

ABSTRACT BOOK

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**Orals - All abstracts that will be presented orally are listed in order that they are scheduled to appear in the agenda for the conference. You can click on any of the titles in the table of contents and that will take you to the full abstract.**

## Elucidation of the enzymatic function of frataxin for drug discovery in Friedreich's ataxia

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Thursday, 14th November - 11:30: Mechanism of Disease - Oral Abstracts Part 1 - Abstract ID: 61

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 61

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**Mr. Sylvain Gervason<sup>1</sup>, Dr. Thomas Botzanowski<sup>2</sup>, Dr. Christina Sophia Müller<sup>3</sup>, Prof. Volker Schünemann<sup>3</sup>, Dr. Sarah Cianferani<sup>4</sup>, Dr. Michel Toledano<sup>5</sup>, Dr. Benoit D'Autreaux<sup>5</sup>**

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Friedreich's ataxia (FA) is a neurodegenerative and cardiac disease caused by defective expression of frataxin (FXN), a mitochondrial protein of elusive function. The main therapeutic strategies currently under development are aiming to 1) restore the level of FXN using gene expression modulators, protein stabilizers and gene therapy or 2) alleviate the effects of FXN deficiency. Although gene therapy and pharmacological approaches appear promising, there is still no efficient treatment to cure or even stop disease progression. A third strategy would be to pharmacologically replace FXN or enhance its activity, which could provide molecules directly targeting the primary defect of FA, thus with high potential in therapeutic treatment. However, the lack of enzymatic assay to measure FXN activity has precluded the development of this strategy. FXN is involved in the biosynthesis of iron-sulfur clusters that are small protein cofactors made of iron and sulfide ions and synthesized *de novo* by multi-protein machineries. Fe-S clusters are essential for a multitude of biological processes including protein and DNA synthesis, ATP production and maintenance of genome integrity. The reduced amount of Fe-S clusters in FXN deficient cells, led to suggest that FXN activates Fe-S cluster biosynthesis, but despite tremendous efforts since its discovery in the mid-nineties, the exact role of FXN is still controversial.

Based on the iron accumulation phenotype of FXN-deficient cells and the ability of purified FXN to bind iron, FXN was initially proposed to operate as an iron storage protein or an iron chaperone providing iron to the Fe-S cluster assembly machinery. These hypotheses were later on challenged, but yet its exact role could not be determined. We will present an *in vitro* reconstitution of the Fe-S cluster assembly machinery that faithfully reproduces physiological conditions, thereby allowing us to elucidate the biochemical function of FXN.

In mitochondria, Fe-S clusters are assembled by the Iron-Sulfur Cluster assembly (ISC) machinery, which comprises the scaffold protein ISCU on which Fe-S clusters are assembled, the cysteine desulfurase complex NFS1-ISC11-ACP providing sulfur as a cysteine-bound persulfide (Cys-SSH) and the ferredoxin 2 – ferredoxin reductase system FDX2 – FDXR supplying electrons. Fe-S cluster assembly is supposed to rely on confined production of sulfide in the vicinity of a still ill-defined iron center in ISCU, but this has never been demonstrated. Moreover, previous *in vitro* reconstitutions of the ISC machinery were shown to produce free sulfide that can bind free iron and promotes Fe-S cluster reconstitution, but this process is not considered physiologically relevant since sulfide production is not confined to ISCU. Thereby, the mechanism of Fe-S clusters assembly is still unclear, which has precluded breakdown of the assembly sequence into elementary steps to determine at which step FXN operates.

We found that purified ISCU contains a zinc ion that hinders iron insertion and promotes non-physiological Fe-S



cluster synthesis from free sulfide. By removing the zinc ion, we managed to insert iron in the assembly site of ISCU. We found that this iron-loaded form of ISCU supports Fe-S cluster assembly in a highly efficient way strictly relying on FDX2, with FXN stimulating the whole process, which thus reproduces the phenotypes of cells lacking FDX2 or FXN. Using a persulfide detection assay, we show that Fe-S cluster assembly proceeds via persulfide transfer from NFS1 to ISCU, followed by persulfide reduction into sulfide by FDX2, leading to formation of a [2Fe2S] cluster most likely by dimerization of ISCU. We found that both, persulfide transfer and reduction, requires iron, which coordinates sulfide production with iron availability in ISCU. Our data thus suggest that the reaction performed by iron-loaded ISCU recapitulates physiological conditions of Fe-S cluster assembly by preventing sulfide leakage, in contrast to the reaction observed with zinc-loaded ISCU that is akin to chemical reconstitution. We used the reconstituted machinery with iron-loaded ISCU to assess the specific role of FXN in Fe-S cluster assembly. We discovered that FXN is not required for iron insertion and is neither able to exchange zinc for iron, but that it stimulates the whole process by accelerating persulfide transfer. We thus propose that FXN operates as a stimulator of persulfide transfer in Fe-S cluster biogenesis (Figure 1). Our data should help progress in the development of an *in vitro* assay to discover drugs that could replace FXN *in vivo*.

## Development of a new class of ferroptosis inhibitors as novel therapeutics for Friedreich ataxia

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Thursday, 14th November - 11:45: Mechanism of Disease - Oral Abstracts Part 1 - Abstract ID: 135

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 135

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***Dr. Grazia Cotticelli*<sup>1</sup>, *Dr. Roberto Forestieri*<sup>2</sup>, *Dr. Shujuan Xia*<sup>1</sup>, *Mr. Taehee Lee*<sup>1</sup>, *Dr. Donna Huryn*<sup>2</sup>,  
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Friedreich ataxia (FRDA) is characterized by a progressive neurodegeneration. Most patients develop a cardiomyopathy in which hypertrophy is accompanied by a replacement of cardiomyocytes with fibrotic tissue. The molecular mechanisms leading to cardiomyocyte cell death are incompletely understood.

Ferroptosis is a recently identified pathway of regulated, iron-dependent cell death, biochemically distinct from apoptosis, which requires lipid peroxidation. In the cells, lipid peroxides can be generated enzymatically or through the Fenton reaction. Lipoxygenase enzymes contribute to the generation of lipid peroxides, which are reduced by glutathione-dependent lipid peroxidase, keeping the total level of lipid peroxides tightly regulated. Perturbations of this equilibrium can result in a catastrophic rise in lipid peroxidation, leading to cell death.

We have previously shown that cell lines commonly used to study FRDA are sensitive to erastin and RSL-3, both known inducers of ferroptosis, and, conversely, ferroptosis inhibitors are efficacious in protecting human and mouse cellular models of FRDA treated with ferric ammonium citrate (FAC) and an inhibitor of glutathione synthesis (BSO). FRDA cells have long been known to be sensitive to treatment with iron, BSO, or a combination of both. Moreover, the ferroptosis inhibitor SRS11-92 decreased the cell death associated with frataxin knockdown in healthy human fibroblasts. Taken together, these data suggested activation of the ferroptosis pathway in FRDA cells and, consequently, the possibility that ferroptosis inhibitors could be used as therapeutics in Friedreich ataxia. Oleic acid is an eighteen carbon monounsaturated omega-9 fatty acid, that has been reported to inhibit imidazole-ketone-erastin induced ferroptosis in G-401 cells through an uncharacterized mechanism. We tested oleic acid in primary, patient-derived fibroblasts, as well as mouse cells with FRDA-associated mutations treated with FAC and BSO and found that it was efficacious in rescuing cell viability.

In an effort to develop structure-activity relationships, and in a preliminary effort to develop compounds that might have improved physical properties (e.g. brain penetration), we tested 25 oleic acid derivatives. Twelve of these were commercially available analogs of oleic acid, or other common fatty acids that differed in the number of carbon atoms, site of unsaturation, geometry of olefin, and number of sites of unsaturation. We designed and synthesized thirteen novel analogs, with a focus on improving metabolic stability and improving physico-chemical properties. Among these, a novel compound, OA-200, was more potent than oleic acid in rescuing cell viability in cells treated with FAC and BSO, or with erastin. Furthermore, separation of the enantiomer of OA200 led to the identification of a eutomer and a distomer. We developed a preliminary understanding of SAR for this series based on preferred carbon chain length, unsaturation sites, and absolute stereochemistry.

Oleic acid has been previously shown to interact with PPAR-gamma and to promote PGC1-alpha transcription, but increased PGC1-alpha expression was unable to rescue viability in the cells treated with FAC and BSO. We also showed that whereas a commercial inhibitor of lipoxygenase-15 (Lox15) enzymatic activity was efficacious in the FAC+BSO assay, our optimized analog did not inhibit Lox15 activity in an *in vitro* assay.

## Identification, construction and analysis of ISC machinery variants allowing to bypass frataxin dependency in mammalian cells

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Thursday, 14th November - 12:15: Mechanism of Disease - Oral Abstracts Part 1 - Abstract ID: 107

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 107

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Iron sulfur (Fe-S) clusters are essential co-factors required for the functioning of a variety of proteins involved in key cellular processes. The conserved multiprotein machinery ISC, localized in the mitochondria in eukaryotes, initiate Fe-S clusters biogenesis by *de novo* assembly of sulfur, provide by a cysteine desulfurase, and iron on a scaffold protein. One of the major actor of Fe-S biogenesis is the frataxin protein (FXN). Even if its function is not yet fully characterized, it is accepted that FXN is an allosteric regulator of the ISC machinery increasing Fe-S clusters biogenesis rate by controlling sulfur production and iron entry. There is a growing number of diseases associated with Fe-S clusters biogenesis dysfunction but the most common is Friedreich's ataxia (FA), an autosomic recessive neurodegenerative disease mainly characterized by a progressive spinocerebellar and sensitive ataxia. FA is due to an expansion of GAA trinucleotide repeat in the 1<sup>st</sup> intron of FXN gene leading to a drastic decrease of FXN protein production. The main consequence of FXN deficiency is a decrease of Fe-S clusters biogenesis which leads to deficit in Fe-S proteins, especially in mitochondrial Fe-S enzymes like aconitase, intracellular iron deposits and sensitivity to oxidative stress. In eukaryotes, FXN is essential, while it is not an essential gene in prokaryotes. Recently, a substitution Met to Ile in position 141 of the scaffold protein *isu1* in yeast, allowing  $\Delta Yfh1$  yeast (frataxin deficient) to grow was identified, demonstrating that this suppressor can bypass frataxin. Interestingly, an Ile residue is found at the same position in the scaffold protein IscU in *E.coli*, which if it is replaced by a Met residue, leads to FXN dependency in bacteria. This discovery raises the hypothesis that modification of ISC machinery components could allow Fe-S clusters biogenesis independently of FXN function. The goal of this work was to use synthetic biology approach to build protein variants of ISC machinery bypassing FXN lethality. By using a FXN dependent *E.coli* bacterial system to search small colony size suppressive mutations, different proteins variants of the scaffold protein have been identified. To validate if these mutations can bypass FXN lethality in mammalian cells, they were introduced using a CRISPR-Cas9 system in NC6 L3/L- mice fibroblasts carrying a conditional allele allowing FXN deletion. We showed that the mutation Met to Ile in position 141 of the scaffold protein ISCU allows the cells to survive without FXN. First evidences suggest that despite this viability, mitochondrial Fe-S enzymes are still affected, and cells are sensitive to oxidative stress. Investigation of these phenotypes in addition to biochemical analysis will permit to understand molecular mechanisms underlying FXN dependency and to better characterize early steps of Fe-S clusters biogenesis, FXN function and the impact of FXN deficiency in FA physiopathology.

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## Tissue-specific variability in epigenetic FXN silencing in the humanized mouse model of Friedreich ataxia

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Thursday, 14th November - 12:30: Mechanism of Disease - Oral Abstracts Part 1 - Abstract ID: 248

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 248

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***Ms. Layne Rodden<sup>1</sup>, Ms. Kaitlyn Gilliam<sup>1</sup>, Ms. Christina Lam<sup>1</sup>, Dr. Sahar Al-Madhawi<sup>2</sup>, Dr. Mark Pook<sup>3</sup>, Dr. Sanjay Bidichandani<sup>1</sup>***

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Friedreich ataxia (FRDA) is a progressive condition characterized by ataxia and cardiomyopathy. FRDA is caused by an expanded GAA trinucleotide repeat (GAA-TR) in intron 1 of the *FXN* gene, which induces repressive heterochromatin and transcriptional silencing of the *FXN* gene. The length of the expanded GAA-TR, which typically ranges from 100-1300 triplets, correlates strongly with the severity of *FXN* gene silencing and the clinical phenotype. The expanded GAA-TR establishes a differentially methylated region (DMR) in intron 1 of the *FXN* gene that is hypermethylated in FRDA. FRDA-DMR methylation is variable among patients, and is a strong predictor of *FXN* silencing. FRDA-DMR methylation also partially explains variability in efficacy of histone deacetylase (HDAC) inhibitors, wherein patients with low DNA methylation show higher *FXN* reactivation. We analyzed several tissues from the YG8sR mouse model (GAA-120 and GAA-450) using bisulfite deep sequencing. FRDA-DMR methylation was noted in all disease-relevant tissues (dorsal-root ganglia, cerebellum, and heart) in the GAA-120 and GAA-450 mice, with DNA methylation being 3-fold higher in the latter mice. Strikingly, FRDA-DMR methylation was much lower in the heart compared to all other tissues in both the GAA-120 and GAA-450 mice. The level of FRDA-DMR methylation in the heart of the GAA-120 mouse was very low, indicating a difference in the repeat length threshold and dynamic range of epigenetic silencing in heart versus other tissues. This variability in *FXN* epigenetic silencing may have implications for tissue-specific genotype-phenotype correlation and variability in response to gene reactivation therapy in FRDA.

# Knockdown of frataxin leads to mitochondrial fragmentation and cerebellar degeneration in an inducible mouse model of Friedreich ataxia

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Thursday, 14th November - 14:40: Neurophysiology and the Cerebellum - Oral Abstracts - Abstract ID: 63

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 63

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**Ms. ELIZABETH MERCADO-AYON<sup>1</sup>, Mr. Nathan Warren<sup>2</sup>, Dr. David Lynch<sup>3</sup>, Dr. Hong Lin<sup>4</sup>**

**1.** University of Pennsylvania, **2.** The Children's Hospital of Philadelphia, **3.** Children's Hospital of Philadelphia, **4.** University of Pennsylvania/ Children's Hospital of Philadelphia

## Introduction:

Friedreich ataxia (FRDA) is a life-shortening autosomal recessive neurodegenerative disorder caused by deficiency of the mitochondrial protein frataxin. The pattern of neurodegeneration leads to progress ataxia, dysmetria and dysarthria. Cerebellar neuropathology in FRDA patients includes loss of large principal neurons and synaptic terminals in dentate nucleus (DN) as well Purkinje cell injury and axonal remodeling. Our previous studies have demonstrated early cerebellar mitochondrial biogenesis and synaptic deficits in the frataxin Knockin-Knockout (KIKO) mouse model of FRDA. However, the correlation of frataxin deficiency with mitochondrial fusion/fission and cerebellar neuropathology remain unclear.

## Methods:

Using a tetracycline promotor-controlled inducible frataxin knockdown (FRDAkd) mouse model, we examined the time-course of alterations in mitochondrial fission protein Drp1 levels and activation, and glutamatergic and GABAergic synaptic marker levels after doxycycline induction by Western blot analysis and Immunohistochemical studies. Electron transmission microscopy was used to examine the morphology and integrity of synapses and mitochondria in mouse cerebellar cortex.

## Results:

Induction of frataxin knockdown at 4, 8-12, and 18 weeks lead to excessive and progressive activation of mitochondrial fission protein Drp1 at serine 616 site (pDrp1S616), while frataxin levels are progressively reduced to 50%, 10% and 0% of control levels in FRDAkd mouse cerebellum. Immunohistochemical studies show excessive high levels of pDrp1(S616) in cerebellar cortex. Furthermore, levels of climbing fiber-specific glutamatergic synaptic marker VGLUT2 are consistently decreased at 4, 8-12 and 18-week of doxycycline induction, whereas levels of parallel fiber-specific synaptic marker VGLUT1 are significantly reduced at 18-week of induction, but remain at the similar levels at 4 and 8-12 week of induction compared to aged-match controls, This finding suggests early selective degeneration of climbing fiber synapses and later degeneration of parallel fiber synapses in the inducible FRDAkd mouse model, which is distinct from synaptic deficits with the neurodevelopmental features in the KIKO mouse model of FRDA. GABAergic synaptic marker GAD65 is consistently decreased at early and late stages of induction in FRDAkd mice, which is also different from GABAergic synaptic changes in the KIKO mouse model of FRDA, while GAD67 levels remain unaltered in both mice, suggesting crucial roles of frataxin in synaptic development and in maintaining synaptic integrity and function during adulthood. Moreover, electron transmission microscopic studies further show defected synapses and fragmented mitochondria in cerebellar cortex of FRDAkd mice. Progressive loss of cerebellar Purkinje neurons and large DN principal neurons are observed in FRDAkd mouse cerebellum after 12-24 week of induction, suggesting synaptic and neuronal degeneration in FRDAkd mouse cerebellum,

## Conclusion:

Taken together, our findings thus demonstrate that frataxin knockdown leads to mitochondrial fragmentation and cerebellar degeneration in FRDA<sup>Kd</sup> mouse model, implicating crucial pathophysiological roles of frataxin in maintaining mitochondrial dynamics and neuronal and synaptic function in FRDA cerebellum.

# Preservation of gait and prevention of ataxia in the neurologic mouse model of Friedreich Ataxia when treated with TAT-Frataxin

Thursday, 14th November - 16:40: Therapeutic Approaches and Drug Discovery - Oral Abstracts Part 1 - Abstract ID: 270

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 270

***Dr. Michael Clark<sup>1</sup>, Ms. Manogna Chintala<sup>2</sup>, Mrs. Melanie Pride<sup>2</sup>, Prof. Ronald Payne<sup>2</sup>***

*1. Nationwide Childrens Hospital, 2. Indiana University School of Medicine*

**Rationale:** Low or absent expression of the mitochondrial protein, Frataxin (FXN), in Friedreich Ataxia (FRDA) causes a progressive and fatal cardiomyopathy, and a progressive and severe spinocerebellar degeneration with ataxia early in life. We have shown that the replacement of FXN using the cell penetrant fusion protein, TAT-FXN, prevents development of cardiomyopathy in the MCK-Cre Knock-Out (KO) FRDA mouse. We reasoned that TAT-FXN would deliver adequate amounts of FXN to neural tissues to be clinically relevant. We used the conditional KO model recapitulating the neurologic features of FRDA recently described by the Puccio lab to test the hypothesis that TAT-FXN would provide adequate levels of FXN in neural tissues to preserve gait and prevent development of ataxia.

**Methods:** Mice expressing Cre under control of the Parvalbumin promoter (Pvalb-Cre) (Jax Labs B6;129P2-Pvalb<sup>tm1(Cre)Arbr</sup>/J) were crossed into the floxed FRDA<sup>f</sup> mouse to generate conditional loss of FXN expression in brain, spinal cord, and dorsal root ganglia (PVKO mice). Three groups of mice were studied for 120 days: wild type mice (WT, n=14), untreated PVKO mice (n=13), and treated PVKO mice (n=10). The treated group received 10 mg/kg ip of TAT-FXN three times a week starting at 7 days of age, whereas the WT and untreated PVKO mice did not receive TAT-FXN. Gait was assessed using a DigiGait treadmill (Mouse Specifics, Inc, Framingham, MA), to digitally capture gait data as the mice walked at 20 cm/sec on a flat incline. A total of 41 variables were thus quantified at each time point for each mouse. Mice were longitudinally evaluated at day of life 30, 45, 60, 75, 90, and 120. Some of the animal measurements were lost at the 90 and 120-day time points due to DigiGait equipment breakdown and thus, later timepoints have smaller groups (no animals died). Human FXN in brain, spine, and dorsal root ganglia (DRG) was measured by ELISA (Abcam #ab176112) and animal weight gain was recorded. Survival to 180 days was also measured.

**Results:** WT animals differed significantly from the untreated PVKO mice for 14 of the 41 analyzed variables at 120 days of life. Twenty-seven variables were not different between WT and untreated KO and were discarded as not informative. When treated PVKO mice were compared to the untreated PVKO mice for those 14 parameters, the treated animals did better than the untreated animals for multiple variables and were highly significant for Stance Width, Ataxia Coefficient, and Midline Distance. The PVKO treated mice were not different from WT mice for these same variables. Treated PVKO mice lived significantly longer than untreated mice but growth was not different between the 2 groups. Significant amounts of human FXN were found in brain, spine and DRG of treated PVKO mice.

**Conclusions:** This data shows significant improvement in behavioral biomarkers of gait and in lifespan in the PVKO mice when they are treated with TAT-FXN. Pharmacologically significant amounts of human FXN was found in the brain, spine and DRG of the treated PVKO mice. TAT-FXN can prevent development of ataxia and preserve gait in the PVKO mouse.



## End targeting oligonucleotides increase stability of FXN mRNA

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 84

Friday, 15th November - 10:45: Therapeutic Approaches and Drug Discovery - Oral Abstracts Part 2 - Abstract ID: 84

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***Dr. Marek Napierala<sup>1</sup>, Dr. Yanjie Li<sup>1</sup>, Dr. Jun Wang<sup>1</sup>, Dr. David Lynch<sup>2</sup>, Dr. Xiulong Shen<sup>3</sup>, Dr. David Corey<sup>3</sup>, Dr. Caroline Woo<sup>4</sup>, Dr. Jonathan Cherry<sup>4</sup>, Dr. Jill Napierala<sup>1</sup>***

*1. University of Alabama at Birmingham, 2. Children's Hospital of Philadelphia, Philadelphia, 3. UT Southwestern, 4. TranslateBio*

Friedreich's ataxia is a severe neurodegenerative disease caused by transcriptional repression induced by expanded GAA repeats located in intron 1 of the *FXN* gene. FRDA is the most common inherited ataxia in humans, with ~1 in 100 people carrying a mutation in the *FXN* gene and the overall population frequency reaching 1 in 30,000 – 50,000. FRDA results from decreased levels of an otherwise functional frataxin protein, thus stabilization of the *FXN* mRNA and/or protein already present in patient cells represents an attractive and unexplored therapeutic avenue. Inhibition of frataxin protein degradation has been investigated, however at the present time results of these studies remain inconclusive. In this work we pursued a novel approach based on oligonucleotide (ON) -mediated targeting of *FXN* mRNA. As cellular mRNAs are subjected to continuous turnover, and extending the half-life of mRNA and therefore its availability as a template for translation will result in increased steady-state levels of the encoded protein. RNA decay occurs predominantly via exonucleolytic cleavage of 5' or 3' mRNA ends, and preventing or slowing down the kinetics of decay processes can increase levels of *FXN* mRNA and ultimately result in upregulation of frataxin levels in FRDA cells. Here we demonstrated that oligonucleotides designed to bind to *FXN* 5' or 3' UTR can increase frataxin mRNA and protein levels. A combined delivery of ONs targeting both ends increases efficacy of the treatment. The approach was confirmed in several FRDA patient derived primary fibroblast lines, iPSC-derived neuronal progenitors and terminally differentiated neurons. Unbiased RNA sequencing and single-cell expression analyses definitively confirmed ON-mediated *FXN* mRNA upregulation. Mechanistically, a significant elongation of the *FXN* mRNA half-life without any changes in chromatin status in at the *FXN* gene was observed upon treatment with end-targeting oligonucleotides, indicating that transcript stabilization is responsible for frataxin upregulation. These results identify a novel approach towards upregulation of steady-state mRNA levels via ON-mediated end protection that may be of significance to any condition resulting from transcription downregulation.

# Generation of a new neuronal model of Friedreich's Ataxia and establishment of a drug screening strategy

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 238

Friday, 15th November - 11:00: Therapeutic Approaches and Drug Discovery - Oral Abstracts Part 2 - Abstract ID: 238

***Mr. Olivier Griso<sup>1</sup>, Mrs. Amélie Weiss<sup>1</sup>, Mrs. Aurelie Eisenmann<sup>2</sup>, Prof. Helene Puccio<sup>1</sup>***

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*Department of Translational Medicine and Neurogenetics, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France ; INSERM, U1258, Illkirch, France ; CNRS, UMR7104, Illkirch, France ; Université de Strasbourg, Strasbourg, France*

Friedreich's Ataxia (FA) is an autosomic recessive neurodegenerative disorder, characterized by a spinocerebellar and sensory ataxia associating cardiomyopathy. The sensory ataxia results from the degeneration of proprioceptive neurons of the dorsal root ganglia (DRG). The major FA-causing mutation is a GAA expansion in the first intron of the *FXN* gene, which leads to the reduced expression of frataxin, a mitochondrial protein involved in iron-sulfur (Fe-S) clusters biosynthesis. The study of mouse models bearing heart-specific frataxin depletion showed a primary decrease of Fe-S clusters biosynthesis followed by mitochondrial dysfunction and iron metabolism dysregulation. Despite the generation of several mouse and cell models aiming at recapitulating the neuronal phenotype of FA, the mechanisms leading to the degeneration of proprioceptive neurons of the DRG remain unknown. Furthermore, proprioceptive neurons represent only 7.5% of the total DRG cell population, limiting specific biochemical and molecular approaches in mouse models. To gain insight into the molecular mechanisms implicated in the neuropathophysiology of FA, we have generated a new cellular model based on primary cultures of mouse DRG sensory neurons fully depleted for frataxin. The characterization of the model revealed typical FA phenotypes: loss of Fe-S clusters, altered mitochondrial activity and structure, cellular iron dysregulation and increased mitochondrial mass. The cells also show mitochondrial oxidative stress. Interestingly, cell survival is not affected by total frataxin depletion, but the size of the sensory neurons soma is greatly reduced, a phenotype highly significant and rescued by human frataxin overexpression.

This new cellular model thereby recapitulates FA classical phenotypes and has become a key tool not only for the identification of new pathways implicated in the neurodegeneration of sensory neurons in FA, but also in the development of a high-throughput screening (HTS) of pharmaceutical compounds. To date, and to our knowledge, there has been no HTS on sensory neurons deficient for frataxin, a cell type primarily affected in FA. Combining optimal culture conditions and a robust readout strategy based on high content imaging, we have been able to set-up a HTS strategy with the model. We will present the characterisation of the model, pathways identified as well as the HTS strategy with results from a pilot study.

## Progressive muscle spindle degeneration in a Friedreich's ataxia mouse model

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Friday, 15th November - 11:50: Cellular and Animal Models of Disease - Oral Abstracts - Abstract ID: 202

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 202

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**Mr. Giuseppe Yañez<sup>1</sup>, Dr. Anna Stepanova<sup>2</sup>, Dr. Joriene de Nooij<sup>3</sup>, Dr. Jordi Magrane<sup>1</sup>**

*1. Feil Family Brain and Mind Research Institute, Weill Cornell Medicine, 2. Department of Pediatrics, Columbia University Medical Center, 3. Department of Neurology, Columbia University*

Located in the dorsal root ganglia (DRG), proprioceptive sensory neurons play a critical role in coordinating complex movements by relaying information between the skeletal muscles and the central nervous system. Type Ia and II sensory afferent nerve fibers accomplish this through innervation of intrafusal muscle fibers (IMFs) to form the muscle spindle complex, sensitive to the speed and extent of muscle contraction. The two types of specialized IMFs with non-contractile regions are the nuclear bag fibers and nuclear chain fibers, divergently innervated by the Ia and II sensory afferents. A single Ia afferent spirals around the central region of all IMFs and serves as the primary sensory ending. A variable number of type II afferents, located adjacent to the central regions of the bag and chain fibers, serve as secondary sensory endings.

Little is known regarding the impact of frataxin (Fxn) deficiency on proprioceptive sensory endings and the effect on IMFs continues to be poorly understood. The essential role of the muscle spindle complex suggests that morphological changes or loss of functionality in type Ia and II sensory afferents may explain the altered sensorimotor function seen in Friedreich's ataxia (FRDA). To address this we investigated the innervation and components of the muscle spindle structure in the Fxn knock-in/knockout (KIKO) mouse model. Analysis of whole-mounted soleus muscles allowed us to examine the impact of Fxn deficiency on proprioceptive sensory endings in their native configuration.

We demonstrate that there is a significant decrease in sensory nerve endings innervating nuclear chain, but not nuclear bag, fibers. While no significant changes in the number of muscle spindles are detected, muscle spindle complexity is altered in 11-month-old KIKO mice. Although the number of Ia afferents remains unchanged, there is a significant loss of type II afferents at this time point. Chain fibers degenerate at 11 months, while type II axonal degeneration is already observed in 3-month-old KIKO mice. Interestingly, nuclear bag fibers are spared of any pathology, perhaps due to increased activity of type Ia afferents. Mitochondrial abnormal accumulation and fragmentation near the afferent ending is detectable as early as 1 month of age, suggesting a causative role in muscle spindle pathology. Interestingly,  $\gamma$ -motor neurons and neuromuscular junctions are devoid of any pathology up to 11 months of age. Moreover, no degeneration of proprioceptive sensory neurons is observed in the DRGs of KIKO mice at 11 months of age, suggesting that neuronal loss may occur later than axonal degeneration during disease process. Fxn-related degeneration of proprioceptive sensory neurons likely alters the pattern and rate of motor neuron activation, thus compromising the initiation and coordination of simple and complex movements.

## The Ataxia Instrumented Measure - Spoon (AIM-S) - A new measure of upper limb function in Friedreich ataxia

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Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 162

Friday, 15th November - 15:10: Natural History, Biomarkers and Endpoints - Oral Abstracts Part 1 - Abstract ID: 162

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**Dr. Louise Corben<sup>1</sup>, Dr. Pubudu Pathirana<sup>2</sup>, Prof. Malcolm Horne<sup>3</sup>, Dr. David Szmulewicz<sup>4</sup>, Mr. Khoa Dinh Nguyen<sup>2</sup>, Ms. Melissa Roberts<sup>1</sup>, Prof. Martin Delatycki<sup>1</sup>**

*1. Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute, 2. Networked Sensing and Control Group, School of Engineering, Deakin University, Geelong. Victoria, 3. Florey Neurosciences Institute, Melbourne. Victoria, 4. Balance Disorders & Ataxia Service, Royal Victorian Eye and Ear Hospital. Cerebellar Ataxia Clinic, Alfred Health. Melbourne. Victoria*

### Introduction

Optimum upper limb function for people with Friedreich ataxia (FRDA) is an essential component of daily function. Evaluation of upper limb dysfunction can be more difficult than that of the lower limbs as the upper limbs are used to perform complex and multidimensional tasks including reaching, grasping and stabilizing, as well as fine manipulation. The most common measures of upper limb function in FRDA are the Nine Hole Peg Test (9HPT)<sup>1</sup>, the Composite Cerebellar Functional Severity (CCFS)<sup>2</sup> score and the upper limb components of the Friedreich Ataxia Rating Scale (FARS)<sup>3</sup>, the Scale for the Assessment and Rating of Ataxia (SARA)<sup>4</sup> and International Cooperative Ataxia Rating Scale (ICARS)<sup>5</sup>. Despite the call from regulatory bodies for outcome measures to reflect changes in function, the capacity for existing measures to reflect functional capacity remains uncertain. Moreover individuals with FRDA question the relationship and meaning of existing measures to daily activities that are important to them. There is a need to identify an upper limb assessment tool that is applicable to all individuals with FRDA throughout the disease trajectory, capturing most aspects of upper limb function and importantly, one that is able to measure a minimally clinically important difference, the capacity of which is vital for future clinical trials. This prospective longitudinal study aims to validate and evaluate the capacity of a new upper limb measure to both quantify upper limb impairment and capture change in function over a 20 and 40 week period in 40 individuals with FRDA.

### Methods

We have developed an objective measure, in the form of an instrumented spoon, which utilizes an important yet challenging intricate upper limb task for individuals with FRDA: the pre-oral phase of eating. The spoon, which we have named the Ataxia Instrumented Measure - Spoon (AIM-S), is equipped with a BioKin wireless motion capture device designed to log complex movements of the hand and upper limb while picking up a spoon from the table, retrieving oats from a bowl, transporting the spoon to the mouth and return. Data related to the movement is recorded on an application on an Android phone. We have used machine learning techniques to quantitatively assess the quality of control, direction and timing of hand movements captured by the AIM-S during task completion. Data has been collected at baseline, on average 20 weeks later (second assessment) and on average 40 weeks after baseline (third and final assessment). In addition, to ascertain test-retest reliability, data will be collected in a cohort of 10 individuals a week apart. Data collection with the AIM-S has been accompanied at each timepoint by scoring on the modified FARS (mFARS), 9HPT, Box and Block (BBT) and a range of patient reported outcomes.

### Results

We have recruited 41 adults and children (target n=40) with FRDA and 20 control participants (target n=20) to base-

line assessments. Twenty individuals have undergone a second assessment, nine have undergone a final assessment and seven participants have undergone test-retest data collection. The remainder of the longitudinal and test-retest data will be collected by October, 2019.

An interim analysis of data from the baseline and second assessment reveals a clear separation of individuals with FRDA and control participants on four distinct features of movement: Dynamic Time Warping, Log Dimensionless-Jerk, Mobility Index, Range of Motion in both the overall movement trajectory and particularly the bowl to mouth component of the trajectory (see Figure 1).

Using the upper limb component of the FARS scale as a comparator, analysis of the longitudinal AIM-S data indicates the AIM-S demonstrates greater sensitivity to change compared to the 9HPT and the BBT (see Figure 2). Final data analysis will be reported at the presentation.

#### Conclusion

We have established that the AIM-S reflects the unique upper limb impairment of FRDA, and has a greater sensitivity to change in upper limb function throughout the continuum of the disease trajectory, than traditional measures. The AIM-S is perceived to be clinically meaningful to individuals with FRDA and importantly will enable inclusion in future clinical trials of those individuals who are currently unable to complete the traditional measures such as the 9HPT or tasks that involve ambulation.

Acknowledgements: This study was funded by L'Association Francaise de l'Ataxie de Friedreich (AFAF)-Friedreich's Ataxia Research Alliance (FARA) rideATAXIA Europe research award.

<sup>1</sup>Grice KO, et al., *Am J Occup Ther.* 2003; 57:570-3. <sup>2</sup>Tezenas du Montcel S. et al., *Brain*, 2008; 131:1352-1361.

<sup>3</sup>Subramony SH, et al., *Neurology* 2005;64: 1261–1262. <sup>4</sup>Trouillas P, et al., *J Neurol Sci* 1997, 145:205-211. <sup>5</sup>Schmitz-Hubsch T, et al., *Neurology*, 2006, 66:1717-1720.

## Extra-Mitochondrial Frataxin and Friedreich's Ataxia

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 256

Friday, 15th November - 17:15: Natural History, Biomarkers and Endpoints - Oral Abstracts Part 2 - Abstract ID: 256

**Dr. Laurent Laboureur<sup>1</sup>, Dr. Liwei Weng<sup>1</sup>, Dr. Qingqing Wang<sup>1</sup>, Ms. Lauren Hauser<sup>2</sup>, Dr. David Lynch<sup>2</sup>,  
Dr. Clementina Mesaros<sup>1</sup>, Prof. Ian Blair<sup>3</sup>**

*1. University of Pennsylvania, 2. Children's Hospital of Philadelphia, 3. Penn SRP Center and Center of Excellence in Environmental Toxicology, Department of Systems Pharmacology and Translational Therapeutics Perelman School of Medicine, University of Pennsylvania*

Friedreich's ataxia (FRDA) is an autosomal recessive disease caused primarily by an intronic GAA triplet expansion in both alleles of the *FXN* gene (homozygous cases). This leads to reduced expression of full-length frataxin protein and the mitochondrial processing peptidase (MPP)-derived mature frataxin (81-210). A small number of cases who are compound heterozygous for GAA repeats on one *FXN* allele and a mutation in the other (< 3 %), also have reduced levels of both full-length and mature mitochondrial mature frataxin. Frataxin is a highly conserved protein, which can be found in both prokaryotes and eukaryotes. Although the exact role of frataxin has not been completely delineated, there is compelling evidence that it is essential for iron-sulfur cluster biogenesis a critical requirement for many mitochondrial enzymes. Consequently, reduced frataxin expression results in decreased activity of the of the mitochondrial enzymes involved in energy metabolism. Surprisingly, mature frataxin (81-210), a mitochondrial protein, was found previously in erythrocytes by both western blot and immunoassays, even though erythrocytes have no ribosomal machinery or mitochondria. Little attention had been given to the paradoxical presence of this mitochondrial protein in erythrocytes, a cell with no mitochondria. This is most likely because aberrant heme formation is not observed in FRDA cases where frataxin levels in erythrocytes are significantly reduced by 65 % compared with normal subjects. We recently made the unexpected discovery that erythrocyte frataxin is in fact an N-terminally acetylated 135 amino acid splice variant of frataxin (isoform E), which lacks the mitochondrial targeting sequence found in full-length frataxin (Guo et al. Sci Rep 2018;8:17043). We now discovered that frataxin isoform E is also expressed in human muscle and that is reduced by similar amounts to mature frataxin in homozygous and most compound heterozygous FRDA cases. This raises the possibility that FRDA is not just a mitochondrial disease. Frataxin isoform E, which cannot translocate to the mitochondria could play a role in assembling iron-sulfur cluster proteins involved in DNA repair and telomere length control (both are lacking in FRDA) rather than those involved mitochondrial metabolism. Significantly, mRNA from the naked mole rat (*Heterocephalus glaber*) codes for a 135 amino acid protein that has 90.3% similarity and 98.5% homology with human isoform E. Furthermore, frataxin mRNA from Brandt's bats (*Myotis brandtii*) codes for a 135 amino acid protein that has even greater similarity (91.9%) and homology (99.3%) with human isoform E. Naked mole rats and Brandt's bats live five times and ten times longer, respectively than would be predicted from their body mass. In contrast, rats and mice, mammals that cannot express frataxin isoform E, live shorter lives than predicted (longevity factors 0.6 and 0.7, respectively) providing additional evidence for the role of extra-mitochondrial frataxin in longevity.

Supported by Penn Medicine/CHOP Friedreich's Ataxia Center of Excellence, and NIH grants P42ES023720, P30ES013508, R01FD006029, and T32ES019851.

## What is the best way to measure speech in clinical trials?

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Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 9

Saturday, 16th November - 11:30: Clinical Trials and Clinical Trial Design - Oral Abstracts - Abstract ID: 9

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***Dr. Adam Vogel***<sup>1</sup>

*1. The University of Melbourne / Redenlab*

Digital biomarkers and wearable technologies continue to make headway in the clinic and clinical trials. Speech is one domain with great promise as a marker of disease progression or treatment response, yet few models of assessment consider how this information may be useful. In most cases there is uncertainty around how to describe speech and what features are important in ataxia.

The task of assessing speech in disease has historically fallen to neurologists or clinicians with expertise in characterizing communication (e.g. speech pathologists). Their well validated and commonly used approach requires a listener to make judgements on features of performance to inform decisions about the patient. While these methods are the mainstay of clinical practice, there are limitations to subjective listener-based judgements.

Here we describe the different approaches to objective analysis of speech considering the speech subsystems (e.g. respiration, phonation, articulation, resonance), broad categories of analysis (timing, voice quality, vocal control) and summative measures (intelligibility, naturalness) and what they might mean to patients, clinicians and clinical trial protocols.

We also describe how we have attempted to align acoustic analysis algorithms with meaningful speech outcomes using a large clinical dataset using sophisticated statistical approaches including machine learning. Data point to a direction of assessment that may enhance our ability to evaluate treatment efficacy in future trials.



# Characterizing the Cardiac Phenotype in Friedreich's Ataxia: Results of the CARFA Study

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 57

Saturday, 16th November - 11:45: Clinical Trials and Clinical Trial Design - Oral Abstracts - Abstract ID: 57

***Dr. Alban Redheuil<sup>1</sup>, Dr. Francoise Pousset<sup>2</sup>, Dr. Stephane Hatem<sup>2</sup>, Ms. Marie Biet<sup>3</sup>, Dr. Jonathan Weinsaft<sup>4</sup>, Dr. Claire Ewencyk<sup>3</sup>, Dr. Charles Perrine<sup>3</sup>, Dr. Anna Heinzmann<sup>3</sup>, Dr. Lise Legrand<sup>2</sup>, Dr. Shraddha Desai<sup>5</sup>, Dr. Brahim Belbellaa<sup>5</sup>, Dr. Richard Pushkin<sup>5</sup>, Prof. Alexandra Durr<sup>6</sup>***

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## Background and Objectives

Friedreich's Ataxia (FA) is a rare genetic disease caused by a defect in the FXN gene, reducing the amount of mitochondrial frataxin in the central nervous system and heart, leading to cerebellar and sensory ataxia and cardiomyopathy. We aimed to measure severity and progression of early stage cardiomyopathy in adult FA patients over one year, and to identify potential clinical endpoints and relevant biomarkers including precise cardiovascular phenotyping over this timeframe. The results of this study will help guide the design of future clinical trials.

