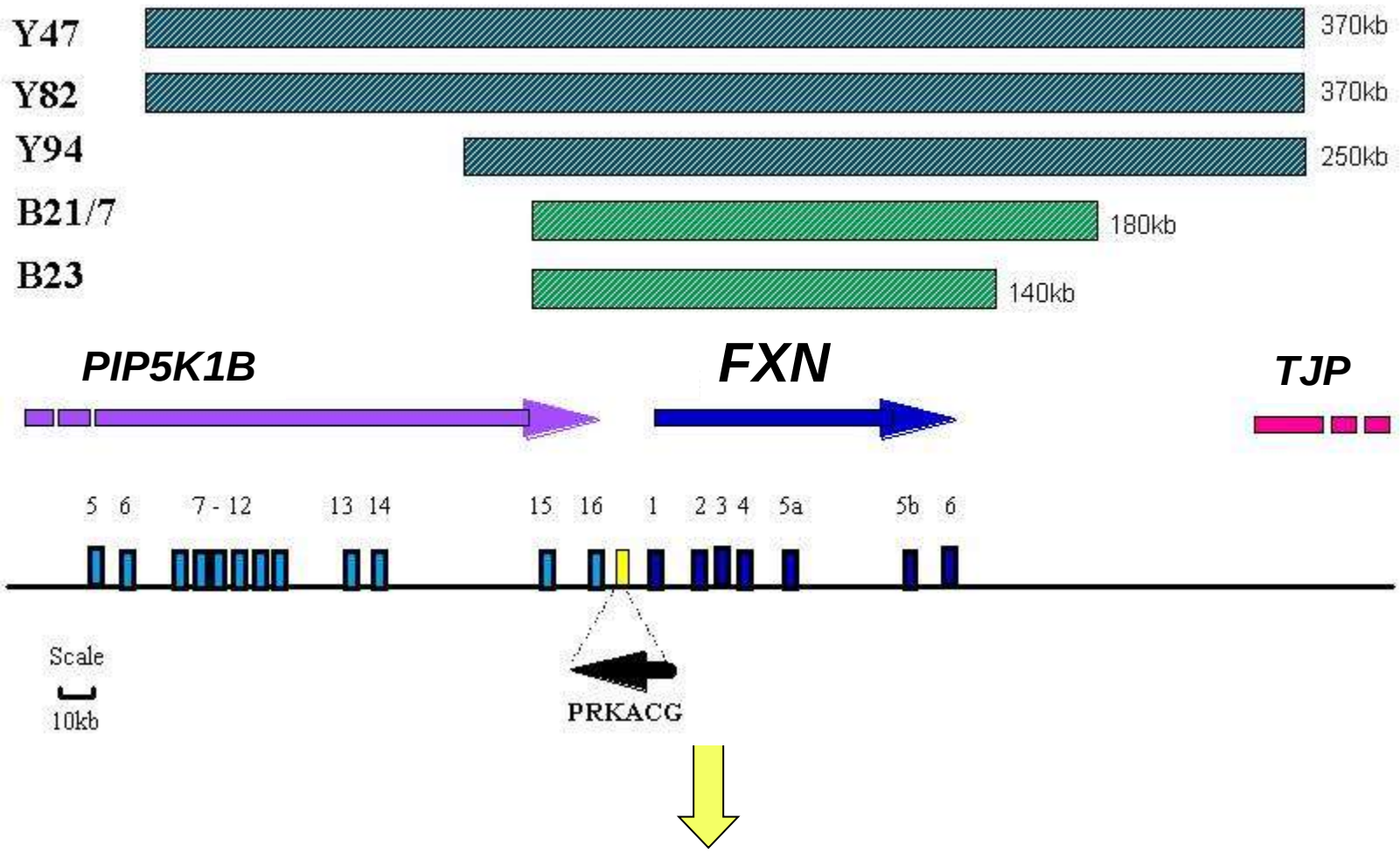
- **To investigate mechanisms of FRDA disease**
  - Molecular genetics – GAA repeat dynamics, epigenetics
    - what causes FXN gene shutdown?
  - Molecular and cellular pathology
    - what causes progressive cell death?
  - Identification of new therapeutic targets



- **Preclinical testing of potential therapies**



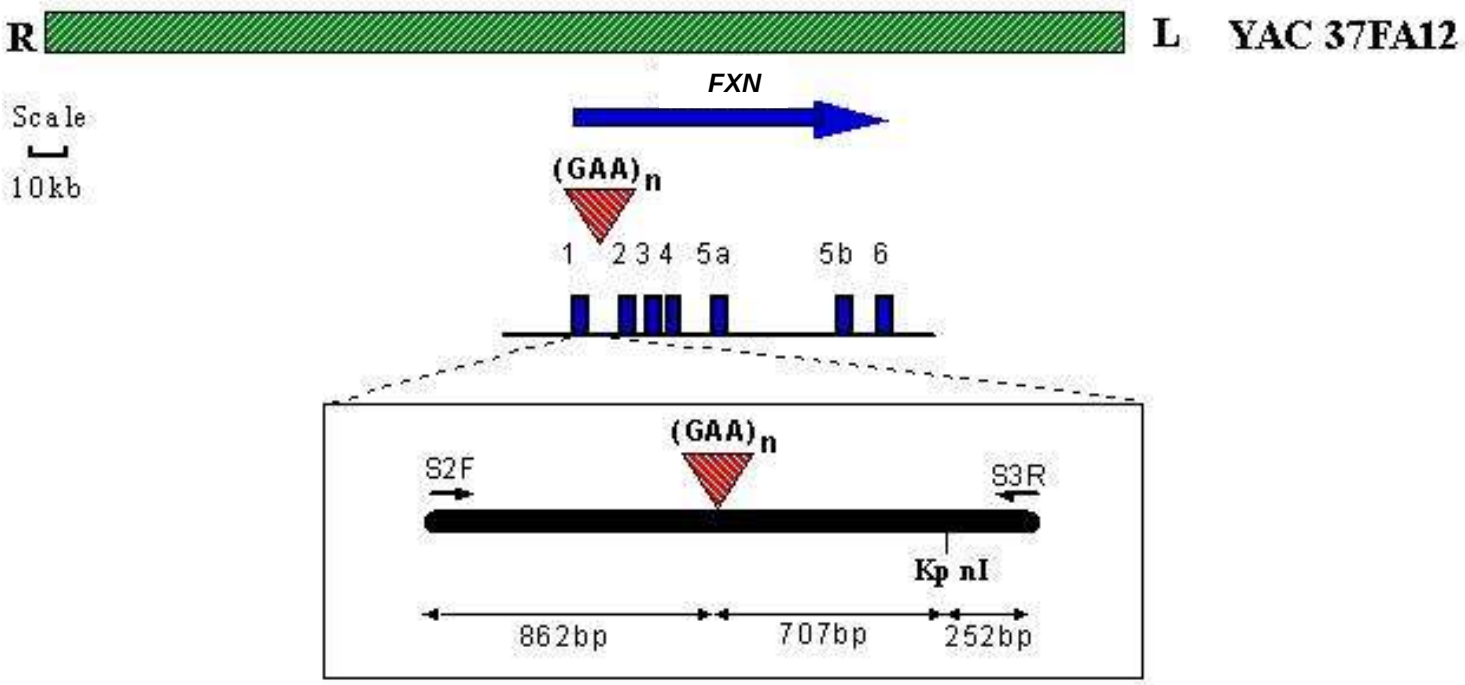
FRDA Mouse Models: Human FXN YAC transgenes



All rescue of *Fxn* KO embryonic lethality

Pook, M.A., et al (2001) Rescue of the Friedreich's ataxia knockout mouse by human YAC transgenesis. *Neurogenetics* 3: 185-193.

