



### **Session 1 (Thursday, 14:00 – 16:00): Clinical Discovery & Impact**

Co-Chairs: Massimo Pandolfo and Mark Payne

#### Oral Presentations

<b>Miriam Cnop</b> (invited)	Pancreatic $\beta$ cell dysfunction and insulin resistance contribute to diabetes in Friedreich's ataxia
<b>Lauren Seyer</b>	Optical Coherence Tomography in Friedreich Ataxia
<b>Louise Corben</b>	Are impairments in planning and online control of movement in Friedreich ataxia an indication of reduced cerebellar-cortico connectivity?
<b>Arnie Koeppen</b>	Friedreich's ataxia: Iron, copper, and zinc in the dentate nucleus
<b>Eric Deutsch</b>	Diagnostic utility of a rapid, noninvasive immunoassay for frataxin in atypical patients
<b>Devin Oglesbee</b>	Development and Validation of a High-Throughput, Quantitative, Luminex Immunoassay for Frataxin in Whole Blood or Dried Blood Spots: An Assay for Newborn Screening, Diagnosis, and Treatment Monitoring
<b>Stéphane Schmucker</b>	Identification of an atypical Friedreich ataxia patient with no GAA expansion but with a homozygous point mutation in the mitochondrial targeting sequence of frataxin

#### Posters

<b>Hamed Akhlaghi</b>	Decreased functional brain activation in Friedreich's ataxia using the Simon effect task
<b>John Anderson</b>	A New Perspective for the Rate of Disease Progression in Friedreich's Ataxia
<b>Claire Bates</b>	A journey from despair to laughter: specialist palliative care in advanced Friedreich's ataxia
<b>Stefania De Mercanti</b>	Anxiety, Depression and Disability in Friedreich's ataxia
<b>Stefania De Mercanti</b>	Evaluating disease progression in Friedreich's ataxia
<b>Joanne Folker</b>	Acoustic measures of the speech disorder associated with Friedreich ataxia: tools for monitoring disease progression and therapy outcomes
<b>Mariana Igoillo-Esteve</b>	Role and Mechanisms of Pancreatic $\beta$ -cell Failure in Diabetes in Friedreich's Ataxia

<b>Arnie Koeppen</b>	Myocardial iron in Friedreich's ataxia: Insights from X-ray fluorescence
<b>Dave Lynch</b>	Frataxin reflects glucose metabolism disorders in Friedreich Ataxia (FA)
<b>RM Pellegrino</b>	Rapid molecular analysis of frataxin transcription and protein
<b>Barbara Polek</b>	Healthcare Resource Utilization of Persons with Friedreich's Ataxia Living in the United States and Canada
<b>Gary Rance (presented by Louise Corben)</b>	Management of listening-in-noise deficits in individuals with Friedreich ataxia
<b>Sean Regner (presented by Dave Lynch)</b>	Single Site Cross-Sectional Analysis of Echocardiograms from Patients with Friedreich Ataxia

## Title: Pancreatic $\beta$ cell dysfunction and insulin resistance contribute to diabetes in Friedreich's ataxia

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Friedreich's ataxia (FA) patients have a high prevalence of impaired glucose tolerance (IGT) and diabetes, the pathogenesis of which remains poorly understood. Our aim was to determine the relative contribution of insulin resistance and pancreatic  $\beta$  cell failure in the pathogenesis of IGT and diabetes in FA.

Forty-one FA patients, 26 carriers and 53 healthy controls not known to have diabetes underwent oral and intravenous glucose tolerance tests. The minimal model-derived insulin sensitivity index ( $S_I$ ) and acute insulin response to glucose ( $AIR_g$ ) were used to calculate the disposition index, a measure of pancreatic  $\beta$  cell function adjusted for prevailing insulin sensitivity.

The FA patients and controls were well matched for age (patients  $36\pm 2$  vs controls  $36\pm 2$  years) and body mass index (patients  $23\pm 1$  vs controls  $25\pm 1$  kg/m<sup>2</sup>). The carriers were older ( $46\pm 3$  years,  $p<0.01$ ) and slightly heavier (body mass index  $26\pm 1$  kg/m<sup>2</sup>,  $p<0.01$  vs patients). Fasting glucose levels were not significantly different in the FA patients (patients  $98\pm 3$  mg/dl vs controls  $93\pm 1$  mg/dl and carriers  $96\pm 2$  mg/dl). However, the 2-hour glucose level during the oral glucose tolerance test was higher in patients compared to controls (patients  $152\pm 7$  mg/dl vs controls  $129\pm 4$  mg/dl,  $p<0.01$ ; carriers  $137\pm 6$  mg/dl). Of the FA patients, 50% had IGT and 10% diabetes, compared to 30% IGT and no diabetes in controls, and 45% IGT and 5% diabetes in carriers.

FA patients were insulin resistant ( $S_I$   $17\pm 2 \times 10^{-5}$  min<sup>-1</sup>/(mU/ml),  $p<0.01$ ) compared to healthy controls and carriers ( $S_I$   $25\pm 2$  and  $26\pm 3 \times 10^{-5}$  min<sup>-1</sup>/(mU/ml), respectively). Under normal physiological circumstances, when insulin resistance develops in an individual it is compensated by increased insulin release by the pancreatic  $\beta$  cells, in order to maintain glucose levels normal. In FA patients, however, insulin resistance was not compensated for by increased  $AIR_g$  (patients  $51\pm 8$   $\mu$ U/ml, controls  $59\pm 8$   $\mu$ U/ml and carriers  $46\pm 10$   $\mu$ U/ml). This resulted in a much reduced disposition index (FA patients  $862\pm 156$  vs controls  $1507\pm 169 \times 10^{-5}$  min<sup>-1</sup>,  $p<0.01$ ) suggestive of  $\beta$  cell failure. The carriers tended to have a lower disposition index ( $1182\pm 136 \times 10^{-5}$  min<sup>-1</sup>,  $p=0.09$ ).

In conclusion, FA patients are insulin resistant without the expected compensatory increase in insulin secretion. This suggests an important role for pancreatic  $\beta$  cell dysfunction in the pathogenesis of IGT and diabetes in FA. The impact of reduced frataxin levels on  $\beta$  cell function and survival is now being evaluated in *in vivo* and *in vitro* models for the disease.

## **Title: Optical Coherence Tomography in Friedreich Ataxia**

Authors: Seyer L, Wilson J, Galetta K, Friedman L, Balcer L, Lynch DR

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### **Abstract**

**Background:** Clinical or subclinical optic neuropathy is found in most people with FRDA, suggesting that the retina may provide a location for measuring neuronal loss. We used Optical Coherence Tomography (OCT) to assess retinal nerve fiber layer (RNFL) thickness in Friedreich Ataxia (FRDA), and its relation to genetic severity and neurological function in FRDA.

**Methods:** OCT was performed on both eyes of FRDA patients to evaluate RNFL thickness and macular anatomy. Most recent Friedreich Ataxia Rating Scale (FARS) and Low Contrast Letter Acuity scores were obtained from the CCRN database. Summary statistics, correlations and linear regressions were calculated using STATA.

**Results:** 43 subjects (53.4% female) with a mean age of 27.9 years participated. Mean length of the shorter GAA allele was 543 repeats (excluding two point mutations). Mean age of onset was 15 years. 55% of eyes from adult subjects (n=30) had RNFL thickness below the 5<sup>th</sup> percentile for age-matched controls. RNFL thickness correlated with disease duration ( $R^2=0.2666$ ). In linear regression analysis, GAA repeat length ( $p=0.019$ ;  $R^2=0.131$ ) and increasing age ( $p=0.006$ ) both independently predicted RNFL thickness. Total low contrast visual acuity also correlated highly with RNFL thickness ( $p=0.0001$ ;  $R^2=0.311$ ), and the contrast letter acuity summary measure of high and low contrast charts using both eyes correlated highly with RNFL thickness ( $p=0.0001$ ;  $R^2=0.316$ ). Average RNFL thickness also correlated with FARS scores ( $p<0.0001$ ;  $R^2=0.429$ ). In one more affected patient, macular imaging demonstrated decreases in the outer plexiform/photoreceptor layer, suggesting that FRDA may also affect retinal cells other than the ganglion cells.

**Conclusions:** RNFL thickness strongly correlates with visual and neurologic function in FRDA, as well as genetic severity and disease progression. This suggests that RNFL thickness is useful as markers of neurologic progression in FRDA. In addition, macular changes suggest the presence of retinal neuronopathy as well as axonopathy in FRDA.

## Title: Are impairments in planning and online control of movement in Friedreich ataxia an indication of reduced cerebellar-cortico connectivity?

Authors: Corben, L.A<sup>1,2</sup>, Georgiou-Karistianis, N<sup>2</sup>, Bradshaw, J.L<sup>2</sup>, Hocking, D.R<sup>1,3</sup>, Churchyard, A.J<sup>4</sup> & Delatycki, M.B<sup>1,5</sup>.

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### Background

We recently suggested people with FRDA may have impairment in cognitive capacity either because of direct cortical pathology or because of pathology affecting the projections from the cerebellum to the cortex (1). To further explore this possibility we designed a movement task incorporating Fitts' Law, a robust description of the relationship between movement time and accuracy in goal directed movements (2). Fitts' Law assumes a log-linear relationship between movement time and task difficulty allowing calculation of an index of difficulty which indicates smaller targets further apart return longer movement times due to increased demands for accuracy. By manipulating task difficulty according to target size and distance we were able to further examine processes related to motor planning in individuals with FRDA.

### Methods

Ten right-handed individuals homozygous for a GAA expansion in intron1 of the *FXN* gene and ten matched control individuals participated in this study. Participants were instructed to draw ten continuous horizontal lines between targets that varied according to size (small, large) and distance (near, far). Kinematic parameters were recorded and submitted to a two-way ANOVA.

### Results

People with FRDA showed a disproportionately greater movement time to targets with increasing levels of difficulty compared to controls [ $F(3,54) = 7.70, p < 0.01$ ]. Kinematic analysis indicated individuals with FRDA spent differential time planning [ $F(3,54) = 4.25, p < 0.05$ ] and in the terminal accuracy phase of movement [ $F(3,54) = 3.75, p < 0.05$ ] as task difficulty increased.

### Discussion

Successful completion of this task requires both *planning of movement and online error detection and correction*. The cerebellum and its connections to frontal regions via cerebro-ponto-cerebello-thalamo-cerebral loops are fundamental to both skills. These results lend further support to our contention that in FRDA these loops are impaired (3), reflecting a failure to access prefrontal/anterior regions necessary for effective management of planning and online control of movement.

### References

1. Corben LA, Delatycki MB, Bradshaw JL, Horne MK, Fahey MC, Churchyard AC, et al. Impairment in motor reprogramming in Friedreich ataxia reflecting possible cerebellar dysfunction. *Journal of Neurology*. 2010;257(5):782-91.
2. Elliott D, Hansen S, Grierson LEM, Lyons J, Bennett SJ, Hayes SJ. Goal-directed aiming: Two components but multiple processes. *Psychological Bulletin*. 2010. DOI:10.1037/a0020958
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## **Title: Friedreich's ataxia: Iron, copper, and zinc in the dentate nucleus**

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Abstract:

**Background/Hypothesis:** Friedreich's ataxia (FRDA) causes progressive atrophy of the dentate nucleus (DN). The normal DN contains abundant iron. Frataxin deficiency impairs iron homeostasis in the DN and triggers oxidative injury. A new non-destructive monochromatic X-ray fluorescence (XRF) technique can "map" and quantify iron and other metals that are known to generate reactive oxygen species.

**Methods:** Fixed DN samples from the autopsies of 7 patients with FRDA and 9 normal controls were embedded in polyethylene glycol (PEG). XRF of iron, copper, and zinc across the physical boundaries of the DN provided distribution and quantitative data. Metalloporphyrins in PEG served as calibration standards. After XRF, tissues were transferred into paraffin for slide techniques, including ferritin immunocytochemistry as a surrogate marker of iron.

**Results:** Iron fluorescence in the normal DN was most intense in the hilar white matter but lower in gray matter. In contrast, peak copper fluorescence occurred in DN gray matter. Zinc distribution was variable. In FRDA, the DN collapsed onto itself, and the separation of iron and copper became less distinct. Concentrations in regions of peak fluorescence, however, did not change. In normal DN, they were, in  $\mu\text{g/ml}$  tissue (mean $\pm$ standard deviation): iron,  $428\pm 296$ ; copper,  $48\pm 27$ ; and zinc,  $43\pm 21$ . Levels in FRDA were: iron,  $396\pm 160$ ; copper,  $45\pm 20$ ; and zinc,  $48\pm 23$ . Immunocytochemical reaction product of ferritin correlated with peak iron XRF, localizing mostly to oligodendroglia. In FRDA, oligodendroglia were unusually small, and ferritin shifted to microglia.

**Conclusions:** FRDA leads to major changes in the distribution of iron, copper, and zinc in the atrophic DN but not to metal depletion. Copper and zinc are neuronal metals whereas iron is concentrated in oligodendroglia and microglia. After release from neurons, copper may be more important than iron in oxidative injury. Redistribution of zinc must be attributed to loss of glutamatergic gluzineric synaptic terminals.

## **Title: Diagnostic utility of a rapid, noninvasive immunoassay for frataxin in atypical patients.**

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### **Background/Hypothesis:**

In this study, we used a lateral-flow immunoassay to measure differences in frataxin levels in buccal cells and whole blood samples from a large cohort of control, known FRDA carriers, and FRDA patients. We also measured frataxin in subjects with "atypical disease", as defined by presence of disease phenotype but with only one identifiable mutation in order to identify new mutations. Our hypothesis is that the lateral flow immunoassay is able to clearly and reproducibly differentiate frataxin levels between controls, carriers, and patients, and may be useful as a complementary diagnostic tool for atypical patients.

### **Methods:**

Frataxin levels were measured via a lateral flow immunoassay (Mitosciences) from whole blood and cheek swab samples collected from controls, carriers, FRDA patients (those with two GAA repeat expansions, or one expansion and one point mutation), and "atypical" patients. Mutation status was evaluated by multiplex ligation-dependent probe amplification (MLPA) analysis. Data analysis was performed using STATA and SAS software.

### **Results:**

MLPA analysis revealed two novel heterozygous deletions in two atypical patients: a deletion of exons 2 and 3 in one patient and an exon 5 deletion in another, widening the known spectrum of FRDA-causing mutations. The immunoassay is therefore not only useful in measuring baseline frataxin levels in large cohorts, but also has some diagnostic capability in assessment of frataxin levels in atypical patients.

In addition to significant deficiencies in carrier and FRDA patient frataxin levels compared to controls (56.1% and 21.5%, respectively), we also measured frataxin from a subset of FRDA patients three times over four weeks and saw a reproducible deficiency relative to controls (coefficient of variation 0.4-16.8%).

### **Conclusions:**

This assay may not only be useful for measuring baseline frataxin levels in cohorts of large, early-phase trials, but it may have diagnostic utility in assessment of frataxin levels in atypical FRDA patients.

## **Title: Development and Validation of a High-Throughput, Quantitative, Luminex Immunoassay for Frataxin in Whole Blood or Dried Blood Spots: An Assay for Newborn Screening, Diagnosis, and Treatment Monitoring**

Authors:

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### **Background:**

A diagnosis of Friedreich ataxia is typically confirmed by DNA-based assays to detect *FXN* GAA-repeat expansions or mutations. However, current DNA-based analyses cannot monitor potential treatment, be multiplexed with tests for other conditions, nor are they efficient for population screening. We hypothesized that a Luminex immunoassay would be suitable for these applications.

### **Methods:**

We designed an xMAP-based assay with an anti-frataxin capture antibody, recombinant human frataxin, and an anti-frataxin detection antibody to measure frataxin levels on a Luminex LX200 in a 96-well format. This assay was validated for 15  $\mu$ L of whole blood (WB) and 3 mm dried-blood-spot (DBS) punches by determining its recovery, accuracy, intra- and inter-assay imprecision, reportable range, reference range (125 adult and 126 pediatric subjects, 38 FA individuals, and 10 FA carriers), as well as pre-analytical variables, such as specimen stability. In addition, we quantified frataxin levels in newborn DBS donated by FA individuals alongside storage-matched controls.

### **Results:**

Mean recovery of frataxin from WB and DBS was 99% (78-112%). Intra-assay imprecision ranged from 4.9-13% CV and inter-assay imprecision ranged from 9.8-15.8% CV. The limit of detection was 0.07 ng/mL and reportable range was 2-200 ng/mL. The reference range for normal adult and pediatric subjects was 15-82 ng/mL (median: 33) for DBS and WB. FA carriers' frataxin levels were 12-22 ng/mL (median: 15) but FA individuals' were lower at: <2-12 mg/mL (median: 4). Retrospective analyses of FA individuals' DBS found frataxin levels at <2-7 ng/mL while controls were 28-110 ng/mL. Frataxin in DBS or WB was stable for over 6 months at ambient, 4 °C and -70 °C.

### **Conclusions:**

We clinically validated a high-throughput, Luminex immunoassay for determining frataxin levels in DBS and WB that is applicable to diagnosis, population screening, and potentially for therapeutic monitoring. Ongoing work includes a prospective newborn screening study for FA.

**Title: Identification of an atypical Friedreich ataxia patient with no GAA expansion but with a homozygous a point mutation in the mitochondrial targeting sequence of frataxin**

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Abstract:

**Background/Hypothesis:** Most Friedreich Ataxia (FRDA) patients are homozygous for an unstable GAA trinucleotide expansion in the first intron of the frataxin gene, leading to a transcriptional impairment. Although rare, a few patients either with typical or atypical clinical presentation are compound heterozygous for the GAA expansion and a point mutation. However, to date, no patient carrying a double point mutation with normal GAA repeat length has ever been reported.

**Methods/Results:** We report the identification of a patient carrying a homozygous point mutation leading to a missense mutation in the mitochondrial targeting signal of the frataxin protein. The patient presents a progressive neurological phenotype compatible with FRDA diagnosis although with an atypical clinical presentation characterized by mild generalized motor deficit with amyotrophy and without neuropathy. Frataxin levels were reduced in the patients' muscle and fibroblasts. In cellulo, we demonstrated that the mutation impairs the mitochondrial targeting of frataxin leading to a reduced level of mature frataxin and the pathologic consequences associated to frataxin deficiency.

**Conclusions:** Together, our results identified the first patient carrying a point mutation on both alleles that causes FRDA by mimicking the physiological consequences of the GAA expansion. This case emphasizes that atypical phenotype due to mutation in the frataxin gene may be under-recognized. We propose that frataxin gene sequencing should be more often performed in cases with isolated first motor neurodegeneration even if pure sensitive neuropathy is lacking.

## **Title: Decreased functional brain activation in Friedreich's ataxia using the Simon effect task.**

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### **Background**

This study applied the Simon effect task, a well-known conflict resolution task, to examine the pattern of functional brain reorganization in individuals with Friedreich ataxia (FRDA) using functional magnetic resonance imaging (fMRI). We hypothesised that in line with previous studies (1,2) disruption of cortico-cerebellar loops in individuals with FRDA would likely diminish access to cortical regions necessary for effective management of executive processing, and would result in different patterns of activation reflecting a compensatory functional mechanisms.

### **Methods**

Thirteen right handed individuals homozygous for a GAA expansion in intron 1 of the *FXN* gene, and fourteen matched controls participated. Participants pressed a button corresponding with the direction of an arrow on a computer screen while undergoing fMRI scanning. The task involved congruent stimuli (e.g. right pointing arrow on right side of screen) or incongruent stimuli (e.g. right pointing arrow on left side of screen).

### **Results**

There was a significant [ $F(1,26) = 6.68, p < 0.05$ ] and disproportionately greater difference between reaction time to congruent and incongruent stimuli in people with FRDA (275ms) compared to controls (162ms). For the Simon effect controls showed significantly *increased* bilateral activation in comparison with individuals with FRDA in regions including the superior, middle and inferior prefrontal cortices, superior temporal gyri, lateral occipital cortices, caudate nuclei, and in the right inferior parietal lobule, left insulae and left lobule V and VI of the cerebellum, with no areas significantly more activated in individuals with FRDA than in controls. Individuals with FRDA showed a reduced network of activation.

### **Discussion**

The greater Simon effect behaviourally in individuals with FRDA, compared with controls, together with concomitant reductions in functional brain activation, suggests an ineffective engagement of the attention/executive function network required for response suppression suggesting lessening of cerebellar articulation of these areas and no compensatory increases of activation of other brain regions.

### **References**

1. Corben LA, Delatycki MB, Bradshaw JL, Horne MK, Fahey MC, Churchyard AC, et al. Impairment in motor reprogramming in Friedreich ataxia reflecting possible cerebellar dysfunction. *Journal of Neurology*. 2010;257(5):782-91.
2. Akhlaghi H, Corben LA, Georgiou-Karistianis N, Bradshaw JL, Storey E, Delatycki MB, et al. Superior Cerebellar Peduncle Atrophy in Friedreich's Ataxia Correlates with Disease Symptoms. *Cerebellum*. 2010. DOI: 10.1007/s12311-010-0232-3

## **Title: A New Perspective for the Rate of Disease Progression in Friedreich's Ataxia**

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Abstract:

**Background/Hypothesis:** Changes in the clinical scores of the neurological state of Friedreich's ataxia over short time periods have been used to characterize the rate of change in FRDA. However, there is no overall framework that could be used to track changes from early to late in the disease process and to account for different rates in different subjects. This study addresses that.

**Methods:** The neurological status of 165 FRDA subjects was evaluated with the Friedreich's Ataxia Rating Scale. The composite score and subscores were analyzed as a function of the duration of symptoms with the Gompertz function. The values for the Gompertz "slope" parameter for each FARS score were used to classify the data into clusters. The number of clusters that gave the greatest value for the average distance from a given point in one cluster to all points in another cluster was identified. A scatter plot of FARS scores vs. duration of symptoms was partitioned on the basis of the clusters.

**Results:** The analysis provided evidence for 5 distinct groups. Each was characterized by a different Gompertz function. The asymmetry of the derivative describes a greater change in disease severity early in the disease, compared to later in the life. There was a seven-fold difference in the peak values for rate of change across the 5 groups.

**Conclusions:** The sigmoid representation for disease progression in FRDA provides a framework for tracking changes in the neurological state over time. The future time course for disease progression, given the current state, can be predicted. The model could be used to (a) evaluate the efficacy of treatments based on changes in the rate of progression (compared to the model-predicted natural time course) and (b) help identify common factors among those within the same group which might possibly modify the underlying disease process.

**Title: A journey from despair to laughter: specialist palliative care in advanced Friedreich's ataxia**

Authors: Dr Claire Bates MA (Cantab) MBBS FRCP, Ms Claire Edwards RGN, Ms Leanne Smith, Prof Leslie Findley MD FRCP, Dr Rajith De Silva MD FRCP

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Abstract:

**Background/Hypothesis:** With the inevitable progression of their neurological disabilities, Friedreich's ataxia (FRDA) patients face significant challenges to their quality of life with advancing disease. The aim of palliative care is the prevention and relief of suffering by means of assessment and focused treatment across physical, psychosocial and spiritual domains. FRDA patients meet eligibility criteria for referral to specialist palliative care (SPC) services, and these services may have a crucial role in their management.

**Methods:** Observational single case study of genetically confirmed FRDA; university teaching hospital and community palliative care settings.

**Results/ Case study:** A 37-year-old female with FRDA was referred by her neurologist in 2008 for SPC assessment. Symptoms had first appeared at 14, a clinical diagnosis made at 17 and genetically confirmed at 31. Mobility was increasingly limited, and wheelchair-dependency was imminent. Referral was precipitated by the patient's decision to join the assisted suicide organisation "Dignitas", and the start of discussions about end-of-life issues. The patient was initially reviewed by a hospital palliative care specialist but later referred for local hospice involvement.

The "journey" from referral to present day from the different perspectives of patient, neurologist and palliative care specialists are described. The patient had preconceptions about hospice care, but later commented "How wrong can a person be?" Hospice staff with no previous experience of FRDA also reported initial professional anxieties. The subsequent shared journey highlights the role of the SPC multidisciplinary team, and the improved quality of life resulting from early hospice involvement. An alternative to assisted suicide emerges for the patient as confidence in palliative care services grows.

**Conclusions:** SPC may have a vital role to play in quality of life improvement for patients with advanced FRDA, and in providing support to face difficult end-of-life issues. Systematic appraisal of this intervention is warranted.

## **Title: ANXIETY, DEPRESSION AND DISABILITY IN FRIEDREICH'S ATAXIA**

Authors: Stefania De Mercanti\*, Marco Iudicello\*, Roberto Ferri\*, Filomena Longo§, Antonio Piga§ e Luca Durelli\*

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### Abstract

**Background/Hypothesis:** Friedreich's ataxia (FRDA), is the most frequent inherited ataxia, characterized by progressive ataxia, dysarthria and cardiomyopathy. The onset of symptoms usually occurs around puberty, although it may start in childhood or in adulthood. The development often results in significant impairment and disability by the late teenage years. The impact of FRDA on life can be dramatic and affect the development of personality. Presently there are still not proven therapies available to slow disease progression: the objective is relieve symptoms, prevent complications, and optimize quality of life.

The objective is to correlate the development of anxiety and depression with the level of disability.

**Methods:** we followed for two years, a cohort of 16 patients with FRDA, (six female), with a mean age of 12.43 at onset, 16.56 years at diagnosis and 25.26 at first visit at our Center. 11 patients (68.75%) use wheelchair (mean 10.22 years after onset). Disease severity was measured by the FARS and SARA scales; the degree of disability in daily life was measured with the Barthel index, the ADL and IADL scales. Anxiety and depression were evaluated with HADS scale.

**Results:** average scores range at second year of evaluation of FARS, SARA, Barthel index, ADL, IADL and HADS scales were respectively 55.29 (26-83), 19.75 (5-30), 65 (25-100), 4.14 (1-6), 5.57 (3-8), HADS-Anxiety 5.31(0-12), HADS-Depression 3.77 (0-7). The comparison tests show the prevalence of anxiety on depression (not statistically significant), particularly in wheelchaired patients (6 vs 3.75,  $p=0.12$ ). We did not observed a significant relation between HADS and disability ( $p=0.40$ )

**Conclusions:** the anxiety component prevails on depression, but only two subjects have a pathological level; there is not a significant association between anxiety, depression and disability. It is useful to provide, regardless of the degree of disability, psychological support from early stages of the disease.

## Title: EVALUATING DISEASE PROGRESSION IN FRIEDREICH'S ATAXIA

Authors: Stefania De Mercanti\*, Marco Iudicello\*, Roberto Ferri\*, Filomena Longo§, Antonio Piga§ e Luca Durelli\*

Institutions: \*SCDU Neurologia, AOU San Luigi Gonzaga, Facoltà di Medicina di Orbassano, Università di Torino, Italy; §SCDU Microcitemie, AOU San Luigi Gonzaga, Facoltà di Medicina di Orbassano, Università di Torino, Italy

Corresponding author email address: [sdemercanti@yahoo.it](mailto:sdemercanti@yahoo.it),

### Abstract

**Background/Hypothesis:** Friedreich's ataxia (FRDA) is the most frequent inherited ataxia, characterized by progressive ataxia, dysarthria and cardiomyopathy. The onset usually occurs around puberty, although it may start in childhood or adulthood. The progression leads to disability in some years. The efficacy assessment of new drugs requires sensitive tools able to quantify small changes in the clinical status of FRDA patients.

Our objective is to evaluate the capacity of the current scales to quantify the disease progression.

**Methods:** we analyzed a cohort of 16 patients with FRDA, (six female), with a mean age of 12.43 at onset, 16.56 years at diagnosis and 25.26 at first visit at our Center. Eleven patients (68.75%) needed wheelchair (mean 10.22 years after onset). Disease severity was assessed by FARS and SARA scales; disability degree was assessed by Barthel index, ADL and IADL scales.

**Results:** average scores and range of FARS, SARA, Barthel index, ADL, and IADL scales were respectively: 55.29 (26-83), 19.75 (5-30), 65 (25-100), 4.14 (1-6), 5.57 (3-8). The mean disease duration was 14.06 years (2-24). Age at onset correlates inversely with the degree of disability in all functional scales (Barthel  $p=0.03$ ; ADL  $p=0.045$ ; IADL  $p=0.02$ ), but not with neurological rating scales (FARS  $p=0.12$ ; SARA  $p=0.38$ ). Both disability and neurological rating scales did not correlate with disease duration.

**Conclusions:** inverse correlation between age at onset and disability expresses the increased probability of achieving more severe disease stages in early onset, whereas lack of correlation with neurological scales shows the limits of current scales in advanced stages of FRDA (plateaux effect). This effect may explain also the lack of correlation of neurological and functional evaluation to illness duration. New tools are needed for integrated assessment correlating the degree of neurological deficit to disability they determine (weighing scale) and taking into account aggravating or complicating conditions to better characterize the stage of disease.

**Title: Acoustic measures of the speech disorder associated with Friedreich ataxia: tools for monitoring disease progression and therapy outcomes.**

Authors: Joanne Folker, Kristin Rosen, Bruce Murdoch, Martin Delatycki, Louise Corben, Adam Vogel

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**Background/Hypothesis:** Measures of the change in speech function are expected to be useful for monitoring functional neurological degeneration associated with FRDA. The objective of this study is to identify a combination of acoustic measures that are sensitive to change in the dysarthria over time. Acoustic measures hold rich potential as clinical assessment tools as they are convenient, objective measures of speech. Due to the heterogeneity of the dysarthria associated with FRDA, acoustic measures that capture a broad range of dysarthric symptoms are expected to be the most sensitive to the disease. Measures of change in the spectra and pause duration are global estimates of speech function. They are unique in that they are calculated automatically and can be applied to conversational or functional speech that has variable phonetic content.

**Methods:** Speech samples from 37 individuals with FRDA and 17 healthy controls were recorded during one structured and one unstructured speaking task. Two measures of spectral variation (rate of change and degree of change) and a measure of pause duration were applied to the speech recordings.

**Results:** The results showed that pauses and rate of spectral change together were able to differentiate between speakers with FRDA and healthy controls. Based on this differentiation, these measures are expected to detect change in the dysarthria over time.