## Methods

CARFA was a single-center, observational, parallel-group, unblinded study to characterize cardiac involvement in FA subjects using cardiopulmonary exercise testing (CPET), echocardiography (TTE), cardiac MRI (CMR), and serum biomarkers. Twenty FA subjects with the diagnosis of left ventricular hypertrophy without symptoms of cardiac failure and 20 healthy (age/gender-matched) control subjects were evaluated at 2 visits over 12 months ( $\pm 1$  month).

## Results

- Baseline characteristics for FA included age  $29 \pm 7$  (mean $\pm$ SD) years, 60% male, 50% wheelchair bound, GAA repeats for alleles 1 and 2 of  $544 \pm 215$  and  $759 \pm 240$ , and SARA score  $21.5 \pm 8.3$ .
- FA subjects showed evidence of altered cardiac function on baseline CPET, TTE and CMR compared to controls, the magnitude of which remained unchanged over one year.
- Baseline CMR in FA versus controls demonstrated increased LV mass ( $153 \pm 42$  vs  $130 \pm 27$  g/m<sup>2</sup>,  $p=0.002$ ), anterolateral ( $1.2 \pm 0.2$  vs  $0.8 \pm 0.2$  cm) and posterolateral ( $1.0 \pm 0.2$  vs  $0.7 \pm 0.2$  cm,  $p<0.001$ ) wall thickness. Despite similar ejection fraction between groups ( $69.8 \pm 8.3$  vs  $67.9 \pm 5.5$  %), both LV and RV stroke volumes were decreased in FA versus controls ( $67.4 \pm 18$  vs  $97.8 \pm 22.3$  and  $66.1 \pm 17.6$  vs  $95.1 \pm 22.2$  mL,  $p<0.001$ ), reflecting smaller biventricular chamber size. Heart rate (HR) in FA versus controls was  $77 \pm 11$  and  $66 \pm 11$  bpm, and cardiac output was  $5.27 \pm 1.14$  versus  $5.99 \pm 1.03$  L/min. Right and left atrial function and morphology were similar between the two groups.
- TTE demonstrated similar results, evidenced by FA-associated increments in LV mass and decreases in LV chamber size and stroke volume (all  $p<0.05$ ).
- Over one year, there were no significant changes in CMR or TTE structural or functional parameters.

- 50% of FA subjects had intramyocardial late gadolinium enhancement (LGE) on baseline CMR, consistent with local LV fibrosis. LGE typically occurred in the LV lateral wall and involved a small amount of LV myocardium ( $1.7 \pm 2.4$  %). FA subjects with LGE showed greater LV concentric remodeling (mass/volume) and increased wall thickness than FA subjects without LGE. In areas without LGE, T1 mapping-derived parameters (markers of diffuse fibrosis) such as native T1 and extracellular volume (ECV) did not differ at baseline between groups. LGE, ECV, and native T1 were all unchanged at one-year follow-up.
- FA subjects performed maximal CPET based on HR increase, not significantly different from controls, meaning CPET values are interpretable and lower values in FA are explained by cardiovascular and/or muscular limitation (baseline visit HR at VO2 max  $146 \pm 14$  and  $156 \pm 18$  bpm in FA vs controls). Change over one year in FA was driven by decreased workload (work VO2 max  $58.1 \pm 21.5$  at baseline and  $44.3 \pm 19.1$  watts at one-year, whereas peak VO2 was maintained ( $911.6 \pm 338.4$  at baseline and  $894.4 \pm 293.1$  mL/min at one-year) suggesting decreased energetic efficiency. Balance of pulmonary ventilation and perfusion measures reflected altered diastolic filling and could be an early indication of cardiac remodeling (delta VE/VC02  $33.6 \pm 5.8$  at baseline and  $31.8 \pm 5.0$  at one-year).
- In FA, mean NT-proBNP and Galectin-3 were normal at both visits ( $74.7 \pm 200$  and  $62 \pm 135$  ng/L; and  $12.2 \pm 2$  and  $12.5 \pm 2.62$  ng/mL). Troponin T was elevated at both visits ( $26.3 \pm 27.7$  and  $19.5 \pm 15.5$  ng/L). These parameters were stable over one year.
- Only CCFS (quantitative evaluation of cerebellar function reflecting upper limb ataxia), not SARA, significantly worsened over one year in FA ( $p=0.003$ ).

## Conclusions

- Concentric LV remodeling is a common feature in FA, evidenced by increased LV mass and decreased chamber size on both CMR and TTE. The magnitude of LV remodeling remained stable over one year.
- CMR tissue characterization demonstrated intramyocardial LGE in half the FA patients, highly suggestive of local dense fibrosis. This feature was associated with more severe remodeling and hypertrophy and remained stable over one year. T1 mapping parameters failed to reach significance probably due to small sample size in this rare disease.
- In the population studied, cardiomyopathy appeared to be slowly progressive; no cardiac parameters were identified that could track significant change over one year. In future treatment clinical trials, while demonstrating disease stabilization would be difficult, showing improvement might be possible. The prevalence of myocardial fibrosis in FA patients and its potential impact on treatment response will require further investigation.

# The Upper Limb Cardiopulmonary Exercise Test in Friedrich Ataxia Patients

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 142

Saturday, 16th November - 12:00: Clinical Trials and Clinical Trial Design - Oral Abstracts - Abstract ID: 142

**Dr. CHIARA PANE<sup>1</sup>, Dr. Andrea Salzano<sup>1</sup>, Dr. Claudia Del Prete<sup>1</sup>, Prof. Giuseppe De Michele<sup>1</sup>, Prof. Alessandro Filla<sup>1</sup>, Prof. Antonio Cittadini<sup>1</sup>, Prof. Francesco Saccà<sup>1</sup>**

*1. University "Federico II"*

**Background:** Friedreich Ataxia (FRDA) is an autosomal recessive disease with no available therapy. Phase II clinical trials are generally purely explorative, whereas phase III trials are usually powered on a meaningful slowing of a clinical scale. Primary endpoints for phase IIb trials, or secondary functional endpoints are currently missing. Aim of our study was to explore the feasibility of upper limbs cardiopulmonary exercise testing (CPX) in FRDA patients and to compare the results with a sex, age and Body Mass Index (BMI) matched cohort of Healthy Controls (HC).

## Methods:

CPX was performed using an upper limbs cycle ergometer (Ergoselect 400, Ergoline GmbH, Blitz, Germany). After a 1-min warm-up period at 0 watts (W) workload, a ramp protocol of 5 or 10 W/min was started and continued until limiting symptoms, chest pain, signs of ischemia, or arrhythmias developed or other indications for exercise termination appeared. For patients, the choice of ramp protocol was based on the score of the Scale for Assessment and Rating of Ataxia (SARA) of each patient. For healthy controls, we used a fixed ramp of 10 W/min. Subjects were instructed to keep pedaling at a constant rate (50 to 60 rpm) during the test. Subjects were advised that they were free to stop whenever they wished but were encouraged to continue for as long as possible.

Respiratory gas exchange measurements were obtained breath by breath by a commercially available system (Vmax 29C; SensorMedics, Yorba Linda, CA, USA). Peak oxygen uptake ( $\text{VO}_{2\text{max}}$ ) was recorded at the mean value of  $\text{VO}_2$  during the last 20 sec of the test. The ventilatory anaerobic threshold was detected by the use of the V-slope method. The ventilation per minute (VE) vs carbon dioxide production ( $\text{VCO}_2$ ) relationship (ventilatory efficiency) was measured by plotting ventilation against  $\text{VCO}_2$  obtained every 10 sec of exercise (VE/ $\text{VCO}_2$  slope). The VE/ $\text{VCO}_2$  slope was calculated as a linear regression function, excluding the non-linear part of the relationship after onset of acidotic drive to ventilation.

Continuous variables were compared between groups using an unpaired t test, categorical variable were compared using a chi-square test. Correlation was performed using the Pearson's correlation coefficient.

**Results:** We studied 54 FRDA patients and 22 healthy controls (HC). Age ( $35.3 \pm 13.8$  vs  $33.4 \pm 9.9$ ;  $p=0.559$ ), gender (M:F 28:27 FRDA vs 8:14 HC;  $p=0.248$ ), and BMI ( $23.1 \pm 4.6$  vs  $23.0 \pm 3.4$ ;  $p=0.928$ ) did not differ between groups.  $\text{VO}_{2\text{max}}$  showed a significant 46% reduction in FRDA patients compared to HC performance ( $13.95 \pm 4.7$  for FRDA and  $25.68 \pm 5.9$  mL/Kg/min for HC;  $p<0.001$ ; Figure 1). Peak workload ( $43.87 \pm 12.0$  for FRDA and  $76.18 \pm 20.0$  watts for HC;  $p<0.001$ ), and test duration ( $4.29 \pm 2.4$  for FRDA and  $10.93 \pm 2.5$  minutes for HC;  $p<0.001$ ) were both significantly reduced in FRDA patients. Sixty-four percent of FRDA patients reached the anaerobic threshold, as opposed to 32% of HC ( $p=0.011$ ). VE/ $\text{VCO}_2$  slope was higher in FRDA as compared to HC ( $32.87 \pm 5.1$  for FRDA and  $28.48 \pm 5.9$  for HC;  $p=0.002$ ).

In FRDA patients,  $\text{VO}_{2\text{max}}$  inversely correlated with BMI ( $R=-0.333$ ;  $p=0.014$ ), interventricular septum thickness ( $R=-0.281$ ;  $p=0.039$ ), SARA score ( $R=-0.434$ ;  $p=0.001$ ; Figure 2), disease duration ( $R=-0.397$ ;  $p=0.003$ ), and 9HPT performance ( $R=-0.437$ ;  $p=0.001$ ).  $\text{VO}_{2\text{max}}$  directly correlated with ADL ( $R=0.536$ ;  $p<0.001$ ), and IADL ( $R=0.385$ ;

p=0.004).

**Conclusions:**FRDA patients showed almost half of the HC's oxygen consumption at the CPX with reduced maximum effort, and reached the anaerobic threshold two times more frequently than HC. This indicates that FRDA patients have a reduced aerobic metabolism that parallels a reduced physical performance.  $\text{VO}_2\text{max}$  correlated with several disease measures, indicating that it may reflect the severity of the disease. Future longitudinal data are needed to describe the CPX performance during disease progression.

**Posters - All abstracts that will be presented as posters are listed by topic area. You can click on any of the titles in the table of contents and that will take you to the full abstract.**

# **Topic: Neurophysiology and the Cerebellum**

# Personality, social skills and psychological health in adults with Friedreich ataxia

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 278

***Dr. Louise Crowe*<sup>1</sup>, *Dr. Louise Corben*<sup>2</sup>, *Prof. Martin Delatycki*<sup>2</sup>**

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**Background:** Friedreich ataxia (FRDA) is a neuromuscular disorder typified by neuropathology predominantly affecting the spinal tracts and cerebellum. Damage to the cerebellum has been linked to difficulties forming social relationships and feelings of depression. Cerebellar damage can also impair attention, processing information and the ability to come up with new and different solutions for problems. This study investigated personality, social skills and psychological health in individuals with FRDA.

**Method:** Adults (aged over 18 years) with FRDA, recruited through the Friedreich ataxia clinic, Melbourne, Australia were invited to complete the Depression Anxiety Stress Scale, the NEO Personality Inventory and the Social Performance Survey Schedule. Results of the questionnaires were compared to the mean of standardized psychological questionnaires. The participants completed questionnaires at a time convenient to them. We also asked participants to nominate a significant other such as a family member/carer/close friend to complete a questionnaire about the person affected by FRDA.

**Results:** Eighteen participants with FRDA and 17 carers completed the questionnaires. Sixty percent of participants with FRDA reported symptoms of depression and 30% significant stress levels. Participants with FRDA also rated themselves high for negative social behaviours. Personality traits including low extraversion and higher neuroticism were more common in individuals with FRDA compared with normative data. There was little difference between the participants with FRDA and their significant other's rating of the social skills of the individual with FRDA.

**Discussion:** People with FRDA have difficulty in elements of social skills, and have poorer psychological health than unaffected adults. Some personality traits may be related to FRDA neuropathology. Implications for physicians include a need to be aware of the psychological impacts of FRDA and manage this appropriately.



# **Topic: Mechanism of Disease**

# Interruptions as disease modifiers and repeat regulators

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 262

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## Background

Friedreich ataxia (FRDA) is the most common recessive ataxia and is predominantly caused by abnormal expansion of GAA repeats in the first intron of the *frataxin* gene (*FXN*). Repeat expansion leads to transcriptional silencing and a subsequent deficiency in frataxin protein. There is an inverse correlation between GAA repeat length of the shorter allele and age of onset, whereby the greater the expansion the earlier the age at onset and more severe disease phenotype. However, GAA repeat length only accounts for 36% of the variability in the age at onset (Reetz *et al.*, 2015). These GAA repeats are unstable and tend expand or contract. Interruption of the GAA repeat may have a stabilising effect on the repeat and lead to a less severe phenotype, as we have observed in other triplet repeat disorders. We have previously shown that large interruptions are very rare in FRDA patients and that the 3' end of the GAA repeat tract is commonly interrupted (Al-Mahdawi *et al.*, 2018).

## Materials and methods

We analysed 133 peripheral blood genomic DNA samples from FRDA patients by triplet repeat primed PCR (TP-PCR). PCR products underwent fragment analysis on a 3730xl DNA analyser. Samples that were deemed interrupted had an alteration in the standard TP-PCR trace.

## Results

We show that in our cohort the GAA repeat tract is interrupted 10% more frequently at the 5' end compared to the 3' end. 56% of the patients are either interrupted at 5' or 3' end of GAA repeats and only 16% of patients have pure GAA repeats. When repeat size and age of onset were plotted in linear regression fit model, nearly 80% of the samples which didn't fit the model were potentially interrupted. The model was significant with a *p*-value less than 0.0001 and *R*<sup>2</sup>-value of 0.3533.

## Conclusions

We confirm that sequence interruptions are present more frequently at the 5' end than the 3' end of the GAA tract. These interruptions should be considered disease modifiers and play a major role in defining the age of onset and

disease severity.

## Discovery of frataxin mitochondrial network reveals key regulators of Friedreich's Ataxia

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 261

***Dr. Peter Vitiello*<sup>1</sup>, *Prof. Seasson Vitiello*<sup>2</sup>, *Dr. Oleksandr Gakh*<sup>3</sup>, *Mr. Diing Agany*<sup>4</sup>, *Ms. Danielle May*<sup>1</sup>, *Ms. Rachel Chrisopulos*<sup>1</sup>, *Mr. Jared Ailts*<sup>1</sup>, *Dr. Grazia Isaya*<sup>3</sup>, *Dr. Kyle Roux*<sup>1</sup>, *Prof. Etienne Gnimpieba Z.*<sup>4</sup>**

*1. Sanford Research, 2. Augustana University, 3. Mayo Clinic, 4. University of South Dakota*

Friedreich's Ataxia (FRDA) is an autosomal recessive disease caused by trinucleotide repeat expansion of GAA in the first intron of the frataxin (FXN) gene. This mutation results in a systemic reduction of FXN protein and a predominantly adolescent onset of neuromuscular degeneration marked by ataxia, fatigue and hypertrophic cardiomyopathy. Although FRDA cells accumulate free iron and reduced oxygen species, the molecular mechanisms underlying FRDA pathogenesis are not known. Therefore, most therapeutic strategies are not designed to alleviate functional consequences of reduced FXN or exhibit specificity because our current knowledge of the FXN protein network is almost exclusively limited to ISC biosynthesis. Since discovery of new FXN protein interactions could provide opportunities to understand essential FXN-dependent functions as well as identify new molecular and functional therapeutic targets in FRDA, here we define the FXN protein network in human cells using two complementary approaches: proximity-based labeling (BioID) and endogenous co-immunoprecipitation (co-IP) in human lung adenocarcinoma A549 cells and human lymphocytes respectively. In total, BioID2 & co-IP methods respectively detected 202 and 114 FXN-interacting proteins with 41 in common. Bioinformatics analysis of these data identified peroxiredoxin 3 (Prdx3), a mitochondrial-targeted thiol peroxidase identified and novel FXN binding protein, as a protein of high interest because: (i) genetic manipulation of Prdx3 functional recapitulates FRDA pathologies in experimental mouse and drosophila models, (ii) Prdx3 expression is altered in dorsal root ganglion of an FRDA mouse model, and (iii) Prdx3 antioxidant function could influence redox signaling and/or mitochondrial ROS detoxification. Reciprocal BioID studies using Prdx3 as bait demonstrated binding with known FXN proteins complexes however, Prdx3 expression and basal activity were not altered in a panel of FRDA fibroblasts or A549 cells following FXN knockdown by siRNA. This study uses a proteomics discovery platform to identify FXN protein networks and novel putative molecular therapeutic targets in FRDA.

# Frataxin deficiency causes excessive Drp1-dependent mitochondrial fragmentation

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 216

***Mr. Joseph Johnson<sup>1</sup>, Ms. ELIZABETH MERCADO-AYON<sup>1</sup>, Dr. Elisia Clark<sup>1</sup>, Dr. David Lynch<sup>2</sup>, Dr. Hong Lin<sup>2</sup>***

*1. University of Pennsylvania, 2. Children's Hospital of Philadelphia*

## *Introduction:*

Friedreich ataxia (FRDA) is an autosomal recessive disease with no treatment to date. FRDA is caused by GAA trinucleotide repeat expansions in the first intron of the frataxin gene which decrease synthesis of this mitochondrial protein. While frataxin is critical for iron-sulfur cluster biosynthesis, other functions of frataxin (if any) have not yet been fully defined. Investigating the effects of frataxin deficiency on mitochondrial biology and function is therefore critical for elucidating the function of frataxin, cellular pathologies of FRDA, and potential targets for therapeutic intervention.

Mitochondria fuse and fragment in response to various energetic and stress stimuli through the homeostatic process of mitochondrial dynamics. Excessive mitochondrial fragmentation may occur in response to mitochondrial dysfunction, but fragmentation itself decreases ATP synthesis and further increases sensitivity to bioenergetic stress. The fragmentation signaling pathway phosphorylates the Ser616 residue of Dynamin related protein 1 (Drp1); this (Ser616) phospho-Drp1 is recruited to the outer mitochondrial membrane where it homo-oligomerizes and mechanically severs the mitochondria via GTPase activity. In this study, we assessed the proclivity of mitochondria to fragment in association with acute and chronic frataxin deficiency.

## *Methods:*

Human fibroblasts derived from healthy individuals or FRDA patients were cultured and transfected following standard protocols. P110 and a scrambled form of this peptide were obtained from Genscript and reconstituted in sterile ddH<sub>2</sub>O. Fibroblasts were incubated with 1μM P110 for 72 hours before staining and immunofluorescence imaging. The cells were fixed to glass coverslips, probed for proteins of interest, incubated with fluorophore-conjugated secondary antibodies, and imaged using a Leica confocal microscope and LasX software.

In addition to the biological significance of our findings, we also present a more rigorous and reproducible method of quantifying mitochondrial fragmentation than the current standard practice. Previously, a blinded individual counted the number of cells in each experimental group that exhibit tubular, intermediate, and fragmented mitochondrial networks. We first analyzed our data using this accepted method and then verified the results using our novel, software-based method. Using this method, a confocal immunofluorescence image of a cell's mitochondrial network is imported to ImageJ software and is converted to black and white. A threshold is set to eliminate any background and is kept constant for all images analyzed in this way. A cell is demarcated, and the software quantifies the number and size of mitochondrial fragments (processed as a group of black pixels surrounded by white pixels). The method is repeated on multiple cells to achieve an appropriate sample size. Analyzing mitochondrial fragmentation in this way is more rigorous than relying on relatively arbitrary human judgement as has been common practice for the field. Using our innovation, other investigators may improve the rigor and reproducibility of their mitochondrial fragmentation studies.

## *Results:*

Significantly more FRDA patient fibroblasts exhibit excessively fragmented mitochondrial networks compared to healthy control fibroblasts. Similarly, acute knockdown of frataxin in healthy fibroblasts results in fragmented mi-

tochondria. Utilizing a novel, software-based quantification method, we show that frataxin knockdown increases the number of mitochondrial fragments per cell while decreasing the average size of each mitochondrion (**Fig. 1**), and frataxin knockdown increases the number of (Ser616) phospho-Drp1 clusters per cell. Incubation with P110, a small peptide that inhibits Drp1 GTPase activity, prevents frataxin knockdown-induced mitochondrial fragmentation but is insufficient to restore the chronically fragmented mitochondria in FRDA patient cells. However, transfection with frataxin or a metabolically inactive (but disease-causing) frataxin point mutant, FXN<sup>W155R</sup>, rescues the mitochondrial networks in FRDA patient cells (**Fig. 2**).

*Discussion:*

These findings establish excessive mitochondrial fragmentation as a cellular phenotype of FRDA. Knockdown of frataxin increases fragmentation in healthy cells while transfecting FRDA cells with frataxin rescues the mitochondrial networks, thereby establishing that frataxin deficiency causes excessive mitochondrial fragmentation. The establishment of fragmentation appears to be Drp1-dependent, but after fragmentation occurs, Drp1 is no longer necessary to maintain the fragmented state. FRDA mitochondria may have an impaired ability to fuse, thus preventing the restoration of previously fragmented mitochondrial networks. However, transfecting FRDA patient cells with frataxin fully rescues the mitochondrial network. Additionally, overexpressing a metabolically inactive form of frataxin partially restores the mitochondrial network in FRDA patient cells, suggesting that non-metabolic functions of frataxin may play some role in modulating mitochondrial dynamics.

*Conclusions:*

Frataxin deficiency causes mitochondrial fragmentation in human fibroblasts through a Drp1-dependent mechanism. Drp1 function is necessary to establish fragmentation but not to maintain previously fragmented mitochondria. Excessive mitochondrial fragmentation is restored by overexpression of wild-type frataxin or a metabolically inactive, yet disease-causing, frataxin point mutant, implying that non-metabolic functions of frataxin may contribute to modulating mitochondrial dynamics.

## Effect of frataxin deficiency on OXCT1 and TID1 protein levels in Friedreich ataxia cellular and animal models

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 201

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**Dr. Yina Dong<sup>1</sup>, Mr. Nathan Warren<sup>1</sup>, Ms. ELIZABETH MERCADO-AYON<sup>2</sup>, Dr. David Lynch<sup>3</sup>**

**1. The Children's Hospital of Philadelphia, 2. University of Pennsylvania, 3. Children's Hospital of Philadelphia**

Friedreich ataxia (FRDA) is an autosomal recessive disorder characterized by progressive ataxia, cardiomyopathy, scoliosis, diabetes, and loss of visual and hearing function. It is caused by homozygous GAA repeat expansions in intron 1 of the *FXN* gene, which leads to the deficiency of frataxin, a mitochondrial protein involved in iron sulphur cluster formation and energy production, yet its precise function remains controversial. Using Co-Immunoprecipitation (Co-IP) combined with mass spectrometry, we identified 3-oxoacid-CoA-transferase 1 (OXCT1), a homodimeric mitochondrial matrix enzyme catalyzing the first, rate-limiting step in ketolysis by transferring the CoA from succinyl-CoA to acetoacetyl-CoA which can then be converted into acetyl-CoA entering the citric acid cycle, and tumorous imaginal disc 1 (TID1), a mitochondrial J-protein cochaperone critical for mitochondrial DNA integrity maintenance, as interacting proteins of frataxin both in vivo in mouse cortex and in vitro in mouse cortical neurons. Acute depletion of frataxin using RNA interference markedly decreases OXCT1 but increases TID1 protein levels in human skin fibroblasts. Similar results were also observed in cerebellar homogenates from doxycycline-inducible frataxin knockdown mice. Doxycycline induction led to a time-dependent frataxin knockdown with 29%, 8% and 0% residual frataxin protein levels observed at 4 weeks, 12 weeks and 18 weeks, respectively. OXCT1 protein levels were decreased at 4 weeks of doxycycline induction but returned to control levels at 12 weeks and stayed unchanged until 18 weeks. Instead, TID1 protein levels were increased at 4 weeks of doxycycline induction but returned to control levels at both 12 weeks and 18 weeks. In contrast, significant increases in OXCT1 protein levels were detected in cerebellar homogenates from frataxin knockin and knockout (KIKO) mice both at asymptomatic and symptomatic age. While no changes in TID1 protein were detected in cerebellar homogenates from KIKO mice, significant decreases in TID1 protein were observed in multiple cell types from FRDA patients including skin fibroblasts, buccal cells and platelets. These results suggest a biphasic effect of frataxin deficiency on OXCT1 and TID1 with acute frataxin deficiency leading to OXCT1 decreases and TID1 increases and chronic frataxin deficiency resulting in OXCT1 increases and TID1 decreases. OXCT1 decreases might lead to a lack of ketone body utilization and energy deficit contributing to the pathogenesis of FRDA, with chronic changes representing a chronic shift toward increased ketone body metabolism. TID1 might converge on energy production with OXCT1 based on its crucial role in mitochondrial DNA integrity maintenance and subsequent alterations in mitochondria proteins and oxidative phosphorylation following TID1 changes. Our results thus provide a molecular link between energy metabolism and FRDA.

## DNA Damage Response, oxidative stress and GAA-repeat instability in Friedreich's Ataxia cells: what regulates what?

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 184

***Dr. Elisa Palumbo<sup>1</sup>, Mrs. Viviana Miglietta<sup>1</sup>, Prof. Antonella Russo<sup>1</sup>***

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To address the mechanisms involved in GAA-trinucleotide expansion in Friedreich's Ataxia (FRDA) families, we are deepening two strictly related issues: 1) whether the GAA-expansion may influence the DNA Damage Response (DDR) and in turn the FRDA phenotype; 2) the impact of transcription, and of potential replication/transcription conflicts<sup>1-3</sup>, in GAA-repeat instability. It is known that frataxin depletion leads to unbalanced production of reactive oxygen species (ROS), a condition that could affect the DNA damage response (DDR) pathway and contribute to somatic GAA-repeat instability as reported in other ataxias<sup>4</sup>. Importantly, despite ROS steady state levels are increased in FRDA, the response to induced oxidative stress remains unexplored. Using fluorogenic probes (CellROX®, Molecular Probes) and fluorescence microscopy we measured ROS in FRDA and normal cells exposed to menadione, a well-known inducer of oxidative stress. ROS were measured simultaneously in nucleus/mitochondria and in the cytoplasm by differentially fluorescent probes. Unexpectedly, oxidative stress induction evaluated at the end of the treatment was less intense in FRDA than in wildtype cells, both in the nucleus/mitochondria and in the cytoplasm. ROS scavenger (N-acetyl-cysteine, NAC) was used to validate our observations. Remarkably, ROS levels were higher in the nucleus/mitochondria than in the cytoplasm only in FRDA cells, where frataxin is depleted. In parallel SOD1 and catalase induction, which are markers of oxidative stress, were assessed by western blot in absence/presence of NAC. Again, FRDA cells showed a reduced response with respect to the control. In this frame, it is interesting that *in vitro* physical interaction between frataxin and SOD1 has been recently reported<sup>5</sup>. Within the general aim of understanding whether DNA damage response may be differentially regulated in FRDA cells, we also found that PARP1-mediated apoptosis might participate in the response of FRDA cells to oxidative stress induction. Indeed, PARP1 cleavage, an apoptotic marker depending directly on Caspase-3 activity, was lower in FRDA than in control cells exposed to menadione; however, a differential activation of Caspase-3 was not observed. We concluded that PARP1-mediated apoptosis or possibly PARP1-mediated DDR, might be affected in FRDA. As this project has stemmed from our previously published data<sup>1</sup> regarding the DNA replication dynamics of *FXN* gene where the GAA-repeat expansion is a source of replication stress<sup>1,2</sup>, it is noteworthy that PARP1 is a major factor regulating the replication stress response and in turn DDR.

On the whole, we found unexpectedly that lymphoblastoid FRDA cells appear less responsive than normal ones to conditions of induced oxidative stress.

The second issue concerns the replication/transcription conflict at the expanded GAA-repeat, which could drive further expansion and somatic instability of the repeat<sup>4,3</sup>. However, the mechanisms involved in GAA-trinucleotide expansion in FRDA families are far from being completely understood<sup>7</sup>. To understand whether *FXN* transcription may be modulated along the cell cycle, lymphoblastoid cells derived from FRDA patients or healthy relatives were FACS-sorted in subfractions of different cell cycle stages. We applied RNA-FISH with multiple pre-mRNA probes (Stellaris®) to monitor RNA initiation/elongation at *FXN*. By applying such approach we are deepening the possible involvement of replication/transcription conflicts in GAA-repeat expansion.

Because of the increasing interest for therapies aimed to reduce the length of the repeat, if FRDA cells have an altered DDR the risk of side-effects might be increased in turn. Moreover, if a link exists between transcription/replication and GAA-repeat instability, a reactivation-based therapy would paradoxically increase the risk of trinucleotide ex-



pansion after transcription rescue<sup>6,7</sup>.

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# Friedreich cardiomyopathy is a secondary desminopathy

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 75

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Cardiomyopathy is the most common cause of death in Friedreich ataxia (FA). Heart pathology includes cardiac enlargement, concentric hypertrophy (occasionally dilated cardiomyopathy), cardiomyocyte hypertrophy, heart fiber necrosis, inflammation, postnecrotic and primary fibrosis, and iron-positive perinuclear inclusions in a small percentage of fibers. Proteomic analysis of lysed myocardium from left ventricular wall (LVW) in 38 patients with FA by an 878-antibody microarray (Kinex<sup>TM</sup> KAM-900) and Western blotting with those antibodies that exhibited alterations in expression or phosphorylation compared to LVW from controls revealed significant up- or down-regulation of 25 proteins. Up-regulated desmin and alpha-B-crystallin are relevant to the cytoskeleton of the heart in diverse cardiomyopathies. In FA, the up-regulation of desmin involved expression of a 40 kDa-cleavage product of the 53 kDa native protein, and immunohistochemistry and immunofluorescence with antibodies to desmin and alpha-B-crystallin revealed extensive co-aggregation among cardiac fibrils and at intercalated discs (fig. 1). The aggregation of the two proteins also corresponded to collections of iron-containing granules. Heart disease is a frequent manifestation of mutations in the desmin gene; and the cardiac phenotype is highly variable. The cardiomyopathy of FA differs from that in desmin mutations because the lesions in FA are most prominent in the LVW. It remains to be established how frataxin deficiency in FA causes secondary desminopathy. The clinical importance of desmin in the heart relates to its biomechanical and putative conductive properties: Desmin-containing intermediate filaments stabilize the contractile apparatus of cardiomyocytes and establish “communications” with intercalated discs, the nuclear membrane, mitochondria, and the plasma membrane of heart fibers. Supported by Friedreich’s Ataxia Research Alliance

## In-vivo identification of Diseases Modifiers in a *Drosophila* model of Friedreich's ataxia

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 306

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**Prof. Stephan Schneuwly<sup>1</sup>, Dr. Juan Antonio Navarro Langa<sup>1</sup>**

*1. Universität Regensburg*

Friedreich's ataxia (FRDA) is the most common recessive neurodegenerative ataxia in the Caucasian population and it is caused by the reduced expression of the protein frataxin. Although the role of frataxin as a key protein in the biosynthesis of iron-sulphur clusters is widely accepted by the scientific community, the knowledge of the downstream consequences of frataxin deficiency still remains sparse. A better understanding of such effects is crucial to develop effective treatments. This is of special importance in FRDA since there is no available cure. We used the well-known model organism *Drosophila melanogaster* and its outstanding genetic tool box to unravel new pathways that are affected in a loss-of-frataxin scenario or to identify potential modifiers of the disease. In our case, we have focussed on the fly model with frataxin deficiency in glia cells (Navarro et al 2010). These flies display, among others, three clear phenotypes: impaired locomotion, loss of brain integrity and accumulation of lipids in the brain. We have performed a genetic screen and classified the genes in groups depending if they are able to counteract only one, at least two or all three defects that we have used as a read-out in our screen. Our analysis has revealed some interesting features that have been already published...

- Manipulation of iron metabolism counteracts most of the defects (Navarro et al., 2015).
- Altering Mitochondrial dynamics failed to suppress loss-of-frataxin defects (Edenharter et al., 2018).
- Reduction of Endoplasmic reticulum stress strongly rescues frataxin-deficiency (Edenharter et al., 2018).

...and it continues providing new insights into the pathology

- Scavengers of reactive oxygen species have a very limited effect. This group is important since antioxidant therapies are still an option for patients. The results from flies argue against this type of treatments.
- Inactivation of the Sterol Regulatory Element-Binding Protein (SREBP) results in a clear improvement. Although expression of lipases does not protect, the effects triggered by the reduction of lipid biogenesis highlights the influence of lipid homeostasis in the development and progression of the disease. A molecular analysis indicated an increased expression of this gene in several fly tissues lacking frataxin.
- Promoting the transport of calcium into the mitochondria is sufficient to restore normal locomotion, brain vacuolization and lipid levels. On the one hand, this result reinforces the hypothesis that the connections between mitochondria and ER are playing a pivotal role in the biology of FRDA. On the other hand, it suggests a shortage of calcium inside the affected mitochondria and it brings some light into the contradictory results observed in cultured cells from mouse (Bolinchés-Amoros et al 2014) and rat (Purroy et al 2017) FRDA models.

All together our results strongly indicate that the unique property of the fly to easily perform genetic screen is still of high importance to unveil new elements involved in the pathology as well as new therapeutic targets.

# Hyperactivation of cofilin and ARP2/3 in frataxin-deficient neurons of YG8R mice and its contribution to a deficit in neurite growth

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 289

***Dr. Pablo Calap*<sup>1</sup>, *Dr. Diana Carolina Muñoz-Lasso*<sup>2</sup>, *Dr. Belén Mollá*<sup>3</sup>, *Prof. Federico Vicente Pallardó Calatayud*<sup>4</sup>, *Dr. Francesc Palau*<sup>5</sup>, *Dr. Pilar Gonzalez-Cabo*<sup>6</sup>**

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Abnormalities in actin cytoskeleton have been linked to the neuropathy of Friedreich's ataxia (FA), a disease characterized by an early loss of neurons in dorsal root ganglia (DRGs). Despite the efforts made, we still do not fully understand which are the molecular events that contribute to the early loss of these neurons in FA. In this work, we have studied the adult neuronal growth cone (GC) at the cellular and molecular level to decipher the connection between frataxin, actin cytoskeleton, and DRGs in the well-characterized YG8R mouse model. Immunofluorescence studies in primary cultures of DRG from YG8R mice show neurons with fewer and smaller GCs, associated with the inhibition of neurite growth. We have also observed an increase of the filamentous (F)-actin/monomeric (G)-actin ratio (F/G-actin ratio) in axons and GCs, linked to hyperactivation of cofilin-1, an increase in the expression of the actin-related protein (ARP) 2/3 complex and chronophin (CIN). Cofilin and ARP2/3 complex are two crucial modulators of filamentous actin turnover, and CIN is a cofilin-activating phosphatase. As a whole, our results show for the first time a link between the deficit of frataxin in adult DRG neurons to the dysbalance of cofilin, a vital regulator of the actin cytoskeleton that could guide to a better understanding of the neuropathy of FA.

# Effects of frataxin on mitobiogenesis in the inducible mouse model of Friedreich's Ataxia

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 269

**Dr. Elena Dedkova<sup>1</sup>, Ms. Claire B. Montgomery<sup>1</sup>, Mr. Chun Kiu Hui<sup>1</sup>, Dr. Sandipan Datta<sup>1</sup>, Dr. Alexey A. Tomilov<sup>1</sup>, Prof. Gino Cortopassi<sup>1</sup>**

*1. UC Davis*

**Background.** Friedreich's ataxia is the most common inherited ataxia caused by recessive mutations that reduce the levels of frataxin (FXN), a mitochondrial iron binding protein. Although sensory deficits put Friedreich's ataxia (FA) patients in wheelchairs, the most common cause of mortality is cardiomyopathy. We have recently demonstrated that there is a mitochondrial biogenesis defect proportional to the FXN defect in FA patient fibroblasts and blood lymphocytes of living FA patients (Hayashi & Cortopassi, 2016, Hayashi *et al*, 2017, Jasoliya *et al*, 2019). In this study, we investigated the contribution of the mitochondrial biogenesis defect to the ataxic pathological mechanism in the inducible mouse model of FA (FXN<sup>KD</sup>) (Chandran *et al*, 2017).

**Methods.** Cardiac function was investigated by serial echocardiography and neurobehavioral phenotypes were examined by standard techniques. Aconitase activity, which is often used as a surrogate measure of frataxin's iron-sulfur biogenesis function, complex IV activity and fumarate levels were measured by ELISA in heart, muscle and brain tissues. Frataxin expression and mitochondrial biogenesis were examined by Western Blot and qPCR, respectively.

**Results.** Mice were given doxycycline (Dox) to suppress FXN expression from 35 days of age and cardiac function was studied in wild-type animals (WT-Dox), knockdown (KD) animals (KD-Dox), and KD animals without Dox (KD-Veh) by guided M-mode and two-dimensional echocardiography. At 100 days of age striking changes in cardiac structure and function were observed including a very clear thickening of the left ventricle, very small stroke volume (50% of controls), very small cardiac output (40% of controls), small end systolic volume (6% of controls), small end systolic diameter (25% of controls), and a lower heart rate (63% of controls). Neurobehavioral deficits were also studied in the same FXN<sup>KD</sup> mice given Dox, and consistent differences were detected in frataxin-deficient mice at 2, 3 and 4 months post Dox. The most sensitive neurobehavioral test was the level beam, i.e. how long it takes the mouse to walk across the 9 mm beam (latency), KD-Dox mice were significantly different from both control groups. Furthermore, FXN-deficient mice made more errors than control mice. The Von Frey test of peripheral sensitivity detected a difference from WT-Dox controls only at 4 months. The number of rotarod falls was significantly higher at 3-4 months. At the same time, fumarate level, aconitase and complex IV activity were significantly decreased.

**Conclusion.** The Frataxin KD mouse has a pronounced cardiac and neurobehavioral phenotype which correlated with down-regulation of FXN and reduced mitochondrial biogenesis. These results demonstrate that FXN<sup>KD</sup> mouse is the best mouse model currently available to test drugs that can potentially reverse multiple levels of FA pathophysiology and eventually improve quality of life in FA patients.

# Identification of miRNAs regulating molecular pathways in cellular models of Friedreich's ataxia

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 255

**Dr. Jose Santiago Ibañez Cabellos<sup>1</sup>, Dr. Marta Seco-Cervera<sup>2</sup>, Mr. Dayme González-Rodríguez<sup>2</sup>, Dr. Pilar Gonzalez-Cabo<sup>2</sup>, Prof. Federico Vicente Pallardó Calatayud<sup>2</sup>, Dr. Jose Luis García-Giménez<sup>1</sup>**

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Friedreich's ataxia (FRDA; OMIM 229300), is the most prevalent hereditary. This autosomal recessive neurodegenerative disease is characterized by gait and limb ataxia, lower limb areflexia and dysarthria. A mixed origin of ataxia results from spinocerebellar degeneration, peripheral sensory neuropathy, cerebellar and vestibular pathology, and the posterior adding of the pyramidal disabilities. Other non-neurological features of FRDA are scoliosis, diabetes and hypertrophic cardiomyopathy. The majority of FRDA patients are homozygous for an unstable guanine-adenine-adenine (GAA) expansion in the first intron of FXN gene that localizes in chromosome 9q21.11 producing decreased protein levels of frataxin. The principal function of frataxin is not clear, however the early lethality in embryos of FXN knock-out mice underscores the importance of frataxin function in cell survival. Previous studies have reported the involvement of the FXN protein in the mitochondrial biogenesis of iron-sulphur clusters (ISC). Mitochondrial respiratory chain dysfunction, mitochondrial iron accumulation, decreased mitochondrial DNA levels, oxidative stress, and reduced generation of ATP, and altered lipid metabolisms are molecular features of Friedreich's ataxia. Most research on FRDA has focused on understanding the role of frataxin in the mitochondria, and a whole molecular view of pathological pathways underlying FRDA therefore remains to be elucidated. miRNAs analysis in cellular models would provide different expression profile signatures that may help to the comprehension of the special function of miRNAs in the physiopathology of Friedreich ataxia.