Insertion of GAA repeat into WT *FXN* YAC



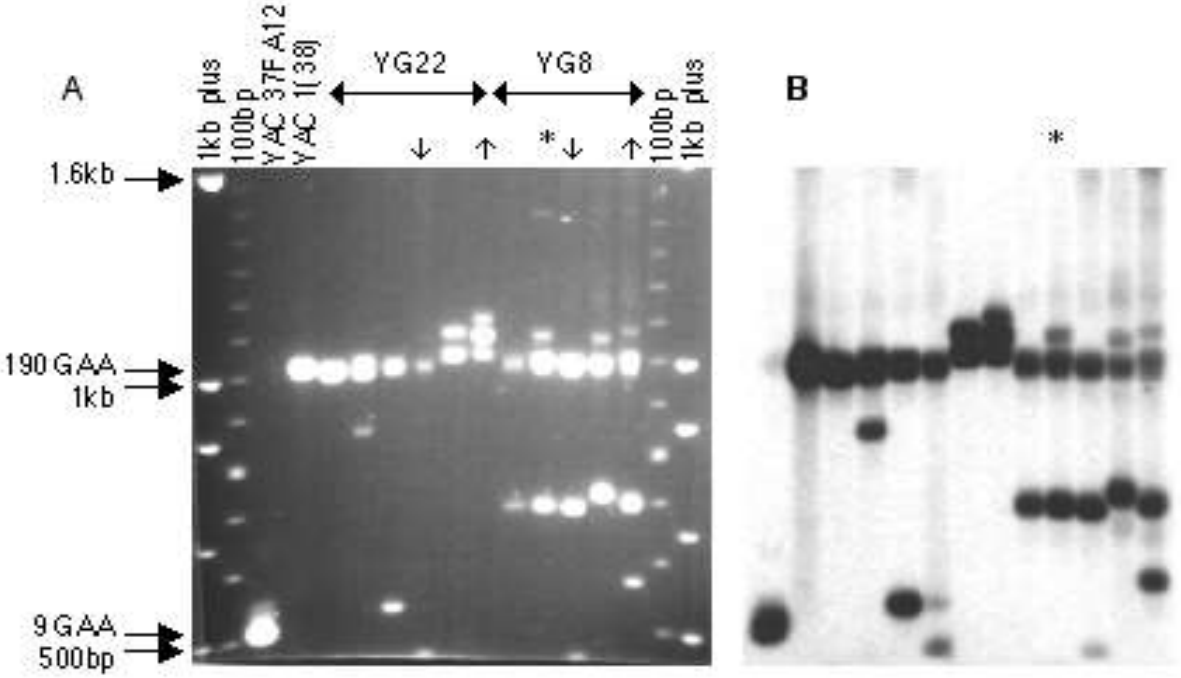
PCR patient's DNA with primers encompassing the GAA repeat (300-700 repeats)

Homologous recombination "Pop-in / Pop-out"

GAA YAC for pronuclear injection

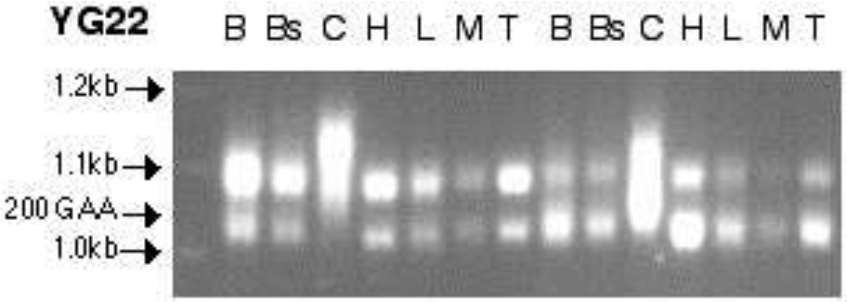
Intergenerational GAA repeat instability

Transgenic line	YAC transgene integrity	Integration sites	*FXN* copy number	Founder GAA repeat length(s)
Y47	Complete 370kb	single	1	9
YG8	Complete 370kb	single	2	190 +90
YG22	Complete 370kb	single	1	190