**Conclusions:** It is anticipated that these acoustic measures will be useful and practical tools to quantify the decline in speech function over time. Appropriate measures of the effects of the disease will enable accurate measurement of the clinical benefits or otherwise of therapeutic intervention.

## Title: ROLE AND MECHANISMS OF PANCREATIC $\beta$ -CELL FAILURE IN DIABETES IN FRIEDREICH'S ATAXIA

Authors: Mariana Igoillo-Esteve<sup>1</sup>, Yasmina Serrouk<sup>1</sup>, Myriam Rai<sup>2</sup>, Chantal Depondt<sup>2</sup>, Audrey Begu<sup>3</sup>, Anyishaï E. Musuaya<sup>1</sup>, Laurence Ladrière<sup>1</sup>, Fabrice Moore<sup>1</sup>, Piero Marchetti<sup>4</sup>, Massimo Pandolfo<sup>2</sup>, Décio L. Eizirik<sup>1</sup>, Françoise Féry<sup>3</sup>, Miriam Cnop<sup>1,3</sup>

Institutions: <sup>1</sup>Laboratory of Experimental Medicine, <sup>2</sup>Laboratory of Experimental Neurology, Université Libre de Bruxelles, <sup>3</sup>Division of Endocrinology, Erasmus Hospital, Brussels, Belgium, <sup>4</sup>Department of Endocrinology and Metabolism, Metabolic Unit—University of Pisa, Pisa, Italy.

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Abstract: 300 word maximum

**Background/Hypothesis:** The pathogenic mechanisms of diabetes are insulin resistance and insulin deficiency. We evaluated the relative contribution of insulin resistance and pancreatic  $\beta$ -cell dysfunction in diabetes in Friedreich's ataxia (FA) and the mechanisms involved.

**Methods:** 41 FA patients and 53 controls, not known to have diabetes, underwent oral and IV glucose tolerance tests. The insulin sensitivity and acute insulin response to glucose (AIRg) were used to calculate the disposition index (DI), a measure of  $\beta$ -cell function adjusted for prevailing insulin sensitivity. The impact of frataxin (Fx) deficiency on  $\beta$ -cell function/survival was studied in KIKO mice and by RNAi in rat  $\beta$ -cells and human islets, treated with oleate (OL) or the endoplasmic reticulum (ER) stressor brefeldin (BR), alone or combined with forskolin (FK).

**Results:** 50% of the FA patients had impaired glucose tolerance and 10% diabetes, compared to 29% and 0% in controls. Patients were insulin resistant but this was not compensated for by the expected increase in AIRg, resulting in markedly reduced DI (FA  $862 \pm 156$  vs controls  $1507 \pm 169 \times 10^{-5} \text{ min}^{-1}$ ,  $p < 0.01$ ) indicating  $\beta$ -cell failure. KIKO mice were insulin resistant and developed impaired  $\beta$ -cell function after 6-month high fat diet. KIKO  $\beta$ -cells showed ER dilation suggestive of ER stress. Fx knockdown by 40-50% increased apoptosis in rat  $\beta$ -cells and human islets (siFx  $27 \pm 1\%$  vs control siRNA (siCT)  $16 \pm 2\%$ ;  $n=4-6$ ,  $p < 0.05$ ). Fx knockdown sensitized rat  $\beta$ -cells to OL and BR (siFx OL  $19 \pm 4\%$  and BR  $43 \pm 2\%$  apoptosis vs siCT  $9 \pm 2\%$  and  $17 \pm 5\%$ , respectively;  $n=4-6$ ,  $p < 0.05$ ). This was partially prevented by FK (siFx OL+FK  $13 \pm 4\%$  and BR+FK  $22 \pm 4\%$ ;  $n=4-8$ ,  $p < 0.05$ ).

**Conclusions:**  $\beta$ -Cell dysfunction plays an important role in diabetes in human FA. KIKO mice also develop  $\beta$ -cell failure after high fat feeding. This may be due to metabolic stress-induced  $\beta$ -cell apoptosis, a process potentially mediated by ER stress. cAMP inducers are protective and may have therapeutic potential.

## **Title: Myocardial iron in Friedreich's ataxia: Insights from X-ray fluorescence**

Authors: R. Liane Ramirez, M.S., Devin Yu, B.S., Jiang Qian, M.D., Ph.D., and Arnulf H. Koeppen, M.D.

Institutions: VA Medical Center, Albany, N.Y., USA and Departments of Neurology and Pathology, Albany Medical College

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Abstract:

### **Background/Hypothesis:**

Cardiomyopathy is the most common cause of death in Friedreich's ataxia (FRDA) and has been attributed to iron-mediated oxidative injury. Routine histochemistry, however, reveals the metal only in a small percentage of cardiomyocytes, and direct chemical assay of extracts yields normal total iron concentrations. A new physical method, X-ray fluorescence (XRF), provides unbiased evidence that myocardial iron is elevated in FRDA and more widely distributed than disclosed by histochemistry.

### **Methods:**

Samples of left and right ventricular walls, interventricular septum, and sinoatrial and atrioventricular nodes of FRDA patients and normal controls were infiltrated with polyethylene glycol (PEG) and scanned for iron, zinc, and copper fluorescence. XRF generated metal "maps" and allowed quantitative analysis of iron and zinc by reference to calibration standards. Tissue samples were transferred into paraffin for subsequent immunocytochemistry of ferritin.

### **Results:**

Two patterns of iron and zinc distribution emerged in FRDA *and* normal controls: diffuse low background signal and regionally elevated fluorescence. Concentrations in the diffusely emitting regions were 67-87  $\mu\text{g/ml}$  tissue for iron and 8.5-19  $\mu\text{g/ml}$  for zinc. FRDA did not differ from controls. FRDA myocardium also showed large confluent regions with maximum iron and zinc levels of 150-237  $\mu\text{g/ml}$  and 16-53.7  $\mu\text{g/ml}$ , respectively. The metals showed partial co-localization. In higher-emitting areas of normal controls, maximum levels of iron were 24-114  $\mu\text{g/ml}$ . Zinc levels remained unchanged. Copper levels were very low. Subsequent ferritin immunocytochemistry of the regions with high iron levels showed the most intense reaction product in the cells of the endomysium in FRDA and controls. In FRDA, however, ferritin was also very prominent in the sarcoplasm of cardiomyocytes.

### **Conclusions:**

X-ray fluorescence technology confirms regional iron and zinc excess in the hearts of patients with FRDA. Intense ferritin reaction product in cardiomyocytes suggests that most of the iron excess in FRDA occurs in the contractile tissue of the heart.

## **Title: Frataxin reflects glucose metabolism disorders in Friedreich Ataxia (FA)**

Authors: Willi S, Deutsch E, Brigatti K, Kucheruk O, Kanhere M, Minnock P, Ratcliffe S ,  
Sciascia T, Lynch D

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Abstract:

**Background/Hypothesis:** Friedreich ataxia (FA) is a neurodegenerative disorder caused by reduced amounts of the mitochondrial protein frataxin. FA is also associated with certain significant systemic complications. One of the most common, but poorly characterized features of this disease is an abnormality in glucose metabolism which contributes to the development of diabetes at a young age. We attempted to examine the relationship between frataxin (as measured by a rapid, noninvasive immunoassay of buccal swabs or whole blood samples) and various disease characteristics related to glucose metabolism.

**Methods:** As part of a clinical trial to evaluate the effects of an investigational medication on glucose metabolism in FA, we evaluated 35 adults diagnosed with FA between the ages of 5 and 47 years. This was a relatively unselected sample, though subjects were excluded from participation if they were known to have diabetes. Parameters assessed included: age at diagnosis, duration of disease, number of GAA repeats, mitochondrial frataxin and a number of indicators of glucose metabolism, including an evaluation of insulin production and insulin resistance from a standard 75 gram oral glucose tolerance test (OGTT). Parameters expected to relate to disease severity were assessed through spearman correlation.

**Results:** Genotype-phenotype interactions were evident from our sample, with clear associations between the number of GAA repeats on the smaller allele and age at diagnosis, ( $r=-0.77$ ;  $p<0.001$ ) as well as insulin production ( $r=0.44$ ;  $p<0.01$ ) and insulin sensitivity ( $r=-0.39$ ;  $p=0.02$ ) from the OGTT. Frataxin demonstrated very high correlation to numerous parameters reflecting disease severity; including: age at diagnosis ( $r=0.83$ ;  $p<0.001$ ), number of GAA repeats ( $r=-0.75$ ;  $p<0.001$ ), insulin production ( $r=-0.81$ ;  $p<0.001$ ) and insulin sensitivity ( $r=0.74$ ;  $p<0.001$ ).

**Conclusions:** Frataxin levels reflect disease severity and the level of disruption in cellular processes, including glucose metabolism. Frataxin may be useful in predicting the progression toward diabetes in FA patients

## **Title: RAPID MOLECULAR ANALYSIS OF FRATAXIN TRANSCRIPTION AND PROTEIN PRODUCTION**

Authors: Pellegrino RM, Longo F, Palmieri A, Piga A and Roetto A

Institutions: University of Torino, Department of Clinical and Biological Sciences, AOU san Luigi Gonzaga, Italy

Friedreich's ataxia (FRDA OMIM 229300) is an autosomal recessive neurodegenerative disease affecting 1 in 50.000 people. The most common causative FRDA mutation is the unstable hyperexpansion of an intronic GAA repeat in the frataxin gene that impairs RNA transcription and reduced protein production. The exact function of frataxin is still unknown and currently no treatment is available.

Up to now, traditional assays to investigate frataxin levels *in vivo* consist in the maintenance in culture of the cells followed by quantitative PCR and/or Western Blot, a quite long procedure.

In this study, qPCR and Western Blot were used to evaluate frataxin RNA and protein levels through a RNA/protein extraction directly from peripheral blood mononucleate cells of FRDA patients, carriers and health controls. Set-up experiments were performed on 10 health subjects and then FRDA patients and their respective relatives were tested for frataxin levels with both RT qPCR and WB. Overall, four families were analyzed with 5 people affected and 8 carriers. The experiments were repeated with different blood samples to evaluate reproducibility of the protocols. In all the affected subjects the levels of both FX RNA and protein were significantly lower (<30%) than respective carriers (40-70%) compared to health controls (stated as having 100% FRDA production), as expected. Although data were preliminary, a correlation between Frataxin RNA/protein amount and patients phenotype was evidenced.

Today there's no therapy able to treat this pathology but in the near future there will be a high demand for rapidly measuring frataxin levels due to the development of therapeutic strategies for FRDA based on manipulating frataxin expression levels *in vivo*. FRDA RNA and protein analysis directly from fresh blood samples could be an effective methodology to decrease time of diagnosis and contamination or loss of sample risks due to *in vitro* culture of patients and relatives cells.

# **Title: Healthcare Resource Utilization and Costs of Persons with Friedreich's Ataxia Living in the United States**

Authors: Barbara Polek, William T Andrews

Institutions: Santhera Pharmaceuticals

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## **Abstract**

### **Background/Objective:**

Friedreich's Ataxia (FRDA) is a rare inherited, debilitating neurodegenerative disease affecting an estimated 10,000 persons in the US. There is limited knowledge about the healthcare cost implications and the cost drivers of FRDA. The objective of the retrospective observational cross-sectional study carried out in the US was to quantify and compare the costs of healthcare resources utilized by FRDA patients.

### **Study Population/Methodology:**

197 persons with diagnosed FRDA were recruited from the patient registry of the Friedreich's Ataxia Research Alliance (FARA). A questionnaire was used to quantify the utilization of products and services of 11 healthcare components during a 12-month period. Cost rates were mainly sourced from Medicare and Red Book, and payer share data from the US HHS Medical Expenditure Panel Survey. Descriptive statistics were applied to characterize total costs and cost shares by the patients and by third-party payers. Regression analysis was applied to assess severity of disease as a cost driver and to test the difference of healthcare costs of persons with FRDA and of adults with two or more chronic conditions.

### **Results/Conclusion:**

In 2010 mean healthcare costs of the adult FRDA population (95% CI: 8,458 - 18,307 USD) were clearly higher compared to 2005 mean healthcare costs of US adults with two or more chronic conditions (95% CI: 4,266 - 4,876 USD). The mean costs of total healthcare services were significantly higher for the severely affected persons than for the less affected persons with FRDA ( $p < 0.01$ ), with the main driver of this difference being paid homecare ( $p < 0.01$ ). Public / private payer shares for FRDA implied healthcare costs (34% / 66%) followed the overall trend in the US (28% / 72%). Measures to reduce or defer the need for paid homecare will alleviate the burden of disease on affected persons and the financial impact on the healthcare system.

**Title: Management of listening-in-noise deficits in individuals with Friedreich ataxia.**

Authors: Rance G, Corben LA, Du Bourg E, King A & Delatycki MB

Institutions: The University of Melbourne, Dept of Otolaryngology  
Murdoch Childrens' Research Institute

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**Background/Hypothesis:** Understanding speech in background noise is a consistently reported problem in individuals with Friedreich ataxia. Recent surveys of auditory function have found that every individual with FRDA (even those relatively early in the disease process) show some degree of perceptual deficit in everyday listening conditions (Rance et al., 2008; 2010).

**Methods:** Ten individuals with FRDA underwent a battery of monaural & binaural speech perception tests and their results were compared with a cohort of matched controls. The FRDA individuals were then fit with personal FM-listening devices and evaluated over a six-week trial period.

**Results:** Speech perception was significantly impaired with FRDA listeners typically only able to access around 50% of the information available to their normal peers. Furthermore, results on a hearing disability questionnaire (Abbreviated Profile of Hearing Aid Benefit [APHAB]) revealed that the FRDA subjects considered their day-to-day listening to be impaired in a wide range of communication circumstances. The mean overall APHAB score (representing the proportion of situations where individuals perceived a difficulty) for this group was  $40.2 \pm 21.1\%$  whereas for the control group, the mean score was only  $8.3 \pm 4.5\%$  ( $P < 0.001$ ).

FM device use produced significant listening and communication improvements for the FRDA cohort. Mean speech perception score (CNC words) increased from  $42.5 \pm 21.1\%$  in the unaided condition to  $69.1 \pm 18.1\%$  in the aided (FM) condition ( $P < 0.001$ ). Furthermore, APHAB scores obtained across the six week trial period, improved from  $39.2 \pm 11.9\%$  in the unaided condition to  $18.8 \pm 6.8\%$  in the aided condition ( $P < 0.001$ ).

**Conclusions:** Functional hearing in everyday listening situations is significantly affected in individuals with FRDA. FM-listening devices, by improving the signal-to-noise ratio at the listener's ear, do however, offer a viable management option.

## **Title: Single Site Cross-Sectional Analysis of Echocardiograms from Patients with Friedreich Ataxia**

Authors: Regner S., Lagedrost, S., Paulsen E., Schadt K., Friedman, L., Seyer, L., Plappert, T., Lynch, DR., St John Sutton, M.

Institutions: Children's Hospital of Philadelphia and the University of Pennsylvania

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**Background/Hypothesis:** While Friedreich's Ataxia (FRDA) is associated with cardiomyopathy, the factors predicting its severity and evolution are poorly understood. In this study, echocardiograms were assessed at a central reading site to investigate the progression of cardiac disease in FRDA.

**Methods:** The most recent echocardiograms from 173 subjects with FRDA were read at a central site. STATA (Ver.10) was used to perform multivariate analysis of disease duration, age of onset, functional disability score, Friedreich Ataxia rating scale (FARS), and repeat length with echocardiogram observations.

**Results:** The mean age of the cohort was  $19.7 \pm 11.6$  years, age of onset was  $10.1 \pm 5.9$  years, duration was  $9.0 \pm 7.7$  years and shorter GAA repeat length was  $686 \pm 181$ . Echocardiograms among this cohort collectively illustrated systolic dysfunction, diastolic dysfunction and hypertrophy. Measures of size and hypertrophy (IVST, PWT) correlated well ( $r > 0.7$ ), but had only moderate correlation with measures of diastolic dysfunction ( $r = 0.2-0.5$ ). The converse was also true, as diastolic measures correlated better with each other ( $r = 0.4-0.6$ ) than with measures of size or hypertrophy. Even though systolic function was decreased in 20-24% of the cohort, measures of systolic function did not reveal a relationship with either diastolic or size measures. In linear regression analysis, increasing age commonly predicted diminishing ejection fraction, reduced ventricular size, and some indices of diastolic dysfunction. In contrast, GAA length predicted only IVST, with few other measures being related to GAA length. FARS score failed to predict cardiac measures when age, sex and GAA length were accounted for.

**Conclusions:** Hypertrophy, diastolic and systolic dysfunction all occur in FRDA and are substantially independent. Increasing age was predictive of systolic dysfunction and loss of hypertrophy, which likely reflects fibrosis, the fundamental progressive component of FRDA-related cardiomyopathy. While GAA length predicted few measures, this may reflect loss to follow-up of severely affected individuals with long repeat length.

## **Session 2 (Thursday, 16:00 – 18:00): Pathways impacted in FA**

Co-Chairs: Pierre Rustin & Tracey Rouault

### Speakers

<b>Armando Moreno-Cermeño</b>	Metabolic rearrangements in conditional yeast frataxin mutant cells
<b>Marcia Haigis</b>	Role of mitochondrial SIRT3 in Friedreich Ataxia
<b>Robert Schoenfeld</b>	Behavioral, oxidative and neuritic defects and microarray of the Friedreich's Ataxia Mouse Model YG8
<b>Gregory Wagner</b>	Cardiac mitochondrial proteins are hyperacetylated in a mouse model of Friedreich's Ataxia
<b>Alain Martelli</b> (invited)	Consequences of Iron Regulatory Protein 1 activation in mouse models of Friedreich's ataxia
<b>Marek Napierala</b> (invited)	Crosstalk between microRNAs and iron metabolism in pathogenesis of Friedreich's ataxia.

### Posters

<b>Aurélien Bayot</b>	The vicious circle hypothesis questioned in Friedreich ataxia
<b>Florent Colin</b>	Identification of new potential Iron-Sulfur Cluster Proteins through a Bioinformatic Approach
<b>Gino Cortopassi</b>	Alterations in Thiredoxin-related antioxidants in Friedreich's ataxia animal and cell models and therapeutic screening
<b>José Luis García-Giménez</b>	Differential expression of PGC-1 $\alpha$ and metabolic sensors suggest age-dependent induction of mitochondrial biogenesis in Friedreich ataxia fibroblasts
<b>Elia Obis</b>	Metabolic rearrangements in a cardiac cellular model of Friedreich Ataxia
<b>Renata Santos</b>	Antioxidant defense and metacaspase are implicated in cell death in yeast frataxin-deficient cells
<b>Dominika Sliwa</b>	Inactivation of mitochondrial aspartate aminotransferase contributes to the deleterious phenotypes in frataxin-deficient yeast cells

## **Title: Metabolic rearrangements in conditional yeast frataxin mutant cells**

Authors: Armando Moreno-Cermeño, Èlia Obis, Joaquim Ros and Jordi Tamarit

Institutions: Departament de Ciències Mèdiques Bàsiques, Facultat de Medicina, IRB-Lleida, Universitat de Lleida, Spain

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Abstract:

**Background/Hypothesis:** In a previous work, we analyzed the primary effects of suppressing the expression of the frataxin homologue in yeast using a conditional *YFH1* mutant (Moreno-Cermeño et al., *J Biol Chem.* 285:41653, 2010). We observed that inactivation of aconitase, an iron-sulfur enzyme, occurred long after the iron uptake system was activated. We also observed a decrease in oxygen consumption which could be the consequence of respiratory failure. In the present work we have performed transcriptomic and proteomic analysis of such conditional *Yfh1* mutant in order to identify novel targets affected by *Yfh1* depletion.

**Methods:** We used a conditional mutant in which *YFH1* expression was placed under the control of a *tetO<sub>7</sub>* promoter. This mutant was able to grow on glycerol in the absence of doxycycline (*YFH1* expressed) and experienced most of the defects found in  $\Delta yfh1$  cells after doxycycline addition (*YFH1* repressed). We analysed the expression profile of *tetO<sub>7</sub>-YFH1* cells by microarray analysis 4 and 10 hours after doxycycline addition. Proteomic analysis were performed by two-dimensional electrophoresis 14 hours after doxycycline addition.

**Results:** Transcriptomic analysis revealed that nearly 150 genes experienced changes in their expression levels after *Yfh1* depletion. Up-regulated genes included proteins involved in iron acquisition, aminoacid metabolism, glucose catabolism and stress response. Down-regulated genes included many glucose-repressed genes. Down-regulation of such group of genes was confirmed by the proteomic analysis. Interestingly, most of the down-regulated genes are dependent on *ADR1*, a key metabolic regulator which control carbon utilization in yeast.

**Conclusions:** Yeast lacking *Yfh1* experience a dramatic metabolic rearrangement which may promote the respiratory failure observed in *Yfh1* deficient cells. This rearrangement may be governed by key metabolic regulators such as *Adr1*. The precise pathway which triggers *Adr1* inactivation in *Yfh1* lacking cells, remains unknown.

## **Title: Role of mitochondrial SIRT3 in Friedreich Ataxia**

Authors: Seung Min Jeong, Hélène Puccio and Marcia Haigis

Institutions: Harvard Medical School

Corresponding author email address: Marcia\_haigis@hms.harvard.edu

Abstract:

### **Background/Hypothesis:**

Sirtuins are a highly conserved family of NAD<sup>+</sup>-dependent deacetylases and ADP-ribosylases with various roles. Mammalian cells have seven sirtuins (SIRT1-7). SIRT1 and SIRT3 reduce cellular oxidative stress and protect against a number of age-related phenotypes.

### **Methods:**

Recently, mammalian cell models for Friedreich Ataxia (FRDA) were established by Helene Puccio. These mutant cells expressed a human frataxin (hFXN) cDNA carrying missense mutations presenting a classical (I154F) and atypical (G130V) FRDA clinical phenotypes. To examine the role of SIRT1 and SIRT3 in FRDA, we overexpressed these sirtuins in the frataxin mutated cells.

### **Results:**

Overexpression of SIRT1 has little effects on mitochondrial reactive oxygen species (ROS) production and proliferation of the frataxin mutant cells. Interestingly, the FRDA-like phenotypes of the hFXN<sup>I154F</sup> mutant cells were partially rescued through transgenic expression of SIRT3. Increased SIRT3 decreases mitochondrial ROS production and rescues proliferation defect of the cells.

### **Conclusions:**

These findings indicate that SIRT3 might be a new target for developing pharmacological intervention for treating frataxin deficiency.

## **Title: Behavioral, oxidative and neuritic defects and microarray of the Friedreich's Ataxia Mouse Model YG8**

Authors: Robert A. Schoenfeld<sup>1</sup>, Yuxi Shan<sup>1</sup>, Eleonora Napoli<sup>1</sup>, Alexey Tomilov<sup>1</sup>, Tasuku Akiyama<sup>2</sup>, Mirela Iodi-Carstens<sup>2</sup>, Earl E. Carstens<sup>2</sup>, Mark A. Pook<sup>3</sup> and Gino A. Cortopassi<sup>1</sup>

Institutions: Univ. California, Davis  
Brunel University, Uxbridge, UK

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Abstract:

**Background/Hypothesis:** The YG8 mouse model of Friedreich's ataxia carries the expanded human frataxin allele in an animal devoid of mouse frataxin, and exhibits behavioral, oxidative, neurodegenerative and demyelinating changes (Al-Mahdawi et al., 2006). We undertook a microarray based study of DRG neurons of YG8 mice to discover whether alteration of Fe/S, iron, or oxidative stress genes occurs in this model of Friedreich's ataxia.

**Methods.** Microarray, behavioral and sensory testing, neuropathology, biochemical analysis, qRT-PCR, Western blotting, DRG neurite outgrowth assays.

**Results.** Analysis of 3-5 month YG8 mice at UC Davis demonstrated multiple movement/sensory deficits: weaker hind limb grip strength, delayed tail flick response, and decreased rotarod performance, as well as DRG neurodegeneration. Body composition changes in YG8 mice included increased adiposity and decreased brain mass and hindlimb muscle mass. Microarray of dorsal root ganglion tissue demonstrated differential regulation of genes related to mitochondrial, oxidative and thiol-stress, axonal transport and myelination, which were verified by quantitative RT-PCR and Western blot analysis. GSSG/GSH levels were elevated in neural tissues of YG8 mice, as we had previously demonstrated in FRDA patient lymphoblasts. DRG neurite outgrowth and sensitivity to oxidants was significantly altered in YG8 mice.

**Conclusions:** The results support frataxin-dependent differences in sensation and movement, transcriptional regulation of thiol-dependent redox poise and outgrowth of DRG neurons in this animal model and suggest that frataxin-deficiency alters thiol-antioxidant status in DRGs to cause demyelination and neurodegeneration.

## **Title: Cardiac mitochondrial proteins are hyperacetylated in a mouse model of Friedreich's Ataxia**

Authors: Gregory Wagner<sup>1</sup>, Melanie Pride<sup>2</sup>, R. Mark Payne<sup>1,2,\*</sup>

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Abstract:

**Background/Hypothesis:** The cardiomyopathy associated with mitochondrial dysfunction in Friedreich's Ataxia (FA) likely causes adaptations in myocardial energy substrate metabolism. Recent studies have demonstrated that numerous enzymes involved in intermediary metabolism are reversibly acetylated in a nutrient-sensitive manner and that this post-translational modification can influence cellular utilization of carbon sources. We hypothesize that there are alterations in cardiac mitochondrial acetylation profiles in FA and these alterations reflect the pathophysiological adaptations to impaired energy homeostasis.

**Methods:** Cardiac mitochondria from 24-28 day-old WT, WT fasted, and frataxin conditional knockout NSE-CRE  $\Delta/L3$  mice were isolated by differential and gradient centrifugation for Western Blotting, redox ratio analysis as defined by [NAD]/ [NADH], and for immunoprecipitation of acetyl-lysine proteins and respiratory complex I. Western Blots were normalized to VDAC and redox experiments were normalized to total protein. Significance was determined using Students t-test.

**Results:** Western blots of Cardiac mitochondrial proteins derived from the NSE-Cre model of FA exhibit markedly increased acetylation as compared to WT and WT fasted mice. Immunoprecipitation of WT and KO mitochondrial acetyl-lysine proteins indicates a known target of SIRT3 is deacetylated. Immunoprecipitation of respiratory complex I indicates hyperacetylated components of the multi-subunit complex and an intact association with SIRT3. Hyperacetylation is associated with a significant ( $p < .01$ ) increase in the ratio of mitochondrial nicotinamide adenine dinucleotide (NAD<sup>+</sup>) to its reduced counterpart (NADH).

**Conclusions:** Hyperacetylation of cardiac mitochondrial proteins in FA is distinct from the fasted state and occurs independently of any dysfunction in the predominant mitochondrial NAD<sup>+</sup>-dependent deacetylase, SIRT3. These findings demonstrate the first report of altered mitochondrial acetylation in a disease state and highlight the importance of putative mitochondrial acetyltransferases in mediating disease-induced adaptations to myocardial energy homeostasis. Studies to identify the hyperacetylated mitochondrial proteins in FA and determine the functional significance of altered acetylation at particular lysine residues are in progress.

## Title: Consequences of Iron Regulatory Protein 1 activation in mouse models of Friedreich's ataxia

Authors: Alain Martelli<sup>1</sup>, Stéphane Schmucker<sup>1</sup>, Laurence Reteunauer<sup>1</sup>, Nadia Messadeq<sup>1</sup>, Hervé Puy<sup>2</sup>, Bruno Galy<sup>3</sup>, Matthias Hentze<sup>3</sup>, Hélène Puccio<sup>1</sup>

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Abstract

**Background/Hypothesis:** Iron dysregulation is a key feature of Friedreich's ataxia, and has also been observed in different cellular and mouse models of the disease. However, the exact molecular mechanism that links frataxin deficiency to iron dysregulation, as well as the implication of iron dysregulation in the pathophysiology are unknown. Using a conditional approach, we have previously generated mouse models that reproduce most of the phenotypic and biochemical characteristics of the disease, and showed that iron-sulfur cluster (Fe-S) deficit precedes mitochondrial iron accumulation.

**Methods:** To further understand the link between frataxin (FXN) deficit and iron dysregulation, we have generated a liver-specific conditional mouse model. Liver is a central organ for iron homeostasis, notably through its high iron storage capacity and its role as an iron sensor that regulates systemic iron availability.

**Results:** The liver specific mice developed a severe phenotype and showed a decreased lifespan. Histopathological analysis revealed steatosis and a strong mitochondrial dysfunction at 4 weeks of age. The primary Fe-S deficit was confirmed as it was the only biochemical feature observed at 2 weeks of age. The expression of genes involved in iron metabolism was measured, as well as Iron regulatory proteins (IRP1 and IRP2) activity. Altogether, the results show that iron dysregulation is secondary to Fe-S deficit and appears to be the result of IRP1 activation. To confirm this hypothesis, *Fxn* conditional mice were crossed with *Irp1* knock-out mice. The double *Fxn/Irp1* knock-out mice, in both liver and heart, are currently under characterization, and preliminary results with the liver mouse model indicate that the absence of IRP1 prevent mitochondrial iron accumulation triggered by FXN deficit.

**Conclusions:** Together, the data enables to define a molecular mechanism, in which IRP1 activation plays a cardinal role that explains the iron dysregulation observed in Friedreich's ataxia.

**Title: Crosstalk between microRNAs and iron metabolism in pathogenesis of Friedreich's ataxia.**

Authors: Elizabeth Mclvor<sup>1</sup>, Urszula Polak<sup>1</sup>, Sherman Ku<sup>2</sup>, Joel Gottesfeld<sup>2</sup> and Marek Napierala<sup>1</sup>

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Abstract:

**Background/Hypothesis:** MicroRNAs (miRNAs) are a class of abundant post-transcriptional modulators of eukaryotic gene expression. Accumulating evidence highlights the importance of miRNAs in a number of biological processes, such as development, cell differentiation, proliferation, and apoptosis, as well as in pathological processes such as tumorigenesis. Recently, the role of miRNAs in the etiology of several neurodegenerative diseases caused by trinucleotide repeat expansions has been demonstrated. However, miRNA processing, expression, and effects on iron metabolism in Friedreich's ataxia (FRDA) have not been studied.