Ferroptosis is a newly term for regulated cell death (RCD) pathways that is remarkably distinct at morphological, biochemical, and genetic levels from other RCD mechanisms. Ferroptosis is characterized by the overwhelming, iron-dependent accumulation of lethal lipid reactive oxygen species (ROS) that has been related to different pathological processes such as neurotoxicity, neuroinflammation, ischemic stroke, and neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, Huntington's disease or Friedreich's ataxia.

In this study, we used small RNA sequencing to identify a series of miRNAs in three cellular models (i.e. fibroblasts, SH-SY5Y-FXN neuroblastoma and Olfactory mucosa stem cells). Then we validated common miRNAs in these cellular models by RT-qPCR. Finally, we checked by RT-qPCR messenger RNA levels which are targets for these miRNAs and evaluated the protein expression by Western-blotting. Also, we evaluated phospholipid hydroperoxides as a mark of ferroptosis using LIPERfluor dye (Dojindo).

Our results showed different miRNAs profile between each affected cellular model and their respective controls. Among them, only miRNAs that showed differential expression in at least two cellular models, were selected. Five of these miRNAs were validated in the three cellular models showing similar expression profile and importantly one of the regulated pathways was Ferroptosis. So, we evaluated mRNA and protein levels of GPX4 and ACSL4, which expression is affected in this RCD pathway, and we observed differences in these three cellular models. In addition, we observed an increase in phospholipid hydroperoxides in FRDA models.

Our results point out the importance of miRNAs regulation in FRDA physiopathology. Furthermore, we provide new evidences that ferroptosis is an event that occurs in this disease, therefore providing a new target to develop new treatments against ferroptosis in FRDA.

# Calcium Deregulation: Novel Insights in Friedreich's Ataxia Pathophysiology.

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 253

**Dr. Rosella Abeti**<sup>1</sup>, **Dr. Alexander Brown**<sup>2</sup>, **Dr. Marta Maiolino**<sup>3</sup>, **Prof. Sandip Patel**<sup>4</sup>, **Prof. Paola Giunti**<sup>5</sup>

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**Background.** Friedreich's Ataxia (FRDA) is a neurodegenerative disorder, characterized by degeneration of dorsal root ganglia, cerebellum and cardiomyopathy. Heart failure is one of the most common causes of death for FRDA patients. Deficiency of frataxin, a small mitochondrial protein, is responsible for all clinical and morphological manifestations of FRDA. Our aim was to investigate the unexplored Ca<sup>2+</sup> homeostasis in cerebellar granule neurons (CGNs) and in cardiomyocytes of FRDA cellular models to understand the pathogenesis of this condition. Ca<sup>2+</sup> homeostasis in neurons and cardiomyocytes is not only crucial for the cellular wellbeing but more importantly to generate action potential in both neurons and cardiomyocytes.

**Methods.** By challenging Ca<sup>2+</sup> homeostasis in CGNs, and in adult and neonatal cardiomyocytes of FRDA models, we have assessed the impact of frataxin decrease on both neuronal and cardiac physiopathology. Using both fluorescence and confocal microscopy we have measured reactive oxygen species (ROS), lipid peroxidation, mitochondrial membrane potential, cytosolic and mitochondrial Ca<sup>2+</sup> alterations in FRDA-like cells. We then used a hypoxia-reperfusion injury to induce cell death and then test the effect of Vitamin E (an antioxidant).

**Results.** Our results showed that oxidative stress is present in our FRDA-like cells and that Ca<sup>2+</sup> homeostasis is altered in CGNs and cardiomyocytes. CGNs showed a Ca<sup>2+</sup> mishandling under depolarizing conditions and this was also reflected in the endoplasmic reticulum (ER) content. In cardiomyocytes we found that the sarcoplasmic reticulum (SR) Ca<sup>2+</sup> content was pathologically reduced, and that mitochondrial Ca<sup>2+</sup> uptake was impaired. In cardiomyocytes, our results showed that ryanodine receptors (RyRs) may be leaking and expel more Ca<sup>2+</sup> out from the SR. At the same time mitochondrial uptake was altered. Similarly to other neurodegenerative conditions we have witnessed in FRDA the increase of oxidative stress. It is known that oxidative stress modulates key players at the SR/ER and mitochondrial level that usually restore the Ca<sup>2+</sup> homeostasis. By using an antioxidant such as Vitamin E these alterations were restored. Moreover, Vitamin E protected from cell death induced by hypoxia-reperfusion injury. Our results revealed novel properties of Vitamin E as potential therapeutic tool for FRDA cardiomyopathy.

**Conclusion.** Our findings demonstrate that in both neurons and cardiomyocytes the decreased Ca<sup>2+</sup> level within the stores has a comparable detrimental impact in their physiology. Moreover, Vitamin E helps protecting cardiomyocytes from the Ca<sup>2+</sup> mishandling found in FRDA like models.

**Acknowledgements:**

We would like to thank FARA, GoFAR and Ataxia UK for supporting this study



## Overexpression of extra-mitochondrial frataxin contributes to improve mitochondrial bioenergetics in a cell model of Friedreich's ataxia

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 226

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Friedreich's ataxia is a predominantly neurodegenerative disease caused by recessive mutations that ultimately lead to a deficiency of frataxin (FXN) protein. In its canonical mature form, frataxin localizes inside the mitochondria, associated with the inner membrane. Over the time, some alternative forms of frataxin protein have been described, with different cellular localization and tissue distribution. Among them, a presumably cerebellum-specific cytosolic isoform called FXN II has been described.

In this work, we aim to explore the roles of FXN II and the potential cross-talk with the mitochondrial FXN isoform in FRDA physiopathology. To achieve this, we transduced FRDA patient fibroblasts with lentiviral vectors overexpressing either the mitochondrial or the cytosolic FXN isoforms and studied their effect on the development of the mitochondrial network, on the bioenergetics of the mitochondria and on the activity of aconitase, an enzyme known to be a direct target of canonical mitochondrial frataxin. We have confirmed the cytosolic localization of FXN Isoform II and the mitochondrial localization of FXN Isoform I. Interestingly both mitochondrial FXN I and cytosolic FXN II have positive effects on mitochondrial respiration measured by Seahorse assay in different cellular models. In contrast, only overexpression of mitochondrial FXN I was able to induce significant changes in the development of mitochondrial network, by increasing both the number of individual and networked mitochondria. In addition, both mitochondrial FXN I and cytosolic FXN II can increase aconitase activity both in basal conditions and after oxidative stress conditions.

Together, these results are pointing to the existence of cross-talk mechanism between cytosol and mitochondria mediated by the different FXN isoforms. A more thorough knowledge of the mechanisms of action behind the extra-mitochondrial FXN II isoform could prove useful to better understand FRDA physiopathology.



# MODELING CARDIAC DYSFUNCTION OF FRIEDREICH'S ATAXIA USING VENTRICULAR SHEETS, TISSUES AND CHAMBERS ENGINEERED FROM HUMAN PLURIPOTENT STEM CELLS

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 227

**Dr. Andy Wong<sup>1</sup>, Dr. Gabriel Wong<sup>1</sup>, Mr. Michael Shen<sup>1</sup>, Ms. Maggie Zi-Ying Chow<sup>1</sup>, Dr. Kennis Tse<sup>1</sup>, Mr. Bimal Gurung<sup>2</sup>, Ms. Erica Mak<sup>1</sup>, Dr. Deborah Lieu<sup>1</sup>, Dr. Bernard Fermini<sup>1</sup>, Prof. Kevin Costa<sup>1</sup>, Dr. Camie Chan<sup>1</sup>, Dr. Alain Martelli<sup>3</sup>, Dr. Joseph Nabhan<sup>3</sup>, Prof. Ronald Li<sup>1</sup>**

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(FRDA) is an autosomal recessive inherited disease that causes progressive damage to the nervous system by a mutation in the frataxin (FXN) gene. FXN, a mitochondrial protein involved in the biosynthesis of iron-sulfur proteins, is essential for oxidative metabolism. Hence, due to its high energy consumption, the heart is one of the first organs presenting pathological symptoms of FRDA. Indeed, cardiac dysfunction is the leading cause of death in FRDA patients. Although transgenic mouse models of FRDA have been previously created, the genotype, severity and disease phenotypes of human patients are not reproduced. To overcome this limitation, human ventricular cardiac anisotropic sheets (hvCAS) and tissue strips (hvCTS) were generated from human embryonic stem cell (hESC)- and induced pluripotent stem cell (hiPSC)-derived ventricular cardiomyocytes (VCMs) for modeling FRDA's electrophysiological and contractile defects, respectively. Lentivirus (LV)-mediated shRNA transduction of hESC- and iPSC-VCMs, as well as reprogrammed FRDA-specific iPSC-VCMs, displayed significantly suppressed FXN transcript and protein levels compared to controls. High-resolution optical mapping of hvCAS revealed such electrophysiological defects of LV-shRNA-transduced hESC and FRDA-iPSC preparations as reduced maximum capture frequency (MCF) and prolonged action potential duration consistent with a T-wave inversion observed in patient electrocardiograms. In the hvCTS assay, developed force at 1-Hz pacing was consistently suppressed (by 55-80%) in LV-shRNA and FRDA groups vs. control, displaying a strong positive correlation with FXN expression. Finally, rescue experiments were performed via forced FXN expression in LV-shRNA-hESC- and FRDA-hiPSC-hvCTS, reversing the reduction in developed force in both FRDA models. As further validation, cardiac organoid chambers (hvCOC; our "human heart-in-a-jar" model), are being tested so that clinically relevant and physiologically complex parameters such as ejection fraction, cardiac output, and pressure-volume loops could be obtained for thorough analysis. We conclude that these human based FRDA models provide a biomimetic platform suitable to facilitate the studies of disease pathogenesis and pharmaceutical testing.

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# Lipid metabolism disruption as a consequence of mitochondrial dysfunction in FA

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 243

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**Ms. Elaine Ho<sup>1</sup>, Dr. Grazia Cotticelli<sup>2</sup>, Dr. Robert Wilson<sup>2</sup>, Dr. David Lynch<sup>2</sup>, Prof. Ian Blair<sup>3</sup>,  
Dr. Clementina Mesaros<sup>1</sup>**

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Friedreich's ataxia (FA) is associated with decreased levels of frataxin mRNA and mature frataxin protein. The best characterized function of frataxin is in the synthesis of iron-sulfur (Fe-S) cluster prosthetic groups for the enzymes of oxidative phosphorylation, the Krebs cycle and fatty acid breakdown ( $\beta$ -oxidation). Increased incorporation of labeled palmitate into HMG-CoA (the intermediate in either sterol synthesis or ketogenesis) of FA patients suggests increased lipid utilization via  $\beta$ -oxidation. The increased  $\beta$ -oxidation produces reduced cofactors, such as FADH<sub>2</sub> and NADH that can be utilized to maintain the electrochemical gradient across the inner mitochondrial membrane required for ATP production. Therefore, the increased lipid metabolism observed in FA could play an important role in cellular homeostasis in times of mitochondrial dysfunction. The metabolism of fatty acids in mitochondria is a key physiological process in humans but has not been investigated as a hallmark of FA. Despite this, abnormal lipid accumulation was described in heart and liver cells in mouse models and in the brains of FA patients. In a *Drosophila Melanogaster* model of FA, increased metabolism of fatty acids was reported. Increased activity of inflammatory pathways was also described in different models: sphingolipid synthesis and Pdk1/Mef2 activation in flies and heart tissues from FA patients.

Frataxin-depleted cells have increased sensitivity to oxidative stress as NRF-2 protein expression is also reduced in FA and suggests a reduced ability to mount an antioxidant response. Further characterization of metabolic abnormalities associated with FA could reveal additional therapeutic targets. Together with decreases in antioxidant genes, microarray data showed increases in inflammatory genes. Eicosanoids are important mediators of inflammation and are formed by different enzymes and reactive oxygen species (ROS) after arachidonic acid is released by cytosolic phospholipase 2 (cPLA2). Two mouse models of FA and human B-lymphocyte cells from FA, showed increased COX-2 expression, together with increased cAMP response element-binding protein (CREB) and activator protein 1 (AP1). Increases in inflammatory cytokines in response to oxidative stress in frataxin-deficient mouse and cell models may contribute to COX-2 overexpression, suggesting that the neurodegeneration could be due to neuroinflammation. If prostanoid synthesis is involved in the pathogenesis of FA, targeting COX, or upstream transcription factors (CREB and AP1) could offer a new avenue for treatment. Inflammatory changes have been noted in the neurodegenerative conditions of Alzheimer's disease, Parkinson's disease, and Amyotrophic lateral sclerosis. Alterations in regulatory and signaling bioactive lipids have been noted in FA models as well as in patients and are likely to indicate responses related to FA pathophysiology. However, the specific eicosanoids dysregulated in FA are poorly described. Therefore, in this work, we examined both systemic and pathway-specific lipid alterations in FA fibroblast cells, to investigate events downstream from frataxin deficiency. This will allow us to test if frataxin levels coincide with changes in the lipidome in subjects with FA.

FA fibroblast cells and age/gender-matched healthy control cells were randomly selected from a larger heterogeneous cohort of subjects with FA and healthy controls (n=10 for each group). Cells were grown to 70% confluence at 1 million cells per plate. Cells were spiked with a panel of stable isotope labeled metabolites and lipids as internal

standards for normalization of extraction and analysis, then scraped in 1 mL of -80 °C 80:20% methanol: water and probe tip sonicated in ice. After protein precipitation the supernatant was divided into 2 equal volumes: one for metabolomics analysis and the other half were used for lipid extraction. Consistent with previous results from FA heart tissues, fibroblast cells showed that sphingosine (**Fig 1A**) and several ceramides (**Fig 1B**) were clearly elevated. Interestingly cholesterol esters were also elevated (**Fig 1C**) and the cholesterol ester corresponding to arachidonic acid (AA) (red insert in **Fig 1C**) was the most abundant one. A similar trend of increased levels of AA (20:4) was observed in other lipid classes (data not shown), suggesting that larger amounts of arachidonic acid could be available as a substrate for COX-2.

For eicosanoid quantification, part of the extracted lipids was derivatized after alkaline hydrolysis with an electron capturing reagent to increase the sensitivity of detection for the chiral-LC-HRMS method. The biosynthesis of eicosanoids can occur enzymatically by COXs, cytochrome P450s, and lipoxygenases (LOXs), or through non-enzymatic free radical reactions. The two routes of biosynthesis result in racemic mixtures of hydroxyeicosatetraenoic acids (HETEs), with one enantiomer predominating if generated enzymatically. The chiral separation was able to differentiate and quantify the enzymatic and non-enzymatic lipid products, together with prostaglandins and isoprostanes.

Supported by Penn Medicine/CHOP Friedreich's Ataxia Center of Excellence, and NIH grant P30ES013508.

# An inducible model of Friedreich's ataxia exhibits preserved cardiac function concealing bioenergetic alterations and mitochondrial stress response

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 210

**Dr. Cesar Vasquez-Trincado<sup>1</sup>, Mrs. Monika Patel<sup>1</sup>, Mrs. Aishwarya Sivaramakrishnan<sup>1</sup>, Mrs. Lauren Anderson-Pullinger<sup>1</sup>, Mr. Nadan Wang<sup>2</sup>, Dr. Erin Seifert<sup>3</sup>**

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**Background:** Friedreich's Ataxia (FA) is a neuromuscular disorder caused by reduced expression of the protein Frataxin (Fxn). This mitochondrial protein participates in the early steps of iron-sulfur (Fe-S) cluster biogenesis. Fe-S clusters are key components of enzymes involved in mitochondrial metabolism, such as aconitase (Krebs cycle), electron transport chain complexes (I, II and III) and the electron transport flavoprotein dehydrogenase ( $\beta$ -oxidation). Here we focused on the pathogenesis of cardiac disease caused by Fxn depletion, considering that cardiac disease is the main cause of mortality of FA. Since cardiac function heavily relies on mitochondrial ATP production, we hypothesized that Fxn deficiency in the heart will suppress mitochondrial ATP synthesis and consequently heart function.

**Methods:** A doxycycline-inducible mice model of Fxn deficiency was used (TG mice). Doxycycline (Doxy) was delivered via the chow starting when mice were 9 weeks of age. Cardiac function was evaluated by echocardiography and electrocardiography (ECG). Oxidative phosphorylation (OXPHOS) was measured in isolated heart mitochondria as oxygen consumption ( $\text{JO}_2$ ). Activity of nutrient (mTORC1 and AMPK) and stress signaling (eIF2 $\alpha$ ) was measured by western blot (WB) as (p)-phospho/total protein ratio. Finally, to evaluate if Fxn-depleted hearts expressed an integrated mitochondrial stress response (ISRmt), qPCR was used to measure transcript levels of ISRmt genes (1C-metabolism, mitochondrial unfolded protein response and amino acid transport/metabolism).

**Results:** WB detection of Fxn protein in heart mitochondria showed 95% depletion after 8 weeks of Doxy and near complete depletion after 12 weeks. Concerning heart function, surprisingly TG mice had normal/higher ejection fraction, though ECG showed abnormalities. The heart was not hypertrophied and was in fact decreased in mass (relative to tibia length). In heart mitochondria, there was a time-dependent suppression of OXPHOS driven by pyruvate/malate (up to ~50% decrease) or succinate (up to ~40% decrease). However, when fatty acids were supplied,  $\text{JO}_2$  was minimally changed. Interestingly, despite almost maintained fatty acid-driven bioenergetics, levels of ISRmt mRNAs and p-eIF2 $\alpha$  were elevated, indicating that Fxn-depleted hearts were not normal. Because a rise in ISRmt mRNAs in other mitochondrial disease models was causally linked to mTORC1 activation, we evaluated this pathway by WB and found evidence for higher activity in TG mice. Finally, few studies have addressed what triggers mTORC1 activity in the context of mitochondrial dysfunction. Because mTORC1 is negatively regulated by AMPK, we evaluated AMPK activity and found p-AMPK levels were decreased in Fxn-deficient hearts. Furthermore, p-eIF2 $\alpha$  was also elevated, which would lead to protein translation and explain the lower heart mass.

**Conclusions:**  $\beta$ -oxidation appears to be preserved, suggesting that  $\beta$ -oxidation could support normal contractile heart function that we observed even after 5 months of Fxn depletion. Differently from  $\beta$ -oxidation that can bypass the TCA cycle, substrate oxidation requiring the TCA cycle was greatly suppressed. This differential effect of Fxn depletion on substrate oxidation may have implications for understanding Fe-S cluster insertion or stability of Fe-

S cluster proteins. Though heart function remained normal after 5 months of Fxn depletion, Fxn-deficient hearts showed alterations in nutrient signaling consistent with an underlying integrated stress response in these hearts.

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## Assessment of Endoplasmic reticulum-mitochondria associated membranes (MAMs) in Friedreich's Ataxia

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 93

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**Mrs. Laura Robles Rodríguez<sup>1</sup>, Dr. Pablo Calap<sup>2</sup>, Prof. Federico Vicente Pallardó Calatayud<sup>1</sup>, Dr. Pilar Gonzalez-Cabo<sup>1</sup>**

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**Background:** Calcium ( $\text{Ca}^{2+}$ ) homeostasis is crucial to maintain a proper physiology in different metabolic processes and signaling pathways. Both mitochondria and the endoplasmic reticulum (ER) contribute to this process through specific protein bridges termed endoplasmic reticulum-mitochondria associated membranes (MAMs). The interaction between these compartments is mainly mediated by the voltage-dependent anionic channel 1 (VDAC1), located in the mitochondrial outer membrane, the inositol-1,4,5-trisphosphate receptor (IP<sub>3</sub>R) in the ER membrane, and the chaperone glucose-regulated protein 75, which links the former two. The wide variety of cellular processes MAMs are involved in (lipid metabolism, autophagy, mitochondrial morphology) are usually altered in several neurodegenerative disorders, such as Friedreich's ataxia (FRDA). We have previously described that frataxin-silenced cells showed an impairment in  $\text{Ca}^{2+}$  buffering, as a consequence of reduced mitochondrial  $\text{Ca}^{2+}$  uptake capacity. Thus, our aim is to elucidate the role of MAMs in the physiopathology of neurodegeneration and their relevance as therapeutic targets for FRDA.

**Methods:** In order to assess MAMs architecture and behavior, using two cellular models with depleted frataxin (FXN-138.1 and FXN-138.2) through FXN gene silencing in the SH-SY5Y neuroblastoma cell line, we have performed three different approaches: i) Proximity ligation assay (PLA) to evaluate MAMs interactions (under baseline conditions and after the addition of Trolox 1mM for 24h); ii) Western blot analysis of the subcellular fractions involved in  $\text{Ca}^{2+}$  flux including ER, mitochondria and MAMs; and iii) Lipidomic analysis of the different subcellular fractions (under baseline and antioxidant conditions).

**Results:** Our findings indicate significant decreased interactions per cell between VDAC-IP<sub>3</sub>R and VDAC-GRP75 in cells with frataxin deficiency as compared to the wild type cell line SH-SY5Y. Addition of the vitamin E mimic Trolox improved the number of interactions per cell in FXN-silenced cells, probably due to stabilization of ER-mitochondria interactions through the reduction of lipid peroxidation. Finally, western blot analysis revealed the subcellular location of frataxin in MAMs, offering new insights about its role in these structures.

**Conclusions:** MAMs architecture is altered in a Frataxin-depleted cellular model, which can be partially reverted with the addition of Trolox. This could also improve  $\text{Ca}^{2+}$  exchange between the ER and mitochondria. These preliminary results open new therapeutic possibilities to evaluate MAMs as potential targets in FRDA.

1. Bolinches-Amorós, A. *et al.* Mitochondrial dysfunction induced by frataxin deficiency is associated with cellular senescence and abnormal calcium metabolism. *Front. Cell. Neurosci.* **8**, 124 (2014).

# INVESTIGATING THE RELATIONSHIP BETWEEN CEREBELLAR ATROPHY AND COGNITIVE IMPAIRMENT IN FRIEDREICH ATAXIA

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 101

***Dr. Sirio Coccozza<sup>1</sup>, Dr. Teresa Costabile<sup>1</sup>, Dr. Giuseppe Pontillo<sup>1</sup>, Dr. Maria Lieto<sup>1</sup>, Dr. Camilla Russo<sup>1</sup>, Dr. CHIARA PANE<sup>1</sup>, Prof. Alessandro Filla<sup>1</sup>, Prof. Francesco Saccà<sup>1</sup>, Dr. Arturo Brunetti<sup>1</sup>***

*1. University "Federico II"*

## **Purpose**

Recent studies have suggested the presence of a significant atrophy affecting the cerebellar cortex in Friedreich ataxia (FRDA) patients, an area of the brain long considered to be relatively spared by the neurodegenerative phenomena occurring in this condition. Cognitive deficits, which occurs in FRDA patients, have been associated to cerebellar volume loss in other conditions. Aim of this study was to investigate the correlation between cerebellar volume and cognition in FRDA.

## **Materials and Methods**

19 patients with genetically confirmed FRDA (M/F:13/6; 28.4±14.1y), along with a group of 20 healthy controls (HC) of comparable age and sex (M/F:11/9; 29.4±9.7y) were included in this study. All subjects underwent an MRI scan including a 3D-T1-weighted sequence and a neuropsychological examination mainly oriented at cognitive domain that are related to cerebellar function (i.e. visuo-perception and visuo-spatial functions, visuospatial memory and working memory).

Cerebellar global and lobular volumes were computed using the Spatially Unbiased Infratentorial Toolbox (SUIT v3.2), implemented in SPM12 (Figure 1). Furthermore, a cerebellar Voxel Based Morphometry (VBM) analysis was also carried out. Correlations between MRI metrics and clinical data were tested via partial correlation analysis, correcting for age and sex.

## **Results**

FRDA patients showed a significant reduction of the total cerebellar volume ( $p=0.004$ ), significantly affecting the Lobule IX ( $p=0.001$ ). At the VBM analysis, a cluster of significant reduced GM density encompassing the entire lobule IX was found ( $p=0.003$ ). When correlations were probed, a direct correlation between Lobule IX volume and impaired visuo-spatial functions was found ( $r=0.580$ ,  $p=0.02$ ), with a similar correlation between the same altered function and results obtained at the VBM ( $r=0.520$ ;  $p=0.03$ ).

## **Conclusions**

With two different and complementary image analysis techniques, we confirmed the presence of cerebellar volume loss in FRDA, mainly affecting the posterior lobe. In particular, Lobule IX atrophy correlate with worst performances at visuo-spatial functions, further expanding our knowledge about the physiopathology of cognitive damage in FRDA.

# **Topic: Therapeutic Approaches and Drug Discovery**



# Exploring the potential protective effects of Sonic hedgehog agonists in frataxin-deficient astrocytes

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 259

**Mr. Andrés Vicente<sup>1</sup>, Prof. Javier Diaz-Nido<sup>1</sup>, Dr. Frida Loria<sup>1</sup>**

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Friedreich ataxia (FRDA) is predominantly a neurodegenerative disease caused in most cases by recessive mutations that lead to a deficiency in a protein called frataxin (Fxn). Although the pathophysiological manifestations of FRDA are mainly observed in neurons, some results indicate that other neural cells such as astrocytes, may be involved in the neurodegenerative process of the pathology.

Under different conditions, astrocytes can undergo a process called reactive astrogliosis. Neuroinflammatory stimuli promotes the formation of A1 reactive astrocytes, which have upregulated many proinflammatory genes, being damaging for neurons. The presence of A1 reactive astrocytes has been detected in post-mortem tissues of patients with different neurodegenerative diseases, so it is postulated that they might exert harmful effects on neurons and therefore contribute to the neurodegenerative process. Besides, it has been recently reported that Sonic hedgehog (SHH) agonists have positive effects in astrocyte viability and proliferation, and also lead to astrocyte-mediated neuroprotective effects. Therefore, in view of these data, we have explored whether Fxn-deficient astrocytes show an A1 reactive phenotype, evaluating as well if SHH agonists affect Fxn-deficient astrocyte viability and exert neuroprotective effects.

For this purpose, we used an in vitro model of Fxn deficiency in human cortical astrocytes transduced with a lentiviral vector carrying a short hairpin RNA for Fxn. We found that Fxn deficiency upregulates A1 reactive astrocyte markers, such as MX1 and the complement component C3. Moreover, the chronic treatment with smoothened agonist (SAG), a SHH agonist, was able to increase Fxn-deficient astrocytes viability, which has been demonstrated to be compromised when astrocytes have low levels of Fxn, and prevented the upregulation of the A1 reactive astrocyte markers previously mentioned. Finally, regarding the possible neuroprotective effects of SHH agonists, previous results showed that Fxn-deficient astrocytes are able to induce neurodegeneration, and we have observed that a chronic treatment with SAG was able to attenuate the neurotoxicity triggered by the treatment of mouse cortical neurons with conditioned medium of Fxn-deficient astrocytes.

Overall, our results suggest that the modulation of the SHH pathway, and in particular the treatment of Fxn-deficient astrocytes with SHH agonists like SAG, could be used as a possible target to reduce neurodegeneration in FRDA.

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## Elucidation of the metabolic signature of Friedreich's ataxia

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 166

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***Dr. Sara Anjomani Virmouni<sup>1</sup>, Dr. Ester Kalef-Ezra<sup>1</sup>, Dr. Adamo Valle Gómez<sup>2</sup>, Dr. Nick Perry<sup>3</sup>, Mr. Aurelien Tripp<sup>3</sup>, Dr. Alice Agliano<sup>4</sup>, Dr. Sahar Al-Madhawi<sup>1</sup>, Dr. Mark Pook<sup>1</sup>***

*1. Brunel University London, 2. Universitat de les Illes Balears, 3. Institute of Cancer Research (ICR), 4. Imperial College London*

We aim to identify differential levels of metabolites and lipids in FRDA cells compared to healthy controls using complementary methods: Rapid Evaporative Ionisation Mass Spectrometry (REIMS), Liquid Chromatography Mass Spectrometry (LC-MS/MS) and Nuclear Magnetic Resonance Spectroscopy (NMR). Using REIMS we have already identified potentially interesting species of ceramide and other metabolites, including phosphatidic acid (PA), phosphatidylserine (PS) and phosphatidylethanolamine (PE), that are significantly different in FRDA cells. We are also analysing the acquired data by NMR and this will be further accomplished by LC-MS/MS analysis to obtain a comprehensive map of the metabolic changes in FRDA samples. These investigations will be extended to biochemical analysis of the key enzymes that are involved in the altered metabolic pathways. Our studies will open up new opportunities for the identification of novel metabolic biomarkers for FRDA and an enhanced understanding of mitochondrial function.

In addition, we have obtained a new FRDA transgenic mouse model that may be more representative of FRDA than all the previous and current transgenic mouse models. We are further characterising this mouse model at molecular, biochemical, histopathological and behavioural levels and will use these mice for our metabolomics studies. Furthermore, we have assessed oxidative stress parameters and mitochondrial function in human FRDA cell lines and found defects in energy metabolism and antioxidant defences and increased reactive oxygen species (ROS) levels in these cells. We investigated whether detoxification of these reactive molecules by antioxidant medicines might substantially alleviate the continuous stress and damage that cells are subjected to. Our preliminary research has led us to identify the naturally occurring ginseng derivative, ginsenoside Rb1, as a particularly promising drug candidate. We will investigate its effects on cell health and metabolism using validated FRDA cell and animal models.

## **Pulling a therapy out of thin air: Hypoxia Rescues Frataxin Loss by Restoring Iron Sulfur Cluster Biogenesis**

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 42

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***Mrs. Tslil Ast*<sup>1</sup>, *Mr. Joshua Meisel*<sup>2</sup>, *Mr. Shachin Patra*<sup>3</sup>, *Mrs. Hong Wang*<sup>1</sup>, *Mr. Robert Grange*<sup>2</sup>, *Ms. Sharon Kim*<sup>1</sup>, *Mrs. Sarah Calvo*<sup>1</sup>, *Mrs. Lauren Orefice*<sup>2</sup>, *Mr. Fumiaki Nagashima*<sup>2</sup>, *Dr. Fumito Ichinose*<sup>2</sup>, *Dr. Warren Zapol*<sup>2</sup>, *Prof. Gary Ruvkun*<sup>2</sup>, *Prof. David Barondeau*<sup>3</sup>, *Prof. Vamsi Mootha*<sup>1</sup>**

*1. The Broad Institute, 2. Massachusetts General Hospital, 3. Texas A&M University*

Friedreich's ataxia (FRDA) is a multisystemic disorder with no known therapies to date, caused by recessive mutations in the mitochondrial protein frataxin (FXN). FXN participates in the biosynthesis of Fe-S clusters and is considered to be essential for viability. Here we report that when grown in 1% ambient O<sub>2</sub>, FXN null yeast, human cells, and nematodes are fully viable. Moreover, in a murine model of FRDA, breathing 11% O<sub>2</sub> attenuates the progression of ataxia, whereas breathing 55% O<sub>2</sub> hastens it. This property is unique to FXN amongst the ISCU-NFS1-LYRM4-FXN complex. In human cells, hypoxia restores steady-state levels of Fe-S clusters in both the mitochondria and cytosol. Cellular studies and *in vitro* reconstitution indicate that hypoxia acts through HIF-independent mechanisms that increase bioavailable iron as well as directly activate Fe-S synthesis, thus overcoming the genetic need for FXN. Our work identifies oxygen as a key environmental variable in the pathogenesis associated with FXN depletion, with important mechanistic and therapeutic implications.

# Leriglitazone, a brain-penetrating PPAR gamma agonist for the treatment of Friedreich's ataxia

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 20

**Dr. Laura Rodríguez-Pascau<sup>1</sup>, Dr. Joaquim Ros<sup>2</sup>, Dr. Pilar Gonzalez-Cabo<sup>3</sup>, Dr. Vijayendran Chandran<sup>4</sup>,  
Dr. Pierre-Gilles Henry<sup>5</sup>, Dr. Christophe Lenglet<sup>5</sup>, Dr. David Lynch<sup>6</sup>, Dr. Marc Martinell<sup>1</sup>, Dr. Pilar  
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*1. Minoryx Therapeutics, 2. IRBLleida, Universitat de Lleida, 3. University of Valencia, 4. University of Florida, 5. University of Minnesota, 6. Children's Hospital of Philadelphia*

Friedreich's ataxia (FRDA) is a rare autosomal recessive neurodegenerative disease characterized by progressive spinocerebellar and sensory ataxia, cardiomyopathy, diabetes mellitus and skeletal deformities. It is the most common inherited ataxia and is caused by mutations in the frataxin (*FXN*) gene resulting in reduced levels of frataxin, a small, nuclear-encoded mitochondrial protein. The pathological consequence of frataxin deficiency is a mitochondrial dysfunction including reduced activity of iron-sulphur cluster containing enzymes, mitochondrial iron overload, defective energy production and calcium metabolism, oxidative stress and dys-regulation of mitochondrial homeostasis including mitochondrial biogenesis. Several studies in both cell and animal models have shown that the peroxisome-proliferator activator receptor gamma (PPAR $\gamma$ )/PPAR $\gamma$  coactivator 1 alpha (PGC1 $\alpha$ ) pathway is dysregulated when there is frataxin deficiency contributing to the disease pathogenesis and supporting PPAR $\gamma$  pathway as a potential therapeutic target for FRDA. Leriglitazone (MIN-102), is a novel selective PPAR gamma agonist with improved profile for CNS diseases. In this study Leriglitazone was tested in vitro in frataxin-deficient dorsal root ganglia neurons, frataxin-deficient cardiomyocytes and fibroblasts from FRDA patients and in vivo in several mouse models of FRDA. The data generated in the different preclinical FRDA related models showed that Leriglitazone is efficacious by increasing DRG neurons survival and decreasing neurite degeneration, compensating the alterations on mitochondrial markers such as PGC1 $\alpha$  and improving mitochondrial function, restoring energy production and calcium dysregulation, and ameliorating motor function. Thus, the results support the use of the compound for the treatment of FRDA. Leriglitazone is currently in a phase 2 clinical trial for the treatment of FRDA in Europe and in a phase 2/3 clinical trial for the treatment of Adrenomyeloneuropathy (AMN) in Europe and US.

## AAV-9 Biodistribution with Different Routes of CNS Administration in Rodents

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 152

*Dr. Jayakrishnan Nair*<sup>1</sup>, *Mr. Ian Debus*<sup>1</sup>, *Mr. David Freeman*<sup>1</sup>, *Ms. Tara McParland*<sup>2</sup>, *Ms. Megan Pope*<sup>1</sup>,  
*Dr. Barbara Perez*<sup>1</sup>, *Dr. Edgardo Rodriguez*<sup>2</sup>, *Ms. Kirsten Coleman*<sup>1</sup>, *Prof. S H Subramony*<sup>1</sup>, *Dr. Barry Byrne*<sup>1</sup>, *Dr. Manuela Corti*<sup>1</sup>

1. University of Florida, 2. Lacerta Therapeutics

**Introduction:** Improved strategies for adeno-associated virus (AAV)-mediated gene transfer have enabled genetic correction of inherited neuromuscular disease (NMD). Recent studies in NHPs and piglets have demonstrated that high doses of AAV injected systemically can lead to toxicities with acute systemic inflammation, coagulation defects, hepatic toxicity and sensory neuron degeneration. Similar to several other neuromuscular diseases, Friedreich's Ataxia (FA) manifest with both systemic and neurological symptoms. In FA, high AAV doses would be required to penetrate the CNS and treat the neurological manifestations by systemic delivery. This approach would likely increase toxicity and immune reactions to the vector capsid. Therefore, successful clinical translation of these therapeutic approaches depends upon the safety and efficacy of more targeted CNS routes of vector administration, at significantly lower doses. **Objective:** The purpose of this study was to determine the optimal CNS route of AAV9 administration at lower doses to achieve widespread bio-distribution and transgene expression needed in FA. **Methods:** Adult wild type C57BL6 mice (n=10/group) & Sprague Dawley rats (n=3/group) were administered AAV9 vector expressing human *FXN* gene (rAAV9-CBA-hFXN or GFP). Assuming the average mouse brain weighs 0.004g, we delivered 6E10 vg/mice (1.5E14 vg/kg brain weight) via either stereotaxic deep cerebellar nucleus (DCN), or intra cerebra ventricular (ICV), or cisterna magna (ICM), or free hand lumbar intrathecal (IT) injection. Animals were sacrificed at 1-month post injection for molecular assays (n=5, vector genome copy and FXN mRNA expression). The remaining animals were trans-cardially perfused, with 4% paraformaldehyde, for immuno-fluorescence (GFAP, IBA), and histochemistry (H & E). Due to high vector related toxicity in the ICM treated group we observed high mortality (n=4/10). Therefore, we recruited another batch of animals for ICM injections (n=3) at a lower vector dose of 5E13 vg/kg brain weight. Statistical analysis was performed using one-way ANOVA with multiple comparisons. **Results:** Preliminary analysis shows no difference (p=0.6) in cerebellar bio-distribution for direct DCN injections and ICM injections, even though ICM injections were performed at 1 log lower doses. Cerebellar distribution was significantly superior with ICM as compared to both ICV (p<0.01) and IT (p<0.001) injections. Similarly, cervical, thoracic and lumbar spinal cord bio-distribution was superior for ICM injections as compared to DCN (p<0.0001), ICV (p<0.0001), and IT (p<0.05) injections. All approaches were significantly better than DCN injections for dorsal root ganglion bio-distribution. However, no group difference in dorsal root ganglion bio-distribution was observed with ICV, ICM or IT injections. Significantly, high amount of vector was detected in systemic circulation with IT injection as measured in heart (p<0.001) and liver (p<0.01). Small amount of vector was also observed in the heart and liver of ICM and ICV treated mice. Parallel studies in rats using rAAV9-GFP as a fluorescent tracker was technically challenging but feasible for ICV and ICM injections. The GFP expression in ICV and ICM injected rats were consistent with the vector bio-distribution profile observed in mice. Further analysis for this study is still ongoing. **Conclusion:** Preliminary results shows vector CNS infusion via ICM injections, at carefully titrated lower doses, may be the most suitable approach to target all the affected regions of CNS in FA.

# Frataxin Function and Quaternary Addition of Small Trojan Tutor Proteins

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 137

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We propose the quaternary addition of a small tutor protein to modulate the frataxin (FXN) structural dynamics and the activity of the supercomplex involved in Fe-S cluster biosynthesis. We used Ribosome Display technology and the Sac7d protein as the scaffold to select affitins exhibiting the capability of interacting with human FXN. Two binders were obtained, Aff186 and Aff224. Affitins were prepared in *E. coli* and purified. The proteins are structured as judged by CD spectroscopy and temperature-induced unfolding experiments. Affitins showed a marked tendency to dimerize (SEC-FPLC). For both affitins, when DTT was absent, an intermolecular disulfide bond was formed, as detected by ESI-MS. Interaction between affitins and FXN was confirmed and investigated in detail *in vitro* using ELISA, SPR and ITC. Additionally, we were able to map the binding site of Aff186 and Aff224 on the region of the acidic ridge of FXN by analyzing chemical shift perturbations (NMR). The interaction between affitins and FRDA variants (G130V, L198R and W155R) or designed stable FXN mutants was also evaluated. Furthermore, we investigated the affitin-induced modulation of desulfurase enzymatic activity of the human supercomplex NFS1-ACP-ISC11-ISCU-FXN. Moreover, we prepared a Trojan variant of Aff224 to deliver the affitin to the mitochondrial matrix to explore the effect of this molecule in the cellular environment. A new screening with a subsequent round of selection is being carried out to expand the diversity of affitins in order to obtain tutor proteins that can interact with FXN: (a) positively modulating FXN function in the context of the supercomplex; (b) maintaining FXN available to promote activation; or (c) stabilizing mutant variants of FXN for enough time to allow FXN-supercomplex interaction and activation.

## Engineered AAVs for efficient delivery to central nervous system

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 125

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***Dr. Nathalie Majeau<sup>1</sup>, Dr. Dominique Ouellet<sup>1</sup>, Mr. Pouire Yameogo<sup>1</sup>, Dr. Catherine Gérard<sup>1</sup>,  
Prof. Jacques P. Tremblay<sup>1</sup>***

*1. Université Laval*

Advances in CRISPR/Cas9 technology that allow efficient gene correction give rise of a lot of expectation for gene therapy. However, *in vivo* delivery of gene editing tools remains challenging in current treatment efforts. Adeno-associated virus (AAV) is the most promising vector as it is considered to be safe and versatile. However, there is an important need to improve AAV efficiency to transduce all targeted organs and more specially to bypass the blood-brain barrier of the central nervous system (CNS). In order to increase the accessibility of the CNS, we engineered AAV9 capsid by introducing 17 amino acid aleatory mutations in 3 different regions of the protein structure that were predicted to be exposed on the surface of the virus. According to the 3D structure, these 3 regions were closed to each other and could interact potentially with a same molecule receptor. The mutated sequences encoding the capsid was enclosed inside the AAV in order to characterize the new viruses that display tissue tropism. Viruses were intravenously administrated in mice and after 10 days, the organs were recovered and DNA extracted. AAV variant sequences present in each tissue were analyzed by deep sequencing. Most AAV sequences recovered from CNS organs were highly divergent from the AAV9 initial sequence, and that for the 3 modified capsid regions. In spite of a wide selection of mutants recovered, we found some conserved motif among the different capsids present in the CNS tissue analyzed.