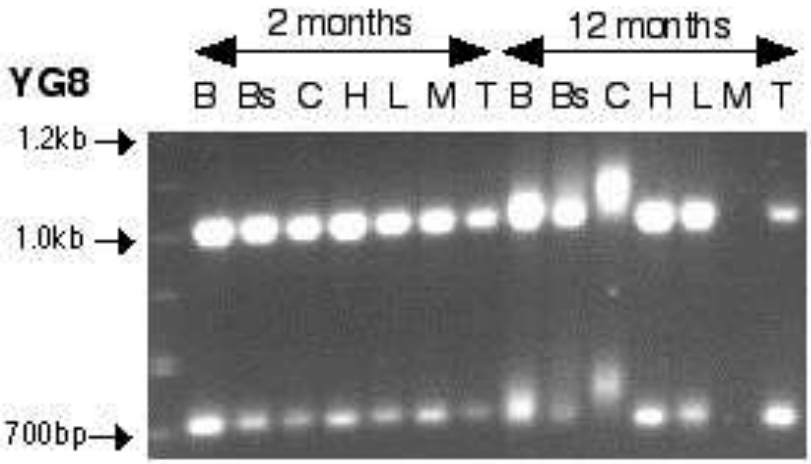


Somatic GAA repeat instability

Expansion in cerebellum



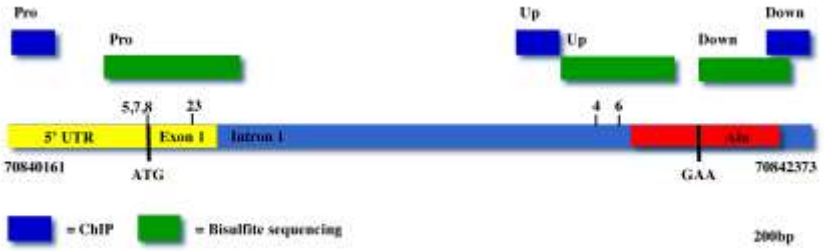
Expansion is age-related

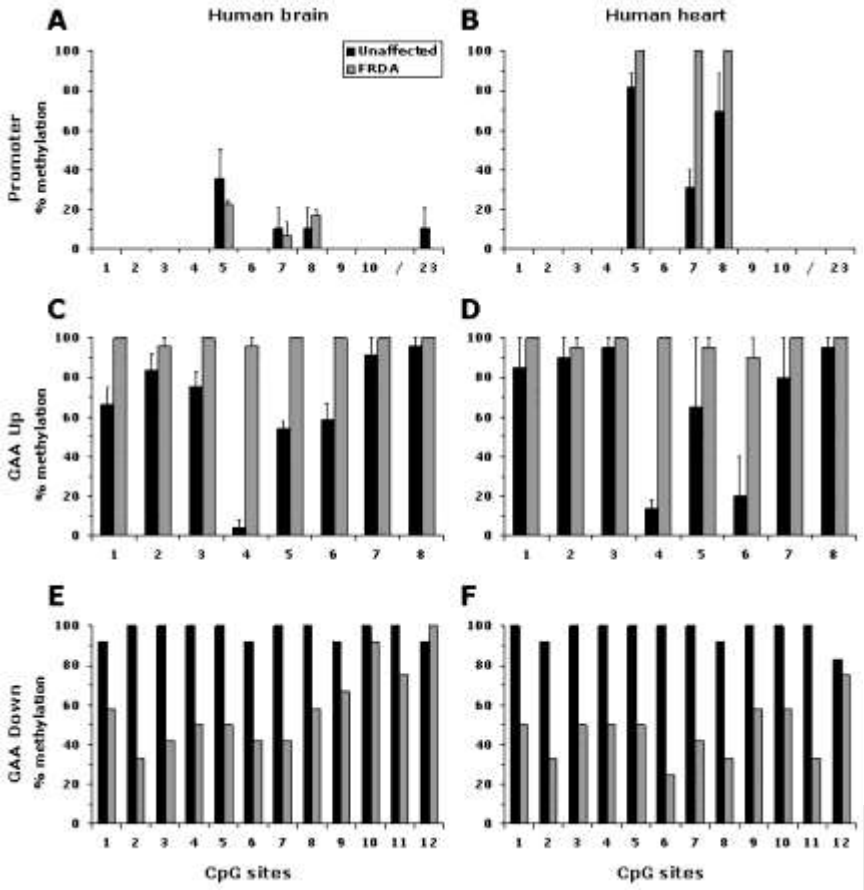


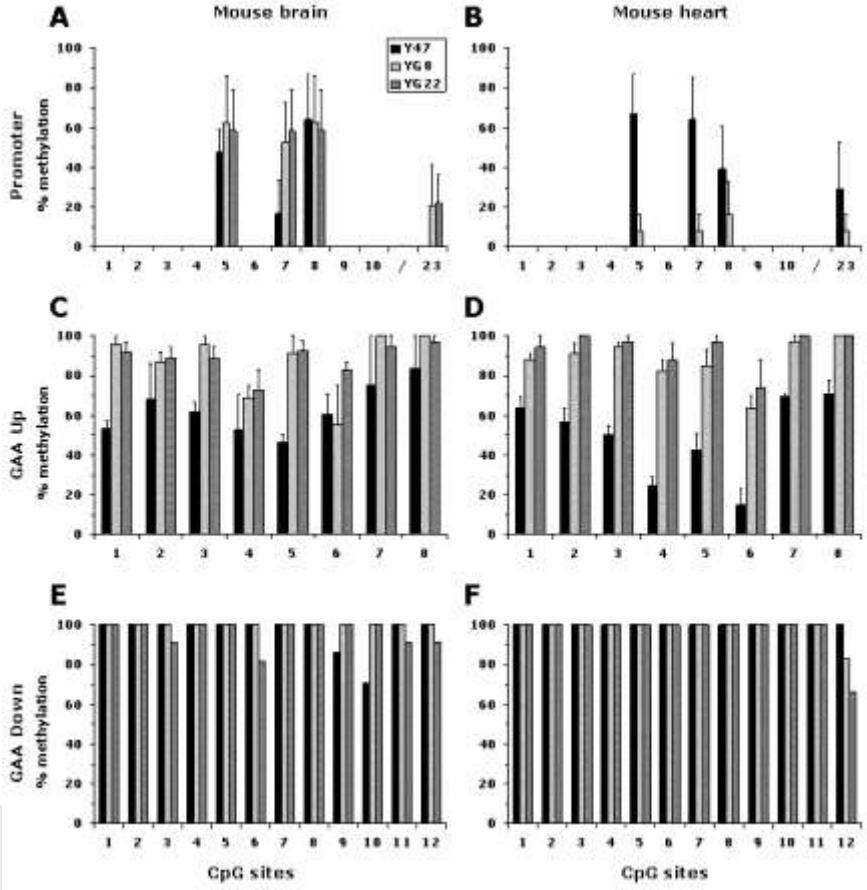
Al-Mahdawi et al. (2004) GAA repeat instability in Friedreich ataxia YAC transgenic mice. *Genomics* 84: 301-10.

Clark, R et al (2007) The GAA triplet-repeat is unstable in the context of the human FXN locus and displays age-dependent expansions in cerebellum and DRG in a transgenic mouse model. *Hum. Genet.* 120: 633-640.

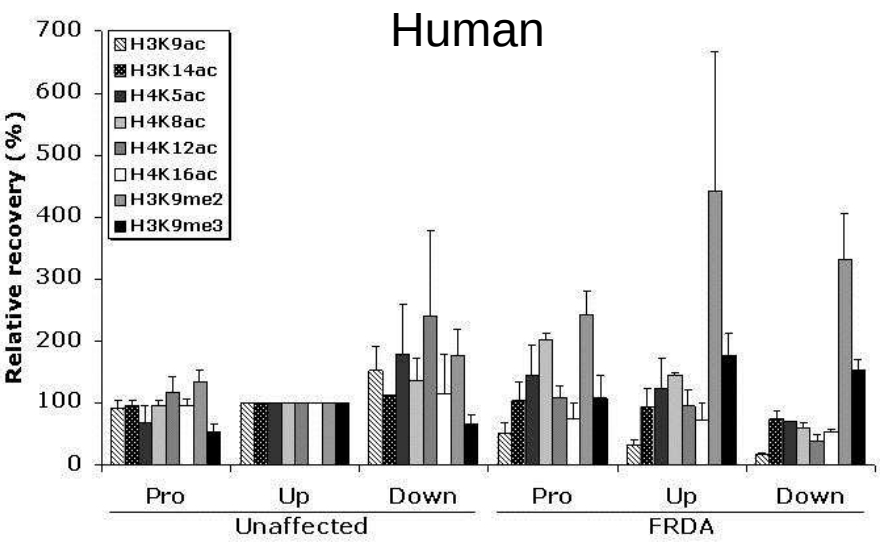
DNA methylation analysis of *FXN* exon/intron 1