**Methods:** MicroRNA expression analyses conducted using microarray in lymphoblasts, fibroblasts, neuronal cells derived from FRDA patients and controls as well as shRNA frataxin deficiency models revealed a subset of 5 miRNAs families differentially expressed between these groups. MicroRNA expression changes were confirmed by quantitative real time PCR analyses.

**Results:** *In-silico* analyses of the mirBase targets database revealed that miRNAs differentially expressed in FRDA and control cells are putative regulators of mRNAs encoding proteins involved in iron metabolism. In order to evaluate whether these mRNAs were indeed targets of the identified miRNAs the 3' UTR regions of these genes were cloned downstream of the luciferase reporter gene. The HEK293 cell line was co-transfected with vectors overexpressing pre-miRNAs and the luciferase reporter constructs. To confirm the specificity of the miRNAs we conducted experiments using miRNA inhibitors alleviating the effect of target miRNAs on mRNA expression. Finally, mutagenesis of the seed sequence proved the miRNA:mRNA interactions. Importantly, the identified miRNAs affected not only the expression of the reporter constructs but also endogenous mRNAs and proteins involved in iron metabolism.

**Conclusions:** We identified miRNA signature of FRDA cells and evaluated the role of differentially expressed miRNAs as potential regulators of the intracellular iron metabolism and important players in FRDA pathogenesis.

## **Titile: The vicious circle hypothesis questioned in Friedreich ataxia**

**Authors: Aurélien Bayot<sup>°+</sup> (PhD), Renata Santos<sup>\*</sup> (PhD), Jean-Michel Camadro<sup>\*</sup> (PhD), and Pierre Rustin<sup>°+</sup> (PhD)**

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### **Abstract:**

Since the involvement of frataxin in Friedreich ataxia (FA) was discovered in the late 1990s, the function of frataxin has been generating vigorous debate. Very early on, we suggested a unifying hypothesis according to which frataxin deficiency leads to a vicious circle of faulty iron handling, impaired iron-sulfur cluster synthesis, and increased oxygen radical production. However, data from cell and animal models now indicate that iron accumulation is an inconsistent and late event and that frataxin deficiency does not always result in impaired iron-sulfur cluster synthesis. In contrast, frataxin deficiency in these models is consistently associated with increased sensitivity to reactive oxygen species, as opposed to increased oxygen radical production. Even though the vicious circle concept may be erroneous, the hypothesized chain of deleterious events related to reactive oxygen species hypersensitivity supports the early use of antioxidants to slow FA disease progression.

## **Title: Identification of new potential Iron-Sulfur Cluster Proteins through a Bioinformatic Approach**

Authors: <sup>1</sup>Florent COLIN, <sup>1</sup>Pierre COLIN, <sup>3</sup>Laurent BIANCHETTI, <sup>2,3</sup>Oliver POCH, <sup>1</sup>Hélène PUCCIO

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### **Background/Hypothesis:**

Frataxin is proposed to be a key regulator of Iron-Sulfur Clusters (ISC) biosynthesis but little is known about recipient ISC-proteins and consequently on misregulated pathways. In superior eukaryotic cells, ISC proteins were thought to be primarily located in mitochondria, but in the past few years new ISC proteins are being uncovered in many essential cellular pathways outside the mitochondria. Only twenty-one different proteins have been clearly characterized as ISC proteins in mammals leading us to the hypothesis that the total number of ISC proteins in superior eukaryotes might be much higher.

### **Methods:**

Our goal is to find new potential ISC proteins using a bioinformatic approach based on the search of ISC characteristic coordination motifs herein called signatures. We have created a program that is able to generate multiple lists of potential ISC-proteins and we are currently creating an open-access database containing all results.

### **Results:**

From a list of seventy known or predicted ISC proteins in superior eukaryotes, we have extracted a permissive list of thirty-one "signatures". As ISC are often coordinated by cysteine residues, most of these signatures have a "CX<sub>n</sub>CX<sub>n</sub>C" format. This list was used to search in Uniprot database for signatures-containing proteins that were considered as new potential ISC proteins. Multiple filtering steps based on cysteine density and clustering were added to the program to eliminate cysteine-rich and zinc-finger proteins. The limits and the quality of the program outputs have been taken into account and allowed us to discriminate between high-potential and low-potential candidates.

### **Conclusions:**

Identifying novel ISC proteins is a fundamental issue as new ISC proteins might uncover new pathways that are implicated in the pathophysiology of Friedreich Ataxia. The creation of a database is the next step and will provide to the Friedreich Ataxia scientific community a tool to search for ISC presence in their favorite proteins, pathways or organisms.

**Title: Alterations in Thiredoxin-related antioxidants in Friedriech's ataxia animal and cell models and therapeutic screening**

Authors: Gino Cortopassi, Yuxi Shan, Robert Schoenfeld, Mark Pook and Sunil Sahdeo

Institutions: University of California, Davis

Corresponding author email address: gcortopassi@ucdavis.edu

Abstract:

**Background/Hypothesis:** Our hypothesis is that frataxin-mediated iron-sulfur dysfunction causes sensitivity to oxidative stress through deficiency of thioredoxin-related antioxidants.

**Methods:** Our methods include microarray of neural tissues, QRTPCR and Western analysis of protein expression, biochemical and neurite extension and toxicity of dorsal root ganglion neurites, and high-throughput drug screening.

**Results.** Microarrays of DRG neurons of the YG8 mouse model indicated decreases in thioredoxin-related transcripts, and in myelination transcripts, and increases in axonal transport transcripts. Biochemical analysis in frataxin-knockdown cells and DRG neurons indicated alterations in peroxiredoxin redox state and expression level. Frataxin knockdown cells have less thioredoxin reductase activity, and frataxin knockdown cells and DRG cells are less viable in the context of challenge with a thioredoxin reductase inhibitor. Screening of 12 inhibitors of different enzymes in the glutathione/thioredoxin/peroxiredoxin pathway in Friedreich's fibroblasts and knockdown cells identified a differential sensitivity to diamide, a specific oxidizer of glutathione. Screening a library of 1060 FDA approved drugs identified several hits, which rescued from diamide sensitivity. These are now being tested in secondary and tertiary screens for function.

**Conclusions:** Frataxin interacts with iron-sulfur related proteins and alters sulfur-amino acid and iron-sulfur biogenesis. How this may impact antioxidant status has not been clear. Our results imply that iron-sulfur deficiency alters redox state of thioredoxin-related antioxidants, decreasing the protection of DRG neurons and Schwann cells from oxidative stress, leading to neurodegeneration. A screen of a 1060-compound library based on diamide sensitivity identifies multiple leads of potential benefit for further testing as Friedreich's therapeutics.

## **Title: Differential expression of PGC-1 $\alpha$ and metabolic sensors suggest age-dependent induction of mitochondrial biogenesis in Friedreich ataxia fibroblasts**

Authors: José Luis García-Giménez<sup>1,2</sup>, Amparo Gimeno<sup>2</sup>, Pilar Gonzalez-Cabo<sup>1,3</sup>, Francisco Dasí<sup>4</sup>, Arantxa Bolinches-Amorós<sup>1,3</sup>, Belén Mollá<sup>1,3</sup>, Francesc Palau<sup>1,3</sup>, Federico V. Pallardó<sup>1,2\*</sup>

Institutions: <sup>1</sup>CIBER on Rare Diseases (CIBERER); <sup>2</sup>Dept. Physiology. Medical School. Universitat de València; <sup>3</sup>Institute of Biomedicine of Valencia (CSIC), and <sup>4</sup>Fundación del Hospital Clínico Universitat de Valencia. FIHCUV-Incliva.

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Abstract:

**Background/Hypothesis:** Generation of reactive oxygen species (ROS) and oxidative stress have been associated with frataxin deficiency. ROS powerfully induce PGC-1 $\alpha$  that in turn, regulates a complex and multifaceted ROS defense system. Here we propose that induction of mitochondrial biogenesis in FRDA fibroblasts might be related with disease evolution in patients.

### **Methods:**

Oxidative stress status and mitochondrial biogenesis was investigated in fibroblasts from FRDA patients (Coriell Institute). Age at biopsy was 32/36 years in two adult siblings and 13 years in a young female. In these cells we have evaluated mitochondrial physiology by measuring ATP production and mitochondrial network morphology. Oxidative stress was evaluated by measuring antioxidants enzymes and ROS levels. To investigate mitochondrial biogenesis we performed Western blot studies of PGC-1 $\alpha$ , mtTFA, and AMPK and p38 MAPK kinases. Expression of both wild-type proteins and phosphorylated proteins was analyzed.

**Results:** All three patients' fibroblasts showed low levels of ATP, increased ROS levels and deficiency of MnSOD. In fibroblasts from the two adult patients we observed increase expression of PGC-1 $\alpha$  and mtTFA and the active phosphorylated forms of the upstream signals modulated by p38 MAPK and AMPK, but not in fibroblasts from the younger patient. Interestingly, the expression of energetic factors and activated kinases correlated with the natural history of disease of the patients, the age when skin biopsy was performed and the size of the GAA expanded alleles. Furthermore, we observed that idebenone inhibits mitochondriogenic responses in FRDA fibroblasts.

**Conclusions:** The increase of ROS and the involvement of the oxidative phosphorylation may be an early event in the cell pathophysiology of frataxin deficiency, whereas increase of mitochondriogenic response might be a later phenomenon associated to the age at onset and natural history of the disease, being more evident as the patient's age increases and disease evolves.

## **Title: Metabolic rearrangements in a cardiac cellular model of Friedreich Ataxia**

Authors: Elia Obis, Veronica Irazusta, Daniel Sanchís, Joaquim Ros and Jordi Tamarit

Institutions: Departament de Ciències Mèdiques Bàsiques, Facultat de Medicina, IRB-Lleida, Universitat de Lleida, Spain

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Abstract:

**Background/Hypothesis:** Cardiomyopathies are the most frequent cause of death in Friedreich Ataxia (FA). They could be caused by alterations in heart myocytes, which are rich in mitochondria and present high oxidative metabolic rates. However, the effects of frataxin depletion on cardiomyocytes are poorly understood. In this context, we have developed a cardiac model of frataxin deficiency using primary cultures of rat neonatal cardiomyocytes. In such cells we have analyzed the effect of frataxin depletion on cellular metabolism.

**Methods:** Rat neonatal cardiomyocytes were transduced with lentiviral particles containing shRNA against frataxin mRNA. Cells transduced with non-interfering shRNA sequences (*scrambled*) were used as a control. Interference was evaluated by quantitative RT-PCR and western blot. The metabolic fingerprint and footprint of both silenced and control cells were analyzed by Liquid Chromatography and Mass Spectrometry-based approaches. Activities of several metabolic enzymes were analyzed by spectrophotometric methods.

**Results:** Frataxin mRNA content decreased to 10-20 % of control levels. This level of interference was stable for at least 9 days. Metabolomic analysis indicated that lack of frataxin triggers metabolic rearrangements, which are related to the carbon source used by cardiomyocytes.

**Conclusions:** Rat neonatal cardiomyocytes are a good model for the analysis of the cardiac effects of frataxin depletion. Metabolic rearrangements observed in our model could be the origin of the altered cardiac status found in most FA patients. Currently, we are investigating the origin of these metabolic alterations and their consequences on cell viability.

**Title: Antioxidant defense and metacaspase are implicated in cell death in yeast frataxin-deficient cells**

Authors: Sophie Lefevre, Caroline Brossas, Dominika Sliwa, Françoise Auchère, Christoph Ruckenstuhl, Emmanuel Lesuisse, Frank Madeo, Jean-Michel Camadro and Renata Santos

Institutions: Institut Jacques Monod (UMR 7592 CNRS – University Paris-Diderot), Mitochondria, Metals and Oxidative Stress Laboratory, Bât. Buffon, 15 rue Hélène Brion, 75205 Paris cedex 13, France

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Abstract:

**Background/Hypothesis:** Oxidative stress is a central pathological feature of Friedreich ataxia (FA) and challenging frataxin-deficient cells with oxidants causes cell death. Our aim is to study the effect of oxidative stress in cell death in a yeast model of FA.

**Methods:** We previously showed that oxygen causes the emergence of deleterious phenotypes of a *Saccharomyces cerevisiae* frataxin-deficient mutant ( $\Delta yfh1$ ), such as loss of aconitase activity, accumulation of oxidized modified proteins, induction of mitochondrial protease Pim1 and decreased activity of the proteasome (Bulteau et al 2007 *Free Radic Biol Med.* 42:1561). Therefore, in this study we used anaerobiosis to aerobiosis transition as an inducer of oxidative stress in  $\Delta yfh1$  cells in addition to treatment of cells with hydrogen peroxide.

**Results:** Our data show that yeast frataxin-deficient cells are in a pro-apoptotic state due to misregulation of antioxidant defenses and increased levels of reactive oxygen species. During transition of  $\Delta yfh1$  cells from anaerobiosis to aerobiosis about 40% of the cell population accumulates reactive oxygen species and dies by apoptosis in the first hour; the remaining cells survive and adapt to oxidative stress conditions by modulating the transcription of genes encoding antioxidant enzymes and glutathione pools. An intricate interaction between frataxin-deficiency and metacaspase was uncovered. Metacaspase protected  $\Delta yfh1$  cells during transition to aerobic conditions. However, in oxygen adapted and damaged  $\Delta yfh1$  cells, deletion of the metacaspase encoding gene resulted in an increase in resistance to oxidants and recovery of heme biosynthesis.

**Conclusions:** Our findings suggest that oxygen and cellular defense to oxidative stress play a major role on the appearance of pathogenic phenotypes in frataxin-deficient cells and imply that searching for new antioxidant molecules is still a valuable therapeutic strategy for FA.

## **Title: Inactivation of mitochondrial aspartate aminotransferase contributes to the deleterious phenotypes in frataxin-deficient yeast cells**

Authors: Dominika Sliwa, Jean-Michel Camadro and Renata Santos

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### Abstract

**Background/Hypothesis:** A defect in iron-sulfur cluster assembly and heme biosynthesis is one of the main phenotypic features of frataxin-deficient yeast cells. The first enzymes of both pathways require pyridoxal phosphate (PLP) as a cofactor for their activity. The aim of this work is to determine whether an alteration in PLP metabolism is implicated in the development of the deleterious phenotypes in frataxin-deficient cells.

**Methods:** We chose to study aspartate aminotransferase (AAT), which belongs to the malate-aspartate NADH shuttle, because this PLP-dependent enzyme has a cytosolic and a mitochondrial isoforms.

**Results:** Here we show that the activity of mitochondrial AAT, but not cytosolic AAT, is severely decreased in the yeast frataxin-deficient ( $\Delta yfh1$ ) compared to wild-type cells. The loss in mtAAT activity is not due to reduced transcription or reduced amount of protein. Analysis of posttranslational modifications that could inactivate the enzyme is under study. Another possibility is that cofactor availability is reduced. Indeed, the PLP and pyridoxal pools are significantly decreased in  $\Delta yfh1$  cells and in patient's fibroblasts. In addition, mtAAT activity is unchanged in yeast frataxin-deficient cells cultured in anaerobic conditions and decreased in wild-type cells treated with menadione, suggesting that mtAAT reduction could be a consequence of oxidative modification. To test the consequences of mtAAT inactivation, the encoding *AAT1* gene was deleted in wild-type cells. The  $\Delta aat1$  mutant shows strong respiration impairment, decreased aconitase and succinate dehydrogenase activities and lack of mitochondrial encoded cytochromes, but no increased sensitivity to oxidants.

**Conclusions:** Our results show that decrease of mtAAT activity in  $\Delta yfh1$  cells contributes to major phenotypes observed in frataxin-deficient cells (respiration impairment and loss of iron-sulfur cluster-dependent enzymatic activities). Inactivation of this enzyme is most probably due to reduction in PLP levels and/or posttranslational modifications resulting from oxidative stress, which is a subject of a current investigation.

### **Session 3 (Friday, 8:30 – 10:30): Genetics, Epigenetics & Regulation (instability)**

Co-Chairs: Joel Gottesfeld & Richard Festenstein

#### Speakers

<b>Ed Grabczyk</b> (invited)	Transcription-coupled GAA•TTC Expansion Via Mismatch Repair in Human Cells
<b>Jintang Du</b> (presented by J. Gottesfeld)	Recapitulation of GAA•TTC Triplet Repeat Expansion in Friedreich's Ataxia iPSCs
<b>Cihangir Yandim</b>	GAA-repeats induce heterochromatinisation, and proteasome-dependent degradation of stalled RNA-PolIII providing a novel way of upregulating <i>FXN</i> in Friedreich's Ataxia
<b>Rodrigo Villaseñor</b>	Long intronic GAA repeats causing Friedreich ataxia impede transcription elongation
<b>Karen Usdin</b>	<i>FXN</i> expression from unaffected and <i>FRDA</i> alleles
<b>Dave Lynch</b>	Association of a Non synonymous <i>SIRT6</i> polymorphism with improved neurological abilities in <i>FRDA</i>
<b>Yogesh Chutake</b>	RNA-mediated transcriptional gene silencing of the <i>FXN</i> gene in Friedreich ataxia
<b>Angela Castro</b>	Deficiency of CTCF-mediated chromatin insulation in Friedreich ataxia results in <i>FXN</i> transcriptional deficiency

#### Posters

<b>Alexandra Henrion-Caude</b>	MicroRNA-mediated regulation of frataxin in Friedreich's ataxia
<b>Vahid Ezzatizadeh</b>	Analysis of intergenerational GAA repeat instability in <i>FRDA</i> transgenic mouse models
<b>Cindy Follonier</b>	GAA repeats induce post-replicative DNA junctions in mammalian cells

## **Title: Transcription-coupled GAA•TTC Expansion Via Mismatch Repair in Human Cells**

Authors: Scott Ditch, Jeffrey Wang, Mimi C. Sammarco, Ayan Banerjee and Ed Grabczyk\*

Institutions: Department of Genetics  
LSU Health Sciences Center  
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New Orleans, LA 70112, USA

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Abstract:

### **Background/Hypothesis:**

We have developed versatile human cell FRDA models that replicate the expansion found in patients. The GAA•TTC repeats expand incrementally, continuously and nearly synchronously. The expansion rate is independent of cell division rate, as in the post-mitotic tissues affected in FRDA. Expansion is linked to the level of transcription through the repeats. Therefore, it is imperative to understand the causes of GAA•TTC repeat expansion: 1) To learn how to halt FRDA disease progression, and 2) to avoid unwanted side effects of potential treatments aimed at increasing FXN gene transcription. Our working hypothesis is that structures formed by transcription in the GAA•TTC repeat attract DNA repair enzymes, which ultimately leads to expansion.

### **Methods:**

We used our human cell models to determine the role of transcription and DNA repair in GAA•TTC expansion. We blocked transcription into the repeat with strong transcription terminators and/or used shRNA to deplete components of Global Genome Nucleotide Excision Repair (GGNER), Transcription Coupled NER (TCNER) and Mismatch Repair (MMR) in the cells and followed the expansion rate.

### **Results:**

Reduced transcription through the GAA•TTC repeats reduced expansion rates. When transcription was blocked from both directions, the repeats were stabilized. DNA repair is secondary to transcription in promoting repeat expansion. Depleting either MSH2 or MSH3 with shRNA in our model cells slowed GAA•TTC expansion. MSH6 had little effect on GAA•TTC expansion. We found no role for either GGNER or TCNER in our system.

### **Conclusions:**

Transcription primes GAA•TTC repeats for expansion by MMR. MSH2 and MSH3 form a hetero-multimer called MutSbeta, which is also essential for CAG•CTG expansion. MSH2 is central to MMR and its absence or reduction predisposes to colorectal cancer. Consequently, MSH2 is not an attractive therapeutic target. However, absence or reduction of MSH3 is not associated with cancer predisposition. Therefore, we suggest targeting MSH3 to halt FRDA progression.

## **Title: Recapitulation of GAA•TTC Triplet Repeat Expansion in Friedreich's Ataxia iPSCs**

Authors: Jintang Du, Sherman Ku, Erica Campau, Elisabetta Soragni, Joel M. Gottesfeld\*

Institutions: The Scripps Research Institute, Department of Molecular Biology, La Jolla, CA 92037

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Abstract:

**Background/Hypothesis:** The genetic mutation in Friedreich's Ataxia (FRDA) is a hyper-expansion of the triplet repeat sequence GAA•TTC within the first intron of *FXN* gene. Normal alleles have 6~34 repeats while FRDA patient alleles have 66~1700 repeats. Although yeast and reporter construct models for GAA•TTC repeat expansion have been reported, studies in FRDA pathogenesis and therapeutic development are limited by the availability of an appropriate human cell model in which to study the mechanism of GAA•TTC triplet repeat expansion in human genome.

**Methods:** Induced pluripotent stem cells (iPSCs) were generated from FRDA patient fibroblasts after transduction with the four transcription factors Oct4, Sox2, Klf4 and c-Myc. These cells were differentiated into neurospheres and neurons, representing a valuable cellular model for FRDA.

**Results:** During the course of these studies, it was observed that during propagation of the FRDA iPSCs, GAA•TTC repeats expand in a manner analogous to the expansion observed in FRDA patient samples, but not in fibroblasts and neurospheres. Importantly, asymptomatic, heterozygous carriers show GAA•TTC triplet repeat expansion on the pathogenic allele, but not the normal allele. The mismatch repair enzyme MSH2, implicated in repeat instability in other triplet repeat diseases, is highly expressed in pluripotent stem cells and occupies *FXN* intron 1. In addition, shRNA silencing of MSH2 impedes GAA•TTC triplet repeat expansion.

**Conclusions:** GAA•TTC triplet repeat expansion is recapitulated in FRDA iPSCs but not fibroblasts or neurospheres. The mismatch repair enzyme MSH2 is involved in the GAA•TTC triplet repeat expansion.

**Title: GAA-repeats induce heterochromatinisation, and proteasome-dependent degradation of stalled RNA-PolIII providing a novel way of upregulating FXN in Friedreich's Ataxia**

Authors: Maria Mayan, Nadine Chapman-Rothe, Raul Torres, Cihangir Yandim, Ana Pombo, Richard Festenstein

Institutions: MRC Clinical Sciences Centre / Imperial College London

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Abstract not available.

## **Title: Long intronic GAA repeats causing Friedreich ataxia impede transcription elongation**

**Authors: Rodrigo Villaseñor, Tanel Punga, and Marc Bühler\***

Institutions: Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

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### **Abstract:**

Friedreich ataxia is a degenerative disease caused by deficiency of the protein frataxin (FXN). An intronic expansion of GAA triplets in the FXN-encoding gene, which plays a crucial role in mitochondrial iron metabolism, causes gene silencing and thus reduced FXN protein levels. Although it is widely assumed that GAA repeats block transcription via the assembly of an inaccessible chromatin structure marked by methylated H3K9, direct proof for this is lacking. In this study, we analyzed different histone modification patterns along the human FXN gene in FRDA patient-derived lymphoblastoid cell lines. We show that FXN mRNA synthesis, but not turnover rates are affected by an expanded GAA repeat tract. Importantly, rather than preventing transcription initiation, long GAA repeat tracts affect transcription at the elongation step and this can occur independently of H3K9 methylation. Our data demonstrate that finding novel strategies to overcome the transcription elongation problem may develop into promising new treatments for FRDA. We are currently setting up high-throughput screens for genes or low molecular weight (LMW) enhancers of frataxin expression. This approach has great potential to reveal novel drug targets or promising compounds that could be further developed into therapeutics to treat FRDA patients.

### **Background/Hypothesis:**

Long intronic GAA repeats can induce the methylation of histone H3 at lysine 9 (H3K9me) at the FXN locus in FRDA cells. This epigenetic modification is a hallmark of heterochromatin and it has therefore generally been assumed that the FXN locus is assembled into a chromatin structure that hinders transcription.

### **Methods:**

Chromatin immunoprecipitation (ChIP), 4-Thiouridine labelling (for RNA turnover analysis), qRT-PCR and Northern blot

### **Results:**

We show that FXN mRNA synthesis, but not turnover rates are affected by an expanded GAA repeat tract. Importantly, rather than preventing transcription initiation, long GAA repeat tracts affect transcription at the elongation step. Moreover, we demonstrate that FXN gene silencing can occur independently of H3K9 methylation. Therefore, the repeat DNA itself seems to be a major obstacle for RNA polymerase II progression at the FXN locus.

### **Conclusions:**

The data demonstrate that long GAA repeats constitute an obstacle to the transcription machinery in FRDA patient-derived cells. The block appears to occur during transcription elongation, irrespectively of the H3K9 methylation status of the FXN gene. Our data demonstrate that finding novel strategies to overcome the transcription elongation problem may develop into promising new treatments for FRDA.

**Title: FXN expression from unaffected and FRDA alleles**

Authors: Usdin, Karen and Kumari, Daman

Institutions: NIDDK, NIH

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**Abstract:**

The region flanking the repeat on FRDA alleles has a histone modification profile typical of repressed genes and shows more extensive DNA methylation in the region flanking the repeat than unaffected alleles. However, it has been suggested that the deficit of mature FXN mRNA arises from a triplex-mediated block to RNA polymerase or aberrant splicing, rather than an epigenetic effect. Using chromatin immunoprecipitation and cell lines from affected and unaffected individuals, we show that while the chromatin changes are most marked in the region of the repeat, they do extend into the promoter region of the *FXN* gene in patient cells. Furthermore, we show that the initiating form of RNA polymerase II and histone H3 trimethylated on lysine 4, a chromatin mark tightly linked to transcription initiation, are both present at lower levels on FRDA alleles. Our data would be most consistent with a model in which repeat-induced chromatin modifications modulate transcription initiation in FRDA. We also see an effect of repeat expansion on transcription elongation, but whether this effect is due to the chromatin changes or problems with transcription through the repeat remains to be seen. We have also found that both affected and unaffected alleles have a block to transcription elongation located upstream of the repeat that may represent a novel target for drug development. Our findings may have implications both for understanding the mechanism responsible for FRDA as well as for therapeutic approaches to reverse the transcription deficit.

## **Title: Association of a Non synonymous SIRT6 polymorphism with improved neurological abilities in FRDA**

Authors: Lynch, D.R., Regner, S., Lagedrost, S., Jespersen, C., Brocht, A., Perlman, S., Bushara, K., Wilmot, G., Gomez, C., Mathews, K., Ravina, B., Murray, S.S., Gottesfeld, J.M.

Institutions: *Children's Hospital of Philadelphia, Scripps Institute, UCLA, Emory, U Chicago, University of Iowa, University of Minnesota, University of Rochester.*

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**Background/Hypothesis:** HDAC inhibition is a potential therapeutic intervention in FRDA based on in vitro and animal data. We investigated whether polymorphisms in HDAC genes influence phenotypic features or severity in FRDA.

**Methods:** We used the DNA repository and clinical database of the CCRNFA to assess the effect of HDAC polymorphisms on features of FRDA including age of onset, FARS score, performance measures, composites Z2 and Z3, and presence of diabetes, cardiomyopathy or scoliosis. Genomic DNA from 250 subjects was genotyped at 49 specific SNPs in HDAC genes (*HDAC3*, *HDAC5*, *HDAC6*, *HDAC10*, *SIRT3*, *SIRT5*, *SIRT6*). Data was analyzed by assessing the ability of individual SNPs to predict phenotype in regression analysis of specific outcomes measures (eg FARS) accounting for the effects of GAA repeat length, sex and age.

**Results:** A specific amino acid changing polymorphism (rs352117) in *SIRT6* predicted FARS, Z2 and Z3 scores with high significance ( $P=0.0012$ ;  $P=0.0008$ ;  $P=0.0012$ , respectively). Effects on individual performance measures were also significant. This SNP, which changes a Ser to Asn, was completely linked with a second SNP (rs352493) that predicted phenotype equally. The improvement in  $R^2$  values with the SNPs was from 31% to 37%. The heterozygous state (Ser/Asn) was associated with a less severe neurological phenotype than the Asn/Asn state. No association was noted with diabetes/scoliosis, and a small association was noted with cardiomyopathy. Other polymorphisms in *SIRT6* produced lower associations. Minimally significant phenotypic effects were seen in a few polymorphisms of *SIRT3* ( $0.01 < p < 0.05$ ), and *SIRT5* SNPs were associated with varying contrast acuity ( $0.007 < p < 0.06$ ).

**Conclusions:** A naturally occurring polymorphism of *SIRT6* was associated with altered phenotypic severity of neurological measures in FRDA. This polymorphism, found in a region of *SIRT6* that is crucial for enzymatic function, is associated with less severe neurological dysfunction. This confirms that HDACs may influence the severity of FRDA.

## **Title: RNA-mediated transcriptional gene silencing of the *FXN* gene in Friedreich ataxia**

Authors: Yogesh K. Chutake<sup>1</sup>, Angela M. Castro<sup>1</sup>, Sanjay I. Bidichandani<sup>1,2</sup>

Institutions: Department of Biochemistry & Molecular Biology<sup>1</sup> and Pediatrics<sup>2</sup>, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

Corresponding author email address: Sanjay-Bidichandani@ouhsc.edu

Abstract:

**Background/Hypothesis:** Epigenetic modification at the *FXN* locus is responsible for transcriptional deficiency in FRDA since it is partially reversed by histone deacetylase inhibitors. FRDA patients have depletion of the chromatin insulator protein CTCF and heterochromatin formation at the +1 nucleosome of the *FXN* gene, suggesting a potential mechanism for transcriptional silencing. Frataxin antisense transcript-1 (FAST-1), which overlaps the +1 nucleosome, is overexpressed in FRDA. However, the mechanism of heterochromatin formation at the +1 nucleosome and the role of FAST-1 remain unclear. Several studies support the role of antisense transcripts in epigenetic regulation of gene expression. Double-stranded RNAs (dsRNA) formed via pairing of sense and antisense transcripts can generate small regulatory RNAs that direct transcriptional gene silencing (TGS) at their target genomic location. We explored a similar role for FAST-1/*FXN* dsRNA.

**Methods:** ChIP to detect epigenetic modifications. RNA immunoprecipitation (RIP) to detect interaction of TGS effector proteins and FAST-1/*FXN* dsRNA. RNase A/T1 assay to detect dsRNA.