# The Novel monomethyl fumarate prodrug IMF dose-dependently induces frataxin, mitochondrial gene expression and aconitase activity more potently than DMF

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 117

***Prof. Gino Cortopassi<sup>1</sup>, Mr. Chun Kiu Hui<sup>1</sup>, Dr. Elena Dedkova<sup>1</sup>, Dr. Sandipan Datta<sup>1</sup>***

*1. UC Davis*

**Background.** Friedreich's ataxia (FA) is an inherited neuro- and cardio-degenerative disease that is ultimately lethal, and for which there is no current FDA/EMA-approved therapy. Prodrugs are chemically modified versions of a drug that are converted to the bioactive form of the drug upon ingestion. For example, DMF=Dimethyl fumarate is a pro-drug of the biologically active Monomethyl fumarate (MMF), and MMF has been shown to engage both Nrf2 and HCA2 drug targets to elicit its pharmacodynamic benefits. We previously demonstrated that DMF dose-dependently increases frataxin and mitochondrial functions in FA cells, and mice, and humans (Hayashi G, 2015; Hayashi G 2017, Jasoliya M 2019), and that mitochondrial number is decreased in Friedreich's cells, mice and humans (Jasoliya M, 2017). Because MMF is the bioactive metabolite, other MMF prodrugs than DMF can be envisioned, that might deliver MMF more effectively. We recently tested a novel MMF prodrug, IMF, with improved pharmacokinetic parameters. Here we show that IMF and DMF prodrugs both dose-dependently induce frataxin levels and mitochondrial activities in C57Bl6 mice, and that IMF is about twice as potent as DMF.

**Methods.** Frataxin expression and mitochondrial gene expression and mitochondrial biogenesis were measured in multiple mouse tissues by Western Blot, QRT-PCR and QPCR. Aconitase activity, which is often used as a surrogate measure of frataxin's iron-sulfur biogenesis function, was measured by activity stain. COXIV activity was also measured by activity stain in tissues. As MMF is metabolized to fumarate, fumarate levels in tissues were measured by ELISA as a measure of MMF delivery to heart, muscle and brain tissues.

**Results.** Dose-escalation experiments were carried out with DMF and IMF in C57Bl6 mice, by intraperitoneal and oral exposure. Dose ranges were 10mg/kg to 120mg/kg, which because of the mouse's 12-fold faster metabolism overlap the human equivalent doses of 10mg/kg DMF currently EMA-approved for human dosing, i.e. 720mg/day. A dose-dependent increase in frataxin expression and mitochondrial COX4 and Ndufs5 expression was observed in heart and brain and liver tissues from 100% to 400% of baseline in C57Bl6/J mice, and dose-dependent increases in aconitase activity and COXIV activity were also observed. Dose-dependent increase in mitochondrial biogenesis was also observed, though to a lesser extent. The pharmacokinetic (PK) parameters of the novel prodrug IMF were measured, and were superior to MMF, in terms of residence time in the gut and ultimately area under exposure curve. Consistent with this observation of 50-100% improved PK parameters of IMF vs DMF, IMF produced about a 2-fold increased pharmacodynamic potency per weight of drug ingested. Thus IMF was about produced about 2-fold more frataxin expression, COX4 expression, mitochondrial biogenesis, and aconitase activity in heart, muscle and brain than DMF per weight.

**Conclusion.** These results demonstrate that oral DMF dose-dependently induces frataxin expression, mitochondrial gene expression, aconitase activity and mitochondrial biogenesis in C57Bl6 mice. Furthermore, the 120mg/kg maximally effective dose of DMF in mice overlaps the human equivalent dose of 10mg/kg *that is currently approved for human dosing*, i.e. 720mg/day. Furthermore, we describe IMF, a novel MMF-prodrug, with improved pharmacokinetic parameters, that is about 2-fold more pharmacodynamically potent than DMF per weight. Because IMF has improved pharmacokinetics and delivers higher fumarate to tissues, we suggest that these improved pharma-



cokinetic parameters underlie IMF's greater pharmacodynamic potency. Because IMF is more potent than DMF, and also releases the safe bioactive molecule MMF, there is a possibility for expedited regulatory approval for IMF. Because IMF is more potent than DMF in raising frataxin and mitochondrial functions, it could be considered as novel small-molecule therapy for Friedreich's ataxia.

# Management of Preexisting Immunity to AAV9 in Friedreich's ataxia

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 106

***Dr. Barbara Perez*<sup>1</sup>, *Ms. Kirsten Coleman*<sup>1</sup>, *Ms. Megan Wichman*<sup>1</sup>, *Ms. Katerina Futch*<sup>1</sup>, *Ms. Kayla Mandolini*<sup>1</sup>, *Mr. Lochlin Cravey*<sup>1</sup>, *Dr. Jayakrishnan Nair*<sup>1</sup>, *Dr. David Lynch*<sup>2</sup>, *Dr. Barry Byrne*<sup>1</sup>, *Dr. Manuela Corti*<sup>1</sup>**

*1. University of Florida, 2. Children's Hospital of Philadelphia*

Friedreich's ataxia (FA) is the most common form of hereditary ataxias. It is caused by an inherited autosomal recessive expansion mutation of GAA repeat in the *frataxin (FXN)* gene. FA manifests between the ages of 8 and 16 as a multisystem disorder, primarily affecting cardiac and nervous system. Preclinical data from our lab and others using AAV-based gene replacement therapy shows promising results. However, a crucial and unresolved challenge for the success of gene therapy is the host immune response to vector capsid proteins. Patients with prior exposure to AAV with a high titer of anti-AAV9 antibodies may have infusion reactions, raising concerns regarding safety, longevity of expression, and loss of therapeutic effect. As a result, patients with anti-AAV antibody titers above 50 U/mL, whether acquired by environmental exposure to wild-type AAV or by participating in a AAV-based clinical trial, are excluded from clinical trials. To understand the extent of preexisting immunity in the FA population, we analyzed serum of 93 patients and found that 53% had preexisting antibody titers to AAV9 that were higher than the inclusion criteria for current AAV-based clinical trials. To overcome this limitation, we evaluated immunomodulatory approaches to decrease preexisting immunity against the AAV capsid and allow for safe administration of AAV. Previous preclinical and clinical studies by our group show that dosing patients with B-cell depleting rituximab and mTor inhibitor sirolimus before the AAV9 infusion resulted in lower levels of circulating anti-AAV antibodies and higher transgene expression. To test this and other immunomodulatory agents (bortezomib and abatacept) in the case of preimmunity, we used empty AAV9 capsids to elicit defined levels of capsid specific preimmunity in mice and compared the effect of treatments on circulating anti-AAV9 antibodies, B and T cell populations, biodistribution and transgene expression. We found that all interventions we used decreased circulating anti-AAV9 by over 50% and that anti-CD20 antibody and sirolimus had the largest effect on levels of B and T cells in circulation and in spleen. Ongoing studies aim to assess the effect of immunomodulatory agents on transgene expression and define the highest level of preimmunity that does not inhibit transgene expression in mice. Overall, this work aims to better understand the relationship between preimmunity to AAV9 and transgene expression, ultimately allowing additional patients access to AAV-based gene therapies.

## Deletion of GAA repeat in FXN in mice using *Campylobacter jejuni* Cas9

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 103

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**Mr. Pouire Yameogo <sup>1</sup>, Dr. Nathalie Majeau <sup>1</sup>, Dr. Catherine Gérard <sup>1</sup>, Prof. Jacques P. Tremblay <sup>1</sup>**

**<sup>1</sup>. Université Laval**

The development of an *in vivo* CRISPR/Cas9 gene editing therapy for FRDA give rise to interesting prospective. The aim will be to remove the expanded GAA trinucleotide repeats within the intron 1 of the frataxin (*FXN*) gene, which is believed to cause transcriptional interference and low protein expression, which lead to oxidative stress and cell death expression. In order to achieve this treatment, we identified two CjCas9 RNA guides that target efficiently FXN gene in the region of the GAA repeat in 293T cells and YG8sR. We then made an AAV construct, which encodes in the same virus the CjCas9 protein and the two guides RNA to optimize DNA editing. The AAV viruses were intravenously administrated in YG8sR mice and after a month, the organs were recovered and DNA extracted from the different tissue. PCR amplification of the FXN gene showed that we were able to remove GAA repeat in different mouse tissues.

# Identification of peptides that target the RNF126-frataxin interaction as potential therapeutics for Friedreich ataxia.

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 92

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Friedreich ataxia (FRDA) is caused by insufficient frataxin levels, therefore the main goal of a specific therapy for FRDA is to increase the amount of the frataxin protein. We have shown that this can be achieved by preventing the ubiquitin-proteasome-mediated degradation of frataxin (Rufini et al., 2011; Rufini et al., 2015). We also found that the ring finger E3 ligase RNF126 ubiquitinates frataxin and promotes its degradation (Benini et al., 2017). Importantly, silencing of RNF126 in cells derived from FRDA patients leads to increased frataxin levels, highlighting the therapeutic relevance of interfering with this pathway. Instead of inhibiting the E3 ligase itself, which could have unpredictable off-targets effect, we focused on the possibility to prevent frataxin ubiquitination by targeting the frataxin-RNF126 interaction. To this aim, we sought to structurally characterize the interaction between these two proteins and define the domains required for the interaction. We have previously shown that the precursor form of frataxin interacts with RNF126 and can be ubiquitinated, while the mature form, lacking the N-terminal part, does not interact and is not ubiquitinated. Based on these data, we investigated the role of the N-terminal portion of frataxin in the ubiquitination process and in the recognition between frataxin and RNF126. Through the generation of a series of frataxin N-terminal deletion mutants and co-immunoprecipitation experiments, we confirmed that the N-terminal domain of frataxin is indeed critical for interaction with RNF126. Accordingly, we have observed that the frataxin N-terminus is also required for frataxin ubiquitination by RNF126 *in vitro*. These data suggested the possibility to use peptides, encompassing the N-terminal part of frataxin and therefore mimicking the recognition surface, to compete and displace the interaction between frataxin and RNF126, eventually resulting in inhibition of ubiquitination. Here we report the identification of the minimal peptide that is indeed able to prevent frataxin ubiquitination *in vitro* in a dose-dependent manner. A fusion between a cell-penetrating sequence and the identified peptide confirmed its ability to displace the frataxin-RNF126 interaction in living cells.

The identification of such peptide provides the proof of principle that it is possible to interfere with the frataxin-RNF126 interaction in order to prevent frataxin ubiquitination in living cells. Moreover, it allows to gain insight into the recognition mode between frataxin and RNF126 and identify surfaces and residues involved in the interaction. This information could eventually be developed into the design of small molecule compounds that, by preventing frataxin degradation and inducing its accumulation, could act as effective therapeutics.

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# Etravirine enhances frataxin translation: a promising candidate for Friedreich ataxia therapy.

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 85

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With the aim to accelerate the development of an effective treatment for Friedreich ataxia, we followed a drug repositioning strategy to identify market-available drugs able to increase frataxin levels. Using a cell-based reporter assay to monitor variation in frataxin amount, we performed a high-throughput screening of a library containing 853 FDA-approved drugs. Among the potentially interesting candidates isolated from the screening, we focused our attention on etravirine, an antiviral drug currently in use as an anti-HIV therapy. Etravirine promotes a significant increase in frataxin levels in several cell types derived from FRDA patients, by enhancing frataxin mRNA translation. This occurs through a mechanism that involves the redistribution of frataxin mRNA to the actively translating heavy polysome fraction (Alfedì et al., 2019). To further confirm that the translation of frataxin mRNA can be enhanced by etravirine, we generated a fusion construct between frataxin and GFP. We observed that, while GFP alone is not upregulated by etravirine in transfected cells, the frataxin-GFP fusion is elevated by etravirine treatment, indicating that some elements in the frataxin transcript can be regulated by etravirine. Moreover, we observed that frataxin accumulation in treated patients cell lines is comparable to frataxin levels in cells from unaffected carrier sibling, suggesting that etravirine effect could be therapeutically relevant. Indeed, etravirine treatment restores the activity of the iron-sulphur cluster containing enzyme aconitase and confers resistance to oxidative stress in cells derived from FRDA patients.

Recently, we obtained evidence that etravirine can upregulate frataxin in cardiomyocytes differentiated from FRDA patient iPSCs. These data prompt to investigate the effects of etravirine in relevant cardiac models and strongly encourage further evaluation of etravirine as a potential therapeutic for Friedreich ataxia.

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## Gene editing of human hematopoietic stem and progenitor cells for Friedreich's ataxia using CRISPR/Cas9 technology

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 23

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Friedreich's ataxia (FRDA) is a multi-systemic autosomal recessive disorder that is predominantly caused by a homozygous GAA repeat expansion mutation within intron 1 of the frataxin gene (*FXN*). This mutation leads to the reduction of frataxin expression, a mitochondrial protein involved in iron metabolism. FRDA is characterized by ataxia, areflexia, sensory loss, muscle weakness, and cardiomyopathy. Symptoms typically begin between 5 to 15 years of age and patients will be in wheelchair within 10-15 years of onset. Currently, there is no treatment for FRDA. In 2017, we showed that transplantation of mouse wild-type hematopoietic stem and progenitor cells (HSPCs) prevents the development of the locomotor deficits, the neuronal degeneration in the dorsal root ganglia and the oxidative damages in brain and muscle in the YG8R mouse model of FRDA. We also showed that the mechanism of rescue is mediated by the transfer of frataxin from the HSPC-derived microglia/macrophages to neurons/myocytes (1). Our goal is now to develop an autologous HSPC transplantation. To achieve our objective, we are developing a CRISPR/Cas9 method to remove the GAA expansion in the intron 1 of the frataxin gene in FRDA patient HSPCs. We first optimized the conditions of in lymphoblasts isolated from FRDA patients and obtained up to 62% of gene correction. At the mRNA and protein levels, frataxin expression reached the same level than their carrier parents' cell lines. In addition, using a very sensitive method developed by BIOLOG, we showed that mitochondrial activity was also improved in corrected cell. We thus moved towards the manufacturing development of the human product using first CD34<sup>+</sup> cells isolated from healthy donor peripheral blood. We successfully gene-corrected 24 to 50% of the CD34<sup>+</sup> cells. The capacity of the gene-modified CD34<sup>+</sup> cells to differentiate into the different hematopoietic lineage cells was tested in vitro by Colony Forming Unit assays and in vivo in NOD *scid*gamma immunodeficient mice. Finally, we gene-edited CD34<sup>+</sup> cells isolated from FRDA patients reaching up 55% correction accompanied by an increased in frataxin expression and normal differentiation in the different hematopoietic lineage cells. With this study, we are laying the foundations for a future clinical trial for autologous HSPC transplantation for FRDA.

# Therapeutic Application of Triplex DNA Binding Agents in Targeted Deletion of Expanded (GAA)<sub>n</sub>•(TTC)<sub>n</sub> Repeats in Friedreich Ataxia

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 167

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**Introduction:** Triplet repeat expansion in genomic DNA is associated with more than 30 human neurodegenerative diseases, including Friedreich Ataxia (FRDA), an autosomal recessive neurodegenerative disease, caused by (GAA)<sub>n</sub>•(TTC)<sub>n</sub> expansion in the intron 1 of the frataxin (FXN) gene in chromosome 9. In FRDA, repeat length shows large somatic and germline instability as well as heterogeneity in the length of the triplet repeats in somatic cells throughout life. Repeat expansion leads to partial silencing of the FXN gene resulting in low levels of frataxin protein, causing mitochondrial failure leading to cell atrophy and ultimately cell death. (GAA)<sub>n</sub>•(TTC)<sub>n</sub> repeats can form DNA triplex structures *in vitro* and in cells. The genetic instability of the repeats increases with the increasing repeat length and the instability may be related to the formation of triplex DNA. Certain DNA intercalating or binding agents can promote the formation of, and stabilization of, triplex DNA structures and increase repeat deletion. This property makes them a target of interest in therapeutic drug design. We hypothesize that treatment with triplex stabilizing agents will stabilize triplex DNA and lead to deletion of the expanded (GAA)<sub>n</sub>•(TTC)<sub>n</sub> repeats in FRDA lymphoblast cells. Stable triplex DNA may cause deletions by normal cellular DNA repair mechanisms acting on triplex DNA or deletion associated with replication restart at triplex DNA, which can form a block to replication.

**Materials and Methods:** Plasmid pBR325 containing (GAA)<sub>n</sub>•(TTC)<sub>n</sub> repeats of varying lengths cloned into the chloramphenicol gene provides a genetic selection for repeat deletion in *E. coli*. The effects of DNA binding or intercalating agents, coralyne, neomycin, and 4-aza-tryptanthrin on cell survival and rates of repeat deletion were measured using standard assays. Agents identified to increase repeat deletion in *E. coli* were then tested in human cells. We are currently performing dose-dependent analysis in three FRDA lymphoblast cell lines from the same family, with two normal alleles (GM15851), a heterozygous line with one expanded allele (GM15849), and a homozygous line with two expanded alleles (GM15850). Cell lines are cultured in RPMI1640 supplemented with Gln, 15% FBS, 1% pen-strep at 37°C in 5% CO<sub>2</sub> for a defined number of generations. Cells are grown in the presence of varied concentrations of different DNA binding agents and samples are taken at different stages of growth for analysis of repeat length by polymerase chain reaction (PCR).

**Results and Discussion:** We have analyzed three compounds coralyne, neomycin, and tryptanthrin that can bind and stabilize triplex DNA. Using *E. coli*, we have shown that coralyne was most effective at increasing the rate of deletion of (GAA) repeats; neomycin showed a smaller increase, and 4-aza-tryptanthrin a minimal, if any, effect. One goal involves identification of concentrations of DNA binding agents that results in repeat deletion with minimal toxicity in cells. In normal and heterozygote FRDA cells, coralyne inhibited the growth rate to similar levels; with no effect on the normal-length alleles. Heterozygote cells treated with 0.5 µg/mL recovered in the absence of coralyne and continued to grow at a regular growth rate when washed and resuspended in fresh medium (Figure 1), while a homozygous cell line was more sensitive to coralyne. In initial dose dependent studies, neomycin selectively reduced the population of expanded alleles, resulting in deletions, with no effect on the normal length alleles.



(Figure 2). The length of a normal allele is ~599 bp. The heterogeneous distribution of expanded allele size is between 2500 and 3000 bp. Analysis of the effect of other triplex binding agents on reducing the length of expanded alleles is on-going. Another goal involves synthetic efforts to produce and evaluate compounds that can act as dual intercalator/groove triplex DNA binders. Groove binding moieties, such as polyamines, are being incorporated into the structures of coralyne and multiple tryptanthrin analogues to increase the degree of triplex stabilization and repeat deletion.

**Conclusion:** Current approaches to treating Friedreich Ataxia include a) improving mitochondrial function; b) increasing frataxin expression; and c) gene therapy. Although, numerous compounds are in varied stages of development and testing as candidate drugs, yet none are approved. No treatments exist for the genetic basis, DNA repeat expansion, of FRDA or many other repeat expansion neurological and neuromuscular disorders. Our novel potential first-generation therapeutic approach involving targeted deletion of expanded  $(GAA)_n \cdot (TTC)_n$  repeats should increase frataxin expression, restoring mitochondrial function, and prevent or delay the onset of FRDA. Targeted repeat deletion may provide a general genome editing approach that may delay or prevent the progression of other repeat expansion diseases at an early stage of development, e.g. Huntington Disease.

# Frataxin-deficient sensory neurons and cardiomyocytes are rescued by calcitriol, the active form of vitamin D

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 198

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*1. Universitat de Lleida, IRBLleida*

**Introduction:** Our group set up two validated cellular models of frataxin deficiency using neonatal rat primary cultures (cardiomyocytes and neurons from dorsal root ganglia-DRG) and lentivirus mediated RNA interference. FXN-deficient cardiomyocytes display disorganized mitochondrial network with evidence of mitochondrial swelling and increased presence of lipid droplets, suggesting impairment of lipid metabolism and/or of respiration [1] FXN-deficient DRGs present decreased mitochondrial membrane potential (MMP), altered calcium homeostasis and signs of apoptotic cell death [2]. Several evidences suggested that vitamin D could be able to protect FXN-deficient DRGs from neurodegeneration because of anti-apoptotic and neuroprotective effects of Vitamin D ( $1\alpha,25(\text{OH})_2\text{D}_3$  or calcitriol) [3,4]. Moreover, the last step in the synthesis of calcitriol depends on CYP27B1, a heme-containing mitochondrial enzyme that hydroxylates  $25\text{-OHD}_3$  (or calcidiol) to the active form  $1\alpha,25(\text{OH})_2\text{D}_3$  and whose expression is repressed by calcitriol. The activity of this cytochrome is dependent on its coupling with adrenodoxin (ferredoxin), an iron-sulfur protein, to transfer electrons from ferredoxin reductase to CYP27B1 [5] and described to interact with frataxin [6].

**Methods:** We tested the effect of calcitriol on FXN-deficient DRG neurons and FXN-deficient cardiomyocytes. Reduction of around 80% of frataxin levels in these cells was achieved by transduction with lentivirus containing shRNA silencing sequences.

**Results:** The results show that frataxin-depleted neurons show decreased cell viability, altered calcium homeostasis and markers of apoptotic cell death. When treated with calcitriol at 10 and 20 nanomolar, a-fodrin cleavage or neurite degeneration which are markers of apoptotic cell death were clearly improved. Also, using JC1 and Rodh5N staining we show that calcitriol restores mitochondrial membrane potential, and mitochondrial calcium level, respectively, to values displayed by control DRG neurons. Using calcidiol at same doses, effects were significantly reduced compared to calcitriol (**Fig. 1A and B**). As a complementary data, supplementing calcitriol (at 20 and 200 nanomolar) to FXN-deficient cardiomyocytes, lipid droplet accumulation and mitochondrial swelling it were both reduced to control levels.

Additionally, we observed a marked increase in CYP27B1 levels observed in frataxin-deficient cultures -thus suggesting low levels of  $1\alpha,25(\text{OH})_2\text{D}_3$ - were reverted to normal values by calcitriol treatment (**Fig. 2A**). Furthermore, frataxin-deficient neurons display decreased amounts of the iron/sulfur protein FDX1, supporting the hypothesis that calcitriol synthesis should be reduced in these cells. Of note, when these cells were supplemented with calcitriol, the normal levels of FDX1 were restored (**Fig. 2B**).

**Conclusion:** These results provide new data on how calcium and iron homeostasis could be altered in frataxin-deficient cells and that they open an easy therapeutic approach to be considered for patients with Friedreich Ataxia.

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**Fig. 1.** Cell survival and neurodegeneration analysis of 5-day-transfected DRGs treated with 20nM 1 $\alpha$ ,25(OH) $_2$ D $_3$  and vehicle, two days after lentivirus transduction. In **A**, the histogram shows the percentage of surviving DRGs treated compared to the vehicle. Survival is analyzed as n $^{\circ}$  of cells at 5 days compared to the initial value for each condition. In **B**, the histogram shows the decreased percentage of neurite degeneration using the treatments.

**Fig. 2.** Total lysates of 5-day-transfected DRGs treated with 20nM 1 $\alpha$ ,25(OH) $_2$ D $_3$  and vehicle were analysed by WB. In **A**, it was used CYP27B1 antibody and anti- $\beta$ -actin as a loading control. In **B**, it was used FDX1 antibody and Coomassie BB stained membrane as a loading control.

## Deconstructing the ketogenic diet to treat Friedreich's Ataxia

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 209

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Ketogenic diets (KD) have emerged as a promising therapeutic strategy to treat an array of neurological disorders. They have been used since the 1920's to treat pharmacoresistant paediatric epilepsy, but their therapeutic potential is currently being evaluated in other neurological conditions, such as Alzheimer's and Parkinson's diseases, as well as other neuromuscular disorders. KDs work by mimicking fasting, reducing drastically the levels of carbohydrates in the diet and replacing them by fat. Fat is then broken down into ketone bodies, which can feed brain cells as an alternative "energy fuel". Replacing carbohydrates by ketone bodies has a number of biological effects that have proven beneficial for the central nervous system. In fact, we consider that KDs could constitute a robust therapeutic approach to treat Friedreich's Ataxia (FA), caused by deficiency of the mitochondrial protein frataxin. However, despite their high potential, the extremely elevated content in fat of KDs (>80%) entails several undesired side effects, in addition to its poor nutritional value. For this reason, the KD is not well tolerated by many patients, which compromises compliance and therapeutic benefits.

Precisely, we have recently started a molecular dissection of the biological effects of the KD using a multidisciplinary approach. With this strategy, we expect to learn what molecular targets of the KD would be valuable to treat FA, so that we can take advantage of the specific benefits of the diet, while avoiding its systemic harmful effects. So far, we have identified mechanisms that instruct neurons to increase their utilization of ketone bodies, which could allow to reduce the severe dietary restrictions of the KD.

In addition, the KD does not only lead to a significant increase in circulating ketone bodies, but it also lowers glucose and alters the levels of several other metabolites. For this reason, we are investigating the extracellular and intracellular effects of ketone bodies, as well as other metabolites and extracellular cues derived from the KD (i.e. specific fasting-associated trophic factors, hormones, etc.).

We are at present working on gaining insight into the biological processes that are triggered by exposure to KD in healthy neurons. We will then test these effects on *in vitro* experimental models of Friedreich's Ataxia. Importantly, our preliminary results show that ketone bodies can affect several signalling networks in neurons. Moreover, we have observed that select manipulation of the metabolic profile in primary cells directly isolated from FA patients can increase frataxin expression. Altogether, these results support the interest in exploring the molecular underpinnings of the KD in FA. In the future, and upon identification of robust mechanisms that improve viability and mitochondrial function in frataxin-deficient neuronal cells, we will aim to test the most successful targets/mechanisms of the KD in experimental animal models of FA. In summary, we expect to be able to develop novel KD-based therapeutic strategies to treat FA.

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## Preclinical Translational Research Opportunities at the NINDS

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 213

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The National Institute of Neurological Disorders and Stroke (NINDS) offers a variety of programs that support the design, implementation, and management of research activities critical to translational challenges in the treatment of neurological disorders. These programs are open to all neurological and neuromuscular disorders, common and rare, that are under the scientific mission of the NINDS, including the spinocerebellar ataxias and Friedreich's ataxia.

The Innovation Grants to Nurture Initial Translational Efforts (IGNITE) Program supports early-stage translational efforts through a suite of funding opportunities to enable the research community to build on their innovative basic research findings and initiate preclinical drug discovery and development. The Blueprint Neurotherapeutics (BPN) Network supports small molecule drug discovery and development through cooperative agreement awards and Small Business Innovation Research (SBIR) fast-track awards. All BPN awards are designed to maintain the grantees' intellectual property while providing non-dilutive funding. The Cooperative Research to Enable and Advance Translational Enterprises (CREATE Bio) Program supports the discovery and development of therapeutic biotechnology products and biologics (e.g., peptides, proteins, antisense oligonucleotides, gene therapies, and cell therapies) through cooperative agreement awards and SBIR fast-track awards. Complementary to these therapy development programs, the NINDS biomarker program is focused on improving the quality and efficiency of neurotherapeutic clinical research toward Phase II, and beyond, by supporting rigorous biomarker validation through cooperative agreement awards.

Data on the NIH investment in ataxia research over the past five fiscal years is also being presented.

## Defining the promoter and cis-regulatory elements of the human FXN gene.

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 223

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Friedreich's ataxia is the most common inherited form of ataxia in humans. It is caused by severe downregulation of Frataxin (FXN) expression instigated by hyperexpansion of the GAA repeats located in intron 1 of the FXN gene. Despite numerous studies focused on identifying compounds capable of stimulating FXN expression, current knowledge regarding cis-regulatory elements involved in FXN gene expression is lacking. Using a combination of episomal and genome-integrated constructs, we defined a minimal endogenous promoter sequence required to efficiently drive FXN expression in human cells. Initially, we generated 13 constructs varying in length of the DNA sequences upstream and downstream of the ATG start codon. Using transient transfection, we evaluated the capability of these constructs to drive FXN expression. Subsequently, selected constructs containing the shortest DNA sequences capable of driving FXN expression were site-specifically integrated into the genome of HEK293 and human induced pluripotent stem cells (iPSCs). These analyses allowed us to identify ~ 250 bp region of the gene indispensable for frataxin expression. Minimal frataxin constructs (miniFXN) containing the defined regulatory region, five exons of FXN and a Flag tag were integrated in both orientations into the AAVS1 safe harbor locus in iPSCs. Expression analyses indicated that when integrated, our miniFXN constructs drove frataxin expression at a level similar to that of the endogenous locus. Expression of the miniFXN constructs persisted after the iPSCs were differentiated to neuronal and cardiac cells, indicating lineage independent function of the selected regulatory DNA sequences. Based on these results, we created AAV expression vectors (AAV9) and generated virions to test whether the endogenous sequences we defined could be used for controlled expression of frataxin in AAV infected cells. Initial results of miniFXN AAV9 testing in cellular models will be presented. Conclusions: Using several episomal and genome-integrated constructs, we defined a minimal FXN control region indispensable for efficient expression of the gene in a lineage-independent manner. Based on these data we generated and tested an AAV expression vector encoding FXN under the control of the endogenous promoter.

# A phenotypic epigenetic probe screen reveals the methyltransferase SUV4-20 as a therapeutic target for Friedreich's ataxia

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 224

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## Abstract

The molecular mechanisms associated with the reduced expression of the frataxin gene (*FXN*) in Friedreich's ataxia (FRDA) have been linked to the epigenetic modification of the *FXN* locus. It has been shown that expanded GAA repeats induce a repressive heterochromatin environment at the *FXN* locus, with accumulation of methylated histones H3K9me2/H3K9me3, H3K27me3 and H4K20me3. These posttranslational histone modifications have been shown to be enriched at silent heterochromatin, suggesting that the epigenetic changes reported are implicated in FRDA pathology. However, little is known about the contribution of histone methylation in the silencing of *FXN*.

Here, we identify that the methyltransferase SUV4-20 plays an important role in the regulation of expression of the frataxin gene. Using a human *FXN*-GAA-Luciferase repeat expansion reporter model of FRDA, we screened the epigenetic probe collection from the Structural Genomics Consortium (SGC). We found that chemical inhibition of the methyltransferase SUV4-20 increased the expression of frataxin protein (FXN) by approximately 1.5 fold in the reporter cell line. Direct down-regulation of SUV4-20 H1 and SUV4-20 H2 by siRNA identified SUV4-20 H1 as the main protein responsible for the up-regulation of FXN. Moreover, chemical inhibition of SUV4-20 also up-regulated FXN in the patient-derived fibroblast line GM04078, and in the lymphoblastoid cell lines GM016220 and GM15850 by approximately 1.5 fold.

We anticipate that the up-regulation of FXN in response to the inhibition of SUV4-20 by a lead compound requires greater efficacy. Therefore, we performed medicinal chemistry on the lead compound to try to achieve greater potency. Finally, considering that the epigenetic modification by a therapeutic compound may have genome-wide effects, we are currently performing RNA Sequencing to determine to what extent the chemical inhibition of SUV4-20 alters genome-wide expression.

Overall, our results suggest that histone methylation, particularly methylation of H4K20, may be important in the regulation of expression of *FXN*, and highlight SUV4-20 as a potential therapeutic target for FRDA.



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# Electrical stimulation to improve upper limb function in people with Friedreich ataxia.

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 282

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**Ms. Tennille Rowland<sup>1</sup>, Dr. John O'Sullivan<sup>2</sup>, Prof. Martin Delatycki<sup>3</sup>, Prof. Jennifer Strong<sup>4</sup>,  
Dr. Louise Corben<sup>3</sup>**

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**Introduction:** Friedreich ataxia (FRDA) typically has a detrimental effect on upper limb function, however there continues to be a paucity of novel motor interventions targeting improving function in individuals with FRDA. Electrical stimulation (ES) and functional electrical stimulation (FES) are interventions regularly utilized to improve function in like neurological conditions. However the efficacy of ES and FES to improve function in FRDA is unknown. This two site, pilot study aimed to evaluate the impact of six weeks of thrice weekly targeted ES and FES on upper limb function of individuals with FRDA.

**Methods:** Participants aged 18 and over, homozygous for a GAA repeat in intron 1 of *FXN* and with carer availability to assist with the programme, were recruited from the FRDA Clinics at Monash Medical Centre, Victoria, Australia and Royal Brisbane & Women's Hospital, Queensland, Australia. They were identified as appropriate to participate according to reduced upper limb strength, range and dexterity as apparent on clinical testing, having identifiable upper limb functional goals and clinical indication for ES. Participants received three weeks of home based ES followed by three weeks of FES (NeuroTrac™Sports CC0120, Verity Medical, UK) applied to the wrist and finger extensors of the dominant upper limb while undergoing specific functional upper limb activities as identified by the Goal Attainment Scale (GAS). The primary outcome was the score on the Box and Block Test (BBT). Secondary outcomes were the score on the GAS, the Nine Hole Peg Test (9HPT), Functional Independence Measure (FIM), Modified Tardieu Scale (MTS), passive and active range of motion, Friedreich Ataxia Impact Scale (FAIS) and the ABILHAND-NMD questionnaire. Clinical parameters including disease severity and results of genetic testing were also recorded.

**Results:** Eleven individuals (two male) with FRDA have participated in the study, one is still to complete the study. Participants had a mean disease duration of 26.8 years (SD: 10.6), an average FARS score of 116 (SD: 13.9) and only 2/11 could complete the 9HPT. Analysis of pre and post intervention data revealed a significant difference in the BBT score for the treated hand ( $t(9)=-5.35$ ,  $p<0.01$ ) whereas there was no difference in BBT scores in the untreated hand ( $t(9)=-0.58$ ,  $p=0.28$ ). In addition there was a significant difference in the GAS score pre and post treatment ( $t(9)=-6.41$ ,  $p<0.05$ ). Final analysis of the full cohort will be presented.

**Conclusion:** Analysis of BBT score pre and post intervention indicates ES and FES may improve upper limb function in some individuals with FRDA, in particular those individuals later in the disease process, for whom treatment options are limited. This preliminary pilot data provides the impetus for further randomized, controlled studies aimed at verifying the effect of ES and FES on improving upper limb functional capacity in individuals with FRDA.

**Acknowledgements:** Data collection at the Queensland site of this study was funded by the Royal Brisbane and Women's Hospital Foundation Grant, 2018.

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# SynTEF1 overrides repressive epigenetic marks at the Frataxin gene

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 291

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***Mr. Christopher Brandon*<sup>1</sup>, *Dr. Asfa Ali*<sup>1</sup>, *Ms. Sam Rider*<sup>1</sup>, *Ms. Sandra Kietlinska*<sup>1</sup>, *Dr. Mangesh Kaulage*<sup>1</sup>, *Dr. Aseem Ansari*<sup>1</sup>**

*1. University of Wisconsin, Madison*

Friedreich's Ataxia (FRDA) is caused by an (GAA)<sub>n</sub> expansion in the first intron of the *FXN* gene. The (GAA)<sub>n</sub> repeat causes silencing of the *FXN* gene by changing the epigenetic landscape, recruiting repressive histone marks to silence transcription. Synthetic Transcription Elongation Factor 1 (SynTEF1) targets this (GAA)<sub>n</sub> expansion and recruits the machinery necessary to activate transcription. SynTEF1 is effective at restoring Frataxin (Fxn) expression in Friedreich's Ataxia patient cells to normal levels. We will present our studies on the impact on SynTEF1 on this repressive epigenetic landscape.

## Impact of SynTEF1 on R-loop stability at microsatellite repeats at FXN

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 294

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***Dr. Mangesh Kaulage<sup>1</sup>, Dr. Asfa Ali<sup>1</sup>, Mr. Christopher Brandon<sup>1</sup>, Dr. Aseem Ansari<sup>1</sup>***

*1. University of Wisconsin, Madison*

Friedreich's ataxia (FRDA or FA) is an autosomal recessive progressive, neurodegenerative disorder caused by an abnormal expansion of (GAA)<sub>n</sub> repeats in the first intron of the frataxin (FXN) gene. The (GAA)<sub>n</sub> repeat expansion triggers R-loop mediated heterochromatinization which leads to frataxin gene silencing. Synthetic Transcription Elongation Factor 1 (SynTEF1) effectively restores the expression of frataxin in Friedreich's Ataxia patient cells by targeting (GAA)<sub>n</sub> repeats with its DNA binding domain and modulating transcription across repressive GAA repeats with the help of its effector domain. We will present our studies on the impact of SynTEF1 on R-loop mediated repression of FXN expression in patient derived cells.

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## CRISPR/Cas9 -based expanded GAA repeat deletion in Friedreich's Ataxia

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 295

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***Dr. Hari Padmanabhan*<sup>1</sup>, *Dr. Nick Pierson*<sup>1</sup>, *Dr. John Leonard*<sup>1</sup>, *Dr. Matthias John*<sup>1</sup>, *Dr. Marek Napierala*<sup>2</sup>, *Dr. Yanjie Li*<sup>2</sup>**

*1. CRISPR Therapeutics, 2. UAB*

Friedreich's Ataxia (FA) is caused frequently by an expansion of trinucleotide GAA repeats in the intron 1 of the Frataxin (*FXN*) gene. Repeat expansion reduces transcription of *FXN* and reduced *FXN* levels result in neurodegeneration and defects in multiple organs. Previous work has demonstrated that deletion of expanded repeats can restore the transcription of *FXN* and therefore is potentially a viable therapeutic strategy. Here we explore CRISPR/Cas9-based approach to identify gRNAs that efficiently delete expanded repeats in patient-derived induced pluripotent stem cell lines (iPSCs). We present results from a screen of various gRNA pairs that couple measurement of deletion efficiency with improvement in *FXN* mRNA and protein expression levels. Going forward, top gRNA pairs identified from this phenotype-based screening strategy will be tested in a mouse model of Friedreich's Ataxia for restoration of *FXN* expression in vivo.

## AI-powered drug repurposing for rare diseases

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 307

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***Dr. Phil Brownjohn*** <sup>1</sup>

*1. Healx, Cambridge, MA*

Friedreich's ataxia is a rare, autosomal recessive condition, which results in neurological and non-neurological impairments, and is currently without a disease-modifying treatment. To address this challenge, Healx, a biotech company focused on accelerating the discovery and development of treatments for rare diseases, is employing its AI-powered drug repurposing platform to deliver new therapeutic candidates for this area of great unmet need. To date, the platform has delivered a pipeline of candidates across a number of disease areas, with one of the most advanced projects, targeting fragile X syndrome, now approaching Phase 2a clinical trials. The process is scalable and hypothesis-free, integrating patient insight, multi-omic data and drug discovery expertise to identify novel treatment opportunities for rare diseases such as Friedreich's ataxia and other ataxias where limited treatment options exist.

# Fumarates rescue frataxin expression and enhance mitochondrial and aconitase enzyme activity in hearts of FXN<sup>KD</sup> mice.

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 305

***Dr. Elena Dedkova<sup>1</sup>, Ms. Claire B. Montgomery<sup>1</sup>, Mr. Chun Kiu Hui<sup>1</sup>, Dr. Alexey A. Tomilov<sup>1</sup>, Prof. Gino Cortopassi<sup>1</sup>***

*1. UC Davis*

**Background.** Friedreich ataxia (FA) is a monogenic recessive ataxia caused by reduction of a single mitochondrial protein, frataxin (FXN), for which there is no approved therapy. Although the name of FA refers to the neurodegenerative ataxia, the lethal event cardiomyopathy, caused by deficient FXN expression in the heart. Here we show that optimally dosed fumarates increase FXN in cardiac tissues of the FXN<sup>KD</sup> mice. FXN's only physiologically proven biochemical function is iron-sulfur cluster biogenesis, and the usual surrogate for frataxin activity is increased activity of the iron-sulfur cluster enzyme aconitase. Through a drug discovery screening program we identified bioactive fumarates as protective in FA cell models and animals. We have subsequently carried out dose-ranging experiments that demonstrate optimal fumarate dosing in mice. Our latest breaking result is that optimally-dosed fumarates, DMF, increase FXN protein, and mitochondrial gene expression and cardiac function in the hearts of the FXN<sup>KD</sup> mouse model. In addition, these fumarates increase mitochondrial enzyme activities, including aconitase and mitochondrial enzyme activity. These data suggest that fumarate therapy could be cardioprotective in Friedreich's ataxia.