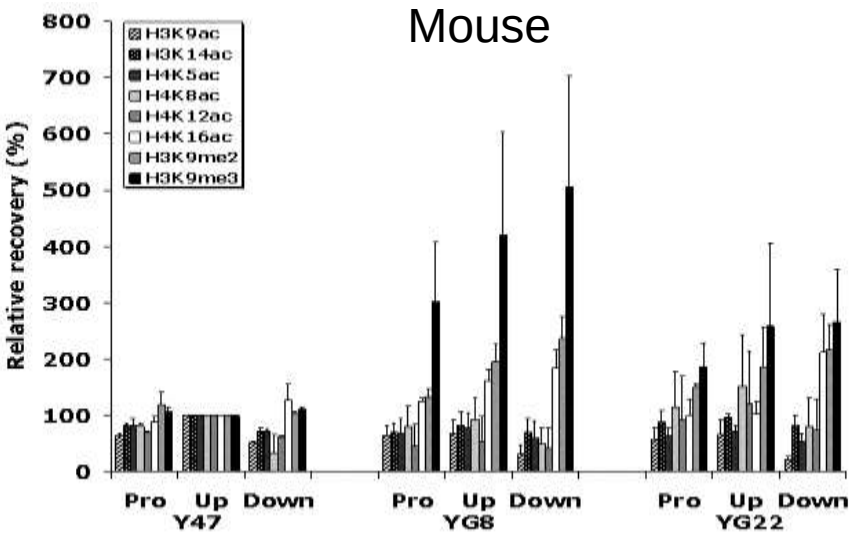


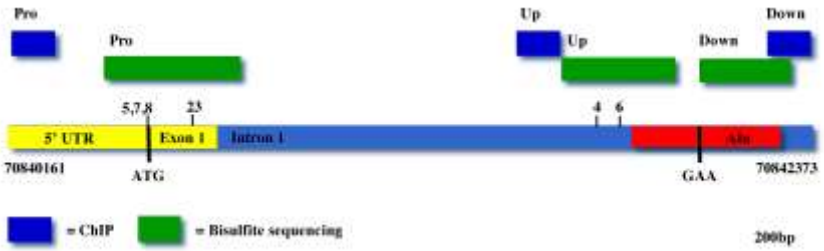


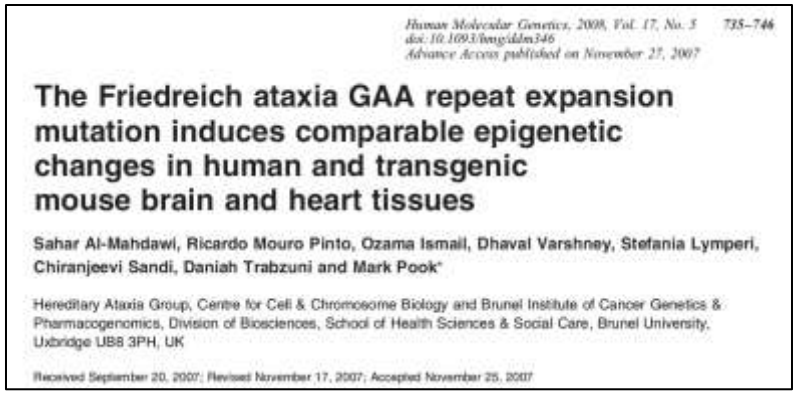


Changes in *FXN* gene histone modifications in human and mouse brain









Human Molecular Genetics, 2008, Vol. 17, No. 5 735–746
doi:10.1093/hmg/ddm346
Advance Access published on November 27, 2007

The Friedreich ataxia GAA repeat expansion mutation induces comparable epigenetic changes in human and transgenic mouse brain and heart tissues

Sahar Al-Mahdawi, Ricardo Mouro Pinto, Ozama Ismail, Dhaval Varshney, Stefania Lymperi, Chiranjeevi Sandi, Daniah Trabzuni and Mark Pook*

Hereditary Ataxia Group, Centre for Cell & Chromosome Biology and Brunel Institute of Cancer Genetics & Pharmacogenomics, Division of Biosciences, School of Health Sciences & Social Care, Brunel University, Uxbridge UB8 3PH, UK

Received September 20, 2007; Revised November 17, 2007; Accepted November 25, 2007

GAA repeat FRDA mouse models: YG8R and YG22R

***FXN* transgenic** (Tg^{GAA} / *Fxn*$^{+/+}$) **X** ***Fxn* heterozygous KO** (*Fxn*$^{+/-}$)

↓

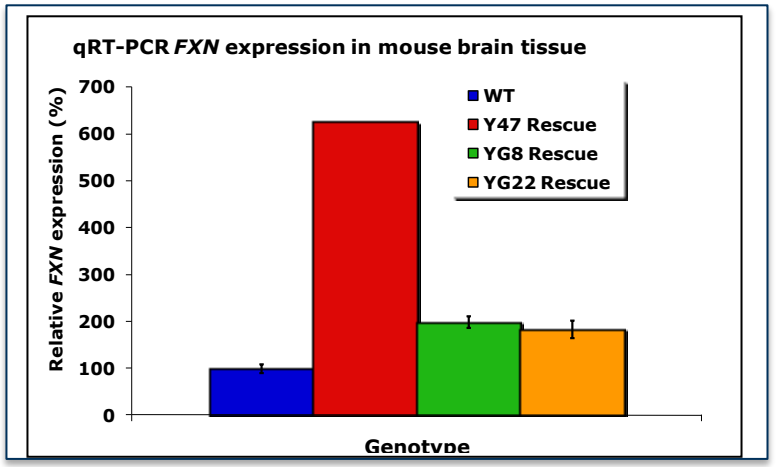
(Tg^{GAA} / *Fxn*$^{+/-}$) **X** (*Fxn*$^{+/-}$)

↓

***FXN* transgene on a null background**

(Tg^{GAA} / *Fxn*$^{-/-}$)

→



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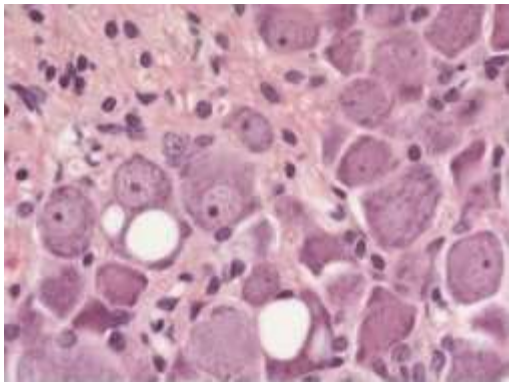
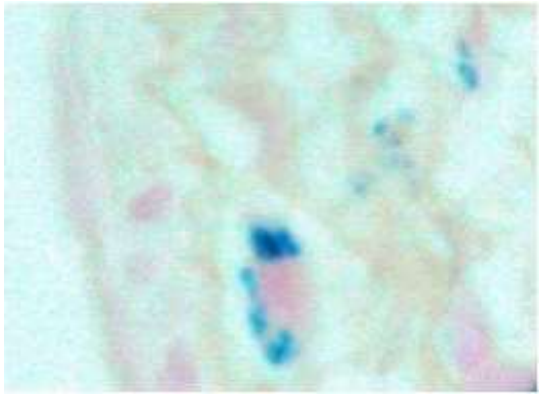
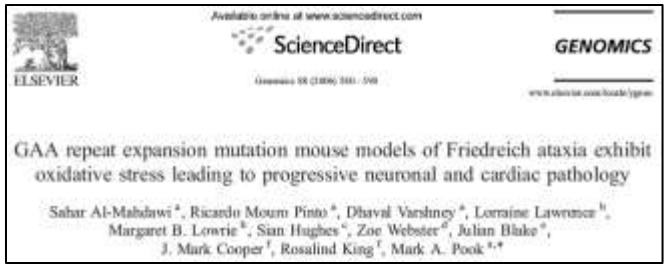