**Results:** The RNase A/T1 assay detected dsRNA overlapping the transcription start sites at the *FXN* locus. RIP revealed association of Argonaute-1 and Argonaute-2, effector proteins in TGS, to sense and antisense RNA strands in the region encompassing the dsRNA. ChIP showed that Argonaute-1 or Argonaute-2 were not enriched at nucleosome +1, supporting their interaction exclusively with the dsRNA. Knocking-down Argonaute-1, Argonaute-2 or Dicer in FRDA lymphoblastoid cells reversed the repressive chromatin signals at nucleosome +1. HDAC inhibitors that reverse the repressive epigenetic changes in the vicinity of expanded GAA repeat in intron 1 also reversed the heterochromatin formation at nucleosome +1, supporting their use as a general strategy to reactivate the *FXN* gene in FRDA.

**Conclusions:** These data support the role of FAST-1 and endogenous dsRNA in epigenetic silencing of the *FXN* gene in FRDA.

**Title: Deficiency of CTCF-mediated chromatin insulation in Friedreich ataxia results in *FXN* transcriptional deficiency**

Authors: Angela M. Castro<sup>1</sup>, Yogesh K. Chutake<sup>1</sup> and Sanjay I. Bidichandani<sup>1,2</sup>

Institutions: Departments of Biochemistry & Molecular Biology<sup>1</sup> and Pediatrics<sup>2</sup>, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

Corresponding author email address: [Sanjay-Bidichandani@ouhsc.edu](mailto:Sanjay-Bidichandani@ouhsc.edu)

Abstract:

**Background:** The expanded GAA•TTC sequence in FRDA is associated with heterochromatin in intron 1 that interferes with transcriptional elongation, which is partially relieved by histone deacetylase inhibitors (HDACi). We and others have noted repressive chromatin changes in the 5'UTR / promoter region of the *FXN* gene in FRDA, consistent with an additional defect in transcriptional initiation. FRDA patients have depletion of CTCF in the 5'UTR and HP1-mediated repressive chromatin involving nucleosome +1. However, it remains unclear if CTCF depletion and repressive chromatin in the upstream regions of the *FXN* gene is a primary event, or secondary to heterochromatin spreading upstream from intron 1.

**Methods:** ChIP to detect repressive chromatin and CTCF occupancy, and qRT-PCR to measure *FXN* transcript. We used FRDA lymphoblasts and CTCF knockdown in HEK293 cells.

**Results:** CTCF knockdown in HEK293 cells resulted in HP1-mediated heterochromatin at nucleosome +1 (but not in intron 1) and *FXN* transcriptional deficiency. Absence of the expanded GAA•TTC sequence in HEK293 cells indicates that transcriptional deficiency is not secondary to upstream spreading of heterochromatin or due to deficient transcriptional elongation. Consistent with this, strand-specific RT-PCR in FRDA lymphoblasts also showed *FXN* transcriptional deficiency upstream of the expanded GAA•TTC sequence. In FRDA lymphoblasts and HEK293-CTCF knockdown cells treatment with HDACi (4b) resulted in complete reversal of the heterochromatin at nucleosome +1, and *FXN* transcript levels in the upstream regions of the *FXN* gene also returned to normal. However, HDACi treatment of FRDA lymphoblasts only partially reversed the deficiency of transcriptional elongation.

**Conclusions:** CTCF functions as a chromatin insulator that is essential for *FXN* transcription, and FRDA patients are deficient in this function. HDACi treatment reversed the deficient transcriptional initiation but only partially normalized transcriptional elongation. These data have important implications for the molecular pathogenesis and the design of therapies in FRDA.

## **Title: MICRORNA-MEDIATED REGULATION OF FRATAXIN IN FRIEDREICH'S ATAXIA**

Authors: Hatem E, Bandiera S, Girard M, Rifai L, Jannot AS, Loiseau C, Munnich A, Lyonnet S, Henrion-Caude A.

Institutions: Inserm U781 – Service de Génétique – Hôpital Necker Enfants Malades – 149 rue de Sèvres, 75015, Paris.

Corresponding author email address: alexandra.caude@inserm.fr

Abstract:

### **Background/Hypothesis:**

Recent studies have uncovered profound and unexpected roles for a family of tiny regulatory RNAs, known as microRNAs (miRs), in the control of gene expression in diverse cellular processes. Interestingly, impairment of miRNA biogenesis in Purkinje cells results in cerebellar degeneration and ataxia in mice. Thus, we hypothesized that miRNA-mediated posttranscriptional regulation of frataxin (FXN) might modulate Friedreich's ataxia neuropathology by affecting the levels of the residual protein.

### **Methods:**

To test this hypothesis, we searched for evolutionarily conserved miRNA binding sites in the 3'UTR of human *FXN* by means of various computational tools. Sequencing analysis of the frataxin 3'UTR region in patients with Friedreich Ataxia was also carried out in search for potential mutations. Finally, the potency of a miR-antisense strategy was assessed by cotransfection experiments together with the frataxin 3'UTR region.

### **Results:**

We identified a subset of putatively critical target sites for microRNA through analysis of the 3'UTR of frataxin using distinct bioinformatic tools. Our analyses prompt us to sequence the 3'UTR region of frataxin in our cohort of patients with Friedreich ataxia (n=57). We identified genetic variants within few miR target sites that may affect the base-pairing between the miR and its target site, and hence affect the miR-mediated gene repression. Targeting by miRs important in neuronal development was specifically characterized.

### **Conclusions:**

Taken together, our results enabled to ascribe -to the 3'UTR region- new regulatory motifs that could modify the severity expression of Friedreich ataxia, thus establishing the rationale to design miR-inhibitory molecules to avoid miR-based downregulation in diseased organs.

## **Title: Analysis of intergenerational GAA repeat instability in FRDA transgenic mouse models**

Authors: Vahid Ezzatizadeh, Ricardo Mouro Pinto, Chiranjeevi Sandi, Sahar Al-Mahdawi and Mark Pook

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Abstract:

**Background/Hypothesis:** We have established two lines of GAA repeat expansion-based human genomic YAC transgenic mouse models of FRDA, termed YG8 and YG22, which initially contained 90+190 and 90 repeats respectively. Studies of GAA intergenerational instability revealed contractions or expansions in approximately 5%-15% of offspring from both YG8 and YG22 parents.

**Methods:** To increase the GAA repeat expansion level and to investigate factors that may influence intergenerational repeat instability, we have cross bred YG8 and YG22 mice (C57Bl6/J background) with other inbred lines (129, CBA, FVB) and genetically modified mice (mismatch repair (MMR) knockout mice). GAA repeat sizes were assessed from mouse tail biopsy DNA samples by PCR and agarose gel electrophoresis.

**Results:** Thus far, GAA repeat sizes in YG8 and YG22 mice have been increased to 135-200 and 230-260, respectively. *Msh2* and *Msh3* knockout mice containing the FXN GAA transgene produce 4-fold and 6-fold increases in the number of offspring showing GAA repeat contractions, whereas *Msh6* knockout mice containing the FXN GAA transgene produce a 4-fold increase in the number of offspring showing GAA repeat expansions.

**Conclusions:** By our breeding studies of YG8 and YG22 mice, we have now significantly increased the GAA repeat sizes within our colonies. Analysis of GAA repeats in offspring of MMR knockout mice compared with WT mice indicate that the MMR system has an effect on GAA repeat instability.

**Title: GAA repeats induce post-replicative DNA junctions in mammalian cells.**

Authors: Cindy Follonier and Massimo Lopes

Institutions: Institute of Molecular Cancer Research Zürich

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Abstract:

**Background/Hypothesis:** Expanded GAA tracts were shown to form stable secondary structures in bacteria and to stall replication forks in yeast. However, the molecular mechanisms leading *in vivo* to repeat expansion and replication interference are still elusive. The aim of this project is to gain insight into the DNA structures and cellular factors mediating GAA instability *in vivo* in human cells.

**Methods:** We established an experimental system to analyse DNA replication intermediates (RI) across GAA repeats in human cells. SV40-derived plasmids containing GAA repeats in different number and orientation are transfected into 293T or U2OS cells and allowed to replicate. Plasmid RI are recovered and analyzed by a combination of bidimensional (2D)-gels, psoralen crosslinking and electron microscopy (EM).

**Results:** We observe that GAA repeats induce a transient fork pausing in a length- and orientation-dependent manner, without affecting the location of replication termination. Our 2D-gels reveal several unexpected GAA-dependent signals that are currently under extensive investigation. In particular, distinctive X shaped molecules accumulate at fully replicated plasmids containing long GAA repeats. These molecules can be distinguished from recombination or termination intermediates and may derive from pre-existing junctions observed at replicating plasmids. By extraction of these molecules from the 2D-gels and direct visualization at the EM we are currently investigating the structural determinants of both control and GAA-specific intermediates.

**Conclusions:** Our studies allowed the identification of alternative DNA structures associated with expanded GAA repeats, which we are further characterizing by direct EM observation. Combining these approaches with depletion (siRNA) of specific factors, we aim to reveal the role of candidate cellular players in GAA replication and stability, thus identifying new potential targets for Friedreich's ataxia therapies.

#### **Session 4 (Friday, 11:00 – 13:00): Frataxin Function**

Co-Chairs: Grazia Isaya & Annalisa Pastore

#### **Speakers**

<b>David Barondeau</b> (invited speaker)	Frataxin-based activation of the human Fe-S assembly complex
<b>Timothy Stemmler</b> (invited speaker)	Molecular details regarding Frataxin's roles in the activation of eukaryotic Fe-S cluster biosynthesis
<b>Stéphane Schmucker</b>	The interaction of mammalian frataxin with a preformed <i>iscu/nfs1/isd11</i> iron-sulfur cluster assembly complex defines the essential function of frataxin in vivo
<b>Clara Iannuzzi</b>	A Mössbauer and resonance Raman study of the role of CyaY in iron sulfur cluster assembly on the <i>E. coli</i> IscU scaffold protein
<b>Andrew Dancis</b>	Mutation in ISU bypasses frataxin
<b>Eva-Christina Ahlgren</b>	The importance of the N-terminus: Structural insights into human frataxin in monomeric and oligomeric forms
<b>Grazia Isaya</b>	Oligomeric frataxin protects NFS1 from oxidative inactivation during Fe-S cluster assembly

#### **Posters**

<b>Salam Al-Karadaghi</b>	Metal-dependent oligomerisation of yeast frataxin: Structural insights from small-angle X-ray scattering (SAXS)
<b>Isaac Amela</b>	A Theoretical Model of the Key Proteins Involved in the Iron-Sulfur Cluster Biogenesis
<b>Ilaria Guccini</b>	Frataxin participates to the hypoxia-induced response in tumors
<b>Hongqiao Li (presented by Grazia Isaya)</b>	FRDA-associated mutations, W155R and I154F, have complex effects on human biogenesis and function
<b>Robert Yan</b>	YFHJ is a modulator of frataxin function in bacteria

**Title: Frataxin-based activation of the human Fe-S assembly complex**

Author: David Barondeau

Abstract:

Cellular depletion of the human protein frataxin is correlated with the neurodegenerative disease Friedreich's ataxia (FRDA) and results in the inactivation of Fe-S cluster proteins. Most researchers agree that frataxin functions in the biogenesis of Fe-S clusters, but its precise role in this process is unclear. Here we provide *in vitro* evidence that human frataxin binds to a Nfs1, Isd11, and Isu2 complex to generate the four-component core machinery for Fe-S cluster biosynthesis. Kinetic analyses coupled to mass spectrometry experiments suggest frataxin functions as an allosteric activator that triggers sulfur delivery from Nfs1 to Isu2 for Fe-S cluster assembly. Structure-function studies of FRDA missense variants reveal a correlation between the severity of the clinical phenotype and loss of the ability to function as an allosteric activator. Spectroscopic data reveal details for iron incorporation into Fe-S clusters, whereas initial structural data provide the basis for protein-protein interactions in the assembly complex. Together our results suggest this *in vitro* system is sensitive and appropriate for deciphering functional defects and mechanistic details for human Fe-S cluster biosynthesis and hint at a role for cellular frataxin in regulating human Fe-S cluster biosynthesis.

**Title: Molecular details regarding Frataxin's roles in the activation of eukaryotic Fe-S cluster biosynthesis.**

Authors: Timothy L. Stemmler

Institutions: Wayne State University

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**Abstract:**

Direct participation of frataxin during iron-sulfur cluster biosynthesis has been widely established. While originally suggested to serve as an iron chaperon, whose metal binding ability has been documented in detail, recent reports suggest frataxin may also modulate the activity of the cysteine desulfurase concurrent with ISC pathway cluster assembly. The negative cysteine desulfurase activity shown in the prokaryotic system is in contrast to the positive activity stimulation recently shown in the human system (and confirmed in our laboratory using the yeast model system) suggesting unique features of the eukaryotic pathway may drive the differences in reactivity seen between cell types. We have performed an extensive characterization of the frataxin-ISC scaffold protein complex using a variety of spectroscopic techniques (NMR, XAS, ITC, Fluorimetry), and in the process provided the molecular details that help explain the energetics that drive interprotein assembly and metal transfer. Additional characterization data of the frataxin Nfs-Iso11 complex in the presence and absence of Iso will be discussed with our recent data to better understand the enhanced reactivity of the macromolecular assembly related to Fe-S cluster assembly in eukaryotes. Based on our molecular characterization of the frataxin-Iso complex and current data regarding the Iso-Nfs binding interface, we believe frataxin interacts with the two protein partners, with the assistance of Iso11, in a manner that provides iron for cluster assembly and allosterically activates eukaryotic cysteine desulfurase activity. A model for these studies will be presented.

**Title: The interaction of mammalian frataxin with a preformed iscu/nfs1/isd11 iron-sulfur cluster assembly complex defines the essential function of frataxin in vivo.**

Authors: Stéphane Schmucker, Alain Martelli, Florent Colin, Adeline Page, Marie Wattenhofer-Donzé, Laurence Reutenauer, Hélène Puccio

Institutions: IGBMC (Institut de génétique et de biologie moléculaire et cellulaire), Illkirch, FRANCE

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Abstract:

**Background/Hypothesis:** Frataxin is thought to be involved in multiple iron-dependent mitochondrial pathways. In particular, frataxin plays an important role in the formation of iron-sulfur (Fe-S) clusters biogenesis.

**Methods/Results:** We present data providing new insights into the interactions of mammalian frataxin with the Fe-S assembly complex by combining *in vitro* and *in vivo* approaches. Through immunoprecipitation experiments, we show that the main endogenous interactors of a recombinant mature human frataxin are ISCU, NFS1 and ISD11, the components of the core Fe-S assembly complex. Furthermore, using a heterologous expression system, we demonstrate that mammalian frataxin interacts with the preformed core complex, rather than with the individual components. The quaternary complex can be isolated in a stable form and has a molecular mass of  $\approx 190$  kDa. We then identified, by directed mutagenesis on frataxin, crucial residues needed for the interaction, defining the interaction surface between frataxin and the ISCU/NFS1/ISD11 complex. Finally, we demonstrate that the mature human FXN<sub>81-210</sub> form of frataxin is the essential functional form *in vivo*.

**Conclusions:** Our results suggest that the interaction of frataxin with the core ISCU/NFS1/ISD11 complex most likely defines the essential function of frataxin. Our results provide new elements important for further understanding the early steps of *de novo* Fe-S cluster biosynthesis. We are currently characterising the complex and the precise role of frataxin within the ISCU/NFS1/ISD11 complex during Fe-S cluster assembly.

**Title: A Mössbauer and resonance Raman study of the role of CyaY in iron sulfur cluster assembly on the *E. coli* IscU scaffold protein**

Authors: Clara Iannuzzi<sup>1</sup>, Salvatore Adinolfi<sup>1</sup>, Barry D. Howes<sup>2</sup>, Ricardo Garcia-Serres<sup>3</sup>, Martin Clémancey<sup>3</sup>, Jean-Marc Latour<sup>3</sup>, Giulietta Smulevich<sup>2</sup>, Annalisa Pastore<sup>1</sup>

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**Abstract:**

Progress in understanding the mechanism underlying the enzymatic formation of iron-sulfur clusters has proven to be difficult since it involves a complex reaction and a multi-component system. We show here that resonance Raman and Mössbauer spectroscopies, two techniques widely used for studying iron complexes, can be successfully applied to follow the succession of events which leads to cluster assembly on the IscU acceptor protein and, thus, to provide new information on the process. Exploiting these tools, we have further characterized the effect of bacterial frataxin CyaY on the enzymatic kinetics of cluster formation. CyaY is a highly conserved protein present in most organisms and responsible in humans for Friedreich's ataxia. Previous studies had suggested a role of CyaY as an inhibitor.

Our new studies independently confirm that CyaY slows down the reaction and suggest that its presence does not significantly alter the relative ratio between  $[2Fe_2S]^{2+}$  and  $[4Fe_4S]^{2+}$ . Taken together this information can help us reconstruct the mechanism of action of frataxin in cluster assembly.

**Title: Mutation in Isu bypasses frataxin**

Authors: Andrew Dancis, Emmanuel Lesuisse, Debkumar Pain

Institutions: University of Pennsylvania, CNRS-Paris, UMDNJ-New Jersey Medical School

Corresponding author email address: Andrew Dancis, adancis@mail.med.upenn.edu

Abstract:

**Background/Hypothesis:**

The function of frataxin in cells has been controversial. The discovery of a protein complex of frataxin and other Fe-S cluster assembly components supports a role in Fe-S cluster assembly. Whereas, Fe-S cluster assembly is an essential process, frataxin is dispensable under some conditions. A possible explanation for this conundrum is that under normal circumstances frataxin interacts with components for Fe-S cluster assembly and regulates their activities. Under special circumstances or in certain mutants, misregulation of these components can bypass the need for frataxin.

**Methods/Results:** Spontaneous suppressors of frataxin deleted strains were selected on non-fermentable (respiratory) substrates and genetically analyzed. The suppressors recovered many of the functions deficient in  $\Delta yfh1$  strains such as Fe-S cluster enzyme activities. The genes involved in Fe-S cluster assembly were sequenced in wild-type, frataxin minus and suppressor strains. A single mutation was discovered in all suppressors that we tested. This was a missense mutation in *Isu1*, altering a conserved amino acid residue, methionine M141 to isoleucine. The genetic work has been done to confirm that this mutation was indeed responsible for the suppression. A plasmid with the mutant form of *Isu1* transformed into a  $\Delta yfh1$  strain conferred wild-type growth on various media. Biochemically, the suppressor exhibited many features of the wild-type, such as aconitase activity. There must be differences between wild-type (frataxin plus) and the frataxin minus (suppressed strain), and this is a subject of study.

**Conclusions:** Frataxin is dispensable for Fe-S cluster assembly. A single point mutation in *Isu1* (M141I) could almost entirely bypass the need for frataxin. Of interest is that all eukaryotes have methionine in this position, while most prokaryotes have isoleucine or valine, suggesting that the mutation is rendering *Isu* prokaryote-like. In crystal structures this amino acid is on the surface and likely altering an interaction site of *Isu*.

## Title: The importance of the N-terminus: Structural insights into human frataxin in monomeric and oligomeric forms.

Authors:

Eva-Christina Ahlgren<sup>1</sup>, Oleksandr Gakh<sup>2</sup>, Alexander Shkumatov<sup>3</sup>, Martin Lindahl<sup>1</sup>, Douglas Y. Smith IV<sup>2</sup>, Grazia Isaya<sup>2</sup> and Salam Al-Karadaghi<sup>1</sup>

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### Abstract

Frataxin is encoded in the nucleus and subsequently imported into the mitochondria where the signalling peptide is proteolytically removed at either amino acid 42, 56, or 81 by the mitochondrial processing peptidase (MPP)<sup>1,2</sup>. In samples from FRDA patients, both the 81-210 (FXN<sup>81-210</sup>) and 42-210 (FXN<sup>42-210</sup>) variants were found, however, the FXN<sup>42-210</sup> variant seems to be depleted<sup>3</sup>.

There are distinctive differences in the behaviour of the different variants of frataxin. The longer versions, FXN<sup>42-210</sup> and FXN<sup>56-210</sup>, have been shown to form oligomeric species, whereas FXN<sup>81-210</sup> was highly soluble and did not show any tendency to oligomerise. The oligomeric FXN<sup>42-210</sup> and FXN<sup>56-210</sup> had the ability to bind both Fe(II) and Fe(III), whereas the monomeric FXN<sup>42-210</sup>, FXN<sup>56-210</sup> and FXN<sup>81-210</sup> could only bind Fe(II)<sup>1,4</sup>. Thus, it appears that the N-terminal part of the protein may play an important role in the regulation of the function of frataxin. The only three-dimensional (3D) structure of human frataxin that has been resolved is that of a truncated monomer<sup>5</sup> and nothing is known about the structure of the oligomeric species. In this work we use single-particle reconstruction from electron microscopy images and solution small-angle X-ray scattering (SAXS) in the study of the structure of human frataxin oligomers. We will present the 3D structure of oligomeric human FXN<sup>42-210</sup> and FXN<sup>56-210</sup> variants, as well as solution SAXS measurements on the FXN<sup>42-210</sup>, FXN<sup>56-210</sup> and FXN<sup>81-210</sup> variants.

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<sup>3</sup> Gakh O, Bedekovics T, Duncan SF, Smith DY 4th, Berkholtz DS, Isaya G. *Normal and Friedreich ataxia cells express different isoforms of frataxin with complementary roles in iron-sulfur cluster assembly*. (2010) J Biol Chem. 285(49): 38486-501

<sup>4</sup> O'Neill HA, Gakh O, Park S, Cui J, Mooney SM, Sampson M, Ferreira GC, Isaya G. *Assembly of human frataxin is a mechanism for detoxifying redox-active iron*. (2005) Biochemistry. 44:537-45.

<sup>5</sup> Dhe-Paganon S, Shigeta R, Chi YI, Ristow M, Shoelson SE *Crystal structure of human frataxin*. (2000) J.Biol.Chem. 275: 30753-30756

## **Title: Oligomeric frataxin protects NFS1 from oxidative inactivation during Fe-S cluster assembly**

Authors: Oleksandr Gakh and Grazia Isaya

Institution: Mayo Clinic, Rochester Minnesota, USA

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Abstract:

### **Background**

We recently reported important functional differences between oligomeric FXN42-210 and monomeric FXN81-210 (Gakh et al. *J. Biol. Chem.* 2010):

- NFS1-ISD11 binds with nanomolar affinity to oligomeric FXN42-210 independent of ISCU.
- Stable complexes between NFS1 and oligomeric FXN42-210 are readily isolated from human cells via size-exclusion chromatography and co-immunoprecipitation.
- Oligomeric FXN42-210 chelates Fe<sup>2+</sup> (>10 atoms/subunit), and promotes its conversion to a stable oxidized form.
- The iron bound to oligomeric FXN42-210 is available for cluster assembly regardless of its oxidation state.

In contrast:

- NFS1-ISD11 binds to FXN81-210 with micromolar affinity only in the presence of ISCU.
- FXN81-210 dissociates from NFS1-ISD11-ISCU during isolation from human cells.
- FXN81-210 does not chelate Fe<sup>2+</sup> or Fe<sup>3+</sup> in a stable manner.
- FXN81-210 catalyzes cluster assembly only under conditions that prevent oxidation of Fe<sup>2+</sup>.

### **Hypothesis**

Tsai & Barondeau (*Biochemistry* 2010) proposed recently that FXN81-210 functions with Fe<sup>2+</sup> to activate NFS1 and cluster assembly. However, in aerobic conditions, NFS1 activation by FXN81-210 was abolished in the presence of Fe<sup>2+</sup>. Based on our previous observations, we hypothesized that oligomeric FXN42-210 might be able to activate NFS1 both in low oxygen and aerobic conditions.

### **Methods**

Biochemical assays were performed with purified recombinant proteins essentially as described by Tsai & Barondeau.

### **Results**

- In low oxygen, oligomeric FXN42-210 activates NFS1 in the presence of ISCU, similar to FXN81-210.
- In aerobic conditions with Fe<sup>2+</sup> present, oligomeric FXN42-210 prevents oxidative inactivation of NFS1, unlike FXN81-210.

### **Conclusions**

- Stable contacts between oligomeric FXN42-210 and NFS1-ISD11-ISCU enable the protected delivery of potentially toxic iron for cluster assembly, while preventing oxidative inactivation of NFS1.
- In mitochondria, FXN42-210 is likely needed to ensure maintenance of Fe-S cluster synthesis within a broad range of oxygen and iron concentrations.

## Title: Metal-dependent oligomerisation of yeast frataxin: Structural insights from small-angle X-ray scattering (SAXS).

Authors: Christopher Söderberg<sup>1</sup>, Oleksandr Gakh<sup>2</sup>, Alexander Shkumatov<sup>3</sup>, Douglas Y. Smith IV<sup>2</sup>, Grazia Isaya<sup>2</sup> and Salam Al-Karadaghi<sup>1</sup>

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### Abstract

Frataxin is encoded in the nucleus and subsequently imported into the mitochondria where the signalling peptide is proteolytically removed at either amino acid 42, 56, or 81 by the mitochondrial processing peptidase (MPP)<sup>6,7</sup>. In samples from FRDA patients, both the 81-210 (FXN<sup>81-210</sup>) and 42-210 (FXN<sup>42-210</sup>) variants were found<sup>8</sup>. The different forms of human frataxin have been demonstrated to behave differently when oligomerisation is considered. The longer versions, FXN<sup>42-210</sup> and FXN<sup>56-210</sup>, have been shown to form oligomeric species, whereas FXN<sup>81-210</sup> was highly soluble and did not show any tendency to oligomerise. It is also known that proteins involved in upholding iron homeostasis in bacterial cytoplasm, like ferritin, bactoferritin, Dps and the bacterial frataxin homologue CyaY, have different propensities to oligomerise. While ferritin oligomerises spontaneously, *E. coli* CyaY forms tetramers when Fe<sup>2+</sup> is added anaerobically, and larger oligomers are formed in the presence of Fe<sup>2+</sup> and atmospheric oxygen<sup>9</sup>. In an attempt to gain an insight into the determinants that control frataxin oligomerization and assembly of functional species, in this work we have studied Co<sup>2+</sup> binding to yeast frataxin Yfh1 trimers by X-ray crystallography and Co<sup>2+</sup>-dependent oligomerization using small angle X-ray scattering, cross-linking and protein-protein docking.

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<sup>6</sup> Condò I, Ventura N, Malisan F, Rufini A, Tomassini B, Testi R. *In vivo* maturation of human frataxin. (2007) Hum Mol Genet. 16:1534-40.

<sup>7</sup> Schmucker S, Argentini M, Carelle-Calmels N, Martelli A, Puccio H. *The in vivo* mitochondrial two-step maturation of human frataxin. (2008) Hum Mol Genet. 17:3521-31.

<sup>8</sup> Gakh O, Bedekovics T, Duncan SF, Smith DY 4th, Berkholz DS, Isaya G. Normal and Friedreich ataxia cells express different isoforms of frataxin with complementary roles in iron-sulfur cluster assembly. (2010) J Biol Chem. 285: 38486-501.

<sup>9</sup> Layer G, Ollagnier-de Choudens S, Sanakis Y and Fontecave M. Iron-sulfur cluster biosynthesis: characterization of *Escherichia coli* CyaY as an iron donor for the assembly of [2Fe-2S] clusters in the scaffold IscU. (2006) J. Biol. Chem. 281: 16256-63.

**Title: Friedreich Ataxia: A Theoretical Model of the Key Proteins Involved in the Iron-Sulfur Cluster Biogenesis**

Authors: Amela I., Cedano J., Delicado P., Gómez A., Querol E.

Institutions: Institut de Biotecnologia i Biomedicina, Universitat Autònoma de Barcelona.

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Abstract:

**Background/Hypothesis:**

Frx1 plays an important role in the assembly/maturation of the mitochondrial Iron-Sulfur Clusters (ISC) through interactions with Isu proteins, Nfs1 and Isd11. Therefore, these proteins might generate the central platform for the ISC biogenesis.

**Methods:**

Bioinformatics analyses of the sequence, structure, function and putative interactors of these proteins have been carried out. Moreover, different protein-protein docking tools have been applied to these putative interactors. Finally, exhaustive clustering analyses were made to the docking outputs to select the best docking representatives.

**Results:**

A putative interaction mechanism among these proteins has been proposed. The structure of the protein complex, the behavior of its components and the position of the iron and sulfur atoms required for the ISC biogenesis are explained.

**Conclusions:**

This model might be a seed to better understand the function and molecular properties of Frx1 contributing to finally solve the exact ISC biogenesis mechanism.

## **Title: Frataxin participates to the hypoxia-induced response in tumors**

Authors: I. Guccini<sup>1</sup>, D. Serio<sup>1</sup>, I. Condò<sup>1</sup>, A. Rufini<sup>1</sup>, B. Tomassini<sup>1</sup>, A. Mangiola<sup>2</sup>, G. Maira<sup>2</sup>, C. Anile<sup>3</sup>, D. Fina<sup>3</sup>, F. Pallone<sup>3</sup>, MP Mongiardì<sup>4</sup>, A. Levi<sup>4</sup>, N. Ventura<sup>1</sup>, R. Testi<sup>1</sup> & F. Malisan<sup>1</sup>

Institutions:

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Corresponding author email address: Dr. Florence Malisan, Email: Malisan@med.uniroma2.it

Abstract:

### **Background/Hypothesis:**

Frataxin is a protein required for cell survival since complete knock-out is lethal. Frataxin protects tumor cells against oxidative stress and apoptosis but also acts as a tumor suppressor. The molecular bases of this apparent paradox are missing. We therefore sought to investigate the pathways through which frataxin enhances stress resistance in tumor cells.