**Methods.** To suppress FXN expression, FXN<sup>KD</sup> mice were fed doxycycline chow (Dox) chow and cardiac function was examined by echocardiography. Aconitase activity, used as a surrogate measure of frataxin's iron-sulfur biogenesis function, complex IV activity and fumarate levels were measured by biochemical assays. Frataxin expression and mitochondrial biogenesis were examined by Western Blot and qPCR, respectively.

**Results. Optimal dosing of DMF.** FXN<sup>KD</sup> and CTL mice were given either vehicle or DMF at 110 mg/kg daily for 3 weeks in peanut butter (vehicle) tabs. This dose of DMF was chosen based on dose-escalation experiments where C57BL/6 mice were fed DMF with a daily dose ranging from 40 mg/kg to 160 mg/kg revealing that 110 mg/kg DMF dose led to maximal upregulation in FXN levels and aconitase activity without toxicity. **Optimally dosed DMF rescues FXN expression, mitochondrial and cardiac function.** We demonstrated that FXN expression and activity of mitochondrial aconitase, complex IV in the heart were significantly decreased in FXN<sup>KD</sup> mice fed dox chow and peanut butter. However FXN<sup>KD</sup> dox mice treated with DMF showed improved cardiac function which correlated with upregulation in FXN expression levels, fumarate content, aconitase and complex IV activities. Dose-dependent increase in mitochondrial biogenesis was also observed, though to a lesser extent. **Frataxin levels decline in FXN<sup>KD</sup> blood and recover with DMF.** Furthermore, we showed that FXN levels decline in FXN<sup>KD</sup> blood in a DOX-dependent way, and FXN levels were rescued in DMF-dosed mice PBMCs. As multiple drugs that used in FA clinical trials do not increase blood FXN, this suggests DMF is unique.

**Conclusions.** These results demonstrate that oral DMF rescues frataxin expression, improves cardiac function, aconitase activity and mitochondrial complex IV activity in FXN<sup>KD</sup> mice. Because cardiomyopathy is the usual lethal event in FA, we suggest that an FDA-approved drug that increases cardiac frataxin could have an important impact on FA mortality, and our data suggest that the drug's effect can be monitored non-invasively in blood.

## **AAV9-mediated frataxin gene replacement reverses cardiac disease features in a conditional knockout mouse model of Friedreich's ataxia**

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 303

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***Dr. Chia-Yen Wu*<sup>1</sup>, *Dr. Barbara Perez*<sup>2</sup>, *Dr. Manuela Corti*<sup>2</sup>, *Dr. Barry Byrne*<sup>2</sup>, *Dr. Cat Lutz*<sup>1</sup>**

*1. The Jackson Laboratory, 2. University of Florida*

Friedreich's ataxia (FA) is the most common form of hereditary ataxias. It is caused by an inherited autosomal recessive expansion of GAA repeats in the promoter region of *frataxin* (*FXN*), resulting in reduced levels of the mitochondrial protein. Hallmark features of the disease include cardiomyopathy and an uncoordinated ataxic gait. There is currently no approved treatment although AAV-mediated gene replacement therapy is a promising strategy. Although many FA mouse models have been developed, most are unsuitable for preclinical studies of gene replacement therapy due to phenotypes too mild to measure. Here, we use a novel conditional knockout mouse model of FA. Mice with floxed exon 2 of the *FXN* gene are crossed with Ckmm-Cre expressing mice, resulting in Cre-mediated deletion of frataxin in heart and skeletal muscle. These mice recapitulate FA cardiac abnormalities on echocardiogram such as decreases in percent ejection fraction and stroke volume as well as hypertrophy. Additionally, mutant mice exhibit peak body weight between 7-8 weeks of age and require euthanasia by 10.5 weeks of age. To evaluate the effect of AAV9-mediated human FXN (hFXN) gene replacement, mice were given a single intravenous dose of AAV9-CBA-hFXN ( $6 \times 10^{10}$  vg) on postnatal day 0-2. AAV9-treated animals had similar body weight to wild type littermates, improvements in echocardiogram parameters such as percent fraction shortening, cardiac output, and stroke volume. Importantly, a single dose of AAV9 was able to extend the lifespan of these animals beyond that of untreated littermates. Additional experiments aim to characterize the percent recovery of frataxin protein levels and functional recovery in heart tissue and the effect of dosing animals after symptom onset. These studies support further development of AAV9-mediated frataxin gene replacement as a strategy for the treatment of FA.

# Novel Nrf2-Inducer Prevents Mitochondrial Defects and Oxidative Stress in Friedreich's Ataxia Models.

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 258

***Dr. Rosella Abeti*<sup>1</sup>, *Ms. Annalisa Baccaro*<sup>1</sup>, *Dr. Noemi Esteras*<sup>2</sup>, *Prof. Paola Giunti*<sup>3</sup>**

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**Background.** Friedreich's Ataxia (FRDA) is an autosomal recessive neurodegenerative disorder, affecting dorsal root ganglia (DRG), cerebellar dentate nuclei and heart. It is caused by a GAA repeat expansion mutation within the frataxin gene (FXN). This impedes FXN transcription resulting in a progressive decrease of the mitochondrial protein, frataxin. Increased oxidative stress leading to a chronic depletion of endogenous antioxidants affects the survival of the cells and causes neurodegeneration. In particular, cerebellar granule neurons (CGNs) show a significant increase of reactive oxygen species (ROS), lipid peroxidation and lower level of reduced glutathione (GSH). In FRDA, one of the major pathways of oxidant scavengers, the Nrf2 antioxidant pathway, is defective. Previous studies on FRDA-like CGNs showed that the reduced level of frataxin and the oxidative stress induce mitochondrial impairments. By triggering the Nrf2 endogenous pathway pharmacologically we determined whether this could promote mitochondrial fitness and counteract oxidative stress. **Methods.** In this work, we sought to investigate the beneficial effect of a promising Nrf2-inducer, omaveloxolone (omav), in CGNs from two FRDA mouse models, KIKO and YG8R, and human fibroblasts from patients. By using confocal microscopy we measured oxidative stress and mitochondrial health with and without treatments. **Results.** We found that CGNs from both KIKO and YG8R presented Complex I deficiency and that omav was able to restore substrate availability and Complex I activity. This was also confirmed in human primary fibroblasts from FRDA patients. Although fibroblasts are not the major tissue affected, we found that they show significant differences recapitulating the disease; this is therefore an important tool to investigate patients' pathophysiology. Interestingly, we found that patient fibroblasts had an increased level of endogenous lipid peroxidation and mitochondrial ROS (mROS), and lower GSH at rest. Omav was able to reverse this phenotype, protecting the cells against oxidative stress. By stimulating the cells with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and looking for potential mitochondrial pathophysiology, we found that fibroblasts could not maintain their mitochondrial membrane potential ( $\Delta\Psi_m$ ). Remarkably, omav was protective to mitochondrial depolarization, promoting mitochondrial respiration and preventing cell death. Our results show that omav promotes Complex I activity and protect cells from oxidative stress.

**Conclusion.** Our results show that omav promotes Complex I activity and protect cells from oxidative stress. We demonstrate that ROS and lipid peroxidation were reduced in FRDA models and mitochondrial membrane potential was restored. **Acknowledgements:** We would like to thank the patients for participating in the study, REATA Pharmaceuticals Ltd., for providing us with the compound (Omaveloxolone; omav) and for supporting our research. Friedreich's Ataxia Research Alliance (FARA) and GoFar for supporting RA.



## A drug combination rescues frataxin-dependent neural and cardiac pathophysiology in FA models.

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 257

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***Dr. Rosella Abeti*<sup>1</sup>, *Dr. Mittal Jasoliya*<sup>2</sup>, *Dr. Mark Pook*<sup>3</sup>, *Mr. Chun Kiu Hui*<sup>2</sup>, *Prof. Gino Cortopassi*<sup>2</sup>,  
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**Background.** Friedreich's ataxia (FA) is an inherited neuro- and cardio-degenerative disease that is ultimately lethal, and for which there is no FDA/EMA-approved therapy. Multiple clinical trials are ongoing, but these treatments may or may not turn out to be effective in FA patients. With the aim of increasing frataxin level, we took a combinatorial approach to screen drugs which have been already proposed for FA. 8 single drug molecules were administered to FA patient cells, and effects on frataxin induction and mitochondrial biogenesis measured: Dimethylfumarate (DMF), Methylene Blue (MB), HDAC109, Epicatechin, Resveratrol (RESV), Betamethosone, Hemin, Nicotinamide. Actives in either assay were then combined in sets of 2 (drug pair) at effective concentrations, to determine whether combinations promoted greater effect than single drug alone. Then, the most effective drug pairs were administered to primary cultures of FA mouse models.

**Methods.** For drug screening frataxin induction and mitochondrial biogenesis were measured in FA patient cells by QRT-PCR and mtDNA/nDNA ratio by QPCR. For physiological measurements, membrane potential, free radicals generation and lipid peroxidation were measured. We then evaluated the beneficial effect on the physiological parameters, described above, in neuronal and cardiac cells from FA animal models, before and after treatments with DMF and RESV.

**Results.** Single-drug testing highlighted DMF, MB, and Resveratrol for frataxin induction and for mitochondrial biogenesis. Paired-drug testing indicated that the drug pair DMF-RESV was the most effective in terms of FXN and mitobiogenesis increase. Thus further physiological testing was carried out with DMF-RESV or DMF. This combination improved physiological functions and reduced mitochondrial reactive oxygen species (ROS) generation in cardiomyocytes and neurons ex vivo. Moreover, the combination of DMF and RESV improved Rotarod performance in the FA mouse model tested. Overall our data suggest that DMF is effective as a single agent, and the addition of RESV provides additional in some assays, but not as consistently as DMF alone. Lastly, the DMF-RESV combination was not toxic to cells. Dose escalation experiments of DMF in mice provide dose-dependent escalation of frataxin and other mitochondrial genes in the absence of toxicity.

**Conclusion.** From our results we can conclude that DMF is proven to be a valuable compound to counteract FA pathophysiology. Further studies will help to understand whether a combined therapeutic strategy could be even more effective on the FA phenotype.

# Friedreich's ataxia neuronal progenitor cells (NPCs) containing an endogenous FXN-luciferase fusion gene as a novel screening platform

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 220

***Dr. Yanjie Li<sup>1</sup>, Dr. Jill Napierala<sup>1</sup>, Dr. Marek Napierala<sup>1</sup>***

*1. University of Alabama at Birmingham*

Friedreich's ataxia (FRDA) is a severe neurodegenerative disease caused by transcriptional repression induced by expanded GAA repeats located in intron 1 of the FXN gene. Rescuing defective transcription of this gene remains a primary goal of potential therapeutic intervention. Thus far a limited number of compounds demonstrated modest stimulation of FXN transcription, however, to date, none of them effectively increased FXN levels in patient clinical trials. In addition, high throughput drug screening (HTS) campaigns using various reporter or model systems failed to identify robust drug candidates capable of boosting FXN transcription. Lack of appropriate reporter systems: (i) derived from patient cells, (ii) representing pathology-relevant cells, and (iii) based on expression of the endogenous FXN gene containing expanded GAAs, are likely to contribute significantly to the limited success of these types of studies to date. Therefore, we decided to utilize FRDA induced pluripotent stem cells (iPSC) to create an endogenous FXN reporter system. To generate and validate a reporter system compatible with HTS and based on disease relevant cells, we reprogrammed FRDA patient fibroblasts to iPSCs. Subsequently, using a CRISPR/Cas9 approach, we engineered an in-frame fusion between the FXN gene and a small luciferase gene (NanoLuciferase, NLuc). Consequently, NLuc is expressed from the endogenous FXN locus and this reporter expression was inhibited by expanded GAA repeats. To validate the platform for HTS, we differentiated the iPSCs into neuronal progenitor cells (NPCs). These cells are early in neuronal commitment and express Sox1, Pax6 and Nestin (i.e. markers of neuronal progenitors). In addition, these cells can be cryopreserved and are capable of proliferation for several passages, thus allowing enough cells and sufficient time for drug screening and expression studies. The FXN-NLuc NPCs were validated using HDAC inhibitor 109 shown previously in different model systems to increase FXN transcription. Treatment with HDACi 109 increased the luminescence signal in FXN-NLuc cells in a concentration-dependent manner. Currently our new HTS screening platform is being tested using a reference library of epigenetic modulators. Conclusions: We established an iPSC-derived, cell model of monolayer NPCs containing one of the FXN alleles tagged at the 3' end with the entire NLuc coding sequence. The luminescence signal as well as expression of the FXN – NLuc fusion protein were affected by the presence of the expanded GAA repeats and could be stimulated by HDACi 109. This new and sensitive reporter cell system can be utilized in HTS campaigns to uncover novel, robust regulators of endogenous FXN expression.

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## Exploring the Patient Experience in Friedreich Ataxia (FRDA) through Qualitative Interviews

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 205

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***Dr. Ankit Shah<sup>1</sup>, Ms. Zaneta Balantac<sup>2</sup>, Dr. Rohini Sen<sup>1</sup>, Dr. Deborah Hoffman<sup>1</sup>, Ms. Anne Skalicky<sup>2</sup>, Dr. Hao Wang<sup>1</sup>, Dr. Colleen McHorney<sup>2</sup>***

*1. Takeda Pharmaceuticals International, Inc., Cambridge, MA, 2. Evidera, Bethesda, MD*

**INTRODUCTION:** Friedreich Ataxia (FRDA) is a hereditary, degenerative rare disorder that leads to loss of coordination, loss of muscle strength, and motor incapacitation and can be associated with numerous functional impairments (Akbar 2015). Currently, there is no approved pharmacological treatment for FRDA. Takeda Pharmaceuticals conducted a Phase 2 study with a D-amino acid oxidase (DAAO) inhibitor (TAK-831) for the treatment of FRDA. An exit interview study was conducted as part of the Phase 2 trial with the following two objectives: (1) to characterize the overall patient experience with FRDA, focusing on symptoms and functional impacts that are most meaningful to patients, and (2) to describe patient-perceived changes in symptoms and impacts (particularly on upper extremities and motor function) due to the study treatments.

**METHODS:** Semi-structured, qualitative exit interviews were conducted as part of a 12-week Phase 2 clinical trial evaluating the efficacy and safety of multiple dose levels of TAK-831 in adult patients with FRDA (2:1:2 ratio for placebo, 75 mg bid, and 300 mg bid of TAK-831). One exit interview was conducted per subject over the telephone within seven days of completion of the last dosing visit or within seven days of notification of early termination. Interviewers were blinded to the subjects' treatment group. Interviews were approximately 60 minutes in duration and were audio recorded. For the analysis purpose, a coding framework, based on the key research objectives and interview discussion guide topics was developed. These codes were applied to concepts in the interview transcripts using ATLAS.ti and resulting concept-frequency tables were created by tabulating the number of patients experiencing the coded concepts.

**RESULTS:** A total of 65 patients (placebo: 27, TAK-831 (75 mg bid): 12 and TAK-831 (300 mg bid): 26) were interviewed. Overall, 57% of patients were female with a mean (SD) age of 31.25 (10.25) years. A majority of the patients enrolled in the trial reported experiencing symptoms related to FRDA such as difficulties with walking or unsteady gait (100%), lack of balance and coordination (100%), inability to control movement in upper extremities (92%), speech difficulties (89%), fatigue (88%), and inability to control movement in the lower extremities (85%). The most commonly-reported functional impacts included the ability to perform household chores (91%), ability to stand unassisted (89%), ability to engage in physical activity (88%), ability to continue in school or work (74%), need to depend on others to carry out regular activities (69%), and ability to drive (69%). While discussing the different impacts of FRDA, patients also mentioned the burden FRDA had on their emotional well-being such as feeling embarrassed for losing balance or using an assist device and feeling miserable or frustrated at being tired all the time. In terms of expectations from treatment in the trial, patients expected improvement in: ability to control movement in upper extremities (66%), balance or coordination (45%), walking or gait (42%), upper-extremity function (41%), speaking clearly (25%), and energy (23%).

Overall, two third of the patients in the trial reported that both TAK-831 and placebo did not help in the management of their FRDA symptoms. There was no difference in the patient perceived changes in the symptoms and functional impacts between the patients receiving TAK-831 and placebo. Thirty-seven percent (37%) of the patients reported an improvement in their upper-extremity motor function and manual dexterity symptoms during the trial. Patients reported they could perform specific activities better, more quickly, and more easily. This included improvements in

eating and food preparation handwriting, brushing their teeth and doing their hair and/or makeup, picking objects up, improved ability to type and using a mouse.

**CONCLUSIONS:** Most patients enrolled in the trial reported experiencing symptoms of FRDA and impacts on their daily living. A majority of the patients reported no improvement in symptoms and functional impacts over the course of the trial which highlights the unmet need among patients. Additionally, no patient reported differences were found between TAK-831 and placebo arms which is consistent with the clinical trial findings. Post-trial interviews provide a unique insight into patient experiences and the opportunity to evaluate patient perception of the same changes as those that are measured with clinical trial endpoints.

## Genome-wide RNAi screen to identify modifiers of transcriptional silencing mediated by the GAA repeat expansion using a *Drosophila* cell-based assay.

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 208

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**Dr. Juan Antonio Navarro Langa**<sup>1</sup>, **Dr. Pablo Calap**<sup>2</sup>, **Dr. Michele Lufino**<sup>3</sup>, **Mr. Enrique Gabriel Zucchet**<sup>2</sup>, **Prof. Stephan Schneuwly**<sup>1</sup>, **Prof. Richard Wade-Martins**<sup>3</sup>, **Dr. Jose Vicente Llorens**<sup>2</sup>, **Prof. María Dolores Moltó**<sup>2</sup>

*1. Universität Regensburg, 2. University of Valencia, 3. Department of Physiology, Anatomy and Genetics, University of Oxford*

Friedreich's ataxia (FRDA) is a neurodegenerative disease caused by an insufficient synthesis of frataxin, an essential and highly evolutionary conserved protein. In the majority of FRDA patients, frataxin deficiency is due to expansion mutation of a GAA repeat in the *FXN* gene that encodes this protein. Pathological GAA expansions show variable size between 66 to >1000 repeats, and can form unusual DNA structures that have the potential to repress the *FXN* expression.

We set up a *Drosophila* cell-based assay to identify genes that may be able to modify the unusual DNA structures associated to the GAA expansion, and thereby to increase frataxin expression. The experimental strategy was based on the use of two cell lines containing a reporter gene (firefly luciferase), under the effect of  $\approx 300$  GAA repeats (the FRDA model cells) or 9 GAA repeats (the control cells). To normalize luminescence measures of firefly luciferase, the two cell lines also express the Renilla luciferase. The experimental design was based on the pACMAN methodology [1] and luciferase expression was controlled by the GAL4-UAS system [2] using a GAL4 driver inducible by copper. Model cells showed 2.5-fold lower luciferase activity (measured as the ratio between firefly luciferase and renilla luciferase luminescence) than the control cells at 1mM CuSO<sub>4</sub> that was the working concentration established for copper.

Next, we interrogated the effect of knocking down the expression of about 13,900 *Drosophila* genes on the luciferase activity of the FRDA model *Drosophila* cells. To do this, we used the DRSC 2.0 library (Harvard Medical School), a collection of double-stranded RNAs (dsRNAs); each one of these dsRNA has the ability to silence a target fly gene through the specific destruction of that gene's mRNA. To narrow the number of candidate hits, a normalized value was assigned to each dsRNA (or well) based on Z-score (a measure of distance in standard deviations), and a series of filtration steps was applied. We removed dsRNA with potential off-target effects and those which genes are more likely not related to the biological question contemplated in our screen. Next, we concentrated on those wells showing Z-score values  $\geq 3$  or  $\leq -2$ . Finally, we selected genes that have human orthologous. Forty-seven genes conform to all these criteria and constitute the final list of potential hits.

The candidate hits are involved in histone modifications, chromatin remodeling, transcriptional regulation and DNA repair. Few hits code for uncharacterized proteins that could be new players of the transcriptional repression mediated by the GAA repeat expansion. These results show a set of *Drosophila* genes that can increase the luciferase expression in the FRDA model cells. The human orthologous of the fly genes are candidates to increase the frataxin level and thereby to the discovery of new therapeutic approaches in FRDA.

This work was supported by FARA and ATAXIA Ireland

## DMF dose-dependently induces frataxin, mitochondrial gene expression and aconitase activity

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 120

**Mr. Chun Kiu Hui<sup>1</sup>, Dr. Sandipan Datta<sup>1</sup>, Dr. Mittal Jasoliya<sup>1</sup>, Dr. Elena Dedkova<sup>1</sup>, Prof. Gino Cortopassi**

<sup>1</sup>

1. UC Davis

**Background.** Friedreich's ataxia (FA) is an inherited neuro- and cardio-degenerative disease that is ultimately lethal, and for which there is no current FDA/EMA-approved therapy. For example, DMF=Dimethyl fumarate is a prodrug of the biologically active Monomethyl fumarate (MMF), and MMF has been shown to engage both Nrf2 and HCA2 drug targets to elicit its pharmacodynamic benefits. We previously demonstrated that DMF dose-dependently increases frataxin and mitochondrial functions in FA cells, and mice, and humans (Hayashi, 2015; Hayashi 2017, Jasoliya 2019), and that mitochondrial number is decreased in Friedreich's cells, mice and humans (Jasoliya, 2017).

**Methods.** Frataxin expression and mitochondrial gene expression and mitochondrial biogenesis were measured in multiple mouse tissues by Western Blot, QRT-PCR and QPCR. Aconitase activity, which is often used as a surrogate measure of frataxin's iron-sulfur biogenesis function, was measured by activity stain. COXIV activity was also measured by activity stain in tissues. As MMF is metabolized to fumarate, fumarate levels in tissues were measured by ELISA as a measure of MMF delivery to heart, muscle and brain tissues.

**Results.** Dose-escalation experiments were carried out with DMF in C57BL6 mice, by intraperitoneal and oral exposure (gavage and peanut butter tabs). Dose ranges were 10mg/kg to 320 mg/kg, which because of the mouse's 12-fold faster metabolism overlap the human equivalent doses of 10mg/kg DMF currently EMA-approved for human dosing, i.e. 720mg/day. A dose-dependent increase in frataxin expression and mitochondrial COX4 and Ndufs5 expression was observed in heart and brain and liver tissues from 100% to 400% of baseline in C57BL6/J mice, and dose-dependent increases in aconitase activity and COXIV activity were also observed. Dose-dependent increase in mitochondrial biogenesis was also observed, though to a lesser extent.

**Conclusion.** These results demonstrate that oral DMF dose-dependently induces frataxin expression, mitochondrial gene expression, aconitase activity and mitochondrial biogenesis in C57BL6 mice. Furthermore, the 120mg/kg maximally effective dose of DMF in mice overlaps the human equivalent dose of 10mg/kg that is currently approved for human dosing, i.e. 720mg/day. Thus, the same pharmaceutical ingredient, DMF, that has been dosed at 480mg/day (as Tecfidera) for Multiple Sclerosis, and at up to 720mg/day (as Skilarence) for psoriasis in more than 200,000 humans worldwide, is effective in raising frataxin and other mitochondrial proteins at the same bioequivalent dose in mice.

## **Delivery of an AAV coding for a shRNA against the frataxin mRNA increased the severity of the phenotype of the YG8sR mouse model of Friedreich ataxia but the phenotype was improved by the delivery of the frataxin gene with another AAV.**

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 124

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***Dr. Catherine Gérard<sup>1</sup>, Dr. Nathalie Majeau<sup>1</sup>, Mr. Pouire Yameogo<sup>1</sup>, Prof. Jacques P. Tremblay<sup>1</sup>***

*1. Université Laval*

Friedreich ataxia is a neurodegenerative progressive disease due to an insufficient quantity of frataxin protein in the tissues. Dr. Pook's group developed the YG8sR mouse model of Friedreich ataxia mouse and the Y47 control mouse. Both mice have no functional mouse frataxin gene but contain a human transgene, the first one from a Friedreich patient and the second from a normal subject. Although the YG8sR mouse produces less frataxin than the Y47 mouse, the phenotype of this YG8sR mouse does not reflect a severe neurodegenerative phenotype. To increase the phenotype severity, shRNAs against the human frataxin mRNA were delivered using an AAV by intravenous injection to the mice. These AAVs containing the shRNA against the frataxin also contained a mCherry gene to permit an easy detection of the AAV infection in different tissues. Different shRNAs were produced a significant reduction of frataxin expression in the tissues of YG8sR and of Y47 mice but also a more clear progressive phenotype characterized with different behavior tests. Indeed, the mice treated with the shRNA were less active in a parallel rod floor (less distance travelled, average speed lower, time freezing increased) and they had a real difficulty to cross narrow beams. These mice also had lower muscle strength and as indicated by a reduced capacity to remain hanging on a wire net (hanging test). To restore the normal quantity of frataxin in the tissues, a second AAV coding for the human frataxin gene (hFXN) was also injected. The injection of the AAV-FXN in the mice also treated with the AAV-shRNA restored the normal quantity of frataxin in the tissues and significantly improved the phenotype of these mice. Indeed, the mice hanged longer, crossed the beams more easily and were more active. The delivery of a shRNA is thus a could method to improve the severity of the YG8sR mouse model and the phenotype of this improved mouse model may be corrected by the delivery the frataxin gene with an AAV. The delivery of the frataxin gene by an AAV is thus a possible treatment for Friedreich ataxia as indicated by previous reports.



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## Modeling cardiomyopathy in Friedreich ataxia with patient-derived cardiomyocytes

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 77

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**Dr. Chicheng Sun<sup>1</sup>, Ms. Deena Qadir<sup>1</sup>, Ms. Vivian Villegas<sup>1</sup>, Dr. Owen Wallace<sup>1</sup>, Dr. John Graef<sup>1</sup>**

**1. Fulcrum Therapeutics**

Friedreich Ataxia (FA) is the most commonly inherited ataxia and is caused by a GAA repeat expansion in the intron of the frataxin gene that results in reduced expression. Heart conditions are prevalent in patients with FA, severely affecting the quality of life and shortening life span. There is currently no treatment for FA, therefore developing small molecule therapeutics that increase frataxin expression is significantly needed. In this study, we aimed to establish an *in vitro* model of FA cardiomyopathy and to develop high-throughput assays for frataxin detection. To this end, we differentiated iPSCs obtained from patients with FA into cardiomyocytes and demonstrated that they express cardiac genes, contract spontaneously, and respond to chronotropic drugs (e.g., isoproterenol and hERG channel blockers). High throughput assays to measure frataxin mRNA and protein levels were established and demonstrated reduced expression of frataxin in FA cardiomyocytes (~20% of healthy control levels). Functional interrogation of FA cardiomyocytes was performed by microelectrode array recordings, plate-based calcium imaging (FLIPR-Tetra), and the Seahorse XF platform. Our data show significant reductions in field potential amplitude, spontaneous calcium transient amplitude, and oxygen consumption rates in FA cardiomyocytes when compared to healthy controls. Such functional deficits in FA cardiomyocytes were phenocopied by antisense oligonucleotide-mediated knockdown of frataxin in healthy controls. In summary, we have established an *in vitro* system to study FA cardiomyopathy with patient-derived cardiomyocytes that could be leveraged to perform chemical probe and genetic knockout screenings for the purpose of identifying novel targets and initiating drug discovery efforts to treat FA.



**Topic: Genetics of Disease**

## SETting the stage in Friedreich's Ataxia. The 'substrate hypothesis' for repetitive DNA gene silencing.

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 277

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***Dr. Sathiji Nageshwaran<sup>1</sup>, Prof. George Church<sup>1</sup>***

*1. Harvard Medical School*

The (GAA)<sub>n</sub> expansion within intron 1 of the frataxin gene causes Friedreich's Ataxia. Epigenetic mechanisms are implicated in gene silencing; DNA secondary structure (non-B DNA conformation and 'sticky' DNA) and transcription-dependent (R-loop, antisense transcription). A connection between length of repeat DNA and extent of gene silencing has not yet been explained. We hypothesise that the GAA tract acts as a 'substrate' on which proteins with GAA affinity can bind, such as zinc finger proteins. We highlight a possible silencing mechanism involving the euchromatic histone lysine methyltransferase SETDB1, known for developmental gene regulation as well as local and distant genome topological organisation, and associated co-repressor complex. We also explain the uncommon chromatin signature and a potential unexplored therapeutic avenue.

## Novel genetic modifiers of somatic CAG repeat instability

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 263

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***Dr. Ricardo Mouro Pinto***<sup>1</sup>

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Ryan Murtha<sup>1</sup>, António Azevedo<sup>1</sup>, Marina Kovalenko<sup>1</sup>, Marissa Andrew<sup>1</sup>, Feng Zhang<sup>2</sup>, Jong-Min Lee<sup>1,3</sup>, Vanessa Wheeler<sup>1,3</sup> and Ricardo Mouro Pinto<sup>1,2,3</sup>

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Identification of genetic modifiers of somatic CAG repeat instability by in vivo CRISPR-Cas9 genome editing  
Polyglutamine diseases are a group of devastating neurodegenerative disorders caused by the expansion of CAG repeats. Such diseases include numerous Spinocerebellar Ataxias (SCAs), Spinal and Bulbar Muscular Atrophy, and Huntington's disease (HD). In HD, somatic expansion of the CAG repeat occurs in a time-dependent and tissue/cell-type specific manner, with high levels in striatum and liver, and is critically dependent on components of the DNA mismatch repair machinery. In HD patients, somatic CAG expansions in the brain are inversely correlated with age of motor onset. Further, in HD and multiple SCAs, recent genetic studies have implicated DNA repair genes as modifiers of disease onset and progression. These data indicate that somatic CAG expansion is a critical disease driver and that therapeutic targeting of this process will either prevent onset or slow the disease course.

Here, with the goal of dissecting the mechanism(s) underlying somatic CAG repeat expansion we report the development of a CRISPR/Cas9-based platform to identify novel instability modifiers *in vivo*. To this end, we generated HD mice (*HttQ111*) that endogenously express Cas9, and targeted known modifier genes, that either enhance or suppress somatic CAG expansion based on genetic knockout studies. Following a single tail vein injection of AAV8 or PHP.B-based viruses carrying sgRNAs against the gene of interest, we confirmed strong liver and brain transduction, detected a high frequency of inactivating mutations at target sites, and successfully suppressed or hastened somatic CAG expansions in HD mice. We also report on using this CRISPR/Cas9-based platform to: 1) investigate new candidate genes identified as part of a large HD age at onset Genome Wide Association Study (GWAS), as potential modifiers of CAG instability; 2) probe more broadly the role of other candidate DNA repair genes in this disease-relevant process; and 3) obtain a better understanding of gene-gene interactions and dependencies. Together, this strategy has already resulted in the successful identification of novel modifiers of the somatic CAG expansion process, as well as providing significant insight into mechanisms of somatic CAG instability and novel targets for therapeutic intervention.

# A CRISPR Knock-Out Genome-Wide Screen identifies novel potential therapeutic targets for the treatment of Friedreich's Ataxia

Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 251

**Dr. Gabriele Corda<sup>1</sup>, Dr. Irene del Molino del Barrio<sup>1</sup>, Mr. Peter Kilfeather<sup>1</sup>, Dr. Gabriela Vilema-Enriquez<sup>1</sup>, Dr. Phillippa Carling<sup>1</sup>, Dr. Saba Saqlain<sup>2</sup>, Mr. Armin Hadzic<sup>1</sup>, Dr. Michele Lufino<sup>2</sup>, Dr. Joseph Nabhan<sup>3</sup>, Dr. Alain Martelli<sup>3</sup>, Prof. Richard Wade-Martins<sup>1</sup>**

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Corda G.1, Del Molino del Barrio I.1 Kilfeather P.1, Vilema Enriquez G.1, Carling P. 1 Saqlain S. 1, Hadzic A.1, Lufino MM.1, Nabhan JF.2, Martelli A.2, and Wade-Martins R.1

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Friedreich's Ataxia (FRDA) is the most common form of inherited ataxia and is caused by a GAA expansion in intron 1 of the frataxin gene. This expansion results in reduced levels of the mitochondrial protein frataxin (FXN), leading to progressive neurodegeneration and severe gait and coordination impairments. Diabetes and cardiomyopathy are also commonly observed in patients, with the latter being the primary cause of premature death. Although progress has been made to elucidate the molecular mechanisms underlying the illness, only a few drugs, which do not cure the disease but only ameliorate the symptoms, are used in the clinic. It is therefore of paramount importance to develop new approaches that permit pharmacological upregulation of FXN, in order to bring forth a disease-modifying therapy.

Here we present the results of a CRISPR genome-wide knockout screen that has allowed us to identify potential targets that, when knocked-out, increase FXN expression in FRDA lymphoblastoid cells. Following the delivery of a CRISPR-KO lentiviral library we used Flow cytometry to measure FXN protein levels and sort those cells with high FXN expression (FXN<sup>high</sup>). Next Generation Sequencing identified several gene KO that were positively enriched in the FXN<sup>high</sup> population, revealing novel potential negative regulators of FXN expression in patient cells. Many of the identified candidate genes are involved in RNA metabolism, splicing and translational regulation, suggesting that FXN RNA processing could have a pivotal role in FRDA. We are confirming findings by knocking out potential targets in multiple FRDA cell lines. Overall our results suggest a novel role of RNA metabolism in controlling FXN expression in FRDA cells and may yield new therapeutic target for FRDA.

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## Screening Study of Spinocerebellar Ataxia-Negative Ataxic Patients for the Presence of the Friedreich's Ataxia Mutations

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 232

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***Dr. Alexander Brown<sup>1</sup>, Dr. Michael Parkinson<sup>1</sup>, Dr. Ese Mudanohwo<sup>1</sup>, Dr. Robyn Labrum<sup>1</sup>, Dr. Mary Sweeney<sup>1</sup>, Prof. Paola Giunti<sup>1</sup>***

*1. University College London*

**Objective:** Patients with suspected genetic ataxia are often tested for Friedreich's ataxia (FRDA) and/or a variety of spinocerebellar ataxias (SCAs). FRDA can present with atypical, late-onset forms and so may be missed in the diagnostic process. We aimed to determine the proportion of patients who were FRDA-positive among two cohorts of patients referred to a specialist ataxia centre either for FRDA testing or for SCA testing, in order to determine whether and to what extent cases of FRDA are missed in the diagnostic screening process. We also aimed to gather clinical information on these cases to inform the clinical definition of FRDA.

**Methods:** A cohort of 2000 SCA-negative ataxia patients, not previously referred for FRDA testing (group A), were tested for the presence of the FRDA expansion in either allele of the FXN gene using Triplet-Primed PCR (TP-PCR). TP-PCR positive cases were then further analysed by Long Range PCR, which determined the allelic status and thus whether samples bore two expanded alleles (FRDA-positive cases). Heterozygous cases were tested for point mutations and large deletions to determine whether they were compound heterozygous FRDA-positive cases, or heterozygous FRDA carriers. Group A was compared with a second cohort of 1768 ataxia patients who had been previously referred for FRDA testing (group B) and were therefore more likely to have a typical presentation. The phenotypes of positive cases in both cohorts were gathered and assessed through review of the clinical case notes.

**Results:** Three patients (0.2%) in group A had the FRDA expansion in both alleles, and thus were FRDA-positive, compared with 207 FRDA-positive patients (11.7%) in group B. Heterozygous carrier rate across both cohorts was of 41 out of 3768 cases or 1.1%, within the expected range. The size of the expansions in the three FRDA positive patients of the former cohort was on average small, and their presentation was atypical with late-onset.

**Discussion:** This study demonstrates that FRDA is very rare among patients referred purely for SCA testing without the clinical suspicion of FRDA. This study confirms that in such cases it is not necessary routinely to extend genetic testing to include FXN mutations. FRDA testing may be considered if SCA testing is negative and if there is any clinical suspicion of late onset FRDA. These results add to the body of evidence supporting the guidelines for the genetic testing and counselling of ataxia patients.

## COMPOUND HETEROZYGOUS PATIENTS WITH FRIEDREICH'S ATAXIA IN A BRAZILIAN COHORT

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 150

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**Mr. Thiago Mazzo Peluzzo <sup>1</sup>, Ms. Luciana Cardoso Bonadia <sup>1</sup>, Ms. Amanda Donatti <sup>1</sup>, Ms. Miriam Coelho Molck <sup>1</sup>, Prof. Laura Jardim <sup>2</sup>, Dr. Wilson Marques Júnior <sup>3</sup>, Dr. Iscia Teresinha Lopes-Cendes <sup>1</sup>,  
Dr. Marcondes Cavalcante França Jr. <sup>4</sup>**

*1. Dept. of Medical Genetics and Genomic Medicine, School of Medicine, University of Campinas – FCM/UNICAMP, 2. HCPA & UFRGS, 3. Dept. of Neurosciences and Behavior Sciences, Ribeirão Preto School of Medicine, University of São Paulo – HCFMRP-USP, 4. Department of Neurology, School of Medical Sciences, University of Campinas (UNICAMP)*

Friedreich's Ataxia (FRDA) is the most common hereditary ataxia worldwide. It's caused by a homozygous GAA expansion in the first intron of the *FXN* gene in 96% of the affected individuals. As a result, there is a dramatic reduction of frataxin expression. The remaining individuals are compound heterozygous for a GAA expansion and a second *FXN* point mutation. FRDA is a neurodegenerative disease characterized by early onset and progressive ataxia, spasticity and scoliosis. Dysphagia, dysarthria, subtle cognitive dysfunction, cardiomyopathy and diabetes are also often found. Looking at *FXN* point mutations is a promising strategy to uncover the metabolic pathways related to frataxin. In addition, it has obvious implications for clinical diagnosis and genetic counseling. Little is known about compound heterozygous populations outside Europe and North America. We therefore designed this study to determine the frequency, mutational spectrum and phenotype of compound heterozygous Brazilian patients with FRDA. To accomplish that, we recruited 183 index patients from 3 national reference centers (UNICAMP, USP-RP and HCPA). Those patients with a single identified expansion underwent sequencing of all 5 exons and exon-intron boundaries at *FXN* (Sanger technique). We also did a CGH-Array test for those patients who had no point *FXN* mutations identified. Compound heterozygosity accounted for 2.87% of all patients. A novel variant (c.482+1G.T), considered pathogenic following ACMG guidelines, was found. In addition, another pathogenic variant previously described in the literature (c.157delC) was observed in 2 unrelated subjects. The phenotype of these patients was heterogeneous when considering age of onset, severity and systemic manifestations. These are novel data in the Brazilian population. From a clinical perspective, they will help in the choice of adequate techniques for diagnosing FRDA and proper genetic counseling in our country. We would like to thank CAPES and FAPESP for the funding's of this research.

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# Quantifying and Mitigating the Acute Toxicity of Antisense Oligonucleotides in the Central Nervous System

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Thursday, 14th November - 17:30: Poster Session 1 - Abstract ID: 239

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***Mr. Feng Wang<sup>1</sup>***

*1. UMass Medical School*

Antisense oligonucleotides (ASOs) are emerging as a promising class of therapeutics for neurological diseases. When injected directly into the cerebrospinal fluid, ASOs distribute broadly across brain regions and exert long-lasting therapeutic effects. However, we show that many phosphorothioate (PS)-modified ASOs show acute toxicity when injected into the cerebrospinal fluid. To enable comparison of the effect of different chemical modifications we developed a scoring assay, called EvADINT (Evaluation of acute drug-induced neurotoxicity), to quantify acute behavioral oligonucleotide-induced toxic phenotypes. Using this assay, we show that both sugar and phosphate modifications influence acute neurotoxicity. Among sugar analogues, PS-DNA induces the highest acute neurotoxicity while 2'-substituted RNA modifications improve the tolerability of PS-ASOs. Reducing the PS content of gapmer ASOs, which contain a stretch of PS-DNA, improves their toxicity profile, but in some cases also reduces their efficacy or duration of effect. Reducing PS content improved the acute tolerability of ASOs in both mice and sheep. The acute toxicity we see is not mediated by innate immune responses. Formulating ASOs with calcium ions before injecting into the CNS further improved their tolerability, but through a mechanism at least partially distinct from the reduction of PS content.