→ No overt ataxia, but rotarod coordination and locomotor deficits

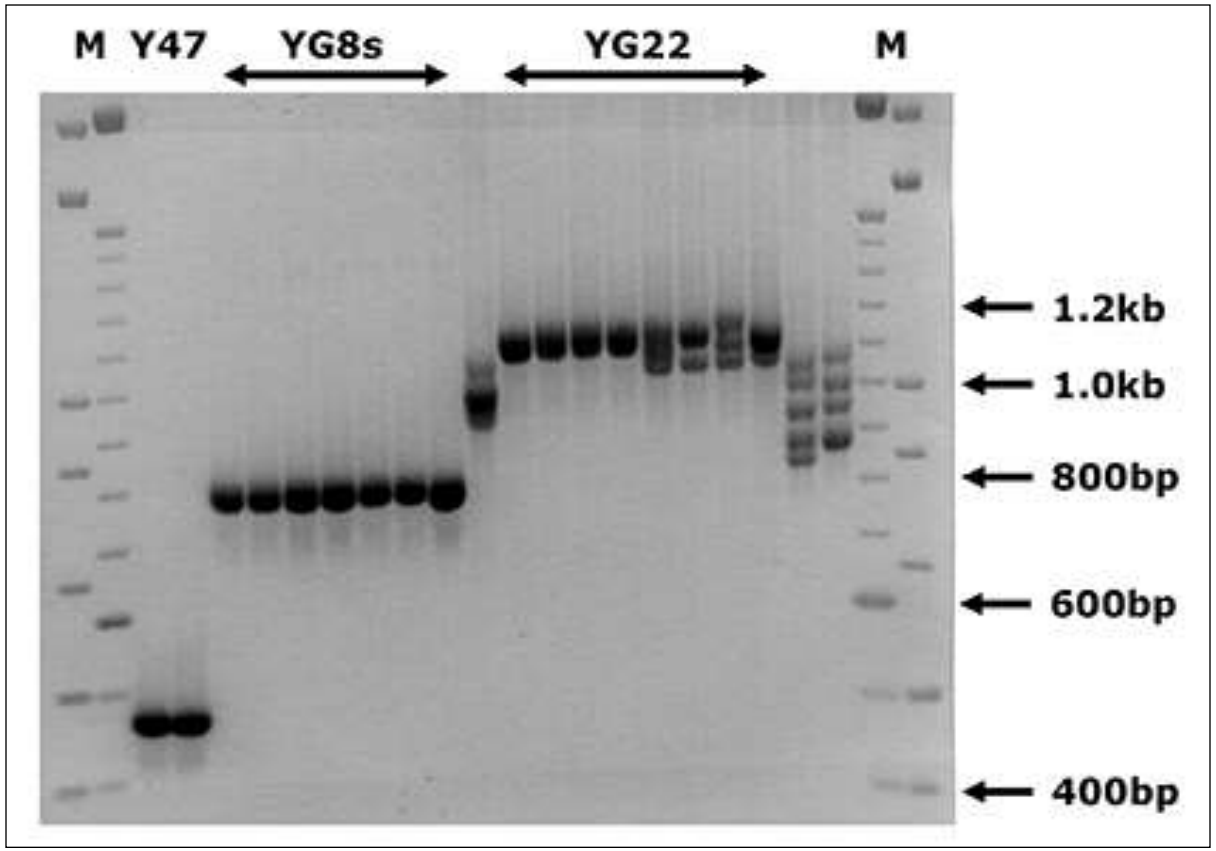
Progressive histopathology: large vacuoles in DRG sensory neurons and iron accumulation in heart