### **Methods:**

Frataxin protein expression and mRNA content in human tumor cell lines was analyzed upon hypoxic stress induction (<1%O<sub>2</sub>) by western blotting and real-time PCR respectively. Hypoxia-Inducible Factors (HIF) involvement in frataxin upregulation was assessed in human glioblastoma cell lines stably expressing shRNA against HIF-1α. Wild type cells and cells stably expressing shHIF-1α cells were subjected to hypoxia and frataxin protein expression was analyzed. Hypoxia-induced p53 activation was examined in immortalized B lymphoblasts derived from a FRDA patient, compared to control matched immortalized B lymphoblasts derived from a healthy brother, and also in HeLa and U118 cell lines interfered with frataxin expression using a shRNA-based approach. Frataxin was also quantitated in human glioblastoma and colon carcinoma tumor samples, comparing central tumor samples with the adjacent healthy tissue of the same patient.

### **Results:**

We found that frataxin expression is upregulated in several tumor cell lines in response to hypoxic stress, a condition often associated with tumor progression. Moreover, frataxin upregulation in response to hypoxia is dependent on HIF expression and modulates tumor suppressor p53 activation. Importantly, we show for the first time *in vivo* increase of frataxin in human glioblastoma and colon carcinoma tumor samples.

### **Conclusions:**

These results show that frataxin participates to the hypoxia-induced stress response in tumors, thus implying that modulation of its expression could play a critical role in tumor cell survival and/or progression.

# Title: FRDA-associated mutations, W155R and I154F, have complex effects on human frataxin biogenesis and function

Authors: Hongqiao Li and Grazia Isaya

Institution: Mayo Clinic, Rochester Minnesota, USA

Corresponding author email address: isaya@mayo.edu

Abstract:

## Background

- Human FXN precursor is sequentially processed to FXN<sup>42-210</sup> and FXN<sup>81-210</sup>.
- Both FXN<sup>42-210</sup> and FXN<sup>81-210</sup> are present in normal and FRDA cells and tissues.
- FXN<sup>81-210</sup> exhibits monomeric configuration, labile iron binding, and labile/dynamic contacts with NFS1-ISD11-ISCU.
- FXN<sup>42-210</sup> exhibits ability to oligomerize, store iron, and bind tightly to NFS1-ISD11 ± ISCU.
- Monomeric FXN<sup>81-210</sup> donates Fe<sup>2+</sup> for Fe-S cluster assembly on ISCU, while oligomeric FXN<sup>42-210</sup> donates either Fe<sup>2+</sup> or Fe<sup>3+</sup>.

## Hypothesis

Cells expressing FXN proteins with the W155R or I154F mutation exhibited loss of viability or FRDA-like phenotype, respectively. W155 was proposed to be essential for interaction of FXN<sup>81-210</sup> with NFS1-ISD11-ISCU; I154 was implicated in FXN<sup>81-210</sup> stability and iron binding affinity (Calmels et al. *PLoS ONE* 2009; Schmucker et al. *PLoS ONE* 2011; Correia *FEBS J* 2008). We hypothesized that W155R and I154F might have as yet uncharacterized effects on FXN precursor and/or FXN<sup>42-210</sup>.

## Methods

Studies were performed with purified recombinant FXN<sup>42-210</sup> or FXN<sup>81-210</sup> (wild type, W155R, I154F) as well as yeast or human cells expressing wild type or mutant FXN precursors.

## Results

- In yeast cells depleted of endogenous frataxin, the W155R mutation blocks mitochondrial import of FXN precursor causing a knock-out-like phenotype.
- In cells with endogenous frataxin, W155R weakens binding of FXN<sup>81-210</sup> to NFS1-ISD11 without altering the binding affinity of FXN<sup>42-210</sup>.
- We are investigating if W155R impairs the ability of FXN<sup>42-210</sup> to activate NFS1 and cluster assembly.
- The I154F mutation severely affects the stability of FXN<sup>42-210</sup> in *E. coli* and *in vitro* but has no obvious effects on FXN<sup>81-210</sup>.
- In cells, I154F causes aggregation of FXN<sup>42-210</sup>; this results in inability to handle excess iron and also impairs production of FXN<sup>81-210</sup>.

## Conclusions

FXN precursor biogenesis and FXN<sup>42-210</sup> function must be investigated to correctly interpret the phenotypes of FRDA cellular models based on frataxin missense mutations.

**Title: YFHJ is a modulator of frataxin function in bacteria**

Authors: Robert Yan, Salvatore Adinolfi, Clara Iannuzzi, Filippo Prischi, Chiara Pastore, Stephan Martin, Annalisa Pastore

Institution: National Institute for Medical Research, The Ridgeway, London (UK)

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Abstract

**Background/Hypothesis:** YfhJ is a protein of unknown function which takes part in the iron-sulfur cluster assembly machinery, a highly specialised and essential metabolic pathway. YfhJ binds to iron with low affinity and interacts with IscS, the desulfurase central to cluster assembly. The YfhJ surface of interaction with IscS is the same involved in iron binding. Pull-down studies have suggested a competition between YfhJ and CyaY, the bacterial ortholog of frataxin, for the same binding surface of IscS. This competition could suggest a link between the two proteins with functional significance.

**Methods and Results:** Using a multi-technique approach, we show here that YfhJ is a modulator of the inhibitory properties of CyaY: by competing for the same site on IscS, the presence of YfhJ rescues the rates of enzymatic cluster formation which are inhibited by CyaY. The effect is stronger at low iron concentrations, whereas it becomes negligible at high iron concentrations.

**Conclusions:** These results strongly suggest that Fe-S cluster assembly is an exquisite example of an enzymatic process which requires a very detailed fine-tuning.

## **Session 5 (Friday, 14:30 – 17:00): Models of FA**

Co-Chairs: Mark Pook & H el ene Puccio

### Speakers

<b>Mirella Dottori</b> (invited)	Using induced pluripotent stem cells to establish neuronal cellular models of Friedreich Ataxia
<b>Alice P�ebay</b>	FRDA-induced pluripotent stem cell potentials for differentiation
<b>Greg Dusting</b>	Stem Cell Signaling and Generation of Heart Tissue – Potential for cardiac repair and test tissue for new therapeutics for Friedreich Ataxia
<b>Marie Wattenhofer- Donz�e</b>	Development of new models for Friedreich ataxia using induced pluripotent stem cells
<b>Sherman Ku</b>	<i>In vitro</i> differentiation of FRDA induced pluripotent stem cells to neurons provides a cellular model for small molecule therapeutic development
<b>Satyan Chintawar</b>	Generation of Purkinje cells from human iPS cells
<b>Nadia Soussi-Yanicostas</b>	Frataxin depletion in zebrafish embryo, toward a novel animal model for Friedreich ataxia
<b>Sirena Soriano</b>	Study of the metal content in a Drosophila model of Friedreich ataxia
<b>Chiranjeevi Sandi</b>	Prolonged Treatment with Pimelic $\alpha$ -Aminobenzamide HDAC Inhibitors Ameliorates the Disease Phenotype of a Friedreich Ataxia Mouse Model
<b>Michele Lufino</b> (invited)	Drug screening based on <i>FRDA</i> genomic-reporter fusion vectors identifies two candidate molecules able to up-regulate FXN expression

### Posters

<b>Arantxa Bolinches-Amor�s</b>	Continuous silencing of frataxin in human neuroblastoma cells reveals an association of abnormal oxidative phosphorylation and oxidative stress with autophagy, but not apoptosis
<b>Fr�d�eric Boyer</b>	Development of a neuronal model for Friedreich's ataxia with total deletion of frataxin
<b>Ana Ferreira da Silva</b>	Analysis of epigenetic mechanisms induced by GAA triplet expansion in a human cell model of Friedreich's ataxia
<b>Javier Diaz-Nido</b>	Silencing of frataxin gene expression triggers p53-dependent apoptosis in human neuron-like cells
<b>Jonathan Jones</b>	Stem cell-neurotrophic treatment in Friedreich's ataxia mouse models
<b>Kevin Kemp</b>	Mesenchymal stem cells restore frataxin expression in Friedreich ataxia fibroblasts

<b>Michael Koob</b>	Expression of frataxin from mitochondrial DNA can compensate for the loss of nuclear frataxin gene expression
<b>JV Llorens</b>	Frataxin overexpression induces similar phenotypes as frataxin reduction without causing abnormal aggregates or misfolding
<b>Cat Lutz</b>	The Jackson Laboratory Mouse Husbandry and Distribution Core for FA Models
<b>Juan Antonio Navarro</b>	Iron homeostasis in a <i>Drosophila</i> model of Friedreich`s ataxia
<b>Joe Sarsero</b>	Analysis of the role of GAA expansion instability in Friedreich ataxia pathology in a humanized mouse model

## Title: Using induced pluripotent stem cells to establish neuronal cellular models of Friedreich Ataxia

Authors: Mirella Dottori<sup>1</sup>, Mark Denham<sup>1</sup>, Brock Conley<sup>1</sup>, Jessie Leung<sup>1</sup>, Duncan Crombie<sup>2</sup>, Karina Needham<sup>3</sup>, Jun Liu<sup>4</sup>, Marguerite Evans-Galea<sup>5</sup>, Martin B. Delatycki<sup>5,6</sup>, Anna Michalska<sup>7</sup>, Joseph P. Sarsero<sup>5</sup>, Robert Williamson<sup>8</sup>, Paul J. Verma<sup>4</sup> and Alice Pébay<sup>1,2</sup>

Institutions:

<sup>1</sup>Centre for Neuroscience, The University of Melbourne, Australia

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Abstract:

**Background/Hypothesis:** There is a very strong need to establish good human cellular models of Friedreich Ataxia (FA), and for this reason we have generated induced pluripotent stem (iPS) cell lines from skin biopsies taken from adult human FA patients. We describe the differentiation of FA iPS towards neurons, and particularly sensory neurons of the dorsal root ganglia. We hypothesize that these differentiated iPS cell types will serve as appropriate neuronal models of FA for drug screening purposes as well as understanding neurodegenerative mechanisms occurring in this disease.

**Methods:** The FA iPS cell lines were differentiated towards neural progenitors, neurons, neural crest progenitors and sensory neurons using protocols established in our laboratory. Neurons derived from FA iPS cells have been characterized for phenotypic and molecular characteristics of FA, as well as their electrophysiology to show functionality.

**Results:** We show that low level of Frataxin expression is maintained in differentiated FA iPS cells. Furthermore, the GAA repeat expansion appears to increase over time in culture. We also show a system whereby FA iPS cells can be differentiated towards sensory neurons, which can then be isolated and analyzed for their molecular profiling.

**Conclusions:** These studies are necessary for establishing high through-put drug screening assays to measure Frataxin levels specifically within neuronal populations that degenerate in FA as well as creating in vitro neuronal models of FA for studying disease pathogenesis.

## **Title: FRDA-induced pluripotent stem cell potentials for differentiation**

Authors: Rodney Dilley<sup>1,2</sup>, Richard Tee<sup>1</sup>, Rowena Aguilar-Sino<sup>2</sup>, Duncan Crombie<sup>1</sup>, Mirella Dottori<sup>3</sup> & Alice Pébay<sup>1,3</sup>

Institutions: <sup>1</sup> O'Brien Institute; <sup>2</sup> Australian Tissue Engineering Centre; <sup>3</sup> Centre for Neuroscience & Department of Pharmacology, University of Melbourne, Australia.

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Abstract:

### **Background/Hypothesis:**

We generated induced-pluripotent stem (iPS) cell lines derived from skin fibroblasts from two FRDA patients. Although iPS cells are able to differentiate into neural and cardiac lineages, the yield of differentiation remains sub-optimal. Our aim is to improve differentiation techniques of iPS cells in order to obtain sufficient cell numbers for drug screening.

### **Methods:**

We combined various approaches to target iPS cell differentiation: 1) *in vitro* differentiation protocols following published protocols for hESC differentiation; 2) *in vivo* differentiation using a vascularized tissue-engineering chamber for evaluation of FRDA-iPS cells survival after implantation, their ability to integrate into engineered tissues and to differentiate *in vivo*, particularly to clinically relevant cardiac muscle tissue and 3) *in vitro* assessment of gap junctional communication.

### **Results:**

We observed very little spontaneous cardiac muscle differentiation of FRDA-iPS cell lines, which was not improved by the use of published cardiac differentiation protocols. Our data also indicate that when compared to control iPS cells or EBs, FRDA-iPS cells and their differentiated EBs maintain low levels of FXN mRNA and that cx43 and cx45 mRNA are modified in differentiated EBs. Our *in vivo* experiments for cardiac differentiation have so far demonstrated that iPS cells can survive implantation and over 4 weeks integrate into engineered tissues and differentiate into teratomas. Similar data were observed when iPS cells were pre-differentiated into EBs prior to transplantation.

### **Conclusions:**

Our combined approaches for differentiation of iPS cells might provide important information for a more efficient differentiation of FRDA-iPS cells into specific cell types.

## **Title: Stem Cell Signaling and Generation of Heart Tissue – Potential for cardiac repair and test tissue for new therapeutics for Friedreich Ataxia**

Authors: Rodney Dilley<sup>1,4</sup>, Hitesh Peshavariya<sup>1</sup>, Guei-Sheung Liu<sup>1</sup>, Alice Pébay<sup>1,2,3</sup>, Gregory J Dusting<sup>1,3,4</sup>

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### Abstract

We have developed a surgical method for growing fully vascularised, beating heart tissue *in vivo*, such that it can be transplanted to replace dead or malfunctioning tissue in a failing heart. Stem cells are sources for the human cardiomyocytes required, either adipose-derived stem cells (ASC) or induced pluripotent stem cells (iPSC) derived from fibroblasts. To grow complex heart tissue on a vascular bed we may need up to 100,000 cardiomyocytes, which can be obtained from differentiated cells of embryoid bodies, themselves derived from human iPSC, and indeed we have generated beating cardiac cells from Friedreich's ataxia patients (see Pébay presentation). In order to expand iPSC and to increase the efficiency of iPSC differentiation down the cardiac lineage, we are now exploring the cell signaling involved. Others have shown oxidant signaling involving the NADPH oxidase system is important for differentiation of embryonic cells to cardiomyocytes. In the FA3 cell line from Friedreich's we found expression of the Nox4 component of NADPH oxidase was 50-fold higher in differentiated embryoid bodies than in the iPS cells from which they were derived, along with the cardiac markers troponin, Nkx2.5 and cardiac actin. We are now manipulating the NADPH oxidase pathway in iPSC and ASC in an attempt to preferentially direct pathways of differentiation into cardiac or other lineages, and to produce appropriate cardiac pacemaker, atrial, conducting or ventricular phenotypes. These are being grown into cardiac constructs in immunocompromised rats, and human ASC have been grown into cardiac constructs which incorporate human cells in chimeric blood vessels and heart muscle. Thus we are on the path to producing fully vascularised, large human cardiac constructs, ideal for transplantation to repair cardiac defects or malfunctions, such as contractile failure or conduction defects. In conclusion, Nox 4 has crucial roles in iPSC differentiation down the cardiac lineage and in angiogenesis, which can be exploited to generate cardiac constructs more efficiently. Human cardiac constructs derived from Friedreich's patients could be used as test beds for developing new therapeutics for cardiac abnormalities, and after genetic correction *in vitro*, iPSC could be grown into replacement cardiac tissues for these patients.

## **Title: Development of new models for Friedreich ataxia using induced pluripotent stem cells.**

Authors: Marie Wattenhofer-Donzé<sup>1</sup>, Aurore Hick<sup>1</sup>, Philippe Tropel<sup>2</sup>, Nadège Vaucamps<sup>1</sup>, Laurence Reutenauer<sup>1</sup>, Cécile André<sup>2</sup>, Stéphane Viville<sup>2</sup>, Hélène Puccio<sup>1</sup>

Institutions:

<sup>1</sup>Translational medicine and Neurogenetics and <sup>3</sup>Department of cellular biology & development, IGBMC (Institut de Génétique et de Biologie Moléculaire et Cellulaire) ; Inserm U964 ; CNRS-UdS UMR7104 ; Illkirch, France

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Abstract:

### **Background/Hypothesis:**

The recent technical advances in the generation of induced pluripotent stem cells (hiPSCs) from somatic cells provides a powerful tool to create (GAA)<sub>n</sub> cells with a capacity to differentiate in various cell types, thus providing cellular models for FA.

### **Methods:**

hiPSCs have been obtained by infection of two patient and two control skin fibroblasts with lentiviral vectors expressing Oct4, Sox2, Nanog and Lin 28. After validation of all standard criteria of bona fide reprogramming (morphology, gene expression, tissues derived from the 3 germ layers by teratoma analysis), hiPSCs were induced to neural lineage by noggin treatment using two different protocols, one directing them towards a cerebellar lineage, specifically to a Purkinje cells (PC) phenotype. Cardiomyocyte differentiation was achieved via embryoid bodies.

### **Results:**

Fibroblasts from two FA patients and two healthy controls were reprogrammed to obtain hiPSC clones. Most clones retained the large expansion initially present in the FA fibroblasts. During reprogramming as well as during hiPSC cell passages, the (GAA)<sub>n</sub> is unstable, showing both expansion and contraction. By q-RT-PCR, the frataxin level of FA hiPSCs is 15-25 % of the one of control hiPSCs. We are currently characterizing our hiPSCs clones in term of any specific FA phenotype such as Fe-S proteins activity, epigenetic modifications at the GAA locus. In parallel, we are differentiating our hiPSC clones into neuronal cultures. After 6 weeks of culture following the "cerebellar" protocol, differentiating cells migrated out of hiPSCs-derived neurospheres and generated a mixed cellular population that included 8-10% of calbindin-D28k and a few expressing L7 (Purkinje cell (PC) markers) expressing neurons. These cells have morphologic features corresponding to different stages of PC maturation. Patch-clamp revealed Na<sup>+</sup> currents and ability to fire action potentials. We recently obtained beating cardiomyocytes from both control and FA and are currently characterizing them.

### **Conclusions:**

Our results demonstrate that reprogramming was successfully performed. We are currently characterizing the hiPSC derived neurons and cardiomyocytes to identify any FA specific phenotype and will study (GAA)<sub>n</sub> stability upon differentiation.

**Title: *In vitro* differentiation of FRDA induced pluripotent stem cells to neurons provides a cellular model for small molecule therapeutic development**

Authors: Sherman Ku, Erica Campau, Elisabetta Soragni, Joel M. Gottesfeld

Institutions: The Scripps Research Institute

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Abstract:

**Background/Hypothesis:** Previous work in developing small molecule therapeutics for Friedreich's ataxia (FRDA) has focused on histone deacetylase inhibitors (HDACi) based on the hypothesis of heterochromatin repression of the frataxin gene. However, these studies were performed in cellular or animal models, which are not of biologically relevant cell types or do not fully recapitulate clinical symptoms observed in humans, respectively. While these models are useful in some cases, in order to better understand the heterochromatin nature of the frataxin locus *in vitro*, a patient-specific neuronal model is needed.

**Methods:** To generate *in vitro* Tuj1+ neurons from previously reported FRDA induced pluripotent stem cells, neural induction to neural stem cells (NSCs) was performed by noggin treatment, and further differentiation of these NSCs on a laminin substrate yielded an enriched population of Tuj1<sup>+</sup> neurons. HDAC inhibitor efficacy was assayed by qRT-PCR of frataxin mRNA, Western analysis of protein levels, and chromatin immunoprecipitation experiments.

**Results:** We show that FRDA Tuj1<sup>+</sup> neurons express appropriate neuronal markers and retain marked heterochromatin repression of frataxin expression as shown by qRT-PCR of frataxin mRNA and chromatin immunoprecipitation experiments analyzing various histone marks along the frataxin locus. Upon treatment with the HDAC inhibitor 109, we observed a dose-dependent upregulation of frataxin mRNA by qRT-PCR. Current efforts include verification of frataxin protein upregulation, reversal of heterochromatin histone marks, recovery of mitochondrial defects, and analysis of global gene expression changes upon treatment with HDAC inhibitors.

**Conclusions:** Our preliminary data suggest that *in vitro* differentiated neurons from FRDA iPSCs recapitulate key molecular defects found in FRDA which can be reversed by HDACi treatment and provides a readily available source of cells in which to further study other possible effects of frataxin protein deficiency.

## **Title: Generation of Purkinje cells from human iPS cells**

Author: Satyan Chintawar, Marie Wattenhofer-Donzé<sup>2</sup>, H  l  ne Puccio<sup>2</sup>, Massimo Pandolfo

Institutions: Universit   Libre de Bruxelles

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The recently developed induced pluripotent stem (iPS) cell technology is a promising approach to derive patient-specific cell types affected in Friedreich's ataxia (FRDA). In FRDA patients, there is a slight reduction in cerebellar Purkinje cell (PC) number, whose axons projecting to deep cerebellar nuclei undergo a degenerative process called granule degeneration. Taking advantage of iPSC technology, we aim to derive PCs from FRDA patients, which will eventually help in revealing pathogenic mechanisms and can be used to screen potential therapeutics. FRDA-iPS cells, obtained by reprogramming skin fibroblasts at IGBMC (Strasbourg, France), were neuralized by noggin treatment and subsequently cultured in mitogens, FGF-2 and EGF to derive neural precursor cells cultured as neurospheres. Inductive signals for cerebellar lineage were introduced by signaling molecules and extracellular factors (BMP4, Wnt3a, FGF8). These neurospheres were co-cultured on postnatal mouse cerebellar feeder and differentiated in presence of morphogens and neurotrophins (Shh, BDNF, NT3 and T3) for 8 weeks. Differentiating cells migrated out of neurospheres and generated a mixed cellular population that included LHX5, calbindin-D28k and L7 (PC markers) expressing neurons. Patch-clamp revealed Na<sup>+</sup> currents and ability to fire action potentials. Additional differentiation protocols without a cerebellar feeder layer are currently being tested, with initial promising result that include generation of cells expressing PC markers and spontaneously firing action potentials.

# **Title: Frataxin depletion in zebrafish embryo, toward a novel animal model for Friedreich ataxia**

Authors: C. Yanicostas<sup>1</sup>, P. Rustin<sup>2</sup> and N. Soussi-Yanicostas<sup>1</sup>

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Abstract:

## **Background/Hypothesis:**

A faithful Friedreich ataxia animal model must display reduced, albeit not full, frataxin depletion to reproduce the situation observed in patients and avoid the embryonic lethality induced by full loss-of-function of the gene.

## **Methods:**

We, and a great number of other teams, have shown that any zebrafish gene can be fully or partially inactivated following microinjection of high or low concentrations of gene-specific modified antisense oligonucleotides, or morpholino-oligonucleotides (MO), respectively. We made use of this technology to generate embryos showing severe frataxin depletion.

## **Results:**

We observed that while complete, or nearly complete, frataxin depletion impairs development and embryo viability, as previously described for other animal models, severe, albeit not full, frataxin inactivation affects several organs or tissues, including the ear, and of particular interest, spinal motor neuron axons. We also analyzed the sensitivity of frataxin-depleted embryos to oxidative stress. Next, we have analyzed whether anti-oxidant molecules, such as vitamin E and Idebenone, can protect frataxin-depleted embryos from oxidative stress toxicity.

## **Conclusions:**

The zebrafish is an ideal model for identifying small molecules showing therapeutic properties. Actually, the possibility to use whole living organisms for drug screening allows complex *in vivo* defects, such as neurological impairments, to be detected. In this context, frataxin-depleted zebrafish embryos appear as a good model system to identify molecules showing therapeutic activity.

**Title: Study of the metal content in a *Drosophila* model of Friedreich ataxia.**

Authors: Soriano S<sup>1</sup>, Gutierrez L<sup>2</sup>, Llorens JV<sup>1,3</sup>, Morales MP<sup>2</sup>, Moltó MD<sup>1,4</sup>, Martínez-Sebastián MJ<sup>1</sup>.

Institutions: <sup>1</sup>Universitat de València; <sup>2</sup>Instituto de Ciencia de Materiales de Madrid, CSIC; <sup>3</sup>Instituto de Biomedicina de Valencia, CSIC; <sup>4</sup>Spanish National Network for Research in Mental Health. CIBERSAM.

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Abstract:

**Background/Hypothesis:** We have previously developed a Friedreich ataxia model in *Drosophila melanogaster* with a reduction of frataxin similar to patients. In addition, these flies show a reduction of lifespan, motor performance and aconitase activity, as well as an enhanced susceptibility to oxidative stress.

**Methods:** We measured the metal content of mutant (*actin-GAL4>UAS-fhIR*) and control (*actin-GAL4>yw*) flies at 7 and 35 days after their emergency from puparium. Flies were freeze dried and acid digested prior to the measure of iron, manganese, copper and zinc by atomic absorption spectroscopy. We also studied the effect of the iron chelator Deferiprone (163 µM dissolved in Instant Medium Blue, Carolina Biological Supply) and the effect of oxidative stress (flies were maintained 48 hours in hyperoxia) on the content of these metals.

**Results:** Although an accumulation of mitochondrial iron has been previously described in animal models of the disease as well as in patients tissues, we have not found significant differences in total iron content between mutants and controls in any of the conditions tested. No reduction of iron was detected in the presence of Deferiprone. However, manganese was significantly increased in both 7 and 35 days old mutant flies with respect to control flies of the same age. Mutants show as well an accumulation in copper levels that, contrary to manganese increase, is absent in the first week and appear with aging. On the contrary, there is an increase in zinc levels in the 7 days old mutants that is not found in older mutant flies. Oxidative stress did not have any specific effect on the metal content of our fly model.

Since in the literature the iron accumulation is found in mitochondria and Deferiprone is supposed to scavenge iron from the mitochondria, we are currently extending this study to mitochondrial extracts from our model flies.

**Conclusions:** Our results indicate that other metals besides iron, such as manganese, copper and zinc could be accumulated in Friedreich Ataxia.

## **Title: Prolonged Treatment with Pimelic $\alpha$ -Aminobenzamide HDAC Inhibitors Ameliorates the Disease Phenotype of a Friedreich Ataxia Mouse Model**

Authors: Chiranjeevi Sandi<sup>1</sup>, Ricardo Mouro Pinto<sup>1</sup>, Sahar Al-Mahdawi<sup>1</sup>, Vahid Ezzatizadeh<sup>1</sup>, Glenn Barnes<sup>2</sup>, Steve Jones<sup>2</sup>, James R. Rusche<sup>2</sup>, Joel M. Gottesfeld<sup>3</sup> and Mark A. Pook<sup>1</sup>

Institutions: <sup>1</sup> Division of Biosciences, School of Health Sciences and Social Care, Brunel University, Uxbridge, UB8 3PH, UK, <sup>2</sup> Repligen Corporation, Waltham, Massachusetts, USA, <sup>3</sup> Department of Molecular Biology, The Scripps Research Institute, La Jolla, California, USA.

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Abstract:

**Background/Hypothesis:** We have established a GAA repeat expansion mutation-based human genomic YAC transgenic mouse model of FRDA, designated YG8R, which exhibits an FRDA-like molecular phenotype, including GAA repeat instability, GAA-induced epigenetic changes and frataxin deficiency, leading to impaired motor coordination, reduced aconitase activity and DRG neuronal pathology. This mouse model is considered a suitable system in which to investigate the long-term effects of potential FRDA therapies.

**Methods:** YG8R mice were treated with class I HDAC inhibitors 106, 109 and 136 for up to 5-months and functional analysis, including rotarod, locomotor and beam-breaker activities, were performed at regular intervals. Following treatment, frataxin mRNA and protein levels, H3 and H4 histone acetylation changes and aconitase activities in brain tissue and DRG vacuolar pathology were assessed.

**Results:** The long-term administrations of 106, 136 and 109 were well tolerated and no toxicity was generally observed in the mice throughout the entire period of study. While the neurological deficits of this model are mild, 109 and 106 both produced an improvement of motor coordination, whereas 109 and 136 produced increased locomotor activity. All three compounds increased global histone H3 and H4 acetylation of brain tissue, consistent with the compounds crossing the blood-brain barrier, but only 109 significantly increased acetylation of specific histone residues at the *FXN* locus. Effects on *FXN* mRNA expression in CNS tissues were modest, but 109 significantly increased frataxin protein expression in brain tissue. 109 also produced significant increases in brain aconitase enzyme activity, together with reduction of neuronal pathology of the dorsal root ganglia (DRG).

**Conclusions:** Each of the three class I HDAC inhibitor compounds ameliorated disease effects observed in the YG8R FRDA mouse model, with 109 emerging as the lead compound. These results support further assessment of HDAC inhibitors for treatment of Friedreich ataxia.

**Title: Drug screening based on *FRDA* genomic-reporter fusion vectors identifies two candidate molecules able to up-regulate FXN expression.**

Authors:

Michele Lufino, Ana Ferreira da Silva, Javier Alegre-Abarrategui, Angela Russell and Richard Wade-Martins.

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Abstract:

**Background/Hypothesis:**

Currently, there is no treatment for FA, although several therapeutic strategies are under development. An efficient selection of candidate drugs for this disease requires the development of a high-throughput screening assay based on a cell model that recapitulates the GAA-mediated repression of frataxin expression.

**Methods:**

We have recently developed *FRDA* genomic-reporter fusion vectors by inserting the Luciferase gene in-frame at the 3' end of the *FRDA* gene (*FRDA*-Luc). These vectors allow rapid quantification of frataxin expression by a Luciferase assay. We introduced ~300 GAA repeats in intron 1 of *FRDA*-Luc, creating *FRDA*-GAA-Luc and we generated human clonal cell lines carrying the *FRDA*-GAA-Luc vector. We used the *FRDA*-GAA-Luc cell line to screen 88 compounds selected from a 22,000 molecule library, based on their similarity to structural motifs present in HDAC inhibitors (HDACi). A blind screening using a Luciferase assay was performed.

**Results:**

The initial screening identified seven compounds which increased FXN-Luciferase expression. These compounds were further analyzed on the *FRDA*-GAA-Luc cell line at different concentrations. We discarded two of the seven compounds, due to a negligible effect on FXN-Luciferase expression and analyzed the effect of the remaining five on FXN expression in FA lymphoblastoid cells. All five compounds successfully increased frataxin protein levels. To eliminate drugs that induce an unspecific increase of gene expression we tested their effect on pCMV-Luciferase. Two of the five compounds showed to be specific. We are currently performing a dose response study on FA lymphoblastoid cells to find the optimal active concentration.

**Conclusions:**

We successfully developed an *in vitro* cell assay based on the *FRDA* genomic DNA locus carrying a GAA expansion, which allows high-throughput screening of drugs for FA. We used this assay to select two candidate drugs which can successfully increase frataxin levels in FA patient-derived cells and do not have an unspecific effect on gene expression.