# **Topic: Cellular and Animal Models of Disease**



## Overview of Friedreich's Ataxia Mouse Models: Comparison of Frataxin Protein Levels and Phenotype

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Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 297

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***Ms. Crystal Davis<sup>1</sup>, Dr. Cat Lutz<sup>1</sup>, Dr. Laure Case<sup>1</sup>, Ms. Emily Lowell<sup>1</sup>, Ms. Elizabeth Rocker<sup>1</sup>, Ms. Anna Vanasse<sup>1</sup>***

*1. The Jackson Laboratory*

Friedreich's Ataxia (FRDA) is an autosomal recessive disorder caused by a GAA trinucleotide repeat expansion in intron 1 of the frataxin (FXN) gene. Frataxin is a ubiquitously expressed mitochondrial protein involved in iron-sulphur cluster and heme synthesis. Reduction in frataxin levels causes oxidative stress, iron accumulation in mitochondria and subsequent cell death in particularly vulnerable sites such as the large sensory neurons in the dorsal root ganglia, and dentate nucleus of the cerebellum. Human patients can demonstrate a range of symptoms such as cardiomyopathy, progressive neurodegeneration, glucose intolerance and skeletal deformities. Mouse models play an integral role in understanding the biology of the disease as well as being one of the most efficient resource tools for pre-clinical testing of therapeutics. Often, multiple mouse models exist for any given condition and such is the case with Friedreich's Ataxia (FRDA). We report here a comparative molecular, behavioral and biochemical study of commonly used mouse models available from The Jackson Laboratory Repository for the study of FRDA. Our findings highlight an under-appreciated caveat to mouse modeling of human disease-in that expression of human genes, even at low-level disease states, can be functionally sufficient to dilute out disease phenotypes within the mouse. Conversely, mutations not specific to the human condition can be made generating robust FRDA phenotypes. These observations do not diminish the value of one mouse model over another, but rather provide us with multiple tools, each contributing different pieces of data with which we can evaluate therapeutic efficacy and contribute sufficient information to our understanding of the models that we can use to optimize and improve the collection.

# Direct neuronal reprogramming for the study of Friedreich ataxia

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 206

***Dr. Saul Herranz-Martin<sup>1</sup>, Dr. Alfredo Gimenez-Cassina<sup>1</sup>, Prof. Javier Diaz-Nido<sup>1</sup>***

*1. Universidad Autonoma de Madrid*

Transdifferentiation or direct conversion of mature cell types into mature neurons is becoming in a more valuable approach in order to get new cellular models for the study or treatment of some diseases. One of the main advantages of this technique is to skip the pluripotent step that cell reprogramming via induced-pluripotent stem cells does, avoiding the reactivation of certain pathological genes and keeping, moreover, the physiological features of the original cells. This second aspect is especially important in the case of many neurodegenerative disorders, which show a progressive decline age-dependent. In this context, we aim to get new cellular models for the study of Friedreich ataxia (FRDA), a neurodegenerative disorder caused by a GAA triplet repeat expansion within the first intron of the gene codifying for the protein Frataxin (Fxn), leading to a decreased expression of Fxn.

In this study, we have used fibroblasts from FRDA patients and age-matched healthy donors from a cell repository, in order to optimize a protocol to directly converting into fully mature inducible neurons (iN) using techniques that combine the use of viral vectors and small molecules. Few days after the induction protocol starts, cells begin to lose the typical fibroblast morphology, silencing some fibroblast specific genes while neural specific genes such as *Ascl1*, *Brn2* or  $\beta$ -III tubulin start to express, as RT-PCR and immunofluorescence (IF) experiments reveal. Later during the differentiation process, markers for mature neurons, such as *MAP2* or *Smi31*, begin to express too. IF assays also show a more axonal distribution of these proteins within the iN when you compare them to undifferentiated cells. We have also been able to differentiate fully mature neurons from fibroblasts carrying the GAA triplet repeat expansion using a similar approach to control cells. In order to validate this new model for the study of FRDA, we are currently assessing the pathophysiology of these iN.

Our results, therefore, show that it is possible to get iN from FRDA patients using protocols of direct differentiation “in vitro”. The absence of good human cellular models with the pathological GAA repeat expansion present, it could make this new model a good candidate for both, a better understanding of the disease and to seek some possible targets in order to treat FRDA patients.

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## A simple and rapid Doxycycline-compounded food-induced mouse model of Friedreich ataxia

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Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 193

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***Ms. ELIZABETH MERCADO-AYON<sup>1</sup>, Mr. Nathan Warren<sup>2</sup>, Dr. David Lynch<sup>2</sup>, Dr. Hong Lin<sup>2</sup>***

*1. University of Pennsylvania / Children's Hospital of Philadelphia, 2. Children's Hospital of Philadelphia*

**Introduction:** The *FXN* gene encodes the essential mitochondrial metabolism protein frataxin, whose deficiency leads to the multi-system neurodegenerative disorder Friedrich's ataxia (FRDA). The neuropathology in FRDA patients includes loss of large DRG cells, atrophy of their axons in the dorsal columns, loss of the dorsal spinocerebellar tract, corticospinal tract degeneration, and loss of the dentate nucleus. While there is currently no cure or treatment for FRDA, genetically engineered mouse models have enhanced our understanding of FRDA. A recently developed tetracycline-inducible mouse model of FRDA recapitulates some of the disease phenotypes including dorsal root ganglion (DRG) neuronal loss, cardiomyopathy, and ataxia as seen in FRDA patients. However, the previously used method of induction was prone to inconsistent doxycycline dosage and was labor intensive.

**Methods:** Six cohorts of transgenic mice at 2-6 months old and matching controls were fed with doxycycline-compounded chow diet (200PPM Doxycycline, Animal Specialties and Provisions, LLC., Quakertown, PA) or regular chow diet. FRDA<sup>k</sup> mice (TG<sup>+</sup>) were harvested after 4, 8, 9, 12, and 18 weeks of doxycycline administration. Reversal mice were withdrawn from doxycycline food after 9 weeks of induction. Mice harboring the shRNA transgene (Tet-O model) were designated the acronym TG while WT refers to Wild-Type mice. This is followed by (+), (-), or (±), reflecting doxycycline feed induction, withdrawal, and rescue/reversal, respectively. Frataxin protein expression was assessed via western blot analysis and tissue pathology was analyzed by performing immunostaining.

**Results:** In the present study, we establish a simple and rapid doxycycline-compounded food-induced mouse model of FRDA showing similar disease phenotypes as seen in the original model, but with some distinctions. These mice exhibit age dependent mortality and body weight loss, but with less early mortality or weight loss compared to the previous method of induction. In addition, Rotarod testing demonstrates severe motor coordination deficits. Furthermore, evaluation of the time course of frataxin deficiency across diverse tissues at 4, 8-12, and 18 weeks of induction demonstrated the tissue specific variability in frataxin knockdown. In the central nervous system: cerebellum, DRG, spinal cord, and cortex, frataxin levels slowly and progressively decreased in cerebellum, DRG, spinal cord, and cortex, while in peripheral tissues: heart, skeletal muscle, and liver [LD1], the frataxin levels declined rapidly. Recovery of frataxin levels by removal of doxycycline for 9 weeks only restored partially in cerebellum, cortex and liver, but not in heart and skeletal muscle. However, heart, skeletal muscle, and liver. After doxycycline removal, frataxin was restored incompletely and inconsistently across some tissues and not at all in other tissues; Frataxin recovery occurs in cerebellum, cortex and liver, but not in heart and skeletal muscle in reversal mice (TG<sup>±</sup>) after 8 weeks off doxycycline. Surprisingly, reversal mice (TG<sup>±</sup>) showed behavioral and size body weight deficits did improvements. In the previous manuscript on this model, frataxin restoration in the liver was incomplete (only 40%) at 12 weeks after removal of the inducing agents, and no data was provided on restoration in other tissues. Moreover, our neuropathological studies identified loss of cerebellar Purkinje neurons and large dentate nucleus principal neurons as well as large DRG sensory neurons mimicking some but not all aspects of the neuropathology seen in patients.

**Conclusion:** The present investigation establishes a simple and rapid induction method of frataxin silencing in an FRDA mouse model, thereby validating an efficient and reproducible mouse model for understanding molecular and cellular pathogenic mechanisms due to frataxin deficiency. However, it also defines the limits of this model in

specific cell types and in variability of the restoration of frataxin. Still, the model should aid in identifying therapeutic strategies for FRDA.

## Genotype and Phenotype Characterisation of an Enhanced Friedreich Ataxia GAA Repeat Expansion Mouse Model *Fxnnull::YG8s(GAA)>800*

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Our lab has previously generated YG8R and YG22R FRDA mouse models that are based upon human *FXNYAC* transgenic mice containing a large *FXN* human genomic transgene with GAA repeat expansions crossed with *Fxn*-knockout mice. These mouse models have exhibited a rather mild, late-onset FRDA-like phenotype. Therefore, over the last few years we have performed selective breeding of these mice to produce larger GAA repeat expansion containing FRDA mouse models with a more representative earlier onset FRDA-like phenotype. We now have YG8-derived FRDA mice lines with GAA repeat expansion sizes of approximately ~300 repeats (YG8sR), ~400bp repeats (YG8LR) and ~600bp repeats (YG8XLR). More recently, we have obtained the latest FRDA humanised model that was derived from our previous YG8sR mice by Jackson laboratory (*Fxnnull::YG8s(GAA)>800*). These mice contain a larger expansion of 800 GAA repeats, designated YG8JR. The YG8JR mice underwent a partially different genetic manipulation in which the *Fxn*-knockout in these models was generated by CRISPR/Cas9 and Cre loxP-mediated deletion of exon 2. These models have the largest GAA repeat sizes of all the current FRDA mouse models. Phenotypically, these mice exhibit a degree of hair loss and have reduced weight compared with Y47JR control mice. In addition, these mice have shown further decreases in frataxin expression levels compared to all our previous FRDA mouse models. We have also detected increased somatic GAA repeat instability in the brain and cerebellum of YG8JR mice, together with reduced aconitase activity and altered *FXN* histone modifications and DNA methylation, compared with the control Y47JR mice. Coordination ability of YG8JR mice, together with Y47JR control mice, was assessed using accelerating rotarod analysis. The results indicated a decline in the motor coordination of YG8JR mice at the older age (6-9m) compared to Y47JR controls. We aim to further characterise these mouse models for neurobehavioral deficits and other biochemical and molecular analysis.

# Neurological deficits in a Friedreich Ataxia mouse model based on the pathological point mutation I154F.

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***Ms. Marta Medina-Carbonero<sup>1</sup>, Dr. Rosa Purroy<sup>1</sup>, Ms. Arabela Sanz<sup>1</sup>, Dr. Joaquim Ros<sup>1</sup>, Dr. Jordi Tamarit<sup>1</sup>***

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Friedreich Ataxia (FA) is a rare, inherited recessive disease caused by mutations in the frataxin gene (FXN) that result in decreased frataxin protein levels. Most patients are homozygous for a GAA triplet expansion in the first intron of the gene, while around 4% of patients are compound heterozygous for a GAA expansion and a FXN point mutation or deletion. Several FA mouse models have been generated. Most of them have provided valuable information to the FA field, but unfortunately the biochemical condition causing this disease (a residual expression of frataxin in all tissues) is difficult to mimic in mice models. Therefore, to obtain mice with such condition, we have developed a new mouse model based on the pathological point mutation I154F. We have selected this mutation because compound heterozygote individuals with this mutation present a clinical phenotype similar to patients homozygous for the GAA repeat expansion. Also, because this mutation results in low frataxin levels, as it compromises the solubility of frataxin intermediate form and blocks its proteolytic processing to mature frataxin. To generate the mouse model, the I151F mutation (equivalent to the human Fxn I154F) was introduced in C57BL/6J mice using the CRISPR approach. A founder female was placed into mating with B6J to generate N1 Het mice. These N1 Het mice were mated to generate N2 Het mice. Then, N2 Het mice were crossed to generate homozygous mice WT/WT (control) and I151F/I151F (mutant). We have followed the evolution of these mice for 39 weeks and we have observed by western blot that mutant mice present a marked loss in frataxin content in heart, brain and cerebellum (Figure 1). In mutant mice, frataxin signal is only detected when SDS-PAGE gels are overloaded and membranes overexposed. Behavioral analysis has revealed marked neurological deficits in mutant mice in comparison to control animals: in open-field test, they exhibited shorter distance travelled and velocity (Figure 2A); in paw print analysis displayed reduced stride length (Figure 2B); in rotarod test they showed a decreased latency to fall. These results suggest that the I151F mutation causes motor deficits indicative of ataxia similar to FA patients. Therefore, this model will be very useful for analyzing the consequences of frataxin deficiency in mice and in therapeutic development.

**Figure 1 Generation and consequences of I151F mutation**(A)Left, human frataxin structure showing the position of I154 (in sphere format, magenta color). Right, aminoacid sequence surrounding the mutated Ile (indicated by an arrow) in human (Hu) and mouse (Ms) frataxin.(B)Nucleotide sequence of the WT and mutant alleles generated by CRISPR targeted mutagenesis. The Ile mutated codon is shown in green. A silent mutation in the Thr146 codon is present in the mutant allele (shown in yellow). (C)Representative frataxin western blots from heart (H), brain (B) and cerebellum (C) lysates from 39 week-old wild type (WT) and mutant (mut) mice. Left, 20 ug of protein lysates were loaded on each lane. Right, in order to detect frataxin in mutant mice, 100 ug of protein were loaded on each lane and membranes were overexposed. Protein loading was verified by post-western CBB staining.

**Figure 2. Behavioral analysis of 39-week old mice.**(A)In open field test, distance traveled, velocity and number of crossings were decreased in I151F mice (mut) compared to wild type mice (WT). (B)Paw prints test. Left, representative paw prints from wild type and mutant mice. Right, histograms show that stride length was reduced in mutant mice compared to wild type mice. Data are presented as mean  $\pm$  SD. \*\*p < 0.01, n=12.

## Role of astrocytes in Friedreich's Ataxia neurodegeneration

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**Dr. Pia Intagliata<sup>1</sup>, Dr. Serena Bellani<sup>2</sup>, Dr. Barbara Bettegazzi<sup>2</sup>, Prof. Fabio Grohovaz<sup>2</sup>,  
Prof. Franca Codazzi<sup>2</sup>**

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Friedreich's Ataxia (FA) is a rare autosomal neurodegenerative disorder caused by a GAA repeat expansion within the first intron of FXN gene (Campuzzano et al., 1996); the consequence is a reduced expression of frataxin, a mitochondrial protein involved in iron homeostasis and Fe-S cluster biogenesis.

The pathophysiology of this disorder is more evident in some cell types, such as cardiomyocytes, pancreatic beta cells and neuronal cells from specific area of the central and peripheral nervous system; specifically, signs of the disease are mainly confined to cerebellum and dorsal root ganglia. However it is possible that frataxin down-regulation could affect also non-neuronal cells that, in turn, might contribute to pathogenesis of the disease; in particular, astrocytes are abundant in the CNS, contribute to neuronal homeostasis and play a role in several other neurodegenerative disorders. Moreover, during inflammatory conditions, astrocytes undergo activation process that can alter the physiological interaction with neurons, therefore potentially contributing to neuronal toxicity; signs of inflammation and astrocyte activation have been recently described not only in FA animal models, but also in FA patients.

In this study, we are first characterizing a model of FA-astrocytes, either under resting or inflammatory conditions. To this purpose, primary rat cerebellar astrocytes were transfected for 72 h with specific FXN siRNA and subjected or not to conditions mimicking inflammation, i.e. 24 h-treatment with a mix of cytokines (CKs; IL-1 $\beta$ , TNF $\alpha$  and INF $\gamma$ ). Both FXN transcript level and protein expression were significantly reduced (up to 30% of WT cells) by siRNA transfection and CK treatment did not alter these effects. FA-astrocytes showed the typical cellular hallmarks of FA, with reduced mitochondrial membrane potential and increased levels of oxidative stress, when compared to control cells. On the other hand, FA-astrocytes appeared activated by CK treatments similarly to control cells, with comparable up-regulation of inducible nitric oxide synthase (iNOS) and the consequent release of nitric oxide. Other markers of activation as well as a panel of several cytokines are under investigation, in order to evaluate whether the activation process might be altered in FA astrocytes, with detrimental effects towards the neurons. Since we aim to understand the role of astrocytes on neurodegeneration in FA, we are also investigating the effects of conditioned medium collected from FA- or control- astrocytes, at rest or activated, on cerebellar granule neurons (CGNs). It appears that the conditions medium from CK-activated FA-astrocytes promoted a significant increase of neuronal death.

The analyses of other neuronal parameters and the setting of neuro-astrocyte co-cultures will allow to understand the detrimental or protective role played by astrocytes towards cerebellar neurons, therefore making them a potential therapeutic cellular target.

# Induced pluripotent stem cell-derived primary proprioceptive neurons as Friedreich ataxia cell model

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 113

***Ms. Chiara Dionisi<sup>1</sup>, Dr. Myriam Rai<sup>1</sup>, Prof. Massimo Pandolfo<sup>1</sup>***

*1. Université Libre de Bruxelles*

## Induced pluripotent stem cell-derived primary proprioceptive neurons as Friedreich ataxia cell model

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Laboratory of Experimental Neurology, Université Libre de Bruxelles, Brussels, Belgium.

**Background.** Human induced pluripotent stem cells (iPSCs) are used to generate models of human diseases that recapitulate the pathogenic process as it occurs in affected cells. Many differentiated cell types can currently be obtained from iPSCs, but no validated protocol is yet available to specifically generate primary proprioceptive neurons. Proprioceptors are affected in a number of genetic and acquired diseases, including Friedreich ataxia (FRDA). In FRDA both a developmental deficit and progressive neurodegeneration are thought to underlie the loss of proprioceptors, though the relative contribution of these two components is unclear. The basis of the high specific vulnerability of proprioceptors in FRDA is also unknown.

In order to address these open questions about FRDA pathogenesis and at the same time develop a cell model that can be applied to other conditions primarily affecting proprioceptors, we set up a protocol to differentiate iPSCs into primary proprioceptive neurons.

**Methods.** We modified the well-known dual-SMAD inhibition/WNT activation protocol to favor differentiation into TrkC<sup>+</sup> proprioceptors rather than TrkA<sup>+</sup> nociceptors as in the original report. After eight days of treatment with small molecules pathway inhibitors, neural precursors were allowed to mature in the presence of NT3 and BDNF, neurotrophic factors specific for proprioceptors and mechanoreceptors, respectively.

**Results.** We succeeded in substantially enriching iPSC-derived primary sensory neuron cultures in proprioceptors to 22-34% of neurons, largely exceeding the ~7.5% in dorsal root ganglia (DRGs). We demonstrated that the process of differentiation in culture resembles what happens *in vivo* during DRG development in terms of cellular behaviour, morphological changes and expression of typical markers of differentiating and mature sensory neurons, as analysed by quantitative RT-PCR (Figure 1) and immunofluorescence (IF).

We then showed that proprioceptors can be purified from these cultures by fluorescence-activated cell sorting (FACS) for high expression of TrkC, which is almost specific for this neuronal type as most TrkC<sup>+</sup> mechanoreceptors (Pacinian, Meissner, Lanceolate endings) express it at lower levels. FACS data matched IF findings, confirming that our differentiation protocol significantly enriched the iPSC-derived primary sensory neuron cultures for TrkC<sup>+</sup> cells, and that most TrkC<sup>+</sup> cells were TrkB<sup>-</sup>, indicating that they were not immature cells expressing both receptors not yet committed to a proprioceptor vs. mechanoreceptor fate.

Maturation of sensory neurons was rapid, with the majority of them presenting a bell-shaped or a pseudo-unipolar morphology and expressing markers of differentiated sensory neurons after only two weeks. Functional maturation was essentially complete at three weeks, when neurons become fully competent in the generation of repeated bursts of action potentials (Figure 2). Biocytin staining of patched neurons demonstrated that they extended long and branched neurites (Figure 2). Our protocol therefore overcomes one of the limitations of iPSC-based models, which often require several weeks for the generation of mature cells.

While accurate phenotypic characterization of FRDA vs. control proprioceptors is ongoing, we confirmed stability of expanded GAA repeats and repression of *FXN* expression in differentiated FRDA cells to about 15% of controls,



consistent with levels found in FRDA patients.

**Conclusion.** Though we developed this model because of our interest in translational research in FRDA, it is of obvious interest to investigate many other conditions affecting proprioceptors, both genetic and acquired. The availability of human proprioceptors carrying the causative genetic mutations, or exposed to pathogenic factors as toxins or autoantibodies, allows to directly explore the relevant pathogenic mechanisms better than primary DRG cultures from animal models that often are only approximations for the human condition.

## Development of FRDA iPSCs-derived cardiomyocytes as a tool to evaluate efficacy of potential therapeutic compounds.

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While Friedreich ataxia (FRDA) is primarily considered a neurodegenerative disease, other non-neurological symptoms, including cardiac dysfunction, affect a vast majority of patients. In particular, the left ventricular hypertrophic cardiomyopathy often leads to premature death in FRDA patients. However, the lack of adequate cardiac models has so far hampered the studies on FRDA-related cardiomyopathy and the possibility to screen a large number of potentially therapeutic compounds. With the aim to generate a cellular model to test the efficacy of new promising therapeutic candidates, we have optimized a robust and reproducible method of differentiation and characterization of cardiomyocytes derived from FRDA patient iPSCs, based on the timely controlled modulation of Wnt/ $\beta$ -Catenin pathway (Lian et al., 2013). FRDA iPSCs were kindly provided by Dr. Marek Napierala (University of Alabama at Birmingham, AL, USA). In detail, temporal application of chemical GSK3 inhibitor, followed by Wnt inhibitor, is able to differentiate iPSCs into functional cardiomyocytes. During the whole differentiation process, the expression of a number of markers was assessed by qRT-PCR, cytofluorimetry, and immunocytochemistry techniques. Twenty-four hours after the induction of the Wnt pathway, mediated by the inhibition of GSK3, the cells turn out to be highly positive for Brachyury, a mesodermal induction marker. Subsequently, the complete inhibition of a family of proteins known as porcupines (PORCN), which in turn leads to Wnt pathway inhibition, drives the mesodermal cells towards a cardiogenic fate, by activating some key genes such as Nkx2.5, Isl1 and GATA4. After 9-10 days of culture, the cardiogenic cells organize themselves to form recognizable beating areas. Expression of differentiation markers, such as MLC2a, cTnT and alpha-actinin, was detected at this stage, indicating terminal differentiation into cardiomyocytes. After day 15, higher expression of MLC2v compared to MLC2a identifies the ventricular fate of cardiomyocytes. Importantly, we confirmed the differences in frataxin expression levels between healthy control and FRDA cells during all the differentiation steps.

We then used the obtained cardiomyocytes to test the effect of etravirine on this cell type. Our results show that etravirine promotes a statistically significant 40% increase in the levels of mature frataxin after 24 hours of treatment. In the future it will be important to functionally characterize these cells and highlight phenotypic differences between healthy and FRDA cardiomyocytes. In particular, we will evaluate sensitivity to oxidative stress-mediated cell death and the iron handling capability using the Calcein-AM quenching assay after exogenous iron overload. Lian X, Zhang J, Azarin SM, Zhu K, Hazeltine LB, Bao X, Hsiao C, Kamp TJ, Palecek SP. 2013 Directed cardiomyocyte differentiation from human pluripotent stem cells by modulating Wnt/beta-catenin signaling under fully defined conditions. Nat Protoc 8:162-175.

## Defining the Frataxin G130V pathogenic mechanism in Friedreich's ataxia

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**Ms. Yu-Yun Chen <sup>1</sup>, Dr. David Lynch <sup>2</sup>, Dr. Marek Napierala <sup>1</sup>, Dr. Jill Napierala <sup>1</sup>**

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Friedreich's ataxia (FRDA) is a neurodegenerative disease caused by reduced expression of the mitochondrial protein frataxin (FXN). Most FRDA patients are homozygous for large expansions of GAA repeat sequences in intron 1 of the FXN gene, while a subset of patients are compound heterozygotes with an expanded GAA repeat tract in one FXN allele and a missense or nonsense mutation in the other. The most prevalent missense mutation changes a glycine to valine at position 130 (G130V). FRDA G130V patients exhibit different clinical symptoms than patients with homozygous GAA expansions, including retained reflexes, spared speech, and slower disease progression. We and others have demonstrated that the total level of mature FXN protein is more prominently reduced in FRDA G130V samples compared to samples harboring homozygous expansions. Moreover, our results suggest that mitochondrial maturation processing of FXN to its final form is perturbed by the G130V mutation, resulting in accumulation of the intermediate isoform. We hypothesize that the FXN-G130V intermediate isoform is functional and contributes to the atypical FRDA G130V clinical presentation. Little is known regarding the expression, maturation, and function of the endogenous FXN-G130V protein due to lack of reagents and models that can distinguish the mutant FXN protein from the wild-type FXN produced from the GAA-expanded allele *in vivo*. We used CRISPR/Cas9 to introduce FXN allele-specific V5 epitope tags in FRDA G130V patient-derived induced pluripotent stem cells, thereby creating novel cellular models of FRDA G130V. The endogenous FXN-G130V protein is detected at a much lower level than the FXN-WT protein expressed from the GAA expansion allele despite high expression of the FXN-G130V transcript. The endogenous FXN-G130V protein localizes to mitochondria in FRDA G130V patient-derived cells, however the ratio of intermediate and mature isoforms differs from that observed for the FXN-WT protein, which is almost completely processed to the mature form. We are continuing with experiments to define mechanisms governing steady state levels and maturation of the endogenous FXN-G130V protein to test whether this mutation confers a gain-of-function phenotype that might directly be linked to the milder clinical presentation of FRDA G130V patients. Taken together, results of our studies will significantly increase our knowledge of a unique FRDA G130V pathogenic mechanism and will facilitate development of therapeutic strategies for all FRDA patients.

## **A *Drosophila* model of Friedreich Ataxia with GAA repeats in the frataxin gene reveals in vivo protective effects of N-Acetyl Cysteine treatment.**

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***1. University of Paris. CNRS UMR8251***

Friedreich Ataxia (FA) is caused by a GAA repeat expansion in the first intron of *FXN*, the gene encoding frataxin, which results in decreased gene expression. Thanks to the high degree of conservation of frataxin throughout evolution, the *Drosophila melanogaster* fruitfly appears as an adequate animal model to study FA disease and to evaluate therapeutic interventions. Here, we have generated a *Drosophila* model of FA, called fh-GAA, with CRISPR/Cas9 insertion of around 200 GAA triplets in the intron of the fly frataxin gene *fh*. These flies exhibit developmental delay and lethality and a decrease in frataxin expression of 70%. We were able to by-pass preadult lethality using genetic tools to overexpress frataxin only during the developmental period. These frataxin-deficient fh-GAA adults are short-lived and present strong locomotor defects. RNA seq analysis identified transcriptomic signatures of frataxin-deficiency that are mainly related to oxidative stress. In particular, we observed a progressive increase of TSPO expression, fully rescued by adult frataxin expression. This shows that TSPO expression constitute a molecular marker of the disease progression in our fly model and might be of interest as a molecular marker in other animal models of FA or even in patients. Finally, in a candidate drug screening, we observed that N-Acetyl Cysteine (NAC) treatment increased the lifespan of fh-GAA flies in a dose-dependent manner with an increase in mean lifespan of up to more than 50%. NAC is an old drug with several established clinical applications and is currently widely used as an antioxidant in clinical trials for various applications. Thus, our fly FA model provides the opportunity to study *in vivo* and to elucidate the protective mechanisms of this molecule of high therapeutic potential.

# **Topic: Natural History, Biomarkers and Endpoints**

# Plasma Neurofilament Light Chain Levels as Biomarkers of Neurodegeneration in Friedreich's Ataxia – Temporal Changes during Disease Progression

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**Introduction** – There is a growing need for the identification of robust and easily accessible biomarkers in Friedreich's Ataxia, to monitor disease activity and eventually therapeutic efficacy (Blair et al. 2019). Recently introduced methodologies such as the single-molecule array technology help increasing sensitivity, allowing the reliable measurement of markers of neurodegeneration in plasma. As a result, especially Neurofilament light chain (Nf-L) is discussed as potential candidate biomarker for Friedreich's Ataxia.

Previous analyses have shown increased levels of Nf-L, GFAP and UCHL1 in FRDA patients compared to controls, but not for tau (Zeitlberger et al. 2018). No correlations with GAA1 repeat length or SARA score were found. We wanted to confirm this in a larger cohort including participants of different geographical origin and with a larger range of age at onset, disease duration and severity as measured by SARA and FARS score.

**Methods** – Plasma samples were obtained from patients participating in the Collaborative Clinical Research Network in Friedreich's Ataxia (CCRN in FA) and the European Friedreich's Ataxia Consortium for Translational Studies (EFACTS) study. Biomarker levels were log-transformed and correlated with age and disease duration, using linear and polynomial models to explore the relationships with typical covariates in FRDA (disease duration, repeat length and age of disease onset).

Biomarker data was available from 98 patients in CCRN (163 samples) and 89 patients from EFACTS (89 samples). In addition, we had 130 age-matched control samples. Patients were grouped by age at onset (AAO, <15, 15-24 and >25 years) in order to capture the different phenotypes, present in FRDA and explore the relationship with these biomarkers.

**Results** – We found elevated levels of Nf-L compared to controls, especially in younger individuals with FRDA. In the youngest patients in our sample, Nf-L levels in FRDA were found to be 3-4 times higher than age-matched controls (Figure 1).

Nf-L levels have been repeatedly shown to increase with age in healthy individuals, by ~2.2% per year (Disanto et al. 2017; Khalil et al. 2018; Mattsson et al. 2017). Our control samples showed a slightly bigger increase with time yet behaved similarly (2.8% increase per year).

Using 2nd order polynomial models over age best described Nf-L progression in FRDA and controls. Over the complete age range, FRDA levels were higher than in controls (2.56 log pm/ml vs 2.01 log pm/ml,  $p < 0.001$ ), confirming previous results (Zeitlberger et al. 2018).

However, evident from Figure 1, after around age 35-40, the differences between FRDA and the control group diminishes. This can be demonstrated by analysing AAO-subgroups with age matched controls. In early onset patients (<15y of age), the difference to the control group was highly statistically significant (2.58 log pm/ml vs 1.89 log pm/ml,  $p < 0.001$ ). A similar result was obtained in the intermediate onset group (15-24y of age, 2.69 log pm/ml vs 2.02 log pm/ml,  $p < 0.001$ ). In the late onset group (>24y of age) however, Nf-L levels were not different from controls (2.63

log pm/ml vs 2.48 log pm/ml,  $p = 0.227$ )

In the overall data, when stepwise increasing the threshold for age, subgroup analyses show that at ages  $> 35$ , the group difference is still elevated in FRDA, but not statistically significant anymore (2.63 vs 2.45 log pm/ml,  $p = 0.077$ ).

*Conclusion* – Our results indicate that Nf-L as a biomarker in FRDA might be highly useful, especially in the early AAO group.

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## Onset symptoms and time to diagnosis in Friedreich's Ataxia

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***Dr. Elisabetta Indelicato*<sup>1</sup>, *Dr. Wolfgang Nachbauer*<sup>1</sup>, *Dr. Andreas Eigentler*<sup>1</sup>, *Dr. Matthias Amprosi*<sup>1</sup>,  
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*Prof. Ludger Schöls*<sup>7</sup>, *Prof. Thomas Klockgether*<sup>8</sup>, *Dr. Katrin Bürk*<sup>9</sup>, *Prof. Massimo Pandolfo*<sup>10</sup>, *Prof. Jörg Schulz*<sup>11</sup>, *Dr. Sylvia Boesch*<sup>1</sup>**

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**Objective:**In rare disorders, correct diagnosis may be set only after several years due to limited awareness and unspecific presenting symptoms. Herein, we studied onset features and their relationship to time to diagnosis in Friedreich Ataxia (FRDA), the most common inherited ataxia in the Caucasians.

**Methods:**We analyzed a cohort of 611 genetically confirmed FRDA patients recruited within a multicentric natural history study conducted by the EFACTS (European Friedreich's Ataxia Consortium for Translational Studies). Age and symptoms at onset, age at suspicion of FRDA and genetic data were collected at time of inclusion in the registry.

**Results:**A classical presentation with early-onset (<25 years of age) and balance/coordination disturbances was reported in 83% and 90.5% of cases, respectively. Fifty-eight patients (9.5%) presented with isolated non-neurological symptoms (83% scoliosis, 17% cardiomyopathy). All but one of them had disease onset <25 years of age. The median time to diagnosis in the entire cohort was 4.7 (1;7) years. Time to diagnosis was significantly longer when FRDA presented with non-neurological features (6.6 (95% CI, 5.4-7.8) vs 4.5 (95% CI, 4.2-4.9)  $p=0.001$ ) as well as when it started  $\geq 25$  years of age (5(2;10) vs 3(1;6),  $U=20036$ ,  $p<0.0002$ ) compared to the classical phenotype.

**Conclusions:**In about 10% of cases, FRDA does not present with neurological features at disease onset. Non-neurological presenting features are rare in late-onset cases. Inaugural non-neurological features and onset in the adulthood are both associated with a significant diagnostic delay in FRDA. Diagnostic delay defers appropriate management, counseling and would bear relevant consequences once effective therapies will be available.



## Development of a high-sensitivity immunoassay for the detection of frataxin in biofluids

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***Mr. Shawn Ciotti<sup>1</sup>, Mr. Justin Aubin<sup>1</sup>, Prof. Massimo Pandolfo<sup>2</sup>, Dr. Steven Hersch<sup>1</sup>***

*1. Voyager Therapeutics, 2. Université Libre de Bruxelles*

Friedreich's ataxia (FA) is an inherited neurodegenerative disorder caused by a triplet repeat expansion mutation in the *FXN* gene that results in low levels of frataxin protein throughout the body. Symptoms include progressive ataxia, weakness, fatigue, and impaired speech, vision and hearing, causing profound functional impairment and increasing assistance needs. Patients are typically dependent on mobility aids by their teens to early 20s, and early death commonly results from cardiomyopathy. Restoration of frataxin in affected tissues is a key therapeutic goal.

**Background:** Frataxin measurement has been performed in cellular tissues, including muscle, buccal cells, and purified blood cell populations. These measurements could enable the assessment of target engagement for frataxin modulating treatments in humans, however their informativeness as biomarkers may be limited with respect to central and peripheral nervous systems, heart and other relevant tissues that cannot be readily biopsied.

**Rationale:** Current assays use either enzyme-linked immunosorbent assay (ELISA) or laminar flow dipstick methods which have lower limits of detection in the picogram range. Measurement of frataxin in cerebrospinal fluid (CSF) could help assess whether brain levels can be increased by a treatment, while measurement of frataxin in plasma or serum could provide an integrated whole-body measure for frataxin and a potential prognostic or response biomarker. Neither of the currently available assays have sufficient sensitivity to detect frataxin in circulating biofluids, including plasma, serum, and CSF. High detection sensitivity to accurately detect small differences in frataxin levels is critical for potential use as a biomarker assay since 50% of normal frataxin expression levels are clinically relevant based on the lack of phenotype in heterozygous carriers of *FXN* mutations.

**Method:** Voyager has developed an assay for the measurement of frataxin in biofluids. The assay allows for sub-picogram levels of detection per milliliter of sample with a detection range of 0.6 – 500pg/mL in homogenized human motor cortex tissue.

**Results:** The optimized assay was tested on a set of control samples including samples of serum, plasma, and CSF. Frataxin was detected in 19 of 22 samples tested at ranges of 2.29 – 282.13 pg/mL in serum, 1.59 – 5.11 pg/mL in plasma, and 1.89 – 4.74 pg/mL in CSF. We will next assess frataxin levels in biofluid samples of both FA patients and age-matched controls to establish the magnitude of difference in frataxin within these matrices and how these levels might relate to biological and clinical features of FA. Additionally, we will expand our sample size to better establish baseline frataxin levels in these biofluids.

**Conclusion:** This assay allows, for the first time, detection of frataxin levels in circulating biofluids. With this information we may be able to better assess the impact of therapies on the brain, and to obtain whole-body data that may potentially establish a new response biomarker.

# Continuous glucose monitoring in adults with Friedreich's Ataxia

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 267

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**Background:** Diabetes (DM) is a frequent co-morbidity in Friedreich's Ataxia (FA) and is associated with worse functional status.<sup>1</sup> However, evidence-supported guidelines for FA-related DM screening and management currently do not exist. Continuous glucose monitoring (CGM) enables detailed assessment of glucose homeostasis that may yield relevant clinical insights and inform screening practices.

**Objective:** The main objective was to describe CGM outcomes in a convenience sample of individuals with FA and healthy volunteers.

**Methods:** Participants were non-diabetic adults with FA and healthy control volunteers who were enrolled in an observational metabolic phenotyping study (NCT02920671), in which CGM outcomes were exploratory and CGM placement was optional. A CGM sensor (FreeStyle Libre Pro flash glucose monitoring system, Abbott Laboratories, Abbott Park, IL) was placed on the upper arm of each participant following an overnight fast in the inpatient setting immediately prior to an oral glucose tolerance test (OGTT). Whole body insulin sensitivity was calculated from the OGTT.<sup>2</sup> Participants wore a CGM for up to 14 days.

The following outcomes were calculated: % sensor glucose values <70mg/dL, % sensor glucose values >140 mg/dL, mean glucose, and coefficient of variation (COV). Wilcoxon rank sum tests were used to compare BMI, fasting glucose, HgbA1c, WBISI, and CGM outcomes between cases and controls in this sub-study. In addition, the "glucotype" of each individual was determined, according to a model for integrated examination of individual-specific glucose excursions via spectral clustering methods.<sup>3</sup> Glucotypes are designed to distill complex CGM patterns into strata that reflect future risk of diabetes. Fisher's exact test for difference of distribution was used to evaluate differences in "glucotype" distribution between FA and healthy control subjects.

**Results:** Individuals with FA [n=9; 5 male, median age 24y (IQI 23, 36); median BMI 26.9 kg/m<sup>2</sup> (IQI, 25.6, 29.3)] and healthy volunteers [n=16; 8 male, median age 31y (IQI 27, 43); median BMI 23.9 kg/m<sup>2</sup> (IQI, 21.3, 26.3)] completed CGM. Individuals with FA had a median HbA1c of 5.0% (vs. 5.1% in controls), fasting glucose of 91 mg/dL (vs. 84 in controls). None of these differences was statistically significant. Median WBISI was 2.8 in FA (vs. 4.6 in controls, p=0.019 for difference).

The following CGM outcomes did not differ between FA and controls: median % of values <70 mg/dL was 23% in FA vs. 3% in controls (p=0.22), median % of values >140 mg/dL was 2% in FA vs. 2% healthy controls (p=0.98), median of the mean plasma glucose was 81 mg/dL in FA vs. 92 mg/dL in healthy controls (p=0.25), median of the COV was 20% in FA vs. 17% in healthy controls (p=0.35).

We did detect a difference in the distribution of "glucotypes" by disease category. In FA, 33% were severe, 22% were moderate, and 44% were low, while in controls, 31% were severe, 63% were moderate, and 6% were low (p=0.04).

**Conclusions:** In this pilot study, we did not identify differences in traditional CGM outcomes between non-diabetic adults with FA and unaffected controls. Additional analysis of CGM outcomes and their association with clinical characteristics are planned. The heterogeneity of glucose responses that allow assignment of "glucotypes" may contain information relevant to diabetes risk beyond usual clinical screening tools. In future studies, we may find

that CGM can improve diabetes risk stratification in FA, in particular when individuals at higher risk for FA-related DM are included (e.g., individuals with more clinically severe disease). Prospective CGM data collection and longitudinal follow up will thus help to determine the best potential uses of this tool.

#### **CITATIONS**

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# Can full-body motion-capture data analysis outperform conventional clinical measures in predicting progression in Friedreich's Ataxia?

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 254

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## Introduction

Gold-standard clinical methods for monitoring the effects of neurodegenerative diseases on patients have been systematically shown to lack objectivity and fail to capture the slow progression of such disorders. This prompts the need for more accurate and sensitive digital biomarkers that can detect even small changes in patient performance, such as wearable digital devices to monitor daily step counts. In a search for objective markers of disease progression, we focus here on a holistic analysis of full-body kinematics that can accurately predict the level of dysfunction occurring in patients with Friedreich's ataxia (FA), when compared to scores obtained following conventional assessment by clinicians.

## Methods

Nine FA participants and three unaffected controls participated in this longitudinal natural history study over nine months. The subjects visited the hospital four times (on day-1, week-3, month-3 and month-9) during the study. Scale for the Assessment and Rating of Ataxia (SARA) and Spinocerebellar Ataxia Functional Index (SCAFI) assessments were done on the subjects while they were wearing a full-body motion-capture suit. We analysed two sub assessments of the SCAFI scale, the 8 Meter Walk (8MW) and the 9 Hole Peg Test (9HPT), for which clinicians only use their duration for estimating the progression of the FA disease. We extracted a range of behavioural features from the full-body suit data and then used the state-of-the-art Gaussian Process (GP) regression algorithm to predict the month-9 SARA and SCAFI scales using the clinical scales themselves and the features of the suit data from 8MW and 9HPT tasks.