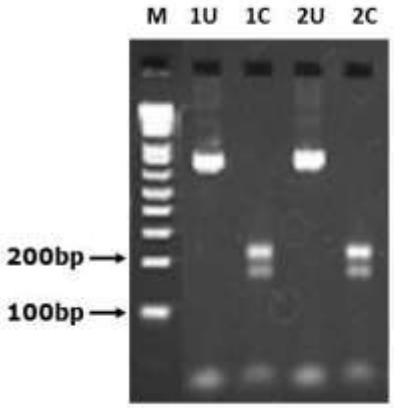
Recent research - New YG8sR FRDA mice

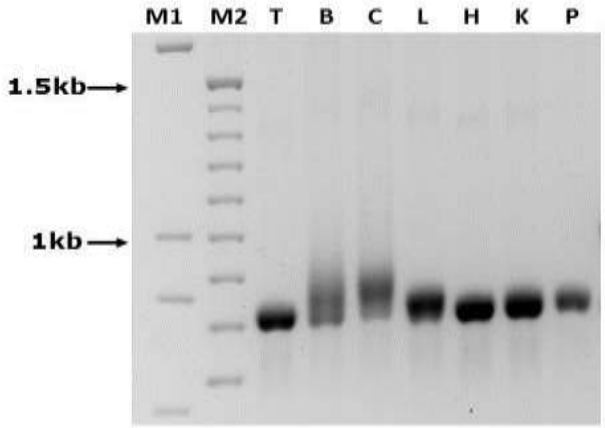
A single GAA repeat of 120 repeats

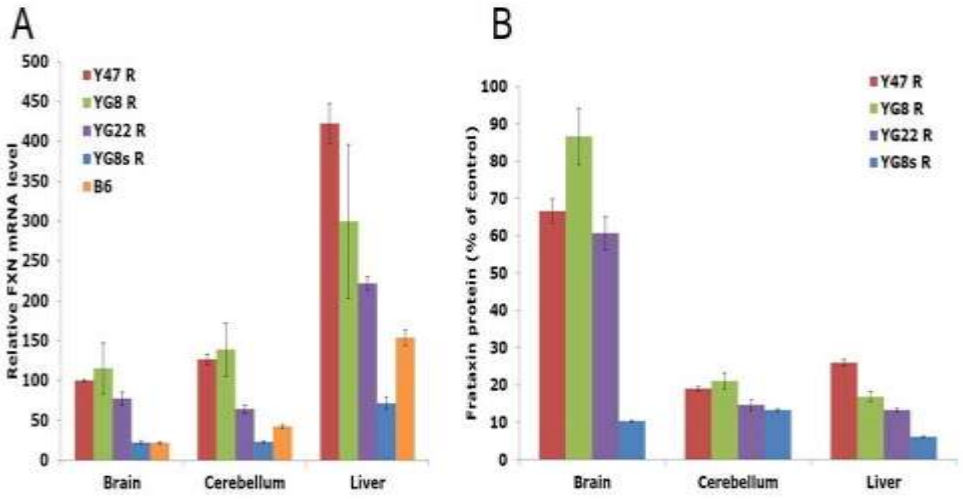
120 GAAs

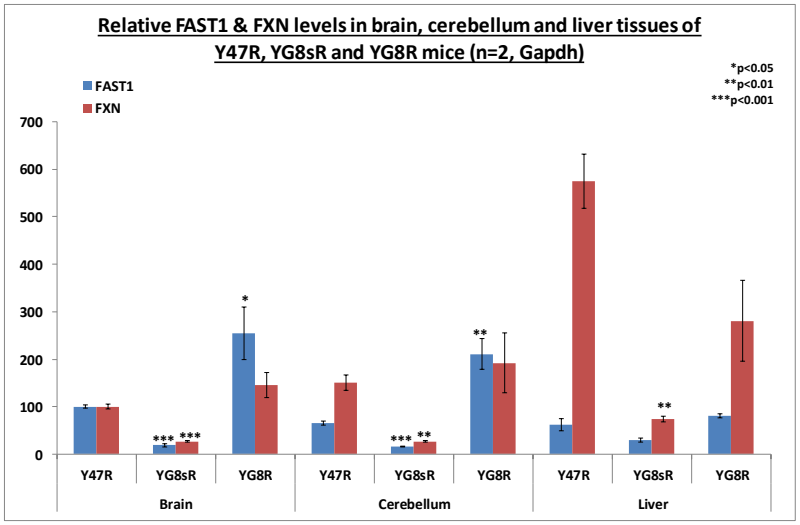


YG8sR mice – molecular characterisation

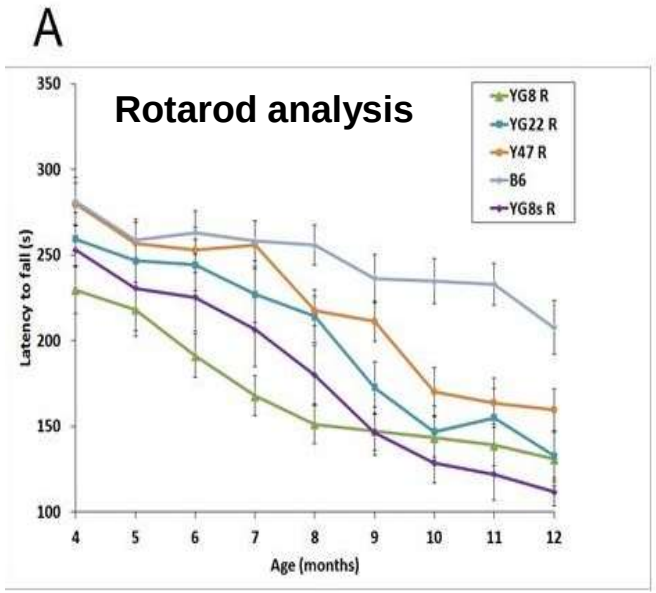


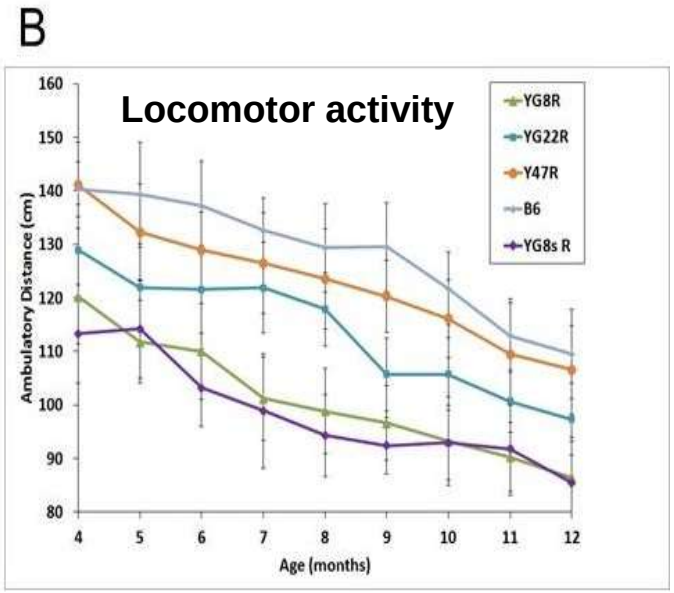


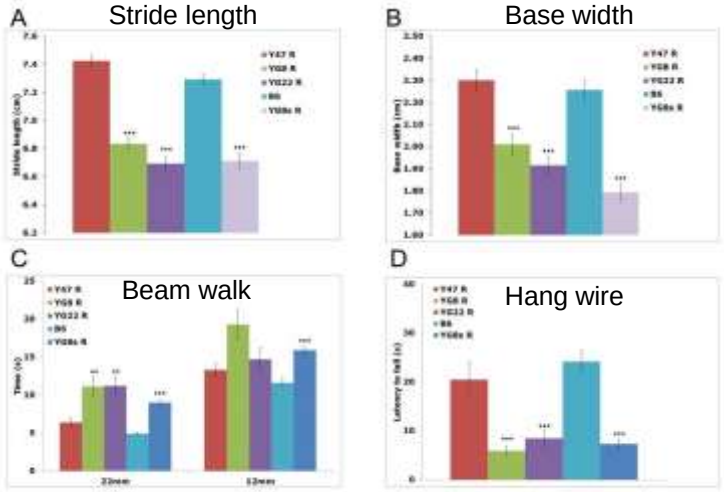




YG8sR mice – functional characterisation

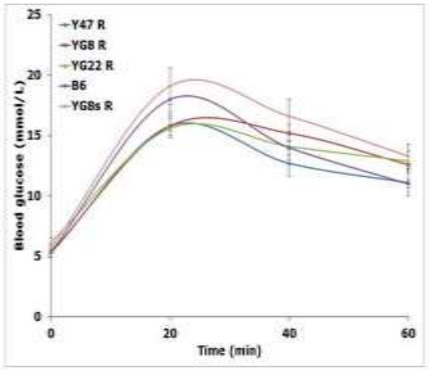




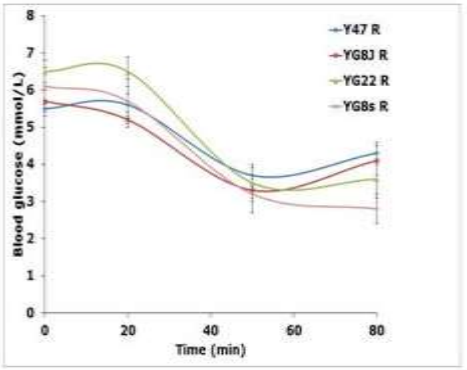


YG8sR mice – Biochemistry and histology

A Fasting Glucose tolerance test



B Fasting Insulin tolerance test



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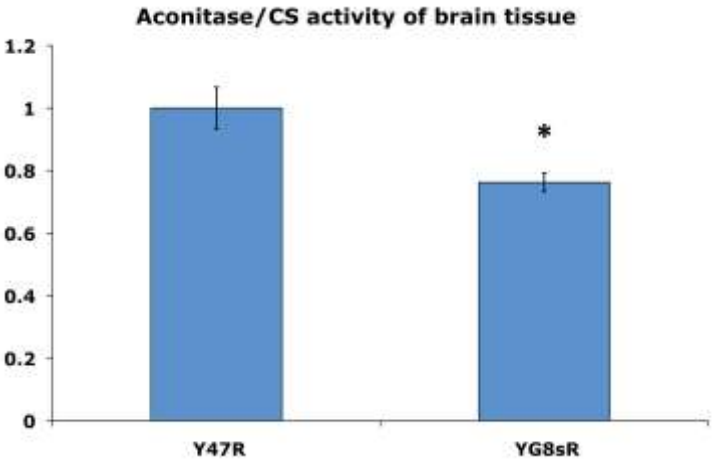
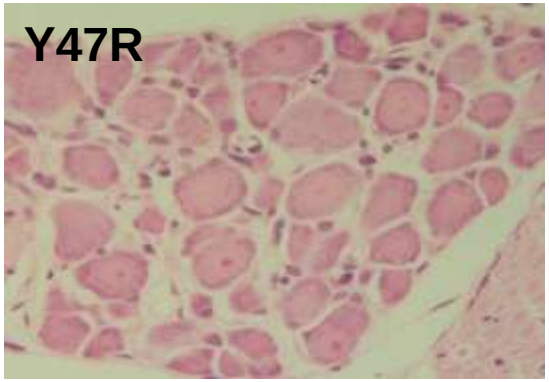
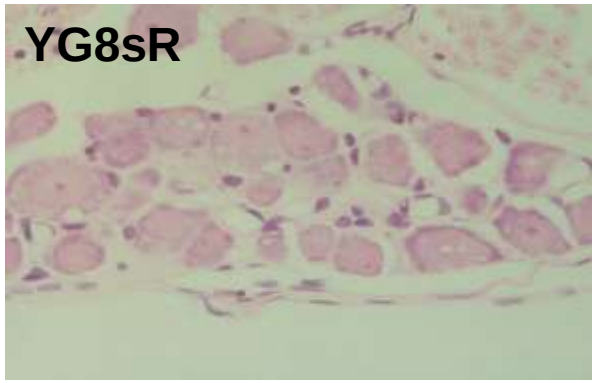