**Title: Continuous silencing of frataxin in human neuroblastoma cells reveals an association of abnormal oxidative phosphorylation and oxidative stress with autophagy, but not apoptosis**

Authors: Arantxa Bolinches-Amorós, Belén Mollá, David Pla-Martin, Maribel Sanchez-Piris, Francesc Palau, Pilar Gonzalez-Cabo

Institutions: Institute of Biomedicine of Valencia, CSIC, and CIBER on Rare Diseases (CIBERER), Valencia, Spain.

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Abstract:

**Background/Hypothesis:** Generation of neuron models deficient for frataxin allows investigating the consequences of the lack of frataxin on mitochondrial metabolism and cell homeostasis. We have established several frataxin RNAi knock-down SH-S5Y human neuroblastoma cell lines, where we have investigated mitochondrial and cell functions. The overall objective is to understand the role of mitochondria pathophysiology of the neurodegenerative process in Friedreich ataxia.

**Methods:** We developed stable SHSY5Y cells lines expressing shRNA against *FXN* transcripts. In this model we have evaluated the mitochondrial function by studying enzymatic activities of the electron transport chain (ETC), and measuring ATP production and mitochondrial membrane potential. We have also analyzed mitochondrial network by measuring mitochondrial morphology, length and number. Oxidative stress was evaluated by determining protein carbonylation, expression of antioxidants enzymes and indirect measure of superoxide anion levels using the MitoSox probe. Finally, we also investigated autophagy and apoptosis by studying specific proteins expression.

**Results:** Two stable cell lines, FXN-138.1 and FXN-138.2, were selected. Silencing efficiency was 68% and 61% reduction of protein, respectively. *FXN* knock-down increased cell growth pace but did not modify cell cycle progression. Frataxin deficiency induced more tubulation of the mitochondrial network. Deficient cells showed reduction of ATP and oxygen consumption, and loss of mitochondrial membrane potential. Enzymatic activities of ETC complexes were normal but complex IV that was reduced. Oxidative stress was present in frataxin-depleted cells but there was no increase of caspase-3 expression suggesting that oxidative damage was not associated with apoptosis. By contrast, we observed increases expression of the autophagy marker LC3-II.

**Conclusions:** Continuous chronic reduction of frataxin in human neuroblastoma SHSY5Y cells affects oxidative phosphorylation function and induces oxidative stress. These mitochondrial changes are associated with induction of autophagy markers but not cell death caused by apoptosis.

**Title: Development of a neuronal model for Friedreich's ataxia with total deletion of frataxin**

Authors: Frédéric Boyer, Laurence Reutenauer, Nadège Vaucamps, Hélène Puccio

Institutions:

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Abstract:

**Background/Hypothesis:**

Mammalian cell models are important to study the molecular mechanisms of the disease and can be used for therapeutic screening approaches. Some cell models have already been developed to reproduce the principal biochemical features of FRDA (ISC enzyme deficit, sensitivity to oxidative stress and mitochondrial iron accumulation) but none of the models developed are neuronal models. Since FRDA is primarily a neurodegenerative disease, a neuronal model that reproduces accurately the major aspects of the pathogenesis is the preliminary condition to study the molecular mechanisms involved in the neuronal loss.

**Methods:**

We have recently developed a neuronal cell model based on neurospheres assay derived from the conditional mouse models. The conditional allele can be deleted by exposure to the Cre recombinase. These neurospheres can be (a) dissociated to form numerous secondary spheres or (b) induced to differentiate, generating the three major cell types of the CNS..

**Results:**

We have developed a protocol that enables us to differentiate the neurospheres reproducibly to a neuronal culture, comprise of about 80% neurons, with the presence of 2-5 % glial cells. These neurons can be maintained in culture for a period of time up to 10 weeks. Using a lentivirus system driving the expression of a Cre-eGFP, we are able to completely delete the frataxin expression into neuronal cells. The neurons completely deleted for frataxin can be maintained in culture for a period of time up to 5 weeks, at time which cellular death appears. This new neuronal model for FRDA, actually in characterization seems to demonstrate some of the biochemical features of FRDA (giant mitochondria, swelling mitochondria, iron deposits...).

**Conclusions:**

The biochemical analysis and phenotyping analysis of this neuronal model will be achieved shortly (activities of the Fe-S enzymes, amount of Iron into mitochondria, neuronal subtype characterization). The further characterization of this model may allow to better understand the function of the frataxin in neurons and the specific death of neurons in Friedreich ataxia and could be useful to test novel therapeutic strategies.

**Title: Analysis of epigenetic mechanisms induced by GAA triplet expansion in a human cell model of Friedreich's ataxia**

Authors: Ana Ferreira da Silva, Michele Lufino, Angela Russell and Richard Wade-Martins.

Institutions:

Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, OX1 3QX, UK.

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Abstract:

**Background/Hypothesis:**

The lack of suitable models for FA has hampered the understanding of the GAA-mediated mechanism of FA pathogenesis. We have developed genomic DNA expression vectors carrying either a ~300 GAA repeats expansion or a normal number of repeats (6 GAA) within the context of the whole *FRDA* genomic locus and generated human clonal cell lines carrying each of the two vectors. The presence of the whole genomic DNA locus provides physiological expression levels and a suitable model to dissect the effect of GAA repeats on the *FRDA* locus.

**Methods:**

We generated *FRDA*-Luc and *FRDA*-GAA-Luc clonal cell lines by site-specific integration, which was confirmed by fluorescence in situ hybridization (FISH) using a *FRDA*-BAC specific probe. To study the GAA repeat-induced gene repression, we analysed the chromatin structure of the *FRDA* promoter and regions flanking the GAA repeats by chromatin immunoprecipitation, using antibodies specific for the human acetylated histones H3K9 and H4K8 and trimethylated histone H3K9. Additionally, we performed bisulfite sequencing studies in these regions to analyse the presence of CpG methylation. Our future work will include the study of GAA repeat instability by small-pool PCR.

**Results:**

FISH experiments using a *FRDA*-BAC specific probe confirmed that both vectors have integrated at the same insertion site located in chromosome 1p, generating directly comparable cell lines, differing only for the GAA repeats insertion. Preliminary epigenetics studies seem to suggest histone acetylation and trimethylation status in accordance with previous studies. Furthermore, we detected increased DNA methylation at specific CpG sites in the studied regions.

**Conclusions:**

The *FRDA*-GAA-Luc cell lines are an invaluable tool to study the mechanisms underlying gene repression, heterochromatin formation and somatic instability caused by expanded GAA repeats.

**Title: Silencing of frataxin gene expression triggers p53-dependent apoptosis in human neuron-like cells**

Authors: GM Palomo, T Cerrato, and J Diaz-Nido

Institutions: Universidad Autonoma de Madrid, Centro de Biologia Molecular Severo Ochoa (UAM-CSIC), CIBER de Enfermedades Raras (CIBERER)

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**Abstract:**

Despite of the importance of neurodegeneration in FA, little is known about the molecular changes that are triggered by frataxin deficit specifically in neurons. We have characterized a human neuron-like cell model for FA based on the use of lentiviral vectors that carry minigenes encoding frataxin-specific shRNAs that silence the expression of the gene. Frataxin gene knockdown triggers large-scale cell death in human neuron-like cells obtained upon differentiation of neuroblastoma cells as well as in primary cultures of rodent neurons. Frataxin-deficient human neuron-like cells die through apoptosis which is preceded by up-regulation of p53, Puma and Bax and is executed by caspase activation. Neuronal apoptosis triggered by frataxin knockdown can be impeded by interference with p53 and caspase inhibition. No significant autophagy is observed in frataxin-deficient neuronal cells, and the pharmacological activation of autophagy does not significantly affect neuronal cell death in response to the frataxin deficit. These results suggest that frataxin gene silencing in human-neuron-like cells may constitute a useful cell model to characterize the molecular changes triggered by frataxin depletion in neurons, as well as to search for therapies that protect against neurodegeneration induced by frataxin knockdown.

## **Title: Stem cell-neurotrophic treatment in Friedreich's ataxia mouse models**

Authors: Jonathan Jones, Jesus Jaramillo-Merchán, Salvador Martínez

Institutions: Neuroscience Institute UMH-CSIC

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Abstract:

### **Background/Hypothesis:**

Stem cells isolated from bone marrow or adipose tissue are an easily obtainable and minimally invasive source of cells that have been proven to be capable of ameliorating and preventing cell death in numerous diseases, including neurodegenerative disorders. There are several methods by which stem cells improve the development of diseases, such as cell fusion processes, transdifferentiation and trophic factor release. Thus, we use these cells in Friedreich's ataxia models in order to confirm if they have a potential beneficial effect, and thus become a possible therapeutic tool in the future.

### **Methods:**

Cultured human Friedreich's ataxia mesenchymal stem cells from the dental pulp of two FA patients, were submitted to oxidative stress (being these cells especially vulnerable to this condition), and grown in human adipose tissue stem cell conditioned medium. Since the dorsal root ganglion is one of the first regions in the AF mouse model to degenerate, resulting in loss of motor skills, mouse bone marrow stem cells were intrathecally injected into the lumbar region of the spinal cord of ataxic mice, in order to avoid the degeneration. After submitting the mice for several weeks in the rotarod, they were sacrificed and analyzed histologically.

### **Results:**

Friedreich's ataxia human cells increased frataxin expression in the presence of stem cell conditioned medium. This expression was further enhanced when the conditioned medium was from stem cells which themselves were submitted to oxidative stress. In the mouse models, the mouse bone marrow stem cells, injected intrathecally, were attached and populated dorsal root ganglia, where they could exert a trophic effect on the ganglionar cells.

### **Conclusions:**

Bone marrow and adipose tissue stem cells exert a local beneficial (neurotrophic) effect on Friedreich's ataxia degenerating cells and tissues, being a possible therapeutic approach for this disease.

## **Title: Mesenchymal stem cells restore frataxin expression in Friedreich ataxia fibroblasts**

Authors: Dr Kevin Kemp, Prof Neil Scolding and Dr Alastair Wilkins

Institutions: Multiple Sclerosis and Stem Cell Group, Institute of Clinical Neurosciences, School of Clinical Sciences, University of Bristol

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Abstract:

### **Background/Hypothesis:**

Dramatic advances in recent decades in understanding the genetic background of Friedreich ataxia (FA) – a GAA triplet expansion causing greatly reduced expression of the mitochondrial protein frataxin – have thus far yielded no therapeutic dividend. There remain no effective treatments that prevent or even slow the inevitable progressive disability in affected individuals. Clinical interventions that restore frataxin expression are attractive therapeutic approaches, as in theory, it may be possible to re-establish normal function in frataxin deficient cells if frataxin levels are increased above a specific threshold. In this study, we therefore performed a series of experiments investigating the effects human bone marrow-derived mesenchymal stem cells (MSCs) have on frataxin expression in fibroblasts derived from patients with FA and healthy controls.

### **Methods:**

Fibroblasts isolated from patients with FA and healthy controls were incubated with MSC conditioned medium. Frataxin mRNA and protein expression levels were then determined along with ability to survive hydrogen peroxide-mediated cytotoxicity. We also examined the link between frataxin expression and sensitivity to hydrogen peroxide in FA fibroblasts through restoring frataxin expression in these cells via transfection of the normal frataxin gene.

### **Results:**

Human bone marrow-derived MSCs increase frataxin protein expression in fibroblasts derived from patients with FA via secretion of soluble factors. Exposure to factors produced by MSCs also increases resistance to hydrogen peroxide mediated toxicity. Furthermore, we have demonstrated there is a direct link between frataxin expression and sensitivity to hydrogen peroxide-mediated toxicity in these fibroblasts.

### **Conclusions:**

We have shown, for the first time that stem cells increase frataxin expression in FA patient-derived fibroblasts and modify their susceptibility to oxidative stress. In time, transplantation of bone marrow-derived MSCs may offer an effective treatment for these patients.

**Title: Expression of frataxin from mitochondrial DNA can compensate for the loss of nuclear frataxin gene expression**

Authors: Young Yoon, Tibor Bedekovics, Yi-Wei Yang, Kellie Benzow, Grazia Isaya, Michael Koob

Institutions: University of Minnesota; Mayo Clinic-Rochester, MN

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**Background/Hypothesis:**

Mitochondrial function is lost in yeast cells when the nuclear frataxin gene is deleted, but can be rescued by nuclear expression of the human frataxin gene. We used this yeast model to test our hypothesis that frataxin expressed from the mtDNA genome can compensate for the loss of frataxin expression from the nuclear genome.

**Methods:**

We recoded the nuclear frataxin genes from both yeast and humans to enable their translation using the mitochondrial genetic code and inserted a wide array of different frataxin cassettes containing these recoded frataxin genes into the yeast mtDNA genome. We then tested the ability of these gene insertions to rescue the growth phenotype of frataxin knock-out strains and evaluated the frataxin expression levels and mitochondrial function in these strains

**Results:**

We found that the most efficient approach for expressing high-levels of frataxin protein from the yeast mitochondrial genome is to fuse the frataxin gene in-frame at the 3' end of a native mitochondrial gene (e.g., *COX2*). A significant proportion of the frataxin peptides are cleaved from the fused protein, presumably via the native frataxin processing mechanisms, and these *COX2-frataxin* fusion constructs restored the growth phenotype and the mitochondrial enzyme activity levels to near wild type levels in frataxin nuclear knock-out yeast strains.

**Conclusions:**

We have demonstrated that expressing frataxin from within mitochondria can restore mitochondrial function in frataxin knock-out yeast. We have used these results as a guide for constructing mouse mitochondrial genomes containing a recoded human frataxin gene, and have devised procedures suitable for transferring mitochondria containing these modified genomes into mouse tissue culture cells. Based on our results from this yeast model system, we anticipate that frataxin expressed from these genomes will be appropriately processed within the mitochondria and will restore mitochondrial function to frataxin knock-out mouse cells.

**Title: Frataxin overexpression induces similar phenotypes as frataxin reduction without causing abnormal aggregates or misfolding.**

Authors: Llorens JV<sup>1,2</sup>., Soriano S<sup>1</sup>., Navarro JA<sup>3</sup>., Botella JA<sup>3</sup>., Schneuwly S<sup>3</sup>., Martínez-Sebastián MJ<sup>1</sup>., Moltó MD<sup>1,4</sup>.

Institutions:1. University of Valencia. 2. Institute of Biomedicine of Valencia, CSIC. 3. University of Regensburg. 4. Spanish National Network for Research in Mental Health. CIBERSAM.

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Abstract:

**Background/Hypothesis:** We have previously studied the effects of reducing and overexpressing the *Drosophila* frataxin gene (*fh*) in *Drosophila*. In both cases the phenotypes obtained were very similar. Afterward we investigated the effect of expressing the human frataxin (*FXN*) in the fly. In the three scenarios we found lethal phenotypes in ubiquitous expression patterns, meanwhile viability in nervous system patterns.

**Methods:** We have carried out lifespan determination and climbing assays of flies overexpressing *FXN* or *fh* using peripheral nervous system (*neuralized*-GAL4), central nervous system (*appl*-GAL4) and glial cells (*repo*-GAL4) drivers, under normoxia and hyperoxia conditions. In addition we used the *mitocat*-GAL4 driver to study the effect of this free radical scavenger on lifespan and locomotor performance in the flies overexpressing frataxins. Finally, we study if the overexpression phenotypes were due to misfold or aggregation of frataxin by means of expressing heat shock proteins and carrying out a size exclusion chromatography analyzing the presence of *FXN* by Western blotting.

**Results:** Overexpression of *fh* or *FXN* in PNS, CNS and glial cells showed similar phenotypes: reduction of lifespan and defects in climbing ability in all cases. *MitoCat* produced significant prolongation of lifespan when frataxin was overexpressed using the *neur*-GAL4 as well as a statistically non-significant increase in the case of *repo*-GAL4. In addition, *mitoCat* could ameliorate the climbing deficiency induced by either *FXN* or *fh* overexpression in glial cells. Co-expression of a human heat-shock protein with human frataxin, and *Drosophila* hsp 70 and hsp 22 in combination to *fh* showed no improvement of climbing ability of frataxin overexpressing flies. In the *FXN* overexpressing larvae the human frataxin was recovered as a monomeric form and no high molecular weight frataxin aggregates were detected in the void volume. Moreover no presence of frataxin was detected in the insoluble cellular protein fraction.

**Conclusions:** This results identify oxidative stress as a key factor of frataxin overexpression phenotype and that frataxin aggregation or misfolding are not involved in these phenotypes. Moderate overproduction could be producing beneficial effects such as protection against oxidative stress, however higher levels of frataxin might induce toxic effects similar to frataxin depletion. We propose that the frataxin function should be such that its increase or reduction produces identical effects. This toxic effect of frataxin overexpression must be taken into account for the use of treatments directed to increasing frataxin levels, such as gene therapy approaches.

**Title: The Jackson Laboratory Mouse Husbandry and Distribution Core for FA models**

Authors: Cathleen Lutz, Melissa Osborne, Kim Huebsch

Institutions: The Jackson Laboratory

Corresponding author email address: [cat.lutz@jax.org](mailto:cat.lutz@jax.org)

**Abstract:**

Friedreich's Ataxia is a neurodegenerative disorder caused by a GAA repeat expansion within the first intron of the gene coding for frataxin, a mitochondrial protein involved in iron homeostasis. Researchers in the field of FA have been developing animal models to aid in the study of the disease. These models are absolutely vital in the process of developing a therapeutic intervention for FA. At The Jackson Laboratory, our mission is to discover the genetic basis for preventing, treating and curing human disease and we enable research and education for the global biomedical community. The Jackson Laboratory has partnered with the Friedreich's Ataxia Research Alliance (FARA) FARA to collect, standardize and distributes FA related mouse strains. This has been made possible by the generous donation of those researchers who engineered the mice and support from the FA Foundation. By providing a centralized distribution resource for FA related strains, The Jackson Laboratory facilitates the process of getting the animal models into the hands that need them most: the biomedical research community.

To model the human disease in mice researchers have taken the approach of knocking down or knocking out the expression of endogenous mouse frataxin and replacing it with human frataxin. The human frataxin often contains repeat expansions that were found in human patients. The data presented here represents our current efforts to monitor repeat expansion, standardize the genetic background across models, and phenotypically compare current FA mouse models.

## **Title: Iron homeostasis in a *Drosophila* model of Friedreich`s ataxia**

Authors: Juan Antonio Navarro<sup>1</sup>, Maria Dolores Moltó<sup>2</sup>, Jose Antonio Botella<sup>1</sup> and Stephan Schneuwly<sup>1</sup>

Institutions: <sup>1</sup>Lehrstuhl für Entwicklungsbiologie, Universität Regensburg and <sup>2</sup>Departamento de Genética, Universidad de Valencia

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Abstract:

**Background/Hypothesis:** It has been shown that ablation of frataxin results in an overload of mitochondrial iron. Recent investigations using the MCK mouse model (Huang M.L-H. *et.al.*, 2009) and a conditional yeast mutant (Moreno-Cermeño A. *et al.*, 2010) have suggested a possible mechanism for this overload, where frataxin depletion leads to a disruption of iron metabolism inducing a decrease in cytosolic iron storage and a stimulation of mitochondrial iron influx.

We have investigated iron dysregulation in *Drosophila* adult flies that show a systemic reduction of frataxin expression (Llorens J. *et al.*, 2007) and extended our investigations by genetic and pharmacological manipulation of iron metabolism in controls and frataxin-deficient adult flies.

**Methods:** We have mimicked an iron overload situation supplementing the fly-food with Ferric Amonium Citrate and analyzed the effect of this iron overload in control and frataxin flies at behavioural, biochemical and molecular levels. Moreover, using the GAL80 technology, we have generated and validated a new *Drosophila* line to perform genetic screenings in order to elucidate new factors that can modify lack-of-frataxin phenotypes.

**Results:** We have found that frataxin deficient flies are more sensitive to iron in terms of shortened life span and a reduction in aconitase and complex II activities. In addition, upon iron treatment, frataxin deficient flies did not show an increased expression of cytosolic ferritin pointing to an iron deficit in the cytosol. Co-expression of cytosolic or mitochondrial ferritin rescued life span but not aconitase activity in normoxia and moderate iron concentration (not in higher amounts). In addition, depheriprone administration failed to rescue aconitase inactivation. Interestingly, a slight overexpression of mitoferrin, the mitochondrial iron transporter, enhanced some of the frataxin deficient phenotypes.

**Conclusions:** Our results show for the first time *in vivo* evidence that an increase in iron transport is a key element in iron toxicity in Friedreich`s ataxia. Furthermore, we have developed a new and powerfull tool to carry out genetic screens which will enable us to identify new interaction parthers in an *in vivo* animal model.

## Title: Analysis of the role of GAA expansion instability in Friedreich ataxia pathology in a humanized mouse model

Authors: Joseph P. Sarsero

Institutions: Murdoch Childrens Research Institute, Royal Children's Hospital, Parkville, Victoria 3052, Australia. Department of Paediatrics, The University of Melbourne, Royal Children's Hospital, Parkville, Victoria 3052, Australia.

Corresponding author email address: joe@sarsero.com

Abstract:

**Background/Hypothesis:** There is evidence that age-dependent and tissue-specific somatic instability of the GAA expansion may be a determinant of the progressive pathology of Friedreich ataxia (FRDA), and is evident in FRDA patients and a YAC-based GAA expansion mouse model. Interruptions in GAA repeat sequences can inhibit the formation of triplex DNA structures, alleviate transcription inhibition, and reduce genetic instabilities. We explored the role of instability of the GAA expansion on FRDA pathology using a humanized mouse model containing an interrupted GAA expansion.

**Methods:** We introduced an interrupted GAA expansion into the appropriate location in the first intron of the human *FXN* gene present on a 188 kb BAC clone by homologous recombination. The genomic insert of the modified BAC clone was used to generate transgenic mice. Breeding with heterozygous *Fxn* knockout mice produced humanized transgenic/KO mice that lacked endogenous mouse frataxin and were able to survive with only human frataxin.

**Results:** The presence of the introduced interrupted GAA repeat expansion resulted in markedly decreased levels of human *FXN* transcript and frataxin protein in humanized mouse tissues. The region immediately upstream of the interrupted GAA expansion region was found to be almost completely methylated and to a much higher degree than that observed in transgenic mice harboring the normal *FXN* gene. The assessment of phenotypic symptoms of FRDA by a series of behavioral, neurological, biochemical and histological tests did not reveal any significant phenotypic differences between humanized and wild type mice. Somatic instability of the interrupted GAA expansion was not detected using the small pool PCR technique.

**Conclusions:** The interruption of the GAA sequence contributes to the somatic stability of the repetitive element, which in turn results in the mice lacking an obvious phenotype despite the low levels of *FXN* mRNA and frataxin protein and repressive epigenetic changes.

## **Session 6 (Saturday, 8:30 – 10:30): Drug Discovery & Development**

Co-Chairs: Sid Hecht & Rob Wilson

### Speakers

<b>Rob Wilson</b> (invited speaker)	High-throughput and Secondary Drug Screening Assays for Friedreich Ataxia
<b>Joe Sarsero</b>	Pharmacological screening for the therapy of Friedreich ataxia
<b>Sunil Sahdeo</b>	A novel cell-based model for high-throughput screening of potential Friedreich's therapeutics
<b>Ping Kei Chan</b>	GAA-repeat induced heterochromatinisation in intron 1 of the Frataxin gene can be decondensed by HDAC inhibitor (HDACi), Nicotinamide
<b>Vincent Jacques</b>	Second generation stable brain-penetrant HDAC inhibitors for Friedreich's Ataxia
<b>Omar Khmour</b>	Multifunctional radical quenchers for the treatment of Friedreich's ataxia
<b>Javier Diaz-Nido</b>	Brain-derived neurotrophic factor (BDNF) inhibits neurodegeneration triggered by frataxin gene silencing

### Posters

<b>Lucia Calatrava</b>	Neuroprotective role of Liver Growth Factor "LGF" in an experimental model of cerebellar ataxia
<b>Ernest Giralt</b>	Novel molecular tools to target the neurodegenerative component of Friedreich's ataxia
<b>Ed Grabczyk</b>	High-Throughput Screen Using a Dual Reporter FXN Minigene in Human Cells
<b>Marek Napierala</b>	A high throughput screen for inducers of frataxin expression using new reporter system
<b>Alexandra Rufini</b>	Preventing the ubiquitin/proteasome-dependent degradation of frataxin, the protein defective in Friedreich's Ataxia
<b>Elisabetta Soragni</b>	Next generation HDAC inhibitors reverse frataxin gene silencing in patient neuronal cells

## **Title: High-throughput and Secondary Drug Screening Assays for Friedreich Ataxia**

Authors: M. Grazia Cotticelli<sup>1</sup>, Jason Melvin<sup>2</sup>, Phillip A. Benedetti<sup>2</sup>, Lynn Rasmussen<sup>3</sup>, Nicole L. Kushner<sup>3</sup>, Sara McKellip<sup>3</sup>, Melinda Ingrum Sosa<sup>3</sup>, Anna Manouvakhova<sup>3</sup>, Shuang Feng<sup>3</sup>, E. Lucile White<sup>3</sup>, Joseph A. Maddry<sup>3</sup>, Jill Heemskerk<sup>4</sup>, Robert J. Oldt<sup>1</sup>, Lea F. Surrey<sup>1</sup>, Rachel Ochs<sup>1</sup>, Amos B. Smith, III<sup>2</sup>, Donna M. Huryn<sup>2</sup>, and Robert B. Wilson<sup>1</sup>

*Institutions: <sup>1</sup>Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA; <sup>2</sup>Department of Chemistry, University of Pennsylvania, Philadelphia, PA; <sup>3</sup>Drug Discovery Division, Southern Research Institute, Birmingham, AL; <sup>4</sup>National Institute of Neurological Disorders and Stroke (NINDS, NIH), Bethesda, MD.*

Corresponding Author: wilsonr@mail.med.upenn.edu

Abstract: Using yeast depleted of Yfh1p, a high-throughput screening (HTS) assay was developed in which mitochondrial function was monitored by reduction of the tetrazolium dye WST-1 in a growth medium with a respiration-only carbon source. Approximately 242,000 compounds were screened, and approximately 460 compounds were identified that rescue mitochondrial function. To confirm activities in mammalian cells, and to begin to understand mechanisms of action, secondary screening assays were developed using primary human Friedreich ataxia fibroblasts, human HepG2 cells, murine C2C12 cells, murine fibroblasts expressing only human I154F frataxin (kindly provided by Dr. Hélène Puccio), and yeast mutants lacking specific complexes of the electron transport chain. The compounds identified in this study have potential relevance for the treatment of Friedreich ataxia.

## Title: Pharmacological screening for the therapy of Friedreich ataxia

Authors: Joseph P. Sarsero

Institutions: Murdoch Childrens Research Institute, Royal Children's Hospital, Parkville, Victoria 3052, Australia. Department of Paediatrics, The University of Melbourne, Royal Children's Hospital, Parkville, Victoria 3052, Australia.

Corresponding author email address: joe@sarsero.com

Abstract:

**Background/Hypothesis:** In Friedreich ataxia (FRDA) there is a correlation between GAA expansion length, the amount of residual frataxin and the severity of disease. As the coding sequence of the *FXN* gene is unaltered, pharmacological upregulation of *FXN* gene expression may restore frataxin to therapeutic levels.

**Methods:** To screen compounds that modulate *FXN* expression, we established a genomic reporter consisting of stable HeLa cells containing an *FXN-EGFP* fusion construct (in-frame fusion of the *EGFP* gene with the entire normal human genomic *FXN* locus on a BAC clone). The cell line was used in manual and high throughput screening (HTS) procedures. Compound hits identified by HTS were further evaluated by flow cytometry in the cellular genomic reporter assay. The effects on *FXN* mRNA and frataxin protein levels were measured in cell lines derived from individuals with FRDA and in a GAA repeat expansion mouse model of the disorder.

**Results:** The anti-cancer drugs cisplatin and camptothecin, the iron chelator deferiprone and the phytoalexin resveratrol were found to elicit increases in *FXN-EGFP* expression. Increases in *FXN* mRNA and frataxin protein were also observed in lymphoblast and fibroblast cell lines derived from individuals with FRDA. Preliminary evaluation of resveratrol in a YAC-based GAA expansion humanized mouse model of FRDA showed an increase in human *FXN* gene expression in mouse tissues.

**Conclusions:** We have identified several compounds that increase human *FXN* gene expression and frataxin protein levels in cellular assays and in an FRDA mouse model. Any compound that specifically increases frataxin levels by several-fold in individuals with FRDA could serve as a potential pharmacological therapy for the disorder.

**Title: A novel cell-based model for high-throughput screening of potential Friedreich's therapeutics.**

Authors: Sunil Sahdeo, Robert Schoenfeld and Gino Cortopassi

Institutions: University of California, Davis

Corresponding author email address: gcortopassi@ucdavis.edu

**Abstract:**

Identification of novel Friedreich's drug therapy has been hampered by the availability of validated, robust high-throughput screens. New drugs are very expensive to develop, and a screen of existing FDA-approved drugs for anti-Friedreich's activity (re-purposing) may be an expedient route to therapy. Microarray of dorsal root ganglion neurons from the YG8 mouse model of FRDA suggested alteration in thiol-related antioxidants, and 11 inhibitors of these antioxidants were tested in Friedreich's patient fibroblasts, which were sensitive to the thiol oxidant diamide. Sensitivity to diamide was the specific result of siRNA-mediated frataxin deficiency in the 50B11 dorsal root ganglion cell line, and could be reversed by DTT and erythropoietin. The cell-based assay was further optimized for high-throughput screening in 96-well plates, with an excellent screening window and low variability, represented by a Z' value of 0.75 (n=5) and was used to screen a library of 1060 drugs that have been approved for clinical use in the USA.

**Background/Hypothesis:** Our hypothesis is that FRDA cells are more sensitive to inhibition of thiol-antioxidants, and this property can be used to screen for novel therapeutics.

**Methods:** Drugs were dispensed to fibroblasts at 10 micromolar, incubated at 37C for 24h after which 200 micromolar diamide was added O/N. Fibroblasts were incubated with Calcein-AM dye and fluorescence read, and hits scored as Basal Median + 3XMAD.