## Results

The results of the prediction of the clinical scales are presented in Figure 1 and Table 1. The predicted month-9 clinical scale is plotted against the actual month-9 clinical scale measured by the clinicians. The extracted features of the suit data from day-1, week-3 and month-3 visits of both 8MW and 9HPT tasks were able to predict the month-9 SARA score (with an  $R^2$  of 0.92) better than predictions using the day-1, week-3 and month-3 SARA score (with an  $R^2$  of 0.56). The difference in the prediction performance between the suit data features and the clinical scales was pronounced in the case of predicting SCAFI scores. While the features of the suit data had an  $R^2$  of 0.9 for the month-9 clinical scale prediction, the SCAFI scales fared poorly with an  $R^2$  of 0.05.

## Conclusion

Our novel methodology captures the subtle changes in patients' behaviour more objectively and predicts the clinical scales with better accuracy than conventional clinical measures. This strongly suggests that full-body kinematics analysis promises to considerably shorten the length or number of patients required for clinical trials in FA and other slowly progressive neurological conditions.

# Understanding the Diagnostic Experience of Individuals with Friedreich's Ataxia

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Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 252

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Background: Friedreich's Ataxia (FA) is an autosomal recessive, neurodegenerative condition characterized by progressive truncal and limb ataxia. The average age of onset for an individual with FA is between 10-15 years old. However, there is a late-onset form of the disease and individuals can present at any age. Receiving a timely diagnosis of FA can be a challenge for various reasons. There is currently no cure for FA but there are clinical trials taking place that are investigating numerous potential therapies. Therefore, a timely diagnosis of FA has greater importance. The purpose of this study is to understand the diagnostic experience of individuals with FA by analyzing different factors contributing to the time to diagnosis. Methods: Two different databases were used, the FA Global Patient Registry (FGPR) and the FARA-funded Collaborative Clinical Research Network (CCRN) to analyze factors contributing to the time to diagnosis in 2,376 individuals with FA. The analyses performed were time to diagnosis based on age at diagnosis and time to diagnosis based on year of diagnosis. Additionally, we examined the initial disease symptom reported by individuals. Results: 70% of participants were diagnosed at or before 19 years of age, corroborating FA as a childhood-onset disorder. 79% of participants from the FGPR and 87% of participants from the CCRN reported ataxia as initial disease symptom. Study participants diagnosed at a younger age had a statistically significant quicker time to diagnosis compared to participants diagnosed at an older age (1.7 years vs. 7.3 years FGPR;  $p < 0.005$  and 1.9 years vs. 8.3 years CCRN;  $p < 0.005$ ). There was no significant change observed in time to diagnosis of individuals with FA over the past twenty years. Conclusions: Study participants diagnosed at a younger age have a quicker time to diagnosis than participants diagnosed at an older age. Although the difference in time to diagnosis between groups is significant, there is still a delay in diagnosis across all age groups. Despite new methodology to detect FA in recent years, the time to diagnosis has not changed. Improved disease recognition among health care providers could help quicken the time to diagnosis in individuals with FA as potential therapies may become available in the near future.

Keywords: Friedreich's Ataxia, ataxia, diagnosis, time to diagnosis, genetic testing, disease recognition

# Characterization of serum cardiac troponin I levels in subjects with Friedreich Ataxia

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 242

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**Background** Patients with Friedreich ataxia may develop cardiomyopathy, a feature of this progressive autosomal recessive neurodegenerative disorder that remains largely uncharacterized. Serum cardiac troponin levels are conventionally undetectable in the non-FRDA population, and their elevation in FRDA suggests they may represent a biomarker of cardiac stress in FRDA. In this study, we characterized serum troponin levels in cross-sectional and longitudinal cohorts of patients with Friedreich Ataxia and evaluated their association with markers of neurological and cardiac severity.

**Methods** Serum cTnI from 236 patients with genetically confirmed FRDA were obtained clinically for cross-sectional analyses, and 540 samples were used from 236 patients for longitudinal analysis. We analyzed these data with Spearman rank coefficients, T-tests, and linear and logistic regressions to investigate the association between detectable troponin and demographic, cardiac and neurologic measures. For cross sectional analysis, data were analyzed using the initial value from a given subject as well as separately using their most recent value.

**Results** 54% of subjects had detectable troponin levels at their most recent evaluation, with 30% of subjects having levels classified as abnormal as defined by their resulting laboratory. Provoked troponin levels were significantly higher than unprovoked levels. The presence of an abnormal troponin was predicted by younger age in logistic regression models in cross sectional analysis. Troponin levels correlated modestly with age of assessment ( $R_s = -0.41$ ,  $N=219$ ,  $p<.001$ ), and weakly but significantly with GAA repeat length on the shorter allele ( $R_s = -0.16$ ,  $N=229$ ,  $p<.01$ ), consistent with lower troponin levels at older ages and shorter repeat lengths. Weak but significant correlations were also observed between selected neurological measures (timed 25 foot walk, ataxia stage) at initial but not most recent troponin evaluation. Matching to proximate (within 1 year) echocardiogram evaluations revealed modest correlations between detectable troponin and intraventricular septal thickness at diastole ( $R_s = 0.39$ ,  $N=64$ ,  $p<.05$ ), and modest correlations with left ventricular mass ( $R_s = 0.51$ ,  $N=36$ ,  $p<.002$ ). Comparison of echocardiograms between subjects less than 18 years of age and 18 years and older revealed no differences in left ventricular septal thickness and posterior wall thickness, whereas internal dimension increased by 10% and left ventricular mass by 19% in the older cohort. 90 subjects had multiple troponin evaluations with detectable troponin values at either the first or final sampling. 30 had changes in detectability over time, with the majority of these (18) becoming undetectable after initially having detectable troponin reading. Of the subjects with detectable troponin at both time points, 34 had a decrease in troponin levels, 1 had no change, and 25 had an increase in troponin levels.

**Conclusion** These results demonstrate that a large number of FRDA patients carry elevations of troponin in the absence of cardiac symptomatology. The presence of detectable troponin levels and the magnitude of such levels was predicted by age, with older ages being associated with lower troponin levels. Cardiac hypertrophy, GAA repeat length, and a few neurological measures also correlated with troponin levels, but not could be independently shown to alter troponin when effects of age were accounted for. In addition, the presence of a detectable troponin rarely changes over time in longitudinal analysis, though there was a minimal tendency of such levels to decrease over time in individual subjects.

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## Tracking Progression in Friedreich's Ataxia (FRDA) to Establish Biomarkers for Clinical Trials.

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**Introduction:** Clinical trials using a variety of promising therapeutic compounds have been carried out in FRDA. The primary endpoints have included well established measures such as clinical rating scales, echocardiography and in one study, MRI of iron deposition in the dentate nucleus of the cerebellum, but none have demonstrated statistically significant improvement despite patients reporting subjective benefits. This has led the scientific community to investigate novel trial designs and explore the identification of new biomarkers that could more reliably capture progression of disease.

This is a longitudinal study assessing the change over time of several candidate imaging biomarkers and correlating them with known measures of disease severity, genetic data and frataxin protein levels. Our candidate biomarkers were selected because they have been shown in cross sectional studies to be significantly different from controls; OCT of retinal nerve fibre layer thickness and MRI metrics including DTI measures and volumetric analyses of the cervical spinal cord.

**Methods:** 63 patients with genetically confirmed FRDA were recruited at two sites, London and Oxford. At each of 3 time points, spanning 24 months, participants had the following data collected; full demographic and medical history, in depth ophthalmological testing, SARA, INAS and ADL scores, peripheral blood mononuclear cells for frataxin protein measurement. A subgroup of 23 participants underwent MRI investigations in addition to the above.

Ophthalmological data collected included visual acuity on a Snellen chart, Ishihara plate testing, Goldmann visual fields and optical coherence tomography images of the peripapillary retinal nerve fibre layer and macula obtained with Fourier Transform Spectral Domain OCT with TruTrack software (Heidelberg Spectralis, Heidelberg, Germany). Quantitative data using DTI, MTR, VBM and T2-relaxometry was obtained using a Philips 3T scanner for both brain and spinal cord imaging and analysis was performed using the FMRIB Software Library (FSL) v5.0, FMRIB, University of Oxford and Statistical Parametric Mapping (SPM) v12.0, Functional Imaging Laboratory, Institute of Neurology, and UCL used in conjunction with MATLAB. All statistics carried out in SPSS.

**Results:** We will have collected and analysed data from the first time point and will report on the following:

- Cervical spinal cord cross sectional area (CSA) is significantly decreased in FRDA patients compared to age and sex matched controls (control  $84.9 \pm 0.32 \text{ mm}^2$ , FRDA  $58.9 \pm 5.33$ ,  $p < 0.0001$ , Mann-Whitney U test). Further to this, we investigated the correlation between cord CSA and the general peripapillary retinal nerve fibre layer thickness in FRDA patients and this was found to be significant  $\rho = 0.491$ ,  $p = 0.024$  (Spearman's rho).



Further correlations between GAA1 and AAO were also significant ( $\rho=0.5$ ,  $p=0.025$  and  $\rho=0.542$ ,  $p=0.011$  respectively), though SARA score was not.

- The DTI metrics fractional anisotropy (control  $0.72\pm0.038\text{mm}^2/\text{s}$  FRDA  $0.62\pm0.034$   $=0.0002$ ) is significantly different between controls and patients. We have further delineated the exact areas of maximal change within the cord using the Spinal Cord Toolbox software and demonstrate that the dorsal columns display the largest degree of separation, followed by all white matter, then lateral columns then grey matter.
- Peripapillary retinal nerve fibre layer thickness correlates with GAA1 size, age at onset and SARA score as well as frataxin protein levels.

**Conclusions:** OCT of the RNFL seems to be the most promising biomarker as it shows correlation with neurological and some MRI parameters, GAA1 length and frataxin protein levels and is easier and cheaper to perform than MRI. Our novel approach to imaging the spinal cord in FRDA demonstrates that we can differentiate specific tracts, allowing us to focus on the areas of known pathology such as the dorsal columns. It also corroborates what is known from neuropathological studies of the spinal cord. Given we are looking at the area of greatest degeneration, we expect that this will improve the standardised response mean when analysed longitudinally. These data build on what has been seen in other MR studies of the spinal cord in different FRDA cohorts.



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## Motor Evoked Potential Input Output Measures and FRDA Disease Burden

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### Rationale:

The process by which frataxin deficiency in Friedreich Ataxia (FRDA) leads to cellular and system dysfunction occurs with different time courses in different nervous system pathways, and effectiveness of therapeutic interventions may partly depend on target sites. Sensory function is often already severely affected at disease presentation, suggesting that these pathways may not be the optimal therapeutic target. Working under the overall hypothesis that corticospinal (primary motor) tract deterioration is the major mediator of functional decline in FRDA *after* diagnosis, and potentially a better therapeutic target, we aimed to develop physiologic measures of corticospinal tract integrity which can track FRDA disease severity of response to therapy over time. Single pulse transcranial magnetic stimulation (TMS) is an established method of assaying the integrity and function of primary motor cortex, based on the motor evoked potential (MEP), a measurable muscle twitch elicited by a single TMS stimulus of motor cortex. As part of a longitudinal study of neurophysiologic biomarkers in FRDA, we present cross-sectional data and longitudinal data on MEP measures and disease burden.

### Methods:

We performed single pulse TMS in 32 individuals with FRDA using a Bistim 200<sup>2</sup> with a 70 mm figure-of-eight coil (Magstim, UK) paired with a frameless stereotaxy system (Brainsight, Montreal, CA) to ensure reliability and precision of stimulation. MEPs were measured with adhesive surface electrodes over the first dorsal interosseous of the dominant hand using recording hardware (CED 1902 and 1401, CED, UK) and Signal 3.13 (CED, UK). After identification of an optimal location to reliably produce MEPs, resting motor threshold was determined using established standards. In patients with RMT greater than machine output, active motor threshold was measured during compression of a small length of plastic tubing to produce a constant contraction. Twenty stimuli at 110% of MT and 120% of MT were recorded while the test subject was engaged in a mental task designed to lessen anticipation of pulses. MEP amplitude was measured as the greatest peak to peak distance for each trial, and all trials not affected by excessive movement or myogenic artifact were averaged to produce a mean MEP at each intensity. The difference in amplitudes obtained with stimulation at 110% MT and 120% MT was used as an input-output measure. The correlation of MEP amplitude difference and age, age at symptom onset, shortest GAA repeat length, product of GAA repeat length and symptom duration (a measure of disease burden), and FARS scores was evaluated by Pearson correlations, and effect of time on MEP amplitude, and amplitude difference was evaluated by repeated measures ANOVA.

### Results:

The cohort included 15 females (47%), and mean age was 18 years (range 7-34). Mean age at symptom onset was 17.2 years (range 2-21). Mean length of the shortest GAA repeat was 734.5 (range 400-1150). Mean FARS score was 58.7 (range 32.5-86.5). Correlations between MEP amplitude at a single stimulation intensity (110% MT or 120% MT) and patient characteristics was modest ( $R = 0.22-0.43$ ), but the correlation between MEP amplitude difference and disease burden was strong ( $R=0.657$ ). Figure 1 illustrates this association across subjects, separated into patients

presenting under age 15 years, and those presenting after age 15 years, with a steeper decline in this cross-sectional example, in the younger presentation age group. In a smaller cohort of 18 patients (ages 11 years – 34 years) who underwent repeat testing at 3 months and 6 months after baseline testing, there was no statistically significant effect of time point on MEP amplitude or amplitude difference. Twelve months after baseline testing, there was a small decline in MEP amplitude difference (0.06 uV SD 0.03) in subjects with baseline MEP difference above 0.5 uV, though the difference was not statistically significant in this small cohort.

#### Conclusions:

While absolute MEP amplitude at a given stimulus intensity is poorly correlated with clinical factors, likely due to the high degree of variability in MEP amplitude between individuals, MEP amplitude difference, a measure which evaluates the increase in MEP amplitude with increasing stimulus intensity, is highly correlated with disease burden. These findings suggest a combined effect of disease duration and GAA repeat length on ability of primary motor cortex to recruit more neurons in response to greater activation, or less synchronous firing, leading to lower increases in MEP amplitude. These findings also illustrate that while changes occurring within an individual are not easily detectable over a period of months, MEP input-output curves may be a sensitive measure of corticospinal tract disease progression over longer periods of time.

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## PET Imaging With [18F]BCPP-EF As A Mitochondrial Complex 1 Biomarker For Friedreich Ataxia

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Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 222

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**Background:** Friedreich Ataxia (FA) is an autosomal recessive disorder caused by the partial deficiency of the mitochondrial protein frataxin (FXN) due to mutations in the FXN gene. Direct measurement of frataxin in tissues of interest (i.e. brain, dorsal root ganglion, heart) is challenging. FXN deficiency impairs iron-sulfur cluster biogenesis and mitochondrial energetics, involving mitochondrial complex 1 (MC1), in both murine model and humans with FA. [18F]BCPP-EF is a radiotracer with specific binding to MC1 detectable by positron emission tomography (PET; Tsukada et al., 2014) and in vivo demonstrates reductions in MC1 density in patients with neurodegenerative diseases such as Alzheimer's, Parkinson's and fronto-temporal dementia, compared to healthy volunteers (MIND-MAPS consortium studies). We set out to determine if MC1 density measurements using [18F]BCPP-EF PET has a potential role as a biomarker in FA.

**Methods:** A conditional frataxin knockout FA mouse model (MCK mouse JAX order 029720, Pedromini et al., 2014) has progressive mitochondrial dysfunction in heart and skeletal muscle (not in brain) with decreased heart rate, ejection fraction and fractional shortening typically seen by 7 weeks of age and the mice have a mean life expectancy of  $86 \pm 5$  days (~12 weeks). At approximately 7 weeks of age (when this phenotype emerges), dynamic PET/CT data was acquired for 60-minutes after delivery of [18F]BCPP-EF, in 6 wild-type (WT) mice and 6 MCK mice. The standardized uptake value relative to plasma (SUV<sub>R</sub>) for the 40 to 60 minutes interval was quantified as an index of MC1 density. Ex-vivo dissection and tissue radioactivity estimation was also performed.

**Results:** Standardized uptake values relative to plasma (SUV<sub>R</sub>) were significantly reduced by ~50% in the heart and 57% in skeletal muscle in MCK mice compared to WT mice with no difference in other tissues measured (Fig.1).

**Conclusions and next steps:** [18F]BCPP-EF PET imaging shows reduced MC1 density in frataxin deficient tissues in the MCK mouse model of FA. We are currently evaluating [18F]BCPP-EF PET as a measure of MC1 density in the heart and brain of healthy volunteers (HV) and FA patients, in an open-label, single center, PET imaging study. The first goal of this work is to determine if [18F]BCPP-EF, which has been demonstrated to be suitable for the quantification of brain MC1 density, can be used for the same purpose in the heart, in 6 HVs. If this is successful, we will proceed to compare cardiac MC1 density between adult FA patients and HVs. The overall goal of this work is to create and characterize translational imaging biomarkers for Friedreich Ataxia.

**References:** Tsukada. *Methods Enzymol.* 2014; 547: 417-31; Perdomini et al. *Nat Med.* 2014; 20: 542-7.

# Fully automated measurement of brainstem and cerebellar volume: imaging endpoint for Ataxia, PSP and FA in multicenter clinical trial studies

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 217

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**Background and Objective:** Automated measurement of brain volumes from MRI data can provide an efficient means to assess local brain neurodegeneration, supporting clinical decision making and clinical trial efficacy analysis as well as patient selection. As new therapies in different ataxias are entering clinical development, the improvement and validation of image analysis pipelines optimized for the specific brain regions affected can critically support trial design and clinical investigation.

**Methods:** LEAP<sup>1</sup> is a fully-automated brain parcellation technology based on machine learning and multi-atlas registration that has been optimized and validated for accurate, localized brain segmentation in different neurodegenerative diseases. Together with careful harmonization of MR imaging parameters across scanner manufacturers and models, standardized assessment of brain volumetry can be realized in multicenter clinical trials. Here, we present a scientific validation of a fully-automated volumetric assessment of cerebellar and brainstem regions as well as operational considerations for successful deployment in clinical trials.

## **Results and discussion:**

The LEAP pipeline has been trained to automatically segment cerebellar and brainstem sub-regions and was applied to a validation set of 17 T1W images with “ground truth” segmentations of the cerebellum using the same parcellation schema and 15 T1Ws with “ground truth” for the brainstem. Test images were inhomogeneity corrected and brain extracted prior to processing with LEAP. Performance results are presented in Figure 1. DICE scores were computed between LEAP generated and “ground truth” segmentations and a mean dice of 0.96 was reported for the overall cerebellum and 0.70 for individual cerebellar regions. For the midbrain, pons, medulla and the superior cerebellar peduncle regions a mean DICE of 0.89, 0.93, 0.88 and 0.61 was observed, respectively.

The LEAP pipeline for cerebellar and brainstem segmentation can be deployed to multicenter clinical trials in combination with protocol harmonization. Harmonizing MR imaging protocols across sites is required to minimize inter-scanner and inter-institute differences in image quality and quantification by means of acquiring data with identical spatial resolution and by harmonizing relevant MRI parameters including sequence type and contrast parameters (such as flip angle, echo time, repetition time) across manufacturers. Exemplarily performance results from applying the proposed method to images from Spinocerebellar Ataxia 3 (SCA3) patients collected across 9 clinical centers are presented in Figure 2 for different brainstem and cerebellar sub-regions.

## **Conclusion:**

We have presented results for a fully-automated segmentation pipeline to segment sub-regions of the brainstem and the cerebellum. The pipeline has been successfully applied to multi-centre studies in Ataxia and PSP (only Ataxia results shown here) but is applicable to other brain diseases that show neurodegeneration in relevant brain regions, including Friedreich’s Ataxia.

## **References:**

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# Circulating miR-323-3p is a biomarker for cardiomyopathy and an indicator of phenotypic variability in Friedreich's ataxia patients

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 204

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## Introduction

Friedreich's ataxia (FRDA; OMIM 229300), an autosomal recessive neurodegenerative mitochondrial disease, is the most prevalent hereditary ataxia. This rare, childhood-onset disease is characterized by a progressive loss of sensory neurons in the dorsal root ganglia (DRG) and posterior columns. The cerebellar dentate nucleus is also affected. Other non-neurological features of FRDA are scoliosis, diabetes and cardiac hypertrophy. The last is the primary cause of death in these patients. FRDA is most often caused by a homozygous GAA repeat expansion mutation (typically between 600 and 1200 repeats) in the first intron of the frataxin gene (FXN), which have been proposed as causes of decreased expression of the mitochondrial protein frataxin. The principal function of Frataxin is not clear yet, although it was described its role in iron homeostasis and Iron-Sulfur Cluster formation. Most research on FRDA has focused on understanding the role of frataxin in the mitochondria, and a whole molecular view of pathological pathways underlying FRDA therefore remains to be elucidated.

MicroRNAs (miRNAs) are noncoding RNAs that measure 18 to 22 nt in length and regulate gene expression by binding to their target mRNAs; miRNAs can be detected in many tissues and even in biological fluids such as serum, saliva or urine, where they are resistant to degradation by RNAases. Although a small number of studies have analyzed miRNAs in FRDA, their regulatory role in this disease has not been clearly reported.

## Methods

In this study, we used smallRNA-sequencing to identify a series of circulating miRNAs with differential expression in blood samples from 25 Friedreich's ataxia patients compared to healthy subjects (n=25). Then we validated miRNAs relative expression by RT-qPCR and performed statistical analysis to evaluate the area under ROC curves for FRDA diagnosis of this miRNA signature and to evaluate the phenotypic variability of FRDA patients according to miRNAs expression.

## Results

SmallRNA-sequencing and bioinformatic miRNAs analysis provided a biomarker signature to differentiate Friedreich's ataxia (FRDA) patients from healthy subjects. We proposed that these seven overrepresented miRNAs (hsa-miR-128-3p, hsa-miR-625-3p, hsa-miR-130b-5p, hsa-miR-151a-5p, hsa-miR-330-3p, hsa-miR-323a-3p, and hsa-miR-142-3p) represent key mechanisms in the modulation of several signaling pathways that regulate the pathophysiology of FRDA (Figure 1). In addition, we found that hsa-miR-323a-3p was significantly upregulated in patients with cardiomyopathy in comparison with other FRDA patients without this clinical feature, so proposing this miRNA as a biomarker for phenotypic differentiation in FRDA patients suffering from cardiomyopathy (Figure 2).

Finally, we found that some circulating miRNAs detected in plasma were also detected in some of the cellular models analyzed (i.e. fibroblasts, neuroblastoma and stem cells), reinforcing the hypothesis that these miRNAs play a relevant role in the pathophysiology of FRDA.

## Conclusion

In this study, we identify miR-323-3p as a candidate marker for phenotypic differentiation in FRDA patients suffering from cardiomyopathy. We propose the use of dynamic miRNAs as biomarkers for phenotypic characterization and prognosis of FRDA.

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## Using the patient voice to capture the clinical relevance of the 9-Hole Peg Test in individuals with Friedreich ataxia

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### Background

Patient-reported outcome and performance-based measures are utilized to assess the impact of Friedreich ataxia (FRDA) on patient functioning. The 9-Hole Peg Test (9-HPT) is frequently used in clinical and research settings to assess upper limb function and manual dexterity. While upper limb function is integral to the FRDA experience, the interpretation of 9-HPT performance as clinically meaningful to real-world outcomes in daily life is needed. Establishing the clinical meaningfulness of performance on the 9-HPT would support use of this assessment for regulatory and clinical monitoring.

### Objective

The aim of this study was to assess the clinical meaningfulness of performance on the 9-HPT in FRDA from the patient perspective. Specific objectives included: 1) assessing the functional impact of the upper limb impairment related to FRDA, 2) determining which functional impacts are considered relevant to the time taken to complete the 9-HPT, and 3) determining the extent to which changes in time to complete the 9-HPT reflects clinically meaningful changes in upper limb-related functional impacts.

### Methods

Semi-structured interviews were conducted with a convenience sample of individuals residing in the US with genetically confirmed FRDA. Participants' experiences related to their upper limb signs, symptoms, and daily functional impacts were explored using a semi-structured interview guide. The 9-HPT was subsequently administered, after which participants were asked to (1) discuss which signs, symptoms, and daily functional impacts were related to their ability to complete the 9-HPT, (2) rank the 9-HPT related functional impacts that were most important to them, (3) discuss whether time to complete the 9-HPT reflected the time taken to complete their most important daily activities, and (4) report whether improvement on 9-HPT scores would accurately reflect cumulative improvement in those activities over the course of the day (in terms of speed and efficiency). The interviews were audio-recorded and analyzed using qualitative and quantitative analytic methods.

### Results

Of the fifteen adults with FRDA participated in this study, 66.7% were female. The mean age was 33.5 years (SD = 9.1). The majority of participants were in the later stages of disease (73.4% in stages 4 or higher); however, all had completed the 9-HPT in under 150 seconds to screen into the study. Participants reported a total of 12 FRDA related upper limb symptoms, eight of which were reported as relating to performance on the 9-HPT. With regard to the overall assessment of the impact of FRDA on patient functioning, participants reported a total of 18 functional impacts across the eight domains. Among these, the physical functioning domain and the activities of daily living (ADL) domain were most impacted.

Four domains, encompassing twelve functional impacts, were reported as being related to the 9-HPT. These included physical functioning (n=5 functional impacts), ADLs (n=4 impacts), household activities (n=2 impacts), and role



functioning (n=1 impact). The functional impacts that were discussed as being related to the 9-HPT were also among the most frequently reported impact concepts overall (Figure 1), including: Handling small objects (n=15, 100.0%), dressing (n=13, 86.7%), eating and writing (n=11, 73.3% each), self-care (n=10, 66.7%), using electronics (n=5, 33.3%) and handling large objects (n=5, 33.3%). Participants most frequently ranked difficulty handling small objects as the most important 9-HPT-related functional impact.

Twelve (80.0%) participants agreed that their performance on the 9-HPT as measured by time to complete the test reflected the overall impact of FRDA on time spent completing their impacted activities throughout the day (Figure 2). All participants rated the importance of time when completing relevant tasks at a 7 or above on a scale from 0 (not important at all) to 10 (extremely important) and that improved time to complete an activity reflects the increased ease associated with the activity.

### **Conclusions**

The results of this study indicate that the functional impacts reported as being most salient to the participants, particularly physical functioning and the ability to perform ADLs, were also perceived to be related to 9-HPT performance. This suggests that even modest improvement in time taken to complete the 9HPT may reflect important real-world consequences associated with patient functioning. These findings serve as preliminary evidence that changes in the time taken to complete the 9-HPT are clinically meaningful in terms of real-world functioning, and that improvements in time to complete the 9HPT may translate into tangible benefits for patients.

Figure 1. FRDA functional impacts and their relevance to the 9-HPT<sup>†</sup>

Figure 2. Participant perception of the extent to which their timed 9-Hole Peg Test score reflects their Friedreich's ataxia (N=15)

# Skeletal muscle oxidative phosphorylation quantitation using creatine chemical exchange saturation transfer (CrCEST) MRI in adults and children with Friedreich's Ataxia

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**Background:** Friedreich's Ataxia (FA) is a neurodegenerative disease caused by reduced expression of the gene *frataxin*, which encodes the protein frataxin.<sup>1</sup> Frataxin regulates the assembly of iron-sulfur clusters necessary for mitochondrial oxidative phosphorylation (OXPHOS).<sup>2</sup> Previously, using <sup>31</sup>P magnetic resonance spectroscopy (<sup>31</sup>P-MRS) based techniques, individuals with FA have been shown to have impaired skeletal muscle OXPHOS capacity.<sup>3</sup> Like <sup>31</sup>P-MRS, creatine chemical exchange saturation transfer (CrCEST) magnetic resonance imaging (CrCEST-MRI) offers a safe, non-invasive, and reproducible method of assessing mitochondrial OXPHOS capacity, with the additional benefit of high anatomic resolution. We have previously used MRI to assess OXPHOS capacity in adults with primary mitochondrial disorders.<sup>4</sup>

**Objective:** To quantitate skeletal muscle OXPHOS capacity in adults and children with FA with CrCEST-MRI in comparison to healthy volunteers.

**Methods:** Affected participants were adults (18-65y) and children (7-18y) with a genetic diagnosis of FA, and healthy controls had a similar distribution of age, sex, body mass index (BMI), and population ancestry. CrCEST data from ongoing adult and pediatric observational studies have been collated for this analysis. OXPHOS capacity of the lateral gastrocnemius (LG) and medial gastrocnemius (MG) muscles were assessed using CrCEST-MRI at 3T using an in-magnet plantar flexion exercise paradigm as per our previous study.<sup>4</sup> Multiple CrCEST-based indices of muscle OXPHOS capacity were calculated, since identification of the most appropriate metric is one focus of this study. Visualization of CrCEST recovery time courses suggested linear fitting may be most appropriate, and these results are reported here. Outcomes include: increase in CrCEST with exercise (%asym) and recovery rate (magnitude of the slope from time=0 to 50% of absolute increase in CrCEST, %asym/sec). Responses with inadequate exercise (post-exercise increase in CrCEST <1%asym or >30%asym) were excluded.

**Results:** This interim analysis includes n=36 adult participants (26 control, 11 FA) and n=14 pediatric participants (5 control, 9 FA). Adult controls were 54% male, with median age 29y (IQI, 25-39), median BMI 24.4 kg/m<sup>2</sup> (IQI, 22.0-26.6). Adult participants with FA were 63% male, with median age 27y (IQI, 23-39), and median BMI 26.9 kg/m<sup>2</sup> (IQI, 24.1-29.4). Median GAA repeat length was 633 bp in the least affected allele (IQI, 467-740).

In adult controls, the median post-exercise increases in CrCEST (%asym) for the MG and LG were 5.0 (IQI, 4.6-5.8) and 8.9 (IQI, 6.0-10.4); in FA, these were 4.6 (IQI, 3.5-6.2) and 6.5 (IQI, 3.8-9.9). In controls, the median recovery rate, for the MG and LG were 0.021 (IQI, 0.009-0.046) and 0.045 (IQI, 0.030-0.054) seconds; in FA, these were 0.013 (IQI, 0.009-0.019) and 0.017 (IQI, 0.008-0.033). Recovery rates were slower in FA relative to controls in both the MG (p=0.025) and LG (p=0.0023), suggestive of decreased OXPHOS capacity in FA.

Pediatric controls were 80% male, with median age 9y (IQI, 7-10), median BMI 29%ile (IQI, 11-91). Pediatric participants with FA were 33% male, with median age 12y (IQI, 10-13y), median BMI 64%ile (IQI, 17-75). Median GAA repeat length was 850 bp in the least affected allele (IQI, 733-900). Six out of 9 completed MRI scans for children

with FA were included in this interim analysis; one participant did not exercise, and the remaining 2 scans were limited by technical difficulties.

In pediatric controls, the median post-exercise increases in CrCEST (%asym) for the MG and LG were 11.4 (IQI, 9.7-12.8) and 8.7 (IQI, 6.6-11.5); in FA, these were 4.0 (IQI, 2.2-6.4) and 4.2 (IQI, 2.6-7.1). Post-exercise increase in CrCEST was nominally greater in pediatric controls in the MG ( $p=0.059$ ). In pediatric controls, median recovery rate (%asym/sec) for the MG and LG were 0.105 (IQI, 0.078-0.146) and 0.038 (IQI, 0.023); in FA, these were 0.0125 (IQI, 0.004-0.025) and 0.025 (IQI, 0.013-0.040). Differences in this small sample did not reach statistical significance.

**Conclusion:** CrCEST MRI can be used to quantify skeletal muscle OXPHOS capacity in adults and children with FA and may indicate decreased OXPHOS capacity relative to healthy volunteers. Substantial limitations in exercise capacity are evident in some individuals with FA; individualizing exercise stimulus may improve the utility of CrCEST estimates. We are developing and evaluating multiple strategies for quantifying post-exercise recovery in CrCEST. Future directions include increased collection of healthy pediatric control data to develop reference ranges, measurement of CrCEST reproducibility and natural history, simultaneous measurements of bone quality, and association with clinical characteristics.

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## Frataxin deficiency in Friedreich's Ataxia is associated with reduced levels of HAX-1, a regulator of cardiomyocyte death and survival

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Our recent study through microarray analysis performed on Friedreich's Ataxia (FRDA) patient's lymphoblastoid cells stably reconstituted with frataxin, indicated HS-1 associated protein X-1 (*HAX-1*) as the highest up-regulated transcript (FC=+2, p<0.0006). This result was further assessed by qRT-PCR and western blot analysis on these lymphoblastoid cells and on HEK293 stably transfected with empty vector compared to wild type frataxin. The association between frataxin and HAX-1 was also observed in lymphoblasts and peripheral blood mononuclear cells (PBMCs) from FRDA patients compared to clinically unaffected heterozygous relatives and non-correlated healthy controls. Low frataxin mRNA and protein expression correspond to reduced levels of HAX-1.

Correlation between frataxin and HAX-1 was further evaluated in a larger group of PBMCs from FRDA patients (n=41) and from non-correlated healthy controls (n=27). A regression model for frataxin which included HAX-1, group membership and group\* HAX-1 interaction revealed that frataxin and HAX-1 are associated both at mRNA ( $R^2=0.55$ , Pearson  $r = 0.54$ , p<0.5) and protein level (model  $R^2=0.6$ , Pearson  $r = 0.81$ , p<0.001).

In FRDA the heart is frequently affected with typical manifestation of hypertrophic cardiomyopathy, which can progress to heart failure and cause premature death. Interestingly, HAX-1 belongs to a family of proteins involved in the protection of cardiomyocytes from apoptotic stimuli and indeed *HAX-1* heterozygous-deficient hearts exhibit increases in infarct size after ischemic injury. Except for frataxin, very few genes correlate with cardiac disease in FRDA.

Thus, in order to evaluate whether the relationship between frataxin and HAX-1 is detected in cardiac tissue, experiments of frataxin overexpression and silencing were performed in AC16 human cardiomyocyte cell line. We observe that HAX-1 protein levels are indeed regulated through frataxin modulation.

Our results suggest that HAX-1 could be considered as a potential candidate for further evaluation of its expression in cardiomyopathies, providing insights into their pathogenesis as well as improving risk stratification strategies.

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## Assessing the value of specialist centres for the diagnosis and management of the ataxias.

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Dr. Julie Vallortigara <sup>4</sup>, Ms. Vinciane Quoidbach <sup>5</sup>**

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**Background:** The European Brain Council (EBC), an organization promoting research on brain health and disorders in Europe, initiated a European study called the Value of Treatment (VoT) for Brain Disorders. Conclusions of this first phase of the VoT project highlighted the need for a more seamless management of brain diseases, and led to a second phase of the project focusing on RNDs (Rare Neurological Diseases). This new phase initiated in 2018 by the EBC is looking at the value of early diagnosis and intervention, with an aim to assess the benefits of coordinated care and multidisciplinary care patterns on patient outcomes. In the context of this second phase of the project, a two-year study focusing on ataxia, dystonia, and phenylketonuria (PKU) was funded. We are presenting here the ataxia case study.

This ataxia case study aims to expand on preliminary evidence for the value of specialist ataxia centres in being able to deliver early interventions in both the diagnosis and in management of patients with the ataxias. Data from the London ataxia centre collected over more than 10 years shows that patients value this service compared with other neurological clinics they may have attended.

**Methods:** This project will explore the patient pathways of individuals with different progressive ataxias and study the health economic effects of specialist ataxia centres compared with care in non-specialist settings. In order to do so, data has been collected in the UK using a patient survey distributed via a patient organization (Ataxia UK); this survey will then be adapted and disseminated in two other European countries: Italy and Germany. The purpose of the survey is to gather information about the diagnosis and the management of the ataxias in specialist and non-specialist settings. Survey questions include: (1) the length of time to get a diagnosis; (2) number, length, reason for hospital admissions; (3) patient satisfaction with services used; (4) management of symptoms.

In addition, we wish to look at the costs and consequences of specialist ataxia centres in terms of the ongoing managing of people diagnosed with ataxia. Therefore the survey also collects data on: 1) attendance at the specialist centres; (2) utilisation of other primary and secondary health care services; (3) out-of-pocket expenses incurred when receiving care (e.g., travel times and need for accommodation). We will use regression analysis to investigate the impact of being treated in a specialist ataxia centre on the consequences and costs, controlling for potential confounding factors, such as age, type and severity of condition and time since diagnosis.

**Outcomes:** This study will provide insight into the value of the specialist centres in terms of diagnosis, management of patients with rare conditions, including cost implications. Ultimately the EBC Value of Treatment project, including several cases studies, aims to influence the policy towards better treatment and care for people with rare neurological diseases across Europe.

**Acknowledgments:** This study is sponsored by EBC. Reata and Takeda are the industry funders. The authors would like to thank all the working group members, the ERN-RNDs (European Reference Network) representatives Dr Carola Reinhard and Dr Holm Graessner, and Prof Antonio Federico from the European Academy of Neurology, for their support towards this study.

# Brain atrophy in Friedreich ataxia preferentially manifests in cerebellar and cerebral motor areas: Results from the ENIGMA-Ataxia consortium

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Friedreich ataxia (FRDA) is the most prevalent inherited ataxia<sup>1</sup>, and is characterised by progressive motor incoordination resulting from neurodegeneration in the spinal cord, cerebellum, and corresponding white matter pathways<sup>2,3</sup>. Magnetic resonance imaging (MRI) has been used to localise and quantify anatomical brain changes *in vivo*, supporting the utility of non-invasive neuroimaging for characterising and tracking brain changes in this disease<sup>4</sup>. However, much of the formative work undertaken to date has relied on inference in small or modestly-sized cohorts. The ENIGMA-Ataxia international neuroimaging working group has been formed to maximise the robustness and reliability of neuroanatomical characterisation of FRDA (and other ataxias) through retrospective multi-site harmonisation and aggregation of MRI data (<http://enigma.ini.usc.edu/ongoing/enigma-ataxia/>).

In this study, harmonised processing of T1-weighted brain structural MRIs from 251 individuals with FRDA (age: median=31 yrs, range=8-66; disease duration: median=15 yrs, range=2-44; onset age: median=17yrs, range=2-44) and 275 non-affected healthy comparators (age: median=33 yrs, range=9-67; CON) was undertaken across 10 sites. Each site included a healthy control group that was age and sex matched to their FRDA cohort. Volumetric differences in grey and white matter were quantified across the whole brain using voxel-based morphometry, implemented using the SUIT toolbox for cerebellum<sup>5</sup>, CAT12 toolbox for cerebrum<sup>6</sup>, and robust quality control procedures developed by the working group. Between-group comparisons (FRDA vs. CON) were performed while controlling for site, age, age-by-cohort interactions, sex, and intracranial volume (ICV). Voxel-level inference was undertaken with family-wise error correction of statistical thresholds ( $p_{FWE} < 0.05$ ) to account for multiple comparisons.

Robust grey matter volume reductions in FRDA, relative to CON, were most evident in the cerebellar anterior lobe (lobules I-V) and adjacent parts of the posterior lobe (lobule VI), with greatest effect sizes ( $d > 0.8$ ) in antero-medial regions (Fig 1A). Sparser findings, with smaller effect sizes ( $d = 0.4-0.6$ ), were also evident throughout the posterior lobe, including in non-motor regions (Fig 1A). In the cerebrum, FRDA was associated with lower grey matter volume in bilateral thalamus, precentral gyri (primary motor area), superior temporal gyri, and cuneus (Fig 1B). White matter volume reductions were evident most strongly throughout the cerebellum, brainstem, and midbrain ( $d > 1.5$ ), although they also extended to cerebral regions underlying the motor and somatosensory cortices (Fig 2). Significant effects were also noted in the splenium of the corpus callosum.

This large, multi-site study of brain alterations in FRDA indicates that volumetric abnormalities are apparent across the cerebro-cerebellar (primarily motor) system, but most prominent in cerebellar and brainstem regions. Further work is underway to investigate inter-individual variability associated with symptom severity and disease progression.

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## How to diagnose ataxia

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One of the most common and medically concerning manifestations of ataxia is gait imbalance. In the case of cerebellar ataxia, there are a number of additional coordination functions which may be affected, and this adds to the disease burden associated with the cerebellar ataxias. There is a lack of 'tools' for readily describing and measuring dysfunction in these systems. This current program of work aims to instrument key aspects of the clinical examination that are utilized in the assessment of the ataxic patient. Customized inertial measurement units, speech recognition and visual-kinematic systems have been applied to a set of functional cerebellar domains.