Anjomani Virmouni, Ezzatizadeh, V., Sandi, C., Sandi, M., Al-Mahdawi, S., Chutake, Y. and Pook, M. A. (2015) A novel GAA repeat expansion-based mouse model of Friedreich ataxia. *Dis. Models. Mech.* 8: 225-235.

Preclinical studies

Histone deacetylase (HDAC) inhibitors (Repligen, Boston) - YG8R mice, 5 months (Sandi et al 2012)

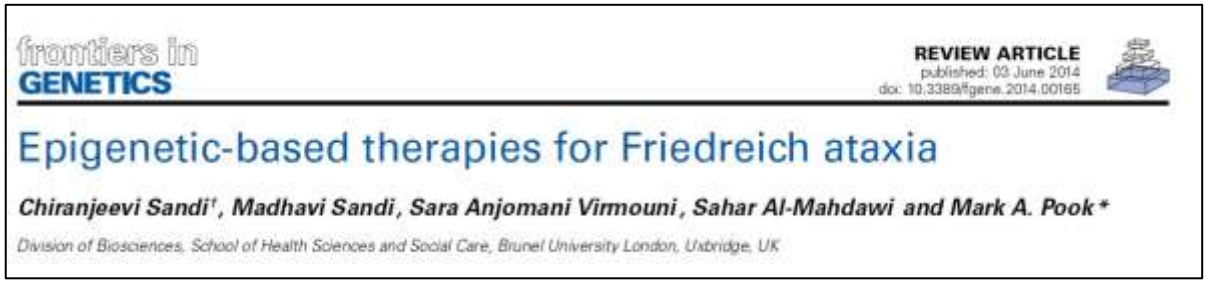
• RG2833 (Repligen) completed a Phase 1 trial – treatment was well tolerated and there was increased frataxin expression.

Interferon gamma (Roberto Testi, Rome) YG8R mice, 3 months (Tomassini et al 2012)

• Placebo controlled Phase 3 studies are underway (sponsored by Horizon)

Nicotinamide (Richard Festenstein, London) YG22R mice, 5 months (Chan et al 2013)

• Phase 2 trial showed increased frataxin and some clinical improvement. A phase 3 trial has recently started (Jorg Schultz, E-Rare 3)