**Results:** Frataxin-deficient patient fibroblasts are more sensitive to diamide. This effect on cell viability can be rescued by both DTT and erythropoietin and some targeted compounds.

**Conclusions:** A FRDA disease-specific cell viability assay has been developed using a thiol stressor, in patient fibroblasts and a DRG neural cell line. Multiple rescue agents have been identified, and thus this assay can be used in the search of translational medicines to treat FRDA patients.

Supported by a FARA/ride ataxia Kyle Bryant award.

**Title: GAA-repeat induced-heterochromatinisation in intron 1 of the Frataxin gene can be decondensed by HDAC inhibitor (HDACi), Nicotinamide**

Authors: Ping Kei Chan, Raul Torres, Cihangir Yandim, Nadine Chapman-Rothe, Richard Festenstein

Institutions: Medical Research Council UK

Corresponding author email address: [r.festenstein@imperial.ac.uk](mailto:r.festenstein@imperial.ac.uk)

Abstract not available.

## **Title: Second generation stable brain-penetrant HDAC inhibitors for Friedreich's Ataxia**

Authors: Jacques V., Plasterer H., Belmonte M., Chen Y., Sharma S., Egan E., Chan A., Soragni E., Gottesfeld J., Rai M., Pandolfo M., and Rusche J.

Institutions: RepliGen Corporation, The Scripps Research Institute, ULB Belgium

Corresponding author email address: vjacques@repligen.com

Abstract:

### **Background/Hypothesis:**

First generation class I- and HDAC3-selective HDAC inhibitors developed at RepliGen have shown efficacy in increasing frataxin mRNA expression and frataxin protein both in animal models and in patient cells. Development candidate RG2833 has been submitted for first-in-human evaluation. However this compound class has shown some limitations including limited CNS penetration and a propensity to chemical and metabolic instability. This can lead to short shelf life and exposure to an undesired metabolite, increasing the risk for side-effects.

### **Methods:**

Chemical synthesis was performed to make 75 derivatives aimed at improving both properties simultaneously. Stability improvements were assessed by a combination of *in vitro* chemical and metabolic stability methodologies. CNS penetration was measured *in vivo* by *iv* injection of mice. Compounds were also tested for their ability to increase frataxin in patient cells.

### **Results:**

Like all HDAC inhibitors, Repligen proprietary compounds can be described by the generic structure cap-connecting unit-linker-ZBG. The connecting unit and linker proved important areas for improving brain penetration and stability respectively. Compounds were obtained that combine improved CNS penetration and increased stability sometimes at the expense of efficacy in cell-based assays, most likely because of high non-specific binding to plasma or serum protein, thereby limiting drug free fraction. Several stable brain-penetrant new analogs dose-dependently increased frataxin protein expression in patient cells. These compounds were also active in neurons differentiated from iPS cells derived from patient fibroblasts.

### **Conclusions:**

Two features of our first series of class-I selective HDAC inhibitors for Friedreich's ataxia were identified as potential limitations. Informed by these observations, we used a combination of medicinal chemistry approaches and adequate experimental tools to identify a series of molecules that, while maintaining class-I and HDAC3-selectivity, show improved brain penetration and stability. We are currently pursuing these as backups for our first-in-class clinical candidate RG2833.

## **Title: Multifunctional Radical Quenchers for the Treatment of Friedreich's Ataxia**

Authors: **Omar M. Khmour**, Pablo M. Arce, David M. Fash, Jun Lu, Xiaoqing Cai, Ruth Goldschmidt, Nidhi Raghav, Sriloy Dey, Jennifer Jaruvangsanti, Jeffrey S. Armstrong, and Sidney M. Hecht

Institutions: Center for BioEnergetics, Biodesign Institute, Arizona State University, Tempe, Arizona

Corresponding author email address: [sid.hecht@asu.edu](mailto:sid.hecht@asu.edu)

Abstract:

### **Background/Hypothesis:**

While oxidative stress is not the cause of Friedreich's ataxia, it is likely to contribute importantly to the progression of the disease. The hypothesis being tested is that coenzyme Q<sub>10</sub> analogues can be designed and prepared which will blunt the effects of oxidative stress, and the progression of Friedreich's ataxia.

### **Methods:**

A number of coenzyme Q<sub>10</sub> analogues were prepared and tested for their ability to quench lipid peroxidation, suppress ROS formation in cultured mammalian cells, protect cultured mammalian cells from the effects of oxidative damage, and restore ATP production in coenzyme Q<sub>10</sub> deficient lymphocytes.

### **Results:**

The coenzyme Q<sub>10</sub> analogues were found to suppress lipid peroxidation both in model membranes and in mitochondrial membranes, and did so more efficiently than alpha-tocopherol. When tested for suppression of ROS formation in CEM leukemia cells treated with diethyl maleate to deplete glutathione, they suppressed ROS formation at low micromolar concentrations, and did so more efficiently than idebenone or idebenol. Representative compounds were tested for their ability to protect cultured CEM cells and FRDA fibroblasts from induced oxidative stress, and did so at nanomolar concentrations. Coenzyme Q<sub>10</sub> deficient lymphocytes, which were shown to have diminished levels of ATP relative to normal lymphocytes, were grown in galactose to minimize non-mitochondrial ATP production and treated with a number of our coenzyme Q<sub>10</sub> analogues. Several of the compounds were found to increase ATP levels, and one compound essentially restored ATP levels to those found in the normal lymphocytes.

### **Conclusions:**

We have succeeded in preparing coenzyme Q<sub>10</sub> analogues that blunt the effects of oxidative stress, and which can restore ATP production in coenzyme Q<sub>10</sub> deficient lymphocytes. The low concentrations at which these coenzyme Q<sub>10</sub> analogues function argues that they must work catalytically to achieve their observed effects.

**Title: Brain-derived neurotrophic factor (BDNF) inhibits neurodegeneration triggered by frataxin gene silencing.**

Authors: Y Katsu, F Loria, F Lim and J Diaz-Nido

Institutions: Universidad Autonoma de Madrid, Centro de Biologia Molecular Severo Ochoa (UAM-CSIC), CIBER de Enfermedades Raras (CIBERER)

Corresponding author email address: J Diaz-Nido; e-mail: [javier.diaznido@uam.es](mailto:javier.diaznido@uam.es)

**Abstract:**

Neurotrophic factors are known to inhibit neurodegeneration in a variety of neurodegenerative diseases. However, the possible impact of neurotrophic factors into the progression and therapy of FA has not been studied so far.

We have used a herpesviral vector carrying the cDNA encoding for brain-derived neurotrophic factor (BDNF) to drive its overexpression in neuronal cells. Gene transfer of BDNF to primary cultures of rodent neurons prevents the apoptosis which is triggered by the knockdown of frataxin gene expression (by transduction with a lentiviral vector carrying a minigene encoding for a frataxin-specific shRNA). The neuroprotective effect of BDNF gene transfer is specific since it can be abolished by the treatment of neurons with a neutralizing antibody to BDNF or a chemical inhibitor of BDNF receptor (TrkB) kinase activity. Interestingly, a small molecule drug which is able to activate BDNF receptor also protects frataxin-deficient neurons against cell death. These results suggest that BDNF is able to compensate for the loss of frataxin and keep the viability of cultured neurons devoid of frataxin.

We are now trying to confirm this neuroprotective effect of BDNF also “in vivo”. Our preliminary results obtained in a mouse model in which frataxin gene knockdown at cerebellar neurons results in a diminished motor coordination suggest that BDNF overexpression is indeed able to rescue the ataxic phenotype.

In addition to their possible use to curb neurodegeneration triggered by frataxin knockdown, we are also exploring the role played by neurotrophic factors in regulating frataxin gene expression. In this respect, we have found that BDNF is also able to up-regulate frataxin gene expression in cultured neuronal cells.

In view of these data, we suggest that BDNF gene transfer may be of therapeutic use in FA since BDNF may increase frataxin expression and also protect frataxin-deficient neurons from apoptosis. A gene therapy approach based on the transfer of BDNF gene has the advantage that the number of “rescued” neurons may be much higher than the number of “transduced” neurons, since BDNF is a secreted protein which may act on neurons other than those directly expressing it.

## **Title: Neuroprotective role of Liver Growth Factor “LGF” in an experimental model of cerebellar ataxia**

Authors: Lucia Calatrava, Rafael Gonzalo-Gobernado, Diana Reimers, Antonio S. Herranz, Juan Jose Díaz-Gil\*, Cristina Miranda, Macarena Rodríguez and Eulalia Bazán

Institutions: Servicio de Neurobiología-Investigación del IRYCIS. Madrid, Spain. \*Servicio de Bioquímica Experimental. Hospital Universitario Majadahonda-Puerta de Hierro. Madrid, Spain

Corresponding author email address: eulalia.bazan@hrc.es

Abstract:

### **Background/Hypothesis:**

Cerebellar ataxias (CA) comprise a heterogeneous group of neurodegenerative diseases characterized by a lack of motor coordination. This group of diseases is caused by disturbances in the cerebellum and its associated circuitries, so the major therapeutic goal is to correct cerebellar dysfunction. Liver growth factor (LGF) is a mitogen for liver cells that shows biological activity in extrahepatic sites and is useful for neuroregenerative therapies. The aim of this work was to investigate the potential therapeutic activity of LGF in the 3-acetylpyridine (3-AP) rat model of CA.

### **Methods:**

The experimental model of CA used consists in the lesion of the inferior olive induced by 40 mg/kg of the neurotoxin 3-AP. Ataxic rats were treated with 5µg/rat LGF or with vehicle during 3 weeks, analyzing a) motor coordination by using the rota-rod test, and b) the immunohistochemical and biochemical evolution of several parameters related with the olivocerebellar function (calbindin and GLUR1 expression, and neuronal and glial markers).

### **Results:**

Motor function, as determined by the rota-rod test, was significantly improved in 3-AP rats that received LGF during 3 weeks. LGF stimulated the expression of the anti-apoptotic protein Bcl2 by 2.3-fold in the inferior olive, but it was unable to rescue the calbindin-positive neurons, which are the target of the neurotoxin. However, in the cerebellum LGF prevented the decrease in calbindin expression promoted by 3-AP, and restored to basal levels the expression of the Class II antigen expressed by activated microglia OX6 that was significantly increased in the cerebellum of 3-AP-vehicle treated rats.

### **Conclusions:**

Alltogether, these results suggest that LGF could be a potential factor useful for the treatment of cerebellar ataxias. Since LGF is also a potent antioxidant agent, its therapeutic activity could be extensive to Friedreich's ataxia.

Supported by: Fundación Ataxias en Movimiento-Caja Navarra and Fibio HRyC

**Title: Novel molecular tools to target the neurodegenerative component of Friedreich's ataxia**

Authors: Roger Prades, Meritxell Teixidó & Ernest Giralt

Institutions: INSTITUTE FOR RESEARCH IN BIOMEDICINE (IRB Barcelona)

Corresponding author email address: [ernest.giralt@irbbarcelona.org](mailto:ernest.giralt@irbbarcelona.org)

Abstract:

**Background and hypothesis:**

Our main objective is to generate novel molecular tools to alleviate the neurodegenerative component of Friedreich's ataxia (FA) using therapeutic approaches that take advantage of active targeting to cross the blood-brain barrier (BBB).

We address the design and development of a nanodelivery system, formed by a nanoparticle core, which once loaded with the macromolecular active agent, will be delivered through the BBB via receptor-mediated transport thanks to peptide BBB-shuttles decorating the nanoparticle surface. Once inside the CNS, other surface-bound homing molecules will target the nanosystem to the specific cell population or intracellular organelle, e.g. mitochondria.

**Methods:**

The work is organized following a double strategy to try to restore frataxin levels. Delivery of DNA-encoding for frataxin to the nucleus of neurons (non viral gene therapy for frataxin production), and delivery of the frataxin protein itself to the mitochondria of neurons (protein replacement therapy). The active drug needs to cross the BBB, target dorsal root ganglion neurons, internalize in these cells and reach the mitochondria. In the case of DNA, a nuclear localization is needed.

**Results:**

In a broader view, we generate novel molecular tools, viral-free systems for DNA and protein delivery that may facilitate gene therapy and protein replacement therapy approaches to treat FA and other rare neurogenetic disorders.

## **Title: High-Throughput Screen Using a Dual Reporter FXN Minigene in Human Cells**

Authors: Ayan Banerjee<sup>1</sup>, Mimi C. Sammarco<sup>1</sup>, Fred Harbinski<sup>2</sup>, Eugene C. Petrella<sup>2</sup> and Ed Grabczyk<sup>1\*</sup>

Institutions: 1Department of Genetics, LSU Health Sciences Center, 533 Bolivar Street, New Orleans, LA 70112, USA 2 Developmental and Molecular Pathways, Novartis Institutes for Biomedical Research, Inc., 250 Massachusetts Ave., Cambridge, MA 02139, USA

Corresponding author email address: egrabc@lsuhsc.edu

Abstract:

### **Background/Hypothesis:**

Expanded GAA•TTC repeats cause FRDA by reducing expression of the *FXN* gene. We have engineered a series of unique dual reporter vectors in human cell lines to dissect the mechanism and to enable high-throughput screening for FRDA therapeutics. A bidirectional promoter drives a different luciferase reporter in each direction. On one side is a splicing *FXN* minigene with GAA•TTC repeats in their native context. The ratiometric luciferase readout provides an accurate measure of the transcriptional impediment presented by an expanded GAA•TTC repeat while controlling for nonspecific effects such as compound toxicity. Reliability is essential to screen reagents for efficacy in tissue culture. Therefore, we integrated a single copy of the test construct within a single chromosomal location. This most closely approximates the *in vivo* disease scenario and provides a high level of confidence in screening results.

### **Methods:**

A cell line engineered to contain ~800 repeats was chosen for HTS as we determined that this range of repeats resulted in less than 10% reporter gene expression. The assay was validated in a 1536-well format using a minigene-50 cell line as the 100% control.

### **Results:**

Proof of concept screening runs showed average Z-factors of 0.52 indicative of excellent assay signal dynamic range and data variation. We then screened a chemical diversity set of 45640 compounds in duplicate and subsequently selected approximately 1200 hits for eight-point dose response validation. Over 600 compounds confirmed for hRLUC/FLUC ratios. Representative compounds are in the process of secondary screening via qPCR.

### **Conclusions:**

We believe our bidirectional dual reporter system is a finely honed tool for translational medicine and ready for a major screening effort. We are optimistic that it will turn up multiple leads for Friedreich ataxia therapeutics.

**Title: A high throughput screen for inducers of frataxin expression using new reporter system.**

Authors: Urszula Polak, Elizabeth McIvor, Marek Napierala.

Institutions: University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd., Houston TX 77030.

Corresponding author email address: mnapiera@mdanderson.org

Abstract:

**Background/Hypothesis:**

We designed, constructed and tested a high-throughput assay that will allow the identification of compounds which alleviate GAA repeat-induced silencing of the frataxin gene.

**Methods:**

To mimic the defect observed in the frataxin gene, we created reporter cell lines harboring a luciferase gene separated by an artificial intron containing varying lengths of GAA repeat tracts: 30 and 850 GAAs. The cell lines were generated by integration of the reporter gene into a unique genomic location in HEK293 cells. The cell line with the long GAA tract corresponds to FRDA patient cells, while cells containing shorter repeats represent unaffected controls.

**Results:**

Similar to the FRDA, the length of the GAA repeats inversely correlates with the expression of the luciferase gene. Importantly, the long GAA tract in the reporter minigene induces deacetylation and methylation of histones at sites associated with silenced chromatin, hence recapitulating epigenetic changes observed FXN gene. To prepare for the high-throughput screening (HTS) campaign we conducted the plate uniformity and signal variability assessment. The assay demonstrated a Z' score in the range of 0.6 – 0.8, a proper response to known stimulators of the frataxin expression, long-term stability of the expanded GAA repeats during culture. As a proof-of concept we conducted pilot screens using NIH Clinical Collection and NCI COMBO compound libraries.

**Conclusions:**

High-throughput screen will be conducted at the Scripps Research Institute and the critical path plan includes two high-throughput screens: a primary screen using Luc\_GAA850 cell line to identify compounds alleviating the transcriptional repression induced by the long GAA repeats, and a counterscreen utilizing Luc\_GAA30 cells to eliminate compounds that increase luciferase levels in a non-GAA repeat-dependent manner. Three secondary assays will allow to analyze effects of HTS-identified hits on expression of the endogenous *FXN* mRNA in FRDA fibroblasts and iPSC-derived neurons.

**Title: Preventing the ubiquitin/proteasome-dependent degradation of frataxin, the protein defective in Friedreich's Ataxia**

Authors: Alessandra Rufini, Silvia Fortuni, Gaetano Arcuri, Ivano Condo', Dario Serio, Ottaviano Incani, Florence Malisan, Natascia Ventura and Roberto Testi

Institutions: Laboratory of Immunology and Signal Transduction, Department of Experimental Medicine and Biochemical Science, University of Rome "Tor Vergata", Italy

Corresponding author email address: roberto.testi@uniroma2.it

Abstract:

**Background/Hypothesis** Since levels of residual frataxin critically affect onset and progression of FRDA, understanding the molecular mechanisms that control frataxin stability may be exploited for the design of new therapeutic strategies. However no information is available on the mechanisms that regulate frataxin turnover and degradation. The ubiquitin-proteasome system is the major pathway for regulated degradation of intracellular proteins in higher eukaryotes. We therefore tested its involvement in the control of frataxin degradation.

**Methods/Results** We found that frataxin is degraded by the ubiquitin-proteasome system and, through systematic lysine mutagenesis, we identified K<sup>147</sup> as the critical residue responsible for frataxin ubiquitination and degradation. Accordingly, the K<sup>147</sup>R frataxin mutant cannot be ubiquitinated and is more stable. By combining computational screening and cell-based functional assays, we identified a set of lead compounds, selected to target the molecular cleft harboring K<sup>147</sup>, which can prevent frataxin ubiquitination and degradation. These compounds can increase frataxin levels in different cellular systems, including cells derived from FRDA patients. Most importantly, these compounds can functionally rescue frataxin deficiency in cells derived from FRDA patients, by restoring aconitase activity and ATP levels.

**Conclusions** We thus provide proof of principle for the therapeutic potential of directly interfering with the frataxin ubiquitination and degradation pathway, eventually increasing levels of functional frataxin. Our work might open new avenues for the search of drugs effective in the treatment of FRDA.

**Title: Next generation HDAC inhibitors reverse frataxin gene silencing in patient neuronal cells**

Authors: Soragni E., Ku S., Jacques V., Xu C., Rusche J. R. and Gottesfeld J. M.

Institutions: The Scripps Research Institute, Repligen Corporation

Corresponding author email address: soragni@scripps.edu

Abstract:

**Background/Hypothesis:**

The hyper-expansion of GAA•TTC triplets in the frataxin gene (*FXN*) interferes with gene transcription, either by forming an unusual DNA structure that impedes transcription elongation or by inducing heterochromatin formation through an unknown mechanism. Based on the hypothesis that the acetylation state of the core histones might be responsible for silencing expanded *FXN* alleles, we identified pimelic  $\alpha$ -aminobenzamide HDAC inhibitors as activators of *FXN* transcription in cell culture and in a mouse model of the disease. While these molecules are promising therapeutics for Friedreich's ataxia, they suffer from two limitations, namely less than optimal brain penetration and formation of an inactive metabolic byproduct in the stomach and serum.

**Methods:**

*To improve on our compounds in terms of pharmacological properties, we used a new synthetic strategy based on Cu(I)-catalyzed click chemistry to combine two structural features that individually improve brain distribution and acid stability of our HDAC inhibitors. To test these new molecules, we differentiated human neuronal cells from patient-derived induced pluripotent stem (iPS) cells.*

**Results:**

Brain penetration is improved by replacement of the "left" amide in the standard pimelic  $\alpha$ -aminobenzamide scaffold with an ether, olefin or ketone and the formation of a benzimidazole metabolic byproduct is avoided by the introduction of a non-saturated  $\alpha$ - $\beta$  linkage adjacent to the "right" amide. When tested on primary lymphocytes from patients and on iPS derived patient neuronal cells, the new HDAC inhibitors reverse frataxin gene silencing as efficiently as the previously published HDAC inhibitor 109. Dose response curves and time course analysis of *FXN* gene reactivation show that in neuronal cells a 16-hour treatment is sufficient to induce a therapeutically significant increase in frataxin gene expression.

**Conclusions:**

The next generation HDAC inhibitors we developed have improved pharmacological properties and are able to reverse frataxin gene silencing in human neuronal cells, thus being promising therapeutics for Friedreich's ataxia.

## **Session 7 (Saturday, 12:00 – 13:00): Clinical Trials**

Co-Chairs: Petra Kaufmann & David Lynch

### Speakers

<b>Francesco Saccà</b>	Delayed effect of Epoetin alfa on frataxin production in Friedreich's ataxia
<b>Antonio Piga</b>	Effects of Deferiprone on cardiac disease in patients with Friedreich's ataxia
<b>Fernando Tricta</b>	A six-month double-blind randomized, placebo-controlled study investigating the safety and tolerability of Deferiprone in subjects with Friedreich's ataxia
<b>Thomas Meier (presented by D. Lynch)</b>	Efficacy of idebenone (Catena®) in pediatric patients with FRDA: data from a 6 month controlled study (IONIA) followed by a 12-month open label extension study (IONIA-E)

### Posters

<b>Katrin Bürk</b>	Do clinical rating scales reflect progression in Friedreich ataxia (FRDA)?
<b>Petra Kaufmann</b>	Developing Common Data Elements for Friedreich's Ataxia Clinical Research

**Title: Delayed effect of Epoetin alfa on frataxin production in Friedreich's ataxia.**

Authors: F. Sacca<sup>1</sup>, R. Piro<sup>1</sup>, G. De Michele<sup>1</sup>, F. Acquaviva<sup>2</sup>, A. Antenora<sup>1</sup>, G. Carlomagno<sup>3</sup>, S. Cocozza<sup>2</sup>, A. Denaro<sup>1</sup>, A. Guacci<sup>1</sup>, A. Marsili<sup>1</sup>, G. Puorro<sup>1</sup>, A. Cittadini<sup>3</sup>, A. Filla<sup>1</sup>.

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Abstract:

**Background/Hypothesis:** Recently it was shown that erythropoietin (EPO) administration increases frataxin expression in lymphocytes of FRDA patients. Two clinical trials showed that Epoetin-beta is able to increase frataxin levels between 20-30% in peripheral blood mononuclear cells (PBMCs) of patients treated with 5000 IU t.i.w. or 2000 IU t.i.w. respectively.

**Methods:** Inclusion criteria were molecular diagnose of FRDA, aged 18-50. Exclusion criteria were treatment with l-deprenone, wheelchair bound patients, renal, hepatic or haematological diseases, history of arterial or venous thrombosis, arterial hypertension, pregnancy or breastfeeding. Patients were treated with 600 IU/Kg s.c. of Epoetin-alfa and frataxin levels were determined at time 0, 24, 48, and 96 hours, 7, 15, 30, and 60 days. 30 days after the last visit, patients were treated with 1200 IU/Kg s.c. of Epoetin-alfa. Endpoints were determined as for the first administration. Frataxin was dosed in PBMCs with lateral flow immunoassay.

**Results:** Patients showed a very small and non-significant acute increase of 9% (first administration) and 16% (second administration) of frataxin in PBMCs at 96 and 48 hours after treatment respectively. On the opposite, a long term increase of frataxin was noted. 3 months after treatment with the first single dose frataxin levels increased of 35% ( $p < 0.05$ ), and six months after the second dose levels reached a 54% increase ( $p < 0.001$ ). All patients showed at least an increase of  $\geq 20\%$  at one time point. Transferrin saturation decreased of 44.6% and 63.2% of baseline value 7 days after each injection. Neurological scale, echocardiography, and hematocrit were not modified after treatment.

**Conclusions:** We confirm the previous observation that EPO is able to increase frataxin expression in PBMCs. In our study frataxin increased up to 54% at 6 months after two high doses of Epoetin alfa, and all patients responded to treatment. Acute treatment with EPO was safe and well tolerated.

**Title: EFFECTS OF DEFERIPRONE ON CARDIAC DISEASE IN PATIENTS WITH FRIEDREICH'S ATAXIA**

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Abstract:

**Background and Objectives.** Iron-related heart disease (hypertrophic cardiomyopathy, dilated cardiomyopathy, and electrophysiological alterations) is a common cause of premature death in patients with Friedreich's ataxia (FRDA). Deferiprone (DFP) is an oral iron chelator that has proved to be effective in removing intracellular iron in many in vitro and in vivo conditions. A recent study in a group of FRDA patients showed a significant improvement in interventricular septum thickness (IST) after 11 months of deferiprone/idebenone combined therapy.

**Design and Methods.** Eleven FRDA patients (mean age: 24 years  $\pm$  6.9) were treated with deferiprone (20 mg/kg/day); 8 of them were on long-term therapy with high doses of idebenone. The patient's heart status before and after the treatment with DFP was evaluated by echocardiography.

**Results.** Nine of 11 patients presented mild to moderate degree of cardiac hypertrophy at baseline. At the end of the period of observation (mean 2.8  $\pm$  0.6 years) heart ultrasound revealed a full normalization of IST in 4/9. A significant improvement was noted in 3/9 patients while 2/9 did not showed any changes. During the follow-up no patient presented worsening of cardiac condition or new onset of cardiac disease. No significant differences were recorded in ejection fraction values that remained within normal range.

**Conclusions.** Findings from this study suggest that long-term therapy with deferiprone is able to induce positive changes in FRDA heart disease, possibly by mitochondrial iron clearing. A randomized controlled setting is warranted to confirm this hypothesis.

## **Title: A Six-Month Double-Blind, Randomized, Placebo-Controlled Study Investigating the Safety and Tolerability of Deferiprone in Subjects with Friedreich's Ataxia**

Authors: J. Arpa<sup>1</sup>, M. Delatycki<sup>2</sup>, A. Munnich<sup>3</sup>, M. Pandolfo<sup>4</sup>, M. Tarnopolsky<sup>5</sup>, F. Taroni<sup>6</sup>, F. Tricta<sup>7</sup>

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Abstract:

### **Background/Hypothesis:**

Deferiprone is a potential treatment for FRDA due to its ability to cross the blood brain barrier and remove mitochondrial labile iron. A pilot study demonstrated normalization of iron levels in the dentate nuclei of FRDA patients and improvement in delicate movements, manipulative dexterity and speech fluency in some patients. The present double-blind, randomized, placebo-controlled study was designed to evaluate the safety and efficacy of deferiprone for the treatment of patients with FRDA.

### **Methods:**

72 patients were randomized to receive 10 mg/kg BID (21-deferiprone; 5-placebo), 20 mg/kg BID (20-deferiprone; 6-placebo) or 30 mg/kg BID (14-deferiprone; 6-placebo). Each study group was stratified by age (7 to <18 years and 18-35 years). Safety was assessed weekly. Efficacy assessments included 9HPT, T25FW, ICARS, FARS, LCLA, ADL and echocardiography.

### **Results:**

The mean ages for children and adults were 12.5 and 25.6 years, respectively. There were no significant differences in baseline to end-of-study changes in non-cardiac scores (each deferiprone-treated group vs control group) except for a significant increase in FARS, ICARS and ADL in the 20 mg/kg BID deferiprone group. Improvements in posture, gait and kinetic function were observed in some patients treated with deferiprone, most notably in patients with mild disease. Deferiprone at 10 mg/kg BID and 20 mg/kg BID was associated with a -20.6 (26.5) and -17.6 (21.5) decrease in LV Mass index, respectively. Deferiprone at 30 mg/kg BID was associated with worsening ataxia in some patients, which improved upon discontinuation of the drug. Low serum ferritin levels occurred in 29% and 45% of subjects in the 10 mg/kg BID and 20 mg/kg BID groups, respectively. There was one case of neutropenia that resolved on drug withdrawal.

### **Conclusions:**

Further studies are required to determine the most appropriate deferiprone regimen that could promote a favourable benefit/risk ratio for the treatment of FRDA patients.

**Title: Efficacy of idebenone (Catena®) in pediatric patients with FRDA: data from a 6 month controlled study (IONIA) followed by a 12-month open label extension study (IONIA-E)**

Authors: Lynch D.R.<sup>1</sup>, Perlman S.L.<sup>2</sup>, Coppard N.<sup>3</sup>, Rummey C.<sup>3</sup> and Meier T.<sup>3</sup>

Institutions:

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<sup>2</sup>David Geffen School of Medicine at University of California at Los Angeles (UCLA), Los Angeles, USA

<sup>3</sup>Santhera Pharmaceuticals, Liestal, Switzerland

**Background/Hypothesis:**

To investigate the efficacy of idebenone on neurological function as assessed by ICARS and FARS scales in pediatric FRDA patients.

**Methods:**

70 pediatric patients were enrolled in a double-blind, randomized, placebo-controlled, study (IONIA) receiving either idebenone (Catena®, 150 mg film-coated tablets) at a weight adjusted dose of 450/900 mg/day or 1350/2250 mg/day or placebo for 6 months. Following completion, 68 patients were enrolled into an open-label extension study where patients received 1350/2250 mg/day idebenone for 12 months (IONIA-E). Changes in ICARS and FARS were recorded during the total of 18 months combined study period.

**Results:**

Data analyzed by a mixed model repeated measures ANCOVA resulted in least square means for the change in ICARS score over the 18 month study period of -1.03 points (SEM 0.68; p=0.132), indicating a trend for improvement in neurological function. When data were analyzed by treatment group as assigned at the beginning of the IONIA study it became clear that only patients who received idebenone 1350/2250 mg/day significantly improved in neurological function over the 18 month combined observational period (change in ICARS: -3.02 ± 1.22, p=0.014). Patients who had been on placebo or 450/900 mg/day idebenone for the 6 month IONIA study and who only received idebenone 1350/2250 mg/day for the 12 month of IONIA-E did not deteriorate over the combined 18-month period, which may also be therapeutically relevant for a progressive disease. The improvement in neurological function over time was best seen when the posture & stance subscore was excluded from the analysis. Comparable data were obtained with the FARS.