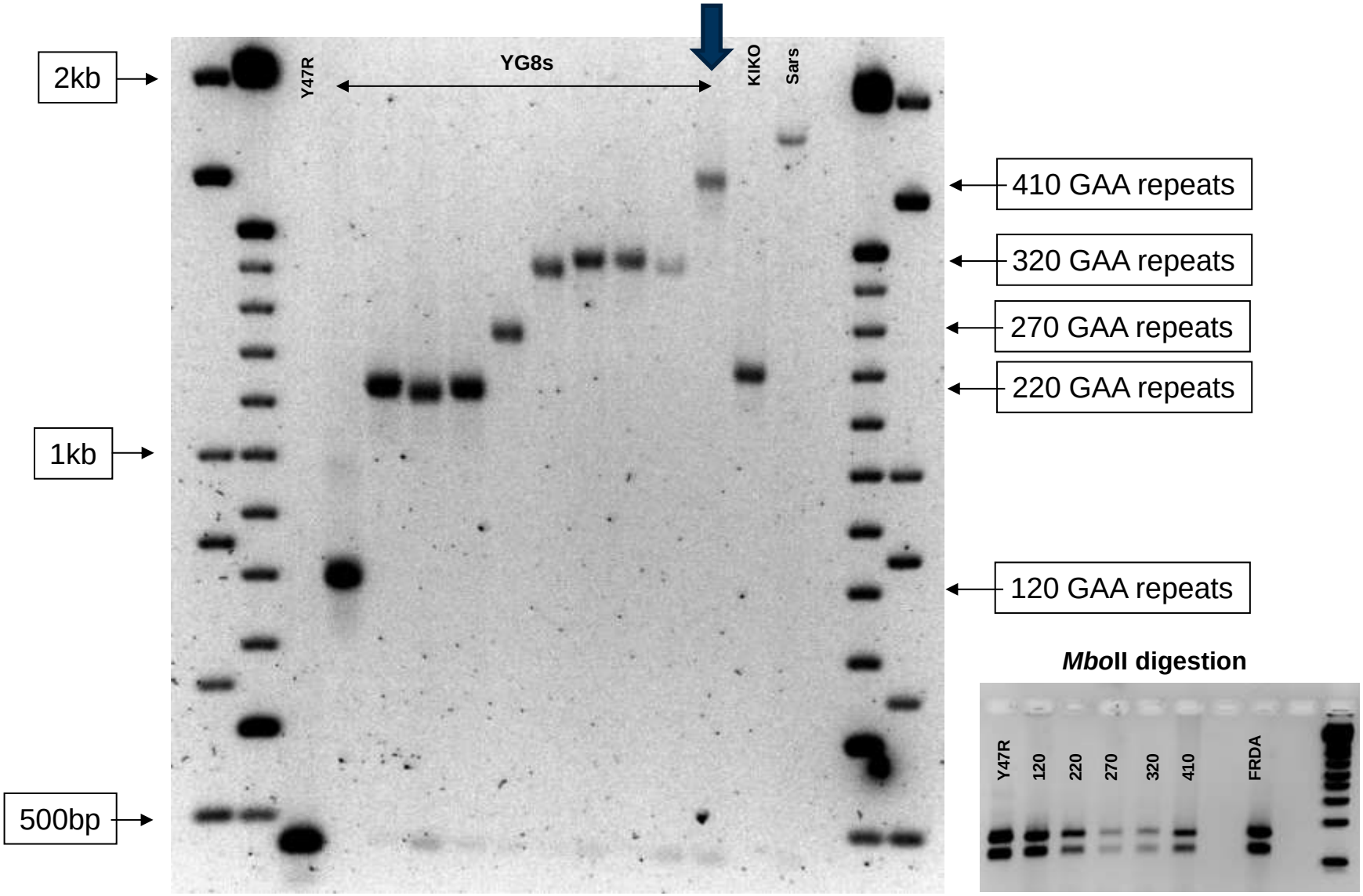
Recent and future collaborative studies

- **Diazoxide** (Carlo Marobbio, Bari, Italy) – mitochondrial K channel activator that increases frataxin expression
- **Dimethyl fumarate (DMF) and Resveratrol** (Gino Cortopassi, UC Davis) - to increase frataxin expression
- **Etravirine** (Alessandra Rufini and Roberto Testi, 'Fratagene', University of Rome Tor Vergata) - to increase frataxin expression

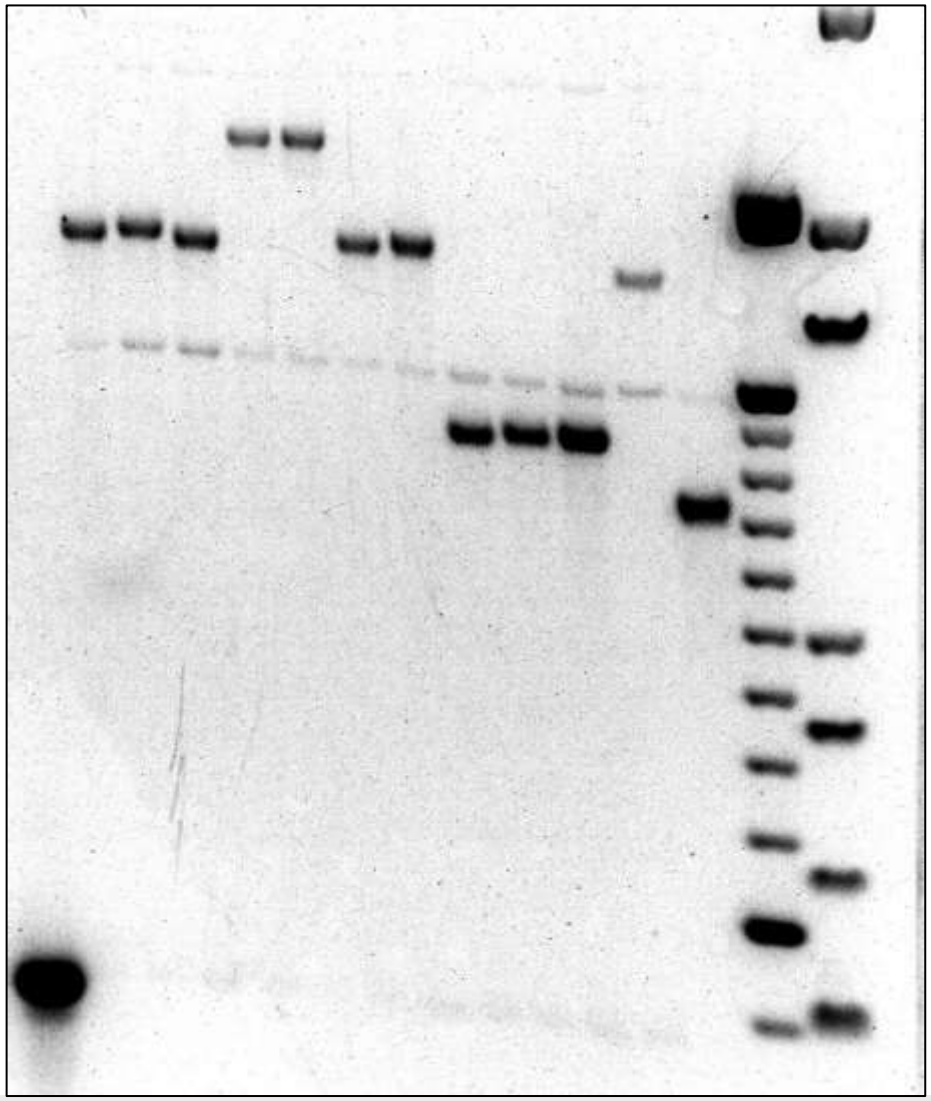
- **D-amino oxidase (DAO) inhibitors** (Takeda, Cambridge) – to decrease cerebellar ataxia

- ***FXN* gene therapy** (Mike Themis, Brunel University London)
- **TAT-Frataxin cell therapy (**Arturo Sala, Brunel University London)
- **Identification of metabolomic-based targets for FRDA therapy** (Sara Anjomani-Virmouni, Brunel University London)

YG8s mutation increases to 410 GAA repeats: YG8L



Mutation further increases to 610 GAA repeats: YG8XL



Our FRDA mice are cryopreserved at The Jackson Laboratory (JAX), Maine, USA, and are distributed by JAX to FRDA investigators worldwide

Acknowledgements:

The Ataxia Group: Sahar Al-Mahdawi, Ricardo Mouro-Pinto, Chiru Sandi, Vahid Ezzatizadeh, Madhu Sandi, Hassan Khonsari, Sara Anjomani Virmouni, Aurelien Bayot, Hajar Mikaeili, Saba Saqlain, Mursal Sherzai, Anastasia Gketsopoulou

Collaborators: Roberto Testi and Florence Malisan, University of Rome Tor Vergata, Gino Cortopassi, UC Davis, Richard Festenstein, Imperial College London, Carlo Marobbio, University of Bari, Jacques Tremblay, Quebec, Mike Themis, Brunel University London..

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