**Conclusions:**

The findings of the open-label IONIA-E study combined with the double-blind IONIA study indicate that idebenone at a dose of 1350/2250 mg/day may offer a therapeutic benefit to pediatric FRDA patients by stabilizing the overall neurological function (as assessed with the ICARS).

## **Title: Do clinical rating scales reflect progression in Friedreich ataxia (FRDA)?**

Authors: Katrin Bürk<sup>1,2\*</sup>, Stefanie Wolf<sup>1\*</sup>, the *GeNeMove consortium* and Jörg B. Schulz<sup>1</sup>  
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Abstract:

### **Background/Hypothesis:**

We here compared clinical scales including FRDA rating scale (*FARS*), the International Cooperative Ataxia Rating Scale (*ICARS*) and the Scale for the Assessment and Rating of Ataxia (*SARA*) for progression in Friedreich ataxia (FRDA).

### **Methods:**

93 FRDA patients enrolled in the *European GeNeMove database* were assessed with FAS, ICARS, FARS, SARA, ADL and HQ. Their mean age at baseline examination was  $27 \pm 13$  years (range 6 – 69 years), their disease duration was then  $14 \pm 10$  years (0 – 43 years) and their age of onset was  $13 \pm 8$  years (0 – 40 years). Each patient had a mean number of  $2.9 \pm 1.0$  (2 – 6) examinations during the study. Investigators were neurologists or senior residents in neurology training.

### **Results:**

There were high intercorrelations between the neurological scales FAS, ICARS, FARS and SARA total scores (all  $r \geq 0.96$ ; all  $p < 0.001$ , one-sided test). They also correlated highly with the ADL questionnaire as one outside criterion (all  $r \geq 0.88$ ). The mean progression of symptoms per year was calculated in two different ways, either as difference between two subsequent examinations or by means of linear regression per patient. All results were very similar, whatever method was used. SARA was found to be the most sensitive scale for detecting longitudinal changes showing outstandingly high values for Cohen's  $d$ , and therefore low values for the estimated test planning number of subjects needed for future t-tests. SARA needed less subjects to prove significant longitudinal changes than the other scales, e.g. when symptom changes pre vs. post treatment need to be examined. Interestingly, the patient questionnaire HQ had the worst results for Cohen's  $d$ , and for the test planning number of subjects  $N$ .

### **Conclusions:**

For longitudinal studies of progression, SARA could be shown to represent a reliable clinical tool in FRDA:

## **Title: Developing Common Data Elements for Friedreich's Ataxia Clinical Research**

Authors: Martin Delatycki,<sup>1</sup> Jennifer Farmer,<sup>2</sup> Kenneth Fischbeck<sup>3</sup>, Paul Kantor<sup>4</sup>, David Lynch<sup>5</sup>, Massimo Pandolfo<sup>6</sup>, Mark Payne<sup>7</sup>, Susan Perlman<sup>8</sup>, Jorg Schulz<sup>9</sup>, Robert Shaddy<sup>5</sup>, Subha Raman<sup>10</sup> (FA CDE Working Group), Wendy Galpern, Petra Kaufmann, Joanne Odenkirchen<sup>3</sup> (NINDS), Lisa Hunegs, Kristy Miller<sup>11</sup>(KAI)

Institutions: <sup>1</sup> Murdoch Institute, Royal Children's Hospital; <sup>2</sup> Friedreich's Ataxia Research Alliance; <sup>3</sup> National Institute of Neurological Disorders and Stroke (NINDS); <sup>4</sup> Hospital for Sick Children; <sup>5</sup> University of Pennsylvania; <sup>6</sup> Université Libre de Bruxelles; <sup>7</sup> Indiana University; <sup>8</sup> University of California Los Angeles; <sup>9</sup> University Medical Center, RWTH Aachen; <sup>10</sup> The Ohio State University; <sup>11</sup> KAI Research, Inc.

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Abstract:

**Background/Hypothesis:** To reduce study start-up time, increase data sharing, and assist investigators conducting clinical studies, the NINDS embarked on an initiative to create common data elements (CDEs) for neuroscience clinical research. The CDE Team has developed general CDEs which are commonly collected in clinical studies regardless of therapeutic area such as demographics and adverse events. In addition to general CDEs, the NINDS CDE Team is now working with experts in Friedreich's Ataxia (FA) to develop CDEs specific to this disease.

**Methods:** To develop FA CDEs, the experts formed a working group and four subgroups to define elements in: Ataxia and Performance Measures; Biomarkers; Cardiac and Other Clinical Outcomes; and Demographics, Laboratory Tests and Medical History. The basic CDE development process includes the following steps:

- Identify international experts in FA clinical research
- Utilize a secure SharePoint Web site to review existing materials [e.g. current study case report forms (CRFs) and publications], and draft documents
- Meet via teleconference to develop a first draft of standardized CDE recommendations from the subgroups
- Vet draft recommendations across the FA WGs
- Release recommendations to the research community for public comment

**Results:** The NINDS CDE Website, <http://www.commondataelements.ninds.nih.gov/>, includes tools to support the use of the general CDEs and several disease-areas, including FA. The tools include: data dictionaries, CRF templates, and procedure manuals to guide use of the CDEs. The FA CDEs will be posted on this website for public comment.

**Conclusions:** Once the FA CDEs are developed and vetted through the research community, the resulting elements will undergo periodic reviews and updates. The NINDS recognizes that the best way to ensure the FA CDEs are useful and the project accomplishes its goals is to periodically refine the CDEs based upon the feedback of researchers who have used them in their clinical studies.

## **Session 8 (Saturday, 14:30 – 16:00): Biomarkers & Functional Measures & Clinical Design**

Co-Chairs: Martin Delatycki & Alexandra Durr

### Speakers

<b>Petra Kaufmann</b> (invited)	Implementation data, endpoints, biomarkers
<b>Martin Delatycki</b>	Measuring the progression of Friedreich ataxia
<b>Cecilia Marelli</b>	Annual changes in Friedreich's Ataxia evaluated by SARA (Scale for the Assessment and Rating of Ataxia) are independent of disease severity
<b>Günther Metz</b>	Rating the disease state of FRDA patients by ICARS: An analysis using a large database of 600 patients
<b>David Lynch</b>	Measures of Neurologic progress in FRDA: The experience of the CCRNFA
<b>Marguerite Evans-Galea</b>	FXN methylation in Friedreich ataxia reveals insight into disease mechanism and new tools for the clinic

### Posters

<b>Paola Giunti</b>	Outcomes of Idebenone Therapy Reported by Patients with Friedreich's Ataxia: Design of the PROTI Study
<b>Thierry Morlet</b>	Auditory, Speech and Vestibular Abilities in Friedreich Ataxia
<b>Roger Peverill (Presenting author – Louise Corben)</b>	Relationship between right ventricular long axis systolic and diastolic function and the <i>FXN</i> gene in early Friedreich ataxia cardiomyopathy
<b>MR Rajesvari</b>	Circulating plasma DNA and protein biomarkers for Friedreich's ataxia
<b>Francesco Saccà</b>	A combined nucleic acid and protein analysis in Friedreich's Ataxia: implications for diagnosis, pathogenesis and clinical trial design
<b>Kim Schadt (Presenting author – Dave Lynch)</b>	Elevation of cardiac biomarkers in asymptomatic Friedreich's ataxia subjects

## Title: Measuring the progression of Friedreich ataxia.

Authors: Geneieve Tai<sup>1</sup>, Louise A Corben<sup>1,2</sup>, Lyle Gurrin<sup>3</sup>, Andrew J Churchyard<sup>4</sup> & Martin B Delatycki<sup>1,5</sup>

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### Abstract:

**Background:** Disease progression in FRDA is commonly captured using rating scales, functional composites and patient-reported outcome measures. This study explored the natural history of FRDA using data collected annually on clinical rating scales and functional measures over 12 years.

**Methods:** The rating scales used were the Friedreich Ataxia Rating Scale (FARS) and the International Cooperative Ataxia Rating Scale (ICARS) (1, 2), neurological examinations with higher scores indicating worse disability. The Functional Independence Measure (FIM) and the Modified Barthel Index (MBI) assess the capacity to complete activities of daily living (ADLs) (3, 4); lower scores indicate increased dependency. We measured disease progression in 118 individuals with disease duration in five-year intervals. Subgroups (GAA1 repeat size  $\leq 674$  and  $> 674$ ; age of disease onset  $< 14$  and  $\geq 14$ , representing  $<$  and  $>$  mean) were created to study the effects of these two clinical variables on performance in the measures.

**Results:** Individuals with GAA1 repeat sizes  $> 674$  had greater FARS (54.6 compared to 45.3) and ICARS scores (27.7 compared to 22.6), and lower MBI (97.6 compared to 99.1) and FIM scores (122.9 compared to 123.5). Individuals with a later age of disease onset had lower FARS (46.2 vs. 55.9) and ICARS (23.3 vs. 28.3) scores and greater MBI (98.8 vs. 97.3) and FIM (124.5 vs. 120.6) scores.

**Conclusions:** Individuals with larger GAA1 repeat sizes had more severe disease and decreased ability to complete ADL's. Measuring disease progression in FRDA is challenging due to its slow progression and variable phenotype. As pharmaceutical agents proposed to delay disease progression have been identified, tools used to measure the progression of FRDA should detect even the smallest clinical change and capture its multi-dimensional nature. This study provides further support for the use of the FARS/ICARS as sensitive tools for measuring disease progression.

### References:

1. Subramony SH, May W, Lynch DR et al. Measuring Friedreich ataxia: Interrater reliability of a neurologic rating scale. *Neurology* 2005; 64: 1261-1262.
2. Trouillas P, Takayanagi T, Hallett M, et al. International Cooperative Ataxia Rating Scale for pharmacological assessment of the cerebellar syndrome. *Journal of the Neurological Sciences* 1997; 145(2):205-211.
3. Mahoney FI, Barthel DW. Functional evaluation: The Barthel Index. A simple index of independence useful in scoring improvement in the rehabilitation of the chronically ill. *Maryland State Medical Journal* 1965; 14:61-65.
4. Granger CV, Hamilton BB, Keith RA et al. Advances in functional assessment for medical rehabilitation. *Topics in Geriatric Rehabilitation* 1986; 1(3):59-74.

**Title: Annual changes in Friedreich's Ataxia evaluated by SARA (Scale for the Assessment and Rating of Ataxia) are independent of disease severity**

Authors: Cecilia Marelli, *MD*, (1,2,3) Julie Figoni, *MS*, (4) Perrine Charles, *MD, PhD*, (3,6), Mathieu Anheim, *MD, PhD*, (1,2,3), Maya Tchikviladze, *MD*, (3), Carlo-Maria Vincitorio, *MD*, (3), Sophie Tezenas du Montcel, *MD, PhD*, (4,5), Alexis Brice, *MD*, (1,2,3,6), Jean Louis Golmard, *MD, PhD*, (4,5), Alexandra Durr, *MD, PhD* (1,2,3)

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Abstract:

**Background/Hypothesis:** To evaluate the sensitivity to change of the Scale for the Assessment and Rating of Ataxia (SARA) in Friedreich's ataxia (FA).

**Methods.** Design: follow-up study in genetically confirmed adult FA patients evaluated at least twice (minimal interval=6 months). Setting: Outpatients at the Reference Centre for Neurogenetics of the Pitié-Salpêtrière University Hospital in Paris.

**Results:** We included 84 adult FA patients; 60% had three or more SARA evaluations. FA population: mean age at onset  $18.7 \pm 11.1$  (5-65) years; mean age at first examination  $36 \pm 13.6$  (14-81) years; mean disease duration  $17.3 \pm 9.2$  (0-44) years; mean SARA score on first assessment  $22.7 \pm 9$ ; 61% of the patients wheelchair-bound. Mean follow up was  $1.84 \pm 1.10$  years. Mean SARA increase was  $1.36 \pm 2.3$  point/year; this variation was not significantly linked to factors known to influence disease severity, such as age at onset, disease duration, length of GAA expansions, and wheelchair use. SARA annual evolution was significantly slower for two of the six raters. All SARA subscores evolved significantly, except item 8.

**Conclusions:** In adult FA patients SARA is able to detect annual changes independently of disease severity. In future therapeutic trials no patients' stratification is required. Association to quantitative scale will be imperative. Validation and sensitivity to change in a pediatric population should still be evaluated.

## **Title: Rating the disease state of FRDA patients by ICARS: An analysis using a large database of 600 patients**

Authors: Metz G.<sup>1</sup>, Coppard N.<sup>1</sup>, Cooper J.M.<sup>2</sup>, Dürr A.<sup>3</sup>, Delatycki M.<sup>4</sup>, Fischbeck K.<sup>5</sup>, Schulz J.<sup>6</sup>, Meier T.<sup>1</sup> and Lynch D.R.<sup>7</sup>

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### **Abstract:**

#### **Background/Hypothesis:**

The International Cooperative Ataxia Rating Scale (ICARS) is a commonly used rating scale to assess symptoms of ataxia with 19 individual items grouped into four subscales. Despite its frequent use in clinical trials the ability of such a scale to measure short-term disease progression in Friedreich's Ataxia has been under dispute. This study analyses the total ICARS, its subscores and individual items for the largest cross-sectional dataset ever compiled.

#### **Methods:**

ICARS ratings and demographic data from 600 Friedreich's Ataxia patients have been collected. Standard metrics such as item-subscale correlations, ceiling effects and Cronbach's alpha have been calculated. For a subset of patients, the influence of age, GAA repeat length and disease duration has been correlated to ICARS subscales. For patients with repeated assessments over a short time period were available, correlation of repeated ratings were analyzed.

#### **Results:**

Our analysis show that neither age nor GAA repeat length but disease duration correlates well with ICARS scores. Item to subscale or total ICARS correlations range from 0.37 to 0.94 and are highest in the Posture & Gait subscale. Ceiling and floor effects are low for subscales but as high as 77% in individual items. Cronbach's alpha is > 0.84 except for the Oculomotor subscale (0.60). Repeated assessments correlate well over the whole range of total ICARS but the standard deviation between ratings was found to be close to four ICARS points.

#### **Conclusions:**

In conclusion, our analysis highlights weaknesses in the ICARS rating as a tool to measure short-term disease progression in a clinical setting in patients with FRDA, particularly when the patient population is heterogeneous with respect to disease history. Our analysis also shows that different ICARS subscales are more sensitive to change depending on the stage of disease as defined by the total ICARS.

## **Title: Measures of Neurologic progress in FRDA: The experience of the CCRNFA**

Authors: Lynch, DR, Farmer, JM, Delatycki, MB., Perlman, SL, Wilmot, G, Gomez, CM, Mathews, K, Bushara, K, Zesiewicz, T, Subramony, SH, Yoon, G, Brocht, A, Ravina, B

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Abstract:

**Background/Hypothesis:** The Collaborative Clinical Research Network in Friedreich Ataxia is conducting a longitudinal natural history study defining the neurologic rate of progression and its relationship to specific disease factors. The present work summarizes ongoing results from this cohort.

**Methods:** Over 500 subjects with FRDA were examined at 11 sites in the United States and Australia for up to 7 years. Annual medical histories, Friedreich ataxia rating scale (FARS), performance measures and their composites (Z2,Z3) were collected and analyzed.

**Results:** The cohort had an age at onset of 13.9, an Age at Baseline of 25.2, and a mean GAA repeat length of 632. The baseline FARS was  $63.5 \pm 21.2$ . Every measure worsened over time. Initially rates of change for all measures were almost linear before floor/ceiling effects were noted 3-6 years after baseline visit. All measures showed evidence of floor/ceiling effects except low contrast letter acuity. The ratio of yearly SDchange/ change decreased over the first 3 years of follow-up for the FARS and Z2 measures (from 2-2.5 at year 1, decreasing to approximately 1 by year 3) with slightly lower values being found for the performance composite Z2 than the FARS exam. Over longer times evaluation was confounded by floor effects and by bias in the group returning for follow-up (with individuals with shorter GAA repeats being more likely to return).

**Conclusions:** The present data show that the FARS and performance measure composites both capture neurologic progression in FRDA. Using the present data, the composite performance measure Z2 would require the lowest sample size in clinical trials of 1-2 years duration. However, such trials would still require >50 subjects per arm presuming a 50% retardation in progression rate. Based on the lower susceptibility of composites to placebo effects in recent trials, such composites may prove optimal for neurological assessment in clinical trials.

**Title: FXN methylation in Friedreich ataxia reveals insight into disease mechanism and new tools for the clinic.**

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**Background/Hypothesis:** The smaller repeat (GAA1) in Friedreich ataxia (FRDA) inversely correlates with age of onset and FXN expression, and directly correlates with sensory neuropathy and left ventricular hypertrophy. However, GAA1 explains only 50% of the phenotypic variability observed. Recent studies show the expansion induces epigenetic changes and heterochromatin formation. HDAC inhibitors being developed for clinical application also correct frataxin deficiency (RAI et al. 2010; RAI et al. 2008). To gain insight into disease mechanism and identify potential biomarkers, we investigated the relationships between the different parameters in a large well-characterized cohort of FRDA individuals.

**Methods:** Samples were obtained from 85 FRDA and 56 control subjects (HREC#22009). The expansion was evaluated by PCR and MbolI digestion. Methylation was measured in three FXN regions – the promoter (PRO), up- (UP) and downstream (DOWN) of the expansion - using EpiTYPER MassARRAY (Sequenom<sup>®</sup>). FXN expression was determined by Q-PCR. Significant correlations were examined using non-parametric Spearman's rank correlation coefficient and linear regression modeling.

**Results:** FRDA samples show hypermethylation in the UP region and hypomethylation in the DOWN region. This significantly correlates with GAA1 and concurs with FRDA heart and brain methylation (AL-MAHDAWI et al. 2008). UP-CpG1 methylation has an independent, inverse correlation with FXN expression ( $R=-0.53$ ,  $p<0.01$ ), while a 2% increase in DOWN methylation variation predicts a 0.2-1.5 year decrease in the age of onset ( $p=0.013$ ). FXN expression inversely correlates with FARS score ( $R=-0.48$ ,  $p<0.01$ ) and together age of onset, disease duration and FXN expression explain 80% of its variability ( $R^2=0.80$ ;  $p<0.001$ ).

**Conclusions:**

- Characteristic methylation indicates a fundamental epigenetic mechanism may be common to all tissues in FRDA.
- Inverse correlation of FXN expression with FARS score has positive implication for developing treatments that increase frataxin.
- Two regions of FXN - in two accessible tissues - could be useful clinical biomarkers.

AL-MAHDAWI, S., R. M. PINTO, O. ISMAIL, D. VARSHNEY, S. LYMPERI et al., 2008 The Friedreich ataxia GAA repeat expansion mutation induces comparable epigenetic changes in human and transgenic mouse brain and heart tissues. *Hum Mol Genet* 17: 735-746. RAI, M., E. SORAGNI, C. J. CHOU, G. BARNES, S. JONES et al., 2010 Two new pimelic diphenylamide HDAC inhibitors induce sustained frataxin upregulation in cells from Friedreich's ataxia patients and in a mouse model. *PLoS One* 5: e8825. RAI, M., E. SORAGNI, K. JENSSEN, R. BURNETT, D. HERMAN et al., 2008 HDAC inhibitors correct frataxin deficiency in a Friedreich ataxia mouse model. *PLoS One* 3: e1958.

## **Title: Outcomes of Idebenone Therapy Reported by Patients with Friedreich's Ataxia: Design of the PROTI Study**

Authors: William T Andrews, MD<sup>1</sup>; Nicholas Coppard, PhD<sup>1</sup>; Paola Giunti, MD, PhD<sup>2\*</sup>

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Abstract:

**Background/Hypothesis:** Clinical studies evaluating the treatment of neurologic symptoms of Friedreich's Ataxia (FRDA) with idebenone have yielded mixed results. However, reports from FRDA patients and their physicians suggest that idebenone may improve fatigue and the ability to perform activities of daily living, raising questions about the sensitivity of assessment tools currently being used in clinical trials. A double-blind, prospective, randomized study will evaluate Patient Reported Outcomes in FRDA patients after withdrawal from Treatment with Idebenone (PROTI) in participants of the ongoing 2-year MICONOS extension study (MES) of high-dose, open-label idebenone.

**Methods:** The approximately 80 FRDA patients who have completed at least 1 year of MES will be invited to participate and will be randomized to receive placebo or continue idebenone at their current MES dose (1350 or 2250 mg/day) for a period of 2 months. The primary endpoint is the proportion of patients who accurately assessed whether they were randomized to idebenone. The key secondary endpoint is the proportion of patients who withdraw because of recurring or worsening FRDA symptoms. Additional secondary endpoints include Modified Fatigue Impact Scale, 9-Hole Peg Test, speech assessments, clinical global impression of change, patient-reported outcomes collected via diary and questionnaires, ICARS, and investigator assessment of double-blind treatment assignment. Eligible patients completing or withdrawing from PROTI will re-enter the MES and may enroll multiple times in PROTI at the 12-, 18-, and/or 24-month time points in MES.

**Results:** This study explores the sensitivity of various clinical assessment tools, including patient-reported outcomes, in the evaluation of idebenone efficacy in patients with FRDA.

**Conclusions:** The results will provide valuable information regarding the potential therapeutic use of idebenone in the treatment of FRDA and the design of a new phase III study of idebenone in FRDA patients.

## **Title: Auditory, Speech and Vestibular Abilities in Friedreich Ataxia**

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Abstract:

**Background/Hypothesis:** The long term goal of this study is to examine if functional measures of auditory, speech or vestibular abilities could be used as potential biological markers of Friedreich Ataxia (FA) for future therapeutic trials.

**Methods:** In pediatric and adult patients with genetically confirmed FA, the following tests are being administered: tympanometry, middle ear muscle reflexes, otoacoustic emissions and their suppression by activation of the medial efferent olivocochlear system, auditory brainstem responses (ABR), pure tone audiometry, speech audiometry in quiet and in noise, gap detection and vestibular evoked myogenic potentials (VEMP). The measures are correlated to the FA characteristics as measured with the Friedreich Ataxia Rating Scale.

**Results:** Among all measures, the ABR appears to be the most sensitive to the progression of hearing abilities in FA. Neural dys-synchrony along the auditory brainstem pathways seems to appear well before changes at a behavioral level are being noticed. Neural dys-synchrony progresses from central to peripheral parts of the brainstem while the cochlear function is being preserved. The middle ear muscle reflex seems to disappear only when neural synchrony at the brainstem level is completely absent. Speech abilities are first reduced in noisy conditions. Pure tone audiometry in quiet is least affected. Suppression of otoacoustic emissions and VEMP are also affected by the disorder in some cases although a clear pattern couldn't be defined.

**Conclusions:** Measure of neural synchrony along the auditory pathways and therefore the emergence of dys-synchrony seems to be a sensitive measure of progression of FA for those individuals affected by hearing loss. Neural dys-synchrony is being observed before hearing and speech abilities become significantly impaired. Although the clinical auditory characteristics of some patients present like those observed in auditory neuropathy spectrum disorder, the pattern of progression from central to peripheral parts of the auditory system in FA probably underlines different mechanisms of impairment.

**Title: Relationship between right ventricular long axis systolic and diastolic function and the *FXN* gene in early Friedreich ataxia cardiomyopathy.**

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Abstract:

**Background/Hypothesis:** Friedreich ataxia (FRDA) is an autosomal recessive condition due to homozygosity of a GAA repeat expansion in the *FXN* gene. FRDA is associated with reduced velocities in systole (S') and early diastole (E') using tissue Doppler imaging (TDI) at the lateral mitral annulus. E' is inversely correlated with the number of small allele (GAA1), but not large allele GAA repeats (GAA2). We have now investigated the effects of FRDA on right ventricular (RV) long axis peak TDI velocities in systole (RVS') and early diastole (RVE') and also the relationship of RV function with GAA1 and GAA2.

**Methods:** We compared 64 FRDA patients (age 31±9 years, 32 men) with normal LV ejection fraction to 48 healthy age matched subjects (24 men). TDI signals were recorded at the tricuspid annulus for measurement of RVS' and RVE'.

**Results:** FRDA patients had a similar blood pressure and body surface area to controls, but a higher heart rate (p=0.001). RVS' and RVE' were lower in FRDA compared to controls (p<0.001 for both) and FRDA was a predictor of lower RVS' and RVE' independent of heart rate (p<0.001 for both). RVS' and RVE' were both negatively correlated with GAA1 (r=-0.32 & -0.33, respectively, p=0.01). RVE' (but not RVS') was also negatively correlated with GAA2 (r=-0.29, p=0.02) and GAA1 and GAA2 were independent inverse correlates of RVE'.

**Conclusions:** RV long axis systolic and diastolic function is reduced in FRDA and GAA repeats on both small and large alleles of the *FXN* gene appear to influence RV long axis diastolic function

## **Title: CIRCULATING PLASMA DNA AND PROTEIN BIOMARKERS FOR FRIEDREICH'S ATAXIA**

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### Abstract:

Friedreich's ataxia (FA) is a progressive, multisystem, degenerative disorder caused by a reduction in frataxin. FA is a fatal disease which is primarily associated with DNA Triplet Repeat Expansion (TRE) in gene. Abnormal expansion of (GAA) triplets in the first intron of *fxn* gene results in triple helical DNA formation which stalls RNA polymerase to transcribe the gene. Normal healthy individuals carry 20-40 (GAA) repeats while repeats are expanded from 66-1800 in 98% of FA patients. Loss of frataxin results in mitochondrial dysfunction and oxidative damage in patients

**Background/Hypothesis:** Our aim of the study was to quantify cell-free circulating DNA and find protein biomarkers from blood plasma of FA patients.

**Methods:** Clinically suspected patients from OPDs and wards of Neurology Department were assessed using ICARS scale and tested for TREs in genomic DNA. Confirmed -homozygous patients of FA (n=10) and normal healthy donors (n=20) were included in the present study. Circulating DNA quantification was done using high purity DNA from 200µl plasma of controls and FA patients. DNA quantification was done by highly sensitive PicoGreen fluorescent assay.

**Results:** We found circulating DNA levels as follows : 167 ±43 ng/ml (range 64-702 ng/ml) in FA patients and only 59±15 ng/ml (40-94 ng/ml) in healthy controls. Significantly high concentrations of circulating DNA in these patients indicate neuronal breakdown in brain and possible compromise of blood brain barrier. Albumin-depleted samples were used in 2D-Difference In-Gel Electrophoresis and proteomics.. We found 15 differentially expressed proteins in FA patients and plasma profiles. Mass spectrometry analysis of differentially expressed protein spots (>2.0 fold, p<0.05) confirmed decreased plasma levels of apolipoproteins A-I, c-II, C-III variant-I, transthyretin etc

**Conclusions:** . In conclusion, we were able to distinguish between FA patients and healthy controls by both levels of circulating plasma DNA and plasma proteins and this will help in developing biomarkers for the prognosis/diagnosis. To the best of our knowledge, this is the first report of this kind in FA.

**Title: A combined nucleic acid and protein analysis in Friedreich's Ataxia: implications for diagnosis, pathogenesis and clinical trial design.**

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Abstract:

**Background/Hypothesis:** FA is the most common hereditary ataxia among caucasians. The molecular defect in FA is the trinucleotide GAA expansion in the first intron of the *FXN* gene, which encodes for frataxin. No studies have yet reported a combined frataxin protein and mRNA levels screening in a large cohort of FA patients, carriers and controls.

**Methods:** We enrolled 24 patients with classic FA phenotype (cFA), 6 late onset FA (LOFA), 5 compound heterozygotes for expansion and point mutations (pFA; I154F, IVS4+3delA, R165P), 33 healthy expansion carriers, and 30 healthy controls. DNA was genotyped for GAA expansion, frataxin mRNA quantified with q-PCR assay, and frataxin protein was measured using lateral-flow immunoassay.

**Results: Conclusions:** We report the first explorative study on frataxin protein and mRNA levels in PBMCs from a cohort of FA patients, carriers and healthy individuals. Lateral-flow immunoassay differentiated cFA and pFA patients from controls. mRNA levels proved to be diagnostic when comparing cFA to controls.

## **Title: Elevation of cardiac biomarkers in asymptomatic Friedreich's ataxia subjects**

Authors: Schadt K, Friedman L, Regner S, Sciascia T, Lynch, DR

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Abstract:

### **BACKGROUND/HYPOTHESIS:**

Serum cardiac troponin I (cTnI) is a sensitive and specific marker of myocardial injury, commonly associated with acute myocardial infarction. Whether increased cTnI levels in Friedreich's ataxia (FRDA) occur chronically, or in clinically silent intervals is unknown. In this study, we analyzed cTnI levels and their relation to other disease traits from a heterogeneous cohort of FRDA subjects without chest pain at the time of sampling.

### **METHOD:**

Serum cTnI levels in FRDA subjects without active arrhythmia or features of acute coronary syndrome were measured and correlated with disease duration, age of onset, functional disability score, serum frataxin levels, and GAA trinucleotide repeat length.

### **RESULTS:**

Upon routine entry screening for the A0001 trial, 11 of 25 subjects had detectable serum cTnI levels greater than population norms (<0.01 ng/ml). Levels typically diagnostic of an acute myocardial event (AME) were found in 1 subject. These unexpected findings prompted baseline testing in 13 other asymptomatic FRDA subjects. Five of those subjects had levels in the range diagnostic for AME. Concurrent chest pain and active arrhythmias were absent in all subjects. Cumulatively, among subjects with abnormal cTnI levels, 6 (22%) were clinically positive for AME, with a mean cTnI value of 0.68 ng/ml. No linear correlations were noted between other disease features. However, younger age predicted an increased risk of abnormal cTnI measures ( $p=0.031$ ) in logistic regression.

### **CONCLUSION:**

Increased serum cTnI levels were present in 22 (58%) of the 38 FRDA subjects. Six subjects displayed levels normally diagnostic of AME. Elevation of cardiac biomarkers in the absence of acute coronary syndrome has unknown prognostic implications. However, it may provide a marker of left ventricular remodeling based on analogies with other cardiomyopathies. Importantly, the present data demonstrate that cTnI levels may not be reliable markers of acute coronary syndromes in patients with FRDA.