# Progression of pyramidal degeneration is a major contributor of motor impairment in advanced Friedreich ataxia

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 146

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**Background.** Loss of hand function and dexterity is a major contributor to continuing loss of autonomy in Friedreich ataxia (FRDA) patients who are wheelchair-bound. Ataxia rating scales like the SARA and the FARS can only partly capture upper limb motor impairment in advanced FRDA, due to ceiling effects. Functional tests as the CCSF, a logarithmic computation of the time needed to realize 10 finger-pointing cycles and a 9HPT by the dominant hand, and “Serious games”, a computer-based game coupled with movement sensors, are more sensitive tools, but they do not discriminate between cerebellar and pyramidal impairment, making their scoring more erratic. We used finger tapping (FT) movement analysis as a way to specifically assess the contribution of corticospinal tracts (CST) dysfunction to upper limb impairment in FRDA, because this kind of rapid repetitive movement is typically affected in pyramidal pathology.

**Methods.** Twenty-one patients with genetically proven FRDA (13 females, 8 males, 1 left-handed) and matched controls for age, sex and handedness performed FT, consisting in repetitive index finger–thumb oppositions with the dominant hand for 90 seconds. Movement frequency and regularity were automatically computed for each cycle. Pearson rank correlation between movement parameters and tapping rank was calculated. Comparisons between groups were assessed with T-tests. Ten patients accepted to MEPs testing. MEPs were measured at the first dorsal interosseous of the dominant hand after cortical and cervical stimulations to determine the motor central conduction time (MCCT).

**Results.** FRDA patients were significantly slower and less regular than controls in FT (Table 1). Ten FA patients showed significant FT frequency reduction during the task (“Slowing” group). Those patients were older, had later age of onset, longer disease duration, and higher SARA score (Table 1). Earlier mean age of onset of the “Non slowing” patients may be a spurious finding due to their markedly younger age that makes it less likely to find later onset patients in this group. All patients of the “slowing” group had MEP testing and 9/10 had prolonged MCCT (median 13 msec, range 7-21; normal <9 msec) while the only patients from the “non-slowng” group who underwent MEP testing had an upper limit of normal MCCT of 9 msec.

## **Conclusion.**

Upper limb motor function significantly worsens in advanced FRDA, as shown by clinical rating scales and specific tests like CCFS and “Serious Games”, contributing to further loss of autonomy and increased dependence for all activities of daily living. While progressive cerebellar pathology is likely to partly underlie such worsening, CST pathology also progresses with advancing disease, causing weakness and impairment in fine motricity. Here we show that older FRDA patients with longer disease duration and more severe ataxia demonstrate significant slowing when performing rapid repetitive movements, a quantifiable sign of CST pathology that may be used as a biomarker. The role of pyramidal symptoms in advanced FRDA is further supported by the finding of prolonged MCCT in these patients.

In conclusion, worsening of CST pathology contributes to upper limb motor impairment in FRDA patients with advanced disease, a major factor in reducing autonomy in wheelchair-using patients. Therapeutic strategies for frataxin restoration should target the CST to prevent progression of motor impairment in advanced FRDA.

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## Age of symptoms onset is a major determinant of intrinsic functional brain architecture in Friedreich ataxia

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Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 144

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**Dr. Gilles Naeije<sup>1</sup>, Dr. Vincent Wens<sup>2</sup>, Dr. Nicolas Coquelet<sup>2</sup>, Dr. Martin Sjøgård<sup>2</sup>, Prof. Serge Goldman<sup>2</sup>, Prof. Massimo Pandolfo<sup>3</sup>, Prof. Xavier De Tiège<sup>2</sup>**

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**Background:** Neurological features of Friedreich ataxia (FRDA) are characterized by afferent ataxia followed by cerebellar and pyramidal symptoms. The underlying neuropathology consists of degeneration of the posterior columns of the spinal cord, spinocerebellar tracts, deep cerebellar nuclei, and corticospinal tracts. Recent studies using MRI volumetry and diffusion tensor imaging also showed involvement of multiple white and grey matter regions in the brain, also reflected in subtly altered neurophysiological and neuropsychological testing. Here, we used magnetoencephalography (MEG) to characterize the main determinants of FRDA-related changes in intrinsic functional brain architecture. Although fMRI is the most commonly used for this kind of studies in humans, this modality might be inappropriate in FRDA because mitochondrial dysfunction and the high prevalence of abnormal carbohydrate metabolism might lead to neurovascular uncoupling. MEG is free of these limitations as it directly records neural activity.

**Methods:** We compared the functional brain connectome at rest (rsFC) of FRDA patients with that of matched healthy subjects, then we then looked for correlation between rsFC changes, genetic and clinical parameters, including GAA1, age at symptoms onset, disease duration and severity of clinical symptoms.

Five minutes of MEG signals were recorded at rest from 18 right-handed FRDA patients and matched healthy individuals (Table 1). The MEG connectome was estimated as resting state functional connectivity (rsFC) matrices involving thirty-seven nodes from six major resting-state networks and the cerebellum. Source-level rsFC maps were computed using leakage-corrected broad-band (3-40 Hz) envelope correlations. Post-hoc median-split was used to contrast rsFC in FRDA patients with different clinical characteristics. Non-parametric permutations and Pearson rank correlation test were used for statistics.

**Results:** Although there was no significant difference in power, rsFC, node strength or global connectivity between the overall cohort of FRDA patients and healthy subjects, FRDA patients who developed symptoms after age 11 showed increased global connectivity and node strength compared with healthy subjects, as well as increased rsFC in 10 connections mainly involving the VAN, the DMN and the cerebellum network. Accordingly, the strength of cross-network interactions between major RSNs (mainly the VAN, the DMN and the cerebellar network) as well as node strength for a large number of the studied resting state networks correlated with the age at symptoms onset. No correlations were found between rsFC and other clinical parameters of disease progression and severity.

**Conclusion:** Decreased rsFC in brain disorders is classically interpreted as reflecting disease-induced disruption of functional integration, while increased rsFC is considered a compensatory mechanism, either promoting functional recovery by helping structurally damaged brain areas to remain functional or to functionally compensate for pathological alterations occurring in distant brain areas.

This MEG study demonstrates a close link between the age of symptoms onset and the intrinsic functional brain architecture of patients with FRDA, in particular the strength of cross-network interactions and the node strength of the cerebellum network, the VAN, and the DMN. It provides empirical findings supporting the existence of compensatory mechanisms or neural reserve in some FRDA patients to foster later (>11 years) age of symptoms onset.

Considering the robustness of neuromagnetic rsFC relying on power envelope correlation at the individual level, this study paves the way for the use of MEG as a potential marker for, e.g., sorting presymptomatic FRDA patients and the determination of optimal time-points for therapeutic trials.

# Quantifying the Cerebellar Cognitive Affective Syndrome in Friedreich Ataxia

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 141

**Dr. Louise Corben<sup>1</sup>, Dr. Adam Vogel<sup>2</sup>, Ms. Hannah Reece<sup>3</sup>, Ms. Eliza Blomfield<sup>4</sup>, Ms. Hiba Bilal<sup>4</sup>, Dr. Ian Harding<sup>5</sup>, Ms. Geneieve Tai<sup>6</sup>, Prof. Martin Delatycki<sup>1</sup>, Prof. Nellie Georgiou-Karistianis<sup>7</sup>**

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## Introduction

Cerebellar pathology is directly related to the disturbance of movement that characterizes Friedreich ataxia (FRDA). However, studies unequivocally demonstrating the role of the cerebellum in cognitive, language and affective regulation have dismissed the singular view of cerebellar control being confined to movement only<sup>1</sup>. This notion is underpinned by extensive neuroanatomical and neuroimaging studies that demonstrate cerebellar connectivity with cerebral areas involved in non-motor functions<sup>2,3</sup>. Accordingly, numerous studies reporting a range of cognitive impairments in individuals with FRDA<sup>4-6</sup> have emerged. However, an ecologically valid and clinically relevant tool to quantify cognitive changes in FRDA remains elusive. In an effort to address this gap, Hoche and colleagues<sup>7</sup> recently validated a scale that is sensitive to cerebellar-mediated cognitive deficits, or the so-called "cerebellar cognitive affective syndrome" (CCAS), which was first described by Schmahmann and Sherman<sup>1</sup>. CCAS is typically characterised by 1) executive deficits apparent as impaired set-shifting, abstract reasoning, working memory, planning, and verbal fluency; 2) visuo-spatial disorganisation and impaired visuo-spatial memory; 3) changes in personality as apparent by flattening or blunting of affect, disinhibition or inappropriate behaviour and 4) linguistic impairments<sup>1</sup>. The CCAS scale, comprising 10 items, was developed to be a bedside measure of the linguistic, executive and visuo-spatial components of cognitive control<sup>7</sup>. The CCAS has been validated in a cohort of individuals with spinocerebellar ataxias, but not in individuals with FRDA<sup>7</sup>. As such this study aims to 1) evaluate the capacity of the CCAS scale to quantify cognitive impairment in individuals with FRDA, 2) explore the relationship between performance on the CCAS and clinical metrics of the disease.

## Methods

The CCAS comprises 10 items and has an overall maximum score of 120. Failure on one item is considered possible CCAS, failure on two items probable CCAS, and failure on three items definite CCAS. Individuals homozygous for a GAA expansion in intron 1 of the *FXN* gene were invited to complete the CCAS.

## Results

Forty-eight adults (28 females) were administered the CCAS. The average age of disease onset was 14.6 years ( $SD=6.8$ ); average disease duration was 23.8 years ( $SD=11.4$ ); and average Friedreich Ataxia Rating Scale score (FARS) was 97.0 ( $SD=27.3$ ).

On average participants scored 85.8 ( $SD=16.0$ ) on the CCAS. Fifteen (31.25%) participants failed one item, four (8.33%) failed two items, and 23 (47.3%) participants failed three or more items. Seven individuals (14.5%) were unable to complete the cube draw item due to the severity of their motor impairment. Items that returned the greatest rate of failure were 1) Category switching (failed by 30 (62.5%) participants), 2) Phonemic fluency (failed by 23 (47.3%) participants) and 3) Semantic fluency (failed by 15 (31.5%) participants). The overall CCAS score negatively

correlated with the FARS score ( $r=-0.55$ ,  $p<0.01$ ) and disease duration ( $r=-0.51$ ,  $p<0.01$ ), and positively with age of disease onset ( $r=0.48$ ,  $p<0.01$ ).

#### Conclusion

Based on the criteria afforded to scoring on the CCAS, nearly half of the individuals with FRDA displayed definite CCAS. Of note is that the three most problematic categories were timed measures of speech output. Given the profound impact of the dysarthria that typifies FRDA, it is difficult to ascertain if a lower score was related to an impairment in category switching, semantic or phonemic fluency, or indeed reflective of the dysarthria that typifies FRDA. It was noted that given our cohort were particularly impaired (as indicated by the mean FARS score) and had a particularly long disease duration, tasks involving timed speech responses may well mask capacity to complete the task effectively. Failure on the task may well reflect significantly slowed production of speech, rather than deficits in verbal fluency per se. Further work exploring the performance of the CCAS by less impaired individuals, the role of dysarthria in performance and comparison with control participants is required prior to confirming that the CCAS measure is a valid and effective measure of cognition in individuals with FRDA.

Acknowledgements: Some of this data was collected in the context of the SpeechATAX project, co-funded by the Friedreich Ataxia Research Alliance (USA) and the Friedreich Ataxia Research Association (Australia).

<sup>1</sup>Schmahmann JD. *J Neuropsych Clin Neurosci*.2004;16(3):367-78.<sup>2</sup>Selvadurai LP et al., *Neurosci Biobehav Reviews*,2018; 84:394-406. <sup>3</sup>Harding IH et al., *Movement Disorders*2017; 32 (8) 1221-1229<sup>4</sup>Wollmann T et al., *J Clin Exp Neuropsych*. 2002;24(5):677-86 <sup>5</sup>Corben LA et al., *Cerebellum* 201716(4):757-763. <sup>6</sup>Corben LA et al., *Neuroscience*. 2011;192(382-390).<sup>7</sup>Hoche, F et al., *Brain* 2018; 141(1), 248-270.

# Ectopic Burden via Holter Monitors in Friedreich Ataxia

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 130

***Dr. Erika Mejia<sup>1</sup>, Ms. Abigail Lynch<sup>1</sup>, Mr. Patrick Hearle<sup>1</sup>, Mr. Oluwatimilehin Okunowo<sup>1</sup>, Dr. Heather Griffis<sup>1</sup>, Dr. David Lynch<sup>1</sup>, Dr. Kimberly Lin<sup>1</sup>***

*1. Children's Hospital of Philadelphia*

## Introduction

Up to 60% of deaths in Friedreich Ataxia (FA) are attributed to cardiac causes, with nearly 1 in 8 deaths due to arrhythmia. While previous studies describe frequent nonspecific ST changes and evidence of hypertrophy by electrocardiogram (EKG), few descriptions of portable heart rhythm monitoring in patients living with FA are available. Therefore, we describe the ectopic burden in patients with FA.

## Methods

Using a prospective natural history study of consecutive patients seen at a single center for FA (10/1998 – 03/2018), we performed a retrospective analysis of cardiac ambulatory arrhythmia monitoring as assessed by 24-48h Holter monitors. For patients with more than one Holter, the most recent Holter monitor was used for this analysis. Exploratory outcome measures included burden of ventricular and supraventricular ectopy, and other arrhythmias. Patient characteristics, arrhythmias and ectopy burden are described here.

## Results

Of 1044 patients from the natural history study, 75 (7.0%) had outpatient Holter data available for this analysis (Table 1). Forty-six (61.3%) were male, the median age of FA symptom onset was 8 years (interquartile range [IQR] 5-13 years, n=69), the median age at the time of ambulatory monitoring was 19.1 years (IQR 14.3-22.8 years, n=74). The median short GAA repeat length was 700 (IQR 546-850, n=70), and the median heart rate was 85 beats per minute (IQR 79-91 beats per minute). With respect to ectopic burden, 45 (60.0%) patients had supraventricular ectopy. 41 (54.7%) had rare (0-5%) supraventricular ectopic beats with 4 (5.3%) having frequent (>10%) ectopy. 15 (20.0%) had supraventricular runs and 1 (1.4%) patient had atrial fibrillation/flutter (n=74). 10 (13.4%) patients had ventricular ectopy with 8 (10.7%) having rare (0-5%) ventricular ectopy. There were no episodes of ventricular tachycardia. 6 (9.1%) patients had a pause of 2.0 seconds or longer (n=66).

## Conclusions

This is the largest retrospective cohort analysis of Holter monitor data in patients with FA. Over half of patients had supraventricular ectopy with four cases of frequent (>10%) atrial ectopic burden. There were rare cases of atrial arrhythmia and no cases of ventricular arrhythmia. One in 11 patients had a pause of 2.0 seconds or longer. While arrhythmias were rare, supraventricular ectopy was more frequently observed than ventricular ectopy. Further investigation is warranted, including correlation of Holter findings with disease specific markers, cardiac function, and degree of hypertrophy when concurrent cardiac imaging data is available. We also plan to investigate whether atrial ectopic burden, sinus pauses, and/or baseline heart rates are predictive of heart failure and other clinically relevant outcomes.



## Longitudinal gait and balance outcome measurement in individuals with Friedreich ataxia

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 127

**Dr. Sarah Milne<sup>1</sup>, Dr. Seok Hun Kim<sup>2</sup>, Dr. Anna Murphy<sup>3</sup>, Prof. Theresa Zesiewicz<sup>2</sup>, Mrs. Mary Danoudis<sup>4</sup>, Ms. Jessica Shaw<sup>2</sup>, Mr. Ritchie Malapira<sup>2</sup>, Dr. Tyagi Ramakrishnan<sup>2</sup>, Ms. Fatemeh Rasouli<sup>2</sup>, Dr. Eppie Yiu<sup>1</sup>, Prof. Nellie Georgiou-Karistianis<sup>5</sup>, Prof. Martin Delatycki<sup>1</sup>, Dr. Louise Corben<sup>1</sup>**

*1. Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute, 2. University of South Florida, 3. Monash Health, 4. Monash Ageing Research Centre, Monash University, 5. School of Psychological Sciences and Turner Institute for Brain and Mental Health, Monash University, Australia*

**Introduction** Individuals with Friedreich Ataxia (FRDA) first experience symptoms at an average of 10-15 years of age. Mobility progressively declines and the capacity to ambulate is lost approximately 10 years after initial onset. Improving mobility is a common goal of therapeutic interventions for individuals; however, there are no gold standard outcome measures that reflect the natural disease trajectory of mobility decline in FRDA. This study aimed to determine the gait and balance outcome measures most responsive to disease progression over 12 months in individuals with FRDA. It was hypothesized that instrumented measures would be more sensitive to change than clinical measures. **Methods** This study was a prospective longitudinal study, measuring gait and balance of ambulatory individuals with FRDA over a 12-month period. Sixty-one individuals (adults=43/children=18) underwent assessment at baseline, 54 individuals repeating the assessment at 12 months. Fifty participants ambulated without and 11 ambulated with a gait aid at baseline. Outcomes administered included: 1) gait parameters at preferred and fast speeds using the GAITRite® instrumented walkway; 2) postural stability test (PST) and limits of stability (LOS) test using the Biodex Balance System™SD with eyes open and eyes closed; 3) Berg Balance Scale (BBS); 4) reciprocal of the Timed 25 Foot Walk Test (25FWT<sup>-1</sup>); 5) Dynamic Gait Index (DGI); 6) daily step and energy activity using the SenseWear MF Armband accelerometer (SWA); and 7) Friedreich Ataxia Rating Scale upright stability subscale (FARS USS). Clinical severity was evaluated via the FARS neurological exam (FARS NEURO), the Scale for the Assessment and Rating of Ataxia (SARA) and disease duration. Correlations between objective measures and clinical severity were examined. The standardised response mean (SRM) was reported as the effect size index for comparison of internal responsiveness. Subgroup analyses of internal responsiveness were conducted to ascertain if there was a difference in change between children and adults; those ambulant with and without an aid; and those with typical onset and late onset FRDA. Sample size calculations were performed to detect a reduction of 50% in the rate of disease progression over a 12-month period for a between-group design. **Results** **Validity:** At baseline, strong negative correlations were seen between clinical gait and balance measures and the SARA, suggesting they reflect a similar underlying construct. Conversely, Biodex PST indices did not correlate with the SARA. Accelerometry and spatiotemporal GAITRite® variables demonstrated significant correlations with disease duration. **Responsiveness:** The Biodex PST eyes closed medio-lateral index had the largest effect size (SRM=0.829,  $p=0.004$ ) of any of the variables at 12 months; however, only 17 participants were able to complete the PST with eyes closed at the final visit. There were no significant changes in PST indices with eyes open. Of the clinical balance measures, the BBS (SRM=-0.720,  $p<0.001$ ), DGI (SRM=-0.692,  $p<0.001$ ) and FARS USS (SRM=0.824,  $p<0.001$ ) had the largest effect sizes over 12 months. Mean velocity at fast speed (SRM=-0.641,  $p<0.001$ ), cadence at fast speed (SRM=0.630,  $p<0.001$ ) and stride length at fast speed (SRM=-0.520,  $p<0.001$ ) were the most responsive spatiotemporal gait variables over this time. In contrast, the 25FWT<sup>-1</sup> did not show a significant change. Heel-to-heel base of support intra-individual variability at preferred speed was the only GAITRite® parameter of variability to demonstrate significant change



( $p=0.024$ ,  $SRM=0.332$ ); and daily step count was the only SWA variable to demonstrate significant change, reducing from (median [IQR]) 5371.1 [2543.9-8623.2] to 3185.0 [2098.6-6226.8] steps per day ( $p=0.031$ ). Over the 12 months, the SARA ( $SRM=0.385$ ,  $p=0.007$ ) and FARS NEURO ( $SRM=0.284$ ,  $p=0.043$ ) demonstrated significant change; however, effect sizes were smaller. The FARS USS and BBS were the only measures to demonstrate significant change in all subgroups. Based on the data, estimated sample sizes using the SARA and FARS NEURO were 432 and 794 participants per group. In comparison, for the FARS USS the estimated sample size was 95 participants per group, or if recruitment was restricted to children, 32 children per group were required with the DGI. **Conclusion** The Biodex PST with eyes closed is a highly responsive gait and balance test; however, most ambulant individuals with FRDA cannot perform this task. Clinical balance measures are able to measure change in a wider range of individuals and are more sensitive to decline in ambulant individuals with FRDA as compared to instrumented measures and the previously validated measures of disease severity, the SARA and FARS NEURO. These measures may provide responsive outcomes for clinical trials and therapeutic interventions. **Acknowledgements** This study is funded by the Friedreich Ataxia Research Alliance (USA) (FARA), PTC Therapeutics and Voyager Therapeutics, as a part of FARA's Biomarker Consortium.

# The Friedreich's Ataxia Integrated Clinical Database

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Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 122

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**Ms. Jane Larkindale<sup>1</sup>, Ms. Jennifer Farmer<sup>2</sup>, Ms. Diane Corey<sup>1</sup>, Mr. Richard Liwski<sup>1</sup>**

*1. Critical Path Institute, 2. FARA*

Drug development for Friedreich's ataxia (FA), like for many rare diseases, is hampered by a lack of clear understanding of disease progression in the population, and specifically in patients who are taking part in clinical trials. This is inherent in any rare disease where the numbers of patients studied is relatively small, and is magnified in diseases such as Friedreich's ataxia where progression is relatively slow (years, not months), and age of onset, rate of progression and specific symptoms vary between patients. This means that design of clinical trials that convincingly show the effect of a potential therapy is challenging, and trials need to be carried out over significant periods of time before a convincing drug effect can be measured. Many clinical trials that have been carried out in FA have failed to meet their primary endpoint, while patients report improvements in their symptoms on drug that could not be quantified in the context of the trial. In many cases, it is unclear if this is due to a placebo effect, trial protocols that are too short to detect differences or choice of the wrong endpoint.

Large collections of patient level clinical data are needed to help model disease trajectories, as well as to inform clinical decisions, economic models and aid in the development of new biomarkers and endpoints. However, developing large collections of data is a challenge for all rare diseases. In FA, the FA Clinical Outcomes Study (FA-COMS) has collected natural history data over 13 years, and has data from over 1,000 patients. This is a valuable database to initiate such modeling efforts, and significant value has been derived from it. However, patients in clinical trials, even on placebo, do not always show progression rates identical to those in natural history studies. This may be due to a placebo effect, due to increased or improved clinical care, more frequent clinical visits or other reasons. Accordingly, the Friedreich's Ataxia Research Alliance (FARA) worked with the Critical Path Institute (C-Path) to develop the FA Integrated Clinical Database (FA-ICD) and make it available to qualified researchers for use.

The FA-ICD contains anonymized patient-level data from the FA-COMS study, and from placebo arm data from four clinical trials (more data is being added as it becomes available). The data is in CDISC format suitable for submission to regulators. The data in the database is available to qualified researchers for free, and has been accessed 8 times in its first three months (anonymized patient-level data may be downloaded in excel or csv format and used for pre-specified research questions). Sharing of the data is directed by a steering committee consisting of the principle investigator of the FA-COMS study, a statistician with a great deal of experience in FA, an industry representative, representatives of two families with FA, as well as representatives from FARA and C-Path. This steering committee also convenes to discuss analysis that may be performed by the collaborators and shared with the community. Increasing the characterization of FA and developing clear quantitative models of patient-level disease trajectories would allow us to better understand how disease progresses as measured by defined outcome measures and/or biomarkers in both natural history studies and in clinical trials. This in turn would aid in understanding the factors that affect the variance in progression rates and placebo affects in trial populations, and help inform development of clinical trial protocols that could more efficiently determine if a new therapeutic was effective or not. This would accelerate clinical development, make it less expensive, and encourage more companies to develop drugs for FA, in addition to many other uses of the database. The database may be accessed through <http://curefa.org/researchresourcesunder FA Integrated Clinical Database>.

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## Objective analysis of syllable repetition tasks in Friedreich Ataxia: is this a valuable speech task?

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Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 91

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**Ms. Hannah Reece<sup>1</sup>, Ms. Geneieve Tai<sup>2</sup>, Ms. Hannah Carter<sup>3</sup>, Dr. Louise Corben<sup>2</sup>, Prof. Martin Delatycki<sup>2</sup>, Dr. Adam Vogel<sup>4</sup>**

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People with Friedreich ataxia (FRDA) present with a mixed spastic-ataxic dysarthria characterised by imprecise articulation of consonants, slow rate of speech, reduced vocal control, reduced breath support for speech and overall reduced intelligibility of speech. Syllable repetition tasks (also known as Diadochokinetic tasks (DDK)) are often used as a proxy of speech function in clinical exams but we know very little about their utility in FRDA. DDK tasks require speakers to repeatedly produce syllables quickly and clearly to assess the rate and regularity of repetitive movements of the oral articulators. Earlier work in small samples and a single timepoint have found that DDK tasks do not correlate with severity of dysarthria in FRDA or overall severity of ataxia in FRDA (Brendel et al, 2013; Brendel et. al, 2014).

We recorded the speech of 82 participants with FRDA collected over a 14-year period from 2005-2019 from the FRDA Clinic in Melbourne Australia. The relationship between speech outcomes and overall dysarthria severity (as perceptually rated by expert listeners), disease severity (Friedreich Ataxia Rating Scale (FARS)), and FRDA-specific diagnostic information (genetic information, age of onset) was explored over time. Data were recorded as part of their annual review within the clinic. In total 170 DDK recordings were included. The majority of participants had one timepoint (n=50), while the remaining had between two and five timepoints. Objective speech outcomes were derived from acoustic analysis.

Preliminary analysis revealed statistically significant associations between DDK variables (DDK rate, DDK period and perturbation of DDK period) and FARS, years post onset, intelligibility of speech as rated by expert listeners and perceptual measures of DDK performance. In-depth analysis will be conducted to determine changes in DDK over time and the relationship to ataxia severity. Additional data and analyses, including change over time, will be available when the poster is presented.

## The Friedreich ataxia impact scale – how do the alternative shorter versions perform over time?

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Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 89

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**Ms. Geneieve Tai<sup>1</sup>, Dr. Sarah Milne<sup>1</sup>, Dr. Eppie Yiu<sup>1</sup>, Prof. Martin Delatycki<sup>1</sup>, Dr. Louise Corben<sup>1</sup>**

*1. Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute*

The Friedreich Ataxia Impact Scale (FAIS) is a quality of life tool that was developed to address specific issues related to disease impact in people with Friedreich ataxia. The FAIS comprises 126 items and three alternative shorter versions of this tool have been proposed. The FAIS-OBS was developed for clinical-based research or observational studies, the FAIS-MORE was designed for studies examining people with a more severe disease, and the FAIS-LESS for studies comprising individuals with a milder disease. The FAIS was previously studied over a period of one and two years and demonstrated limited responsiveness to change<sup>2</sup>. This finding could be attributed to the FAIS losing its sensitivity when administered to a heterogeneous population of varying disease severity.

The aim of the current study is to therefore examine the change over one year in the three alternative versions of the FAIS in the respective groups that they were designed for. To determine the disease severity in participants, we used the FARS score: participants who scored >80 on the FARS were administered the FAIS-MORE and participants who scored ≤80 were administered the FAIS-LESS. The FAIS-OBS was used on all participants.

The FAIS-OBS was completed by 75 participants over one year. Subscales measuring symptoms and physical functioning (body movement ( $p=0.03$ ), speech ( $p=0.01$ ), upper limb functioning ( $p=0.04$ ), complex tasks ( $p<0.01$ )) showed significant change over this time, with participants reporting greater impact of these aspects of health at Year 1 compared to baseline. Subscales measuring psychological and social impact did not show significant change. The FAIS-MORE was administered on 51 participants. For this version of the FAIS, the complex tasks ( $p<0.01$ ) and lower limb functioning ( $p=0.04$ ) subscales showed a significant change over time. Twenty-one people completed the FAIS-LESS – there were no significant changes over one year in any of the subscales in this scale.

Both the FAIS-OBS and FAIS-MORE show greater responsiveness over time when compared to the full item FAIS. The FAIS-LESS did not demonstrate any significant change, although this finding may be due to the smaller number of individuals studied. Further longitudinal studies on a larger cohort with the three versions to determine their sensitivity are required.

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# Health Related Quality of Life in Friedreich Ataxia

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 87

**Ms. Emily Xiong<sup>1</sup>, Ms. Abigail Lynch<sup>1</sup>, Ms. Jennifer Farmer<sup>2</sup>, Dr. Christian Rummey<sup>3</sup>, Dr. David Lynch<sup>4</sup>**

**1. CHOP, 2. FARA, 3. Clinical Data Science GmbH, 4. Children's Hospital of Philadelphia**

**Introduction:** Friedreich Ataxia (FRDA), an autosomal recessive neurodegenerative disease, leads to progressive difficulty with coordination, gait, and speech. It is caused by mutations in the *FXN* gene, which codes for the protein frataxin. In clinical studies, a variety of measures such as exam based scales and performance measures capture progressive neurological dysfunction in FRDA. However, an equally important aspect of understanding the disease is assessment of health related quality of life (HRQOL), the patients' perspective on his/her disability. This study assessed the HRQOL of FRDA patients through participant's responses to HRQOL questionnaires.

**Methods:** The SF-36, a generic HRQOL instrument, and symptom specific scales from the MSQLI were administered to adult FRDA patients at the time of their enrollment in a large natural history study. 501 subjects participated. Responses were scored based on each instrument's standard scoring, and analyzed by comparison with population norms and correlation with disease features such as age at onset, GAA repeat length, disease duration and age. Multiple linear regression models were also used to account for independent effects of genetic severity and age. Assessments were performed at baseline and every other year thereafter.

**Results:** Subjects were on average young adults (mean age=32.4 ±13.6 years), with disease onset as teenagers (16.2 ± 10.2 years), and disease duration of 16.1 years. In cross sectional analysis of the SF36, the subscale with the lowest HRQOL score was the physical function scale (27.6 ±26.6). The emotional well-being score was the highest among SF36 subscores (67.5 ±17.8). We also examined correlations of SF36 scores with disease features. The physical function scale correlated with age of onset ( $P<0.0001$ ), duration ( $P<0.0001$ ) and age ( $P<0.0001$ ). The physical role limitations-physical scale correlated with age of onset and duration; the role limitations/emotional scale correlated with disease duration ( $P=0.0249$ ) and age ( $P=0.0151$ ); the pain scale with duration ( $P=0.002$ ) and age ( $P=0.0382$ ); and the change in health correlated only with age of onset ( $P=0.0046$ ). In assessment of symptom specific scales, bladder control scores (BLCS) correlated with duration and age, while impact of visual impairment scores (IVIS) correlated with duration. In linear regression models, BLCS, Pain Effect Score (PES), and IVIS scores were predicted by age and GAA repeat length; Modified Impact Fatigue Scale (MFIS) scores were predicted only by GAA repeat length. We also examined the change in SF-36 and symptom specific scores over time. Physical function and role limitation scores (both emotional and physical) declined over time. Although initial scores for these subscores were slightly lower in subjects with early onset, the rate of change was similar in subjects with different ages at onset. Essentially no change was seen over time in any of the other subscores. Symptom specific scales also worsened over time; this was most notable for the IVIS and BLCS, with less consistent change in the MFIS and PES. For the IVIS, the decline was far more prominent in individuals with onset at age 14 or below. For other scores progression was similar in subjects stratified by age of onset.

**Conclusion:** The present study demonstrates that HRQOL instruments capture patient reported dysfunction in FRDA in a manner that reflects disease status. HRQOL dysfunction was greatest on physically related scales, and such scales correlated with disease duration, indicating that they worsen with progressing neurologic disease. In addition, a limited number of subscores also reflect genetic severity as measured by age of onset or shorter GAA repeat length. In longitudinal analysis, physical functioning scores declined over time as did vision scores on the IVIS scale, demonstrating the value of such scales in serial evaluation of FRDA.

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## Predictors of Loss of Ambulation in Friedreich's Ataxia

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Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 60

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***Dr. Christian Rummey<sup>1</sup>, Ms. Jennifer Farmer<sup>2</sup>, Dr. David Lynch<sup>3</sup>***

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**Introduction** – Loss of independent ambulation (LoA) is the crucial event in the disease course of patients affected by FRDA. Like in other progressive diseases it is also in FRDA a slow, gradual transition rather than an acute event, which complicates its analysis and limits its direct use as an outcome measure. We analyzed items in the mFARS exam, a well-established neurological rating scale in FRDA, for their potential to more clearly characterize the process of LoA in FRDA.

**Methods** – In a dataset derived from the Friedreich's ataxia clinical outcome measures study (FA-COMS, 4606 yearly follow up visits in 1021 patients), time to event analyses were conducted to estimate disease duration at LoA, as well as loss of other walking and stance functions. We used interval censoring, including left censored observations to avoid potential truncation bias. Age of Disease Onset was used for stratification.

**Results** – In early onset FA (onset <15y of age) during the follow up time 148 subjects experienced LoA at a median disease duration of 11.5y (95%CI 10.5-12.0). Estimates for LoA in later onset groups were markedly higher (18.3y for onset between 15-24y and 23.5y for onset >24y) and fewer events were observed during the follow up time (42 and 21, respectively).

Similar time to loss of function analyses for the stance items in FARS E subscore revealed a unique sequence of function loss in these items (Figure 1). Less than 5% of patients did not follow this general rule. With the help of this pattern, we can further stratify patients into groups with more defined probabilities for LoA. This subsequently allowed to estimate future event rates, facilitating the calculation of sample sizes for *feasible* clinical studies in FRDA, with LoA as specific outcome.

**Conclusions** – This work presents the first reliable estimates for Disease Duration at Loss of Ambulation in early onset (<15y of age) FRDA patients. In addition, we found a specific sequence of stance function loss prior to LoA, with each step being predictive of increased risk of losing ambulation. These clearly defined steps before LoA will help selecting subjects with defined risks of progression, e.g. to examine LoA directly, or studies looking at earlier phases of the disease could select patients at lower risk for LoA, avoiding ceiling effects observed with the mFARS in studies using more advanced patients.

Figure 1 - Stepwise function loss in individual Stance items (from the FARS-Upright Stability Subscore), as well as LoA/E7.

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## Psychometric Properties of the Friedreich Ataxia Rating Scale

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Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 62

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***Dr. Christian Rummey<sup>1</sup>, Dr. Louise Corben<sup>2</sup>, Prof. Martin Delatycki<sup>2</sup>, Prof. S H Subramony<sup>3</sup>, Ms. Jennifer Farmer<sup>4</sup>, Dr. David Lynch<sup>5</sup>***

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**Introduction** – Historically, ataxia was measured using the International Cooperative Ataxia Rating Scale (ICARS), including early trials in Friedreich's Ataxia. Today ICARS has been replaced by either the Scale for the Assessment and Rating of Ataxia (SARA) and the Friedreich's Ataxia Rating Scale (FARS), in part due to unfavorable psychometric properties. In contrast to ICARS and SARA, a detailed psychometric analysis of the FARS has not been reported. Specifically, the consequences on the overall scale characteristics due to reduction in items from the initial full neurological FARS exam (FARSn) to the newly formed and now widely used modified FARS scale (mFARS) is of major importance.

**Methods** – Based on cross-sectional FARS data from 1011 patients recruited into the FA-Clinical Outcome Measures study (FACOMS), correlation-based psychometric analyses were conducted to investigate the interplay of items and sub scores within the FARSn/mFARS constructs. Potential floor or ceiling effects were analyzed, and Cronbach's alpha values calculated for sub- and total scales. Clusters of intercorrelating items were evaluated for their clinical value and interpretation by exploratory principal component analysis (PCA).

**Results** – Our analysis confirmed the validity and structure of the FARSn exam, but specifically endorses the modifications leading to the mFARS scale. Overall, both scales showed appropriate item-subscale groupings, inter-subscale correlations, and internal consistency (Cronbach's alpha >0.90). However, two sets of items within the complete 125-points FARSn scale showed clear weaknesses (See Table 1). First, items in the peripheral nervous system (D) sub score have inefficient within subscale correlations and form a weak construct. Second, the A1 (facial atrophy)/A2 (tongue atrophy) items correlate weakly with other items in the bulbar (FARS A) sub score as well as other parts of the FARS exam.

In addition, within the FARS-E/Upright Stability sub score, specifically the analysis of the stance related items revealed interesting features. While the easier, initial three stance items (E2A, E3A and E2B) show preferable inter-item correlations and should function well within the item response theory, the increasing difficulty in the second three items (E3B, E4 and E5) resulted in only very few patients being able to perform these tests. These items did not provide useful information for patients in our cohort but may behave differently in clinical studies targeted to individuals with FRDA who are very early in the disease process.

**Conclusions** – Overall, the results provide support for both the FARSn and the mFARS constructs, as well as individually for their upper limb- and lower limb coordination components. The omission of the peripheral nervous system subscore (D) and two items of the bulbar subscore (A) in mFARS strengthens the overall construct compared with the complete FARS. Such information is crucial to the ongoing application of the mFARS in natural history studies and clinical trials. Additional analyses of longitudinal changes will be necessary to fully ascertain its utility, especially in non-ambulant patients.

Table 1 - Item-Sub score Correlations in the Neurological FARS Scale. Beneficial correlations are depicted in blue, problematic, low correlations are colored in red (bulbar, peripheral and some of the stance items)



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# The Identification of the Most Important Friedreich's Ataxia Symptoms: Results From a Patient and Caregiver Study

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Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 69

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**Ms. Christine Zizzi<sup>1</sup>, Ms. Ellen Wagner<sup>1</sup>, Ms. Brittany Greco<sup>1</sup>, Ms. Jane Larkindale<sup>2</sup>, Dr. Louise Corben<sup>3</sup>, Prof. S H Subramony<sup>4</sup>, Dr. Chad Heatwole<sup>1</sup>**

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**Background:** Friedreich's ataxia (FA) is a progressive, neurodegenerative disorder. Individuals with FA face a variety of symptoms that significantly impact their lives. As therapeutic trials are planned for individuals with this disorder, it is important to have a clear understanding of the symptoms that have the greatest impact on the lives of individuals with FA.

**Objectives:** To utilize interviews with individuals with FA and caregivers of young people with FA to identify the symptoms that generate the greatest disease burden in the FA population.

**Methods:** We interviewed individuals with FA aged 11 years and older and caregivers of children aged 0 to 10 years with FA. Using open-ended questions, participants with FA identified the symptoms and issues that have the greatest impact on their lives. Caregivers were asked to identify the symptoms and issues that they observe to have the greatest impact on their children's lives. Interviews were recorded, transcribed, and will be analyzed using a qualitative framework technique, triangulation, and a three investigator consensus approach.

**Results:** We interviewed 23 individuals with FA and 7 caregivers of young people with FA. Eight of the individuals with FA were minors. Participants represented 19 states across the United States. Additional interviews are currently underway and will be conducted until concept saturation has been reached. The collective data from all patient and caregiver interviews, including quote and symptom frequency, will be reported at the time of the meeting and will include insight into disease burden from caregivers and individuals with FA of different ages and demographics.

**Conclusions:** There are many symptoms and symptomatic themes that impact the lives of individuals that live with FA. Affected individuals and caregivers are uniquely suited to provide granular input into the symptomatic burden experienced by individuals with FA. Data regarding what symptoms generate the greatest burden is useful as researchers plan studies in FA, clinicians take care of individuals with FA, and as relevant disease-specific outcome measures are developed for this population.

**Acknowledgements:** Funding for this project was provided by the Friedreich's Ataxia Research Alliance (FARA) and the project was developed in collaboration with the FARA PRO working group.



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## Design of Self Configuring Optical Modules for Accurate Walk Timing in a Clinical Setting

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Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 74

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***Dr. Harry Saal<sup>1</sup>, Dr. Leonard Shustek<sup>2</sup>***

*1. Retrotope, Inc., 2. Computer History Museum*

Measuring the time for a patient to walk a fixed distance is a broadly adopted clinical measure in ataxic indications. Studies of longitudinal changes in the time (traditionally converted to speed by inversion) demonstrate increased time as the disease progresses and have been employed as a endpoint in several clinical trials. The change can be quite gradual, particularly in diseases that take decades to progress from diagnosis to end of life. Traditional tools, such as a stopwatch or smart phone manually operated by a clinician observing a patient crossing a floor marked with start and stop lines, introduce uncertainties that may exceed the underlying longitudinal change. To more accurately measure walk time sub-second changes, we have developed a self-configuring wireless hardware software system (Figure 1) wherein the subject's leg breaks an IR beam at the starting and ending positions. The system reports the elapsed time with an accuracy better than 0.1 seconds, which we believe is several times better than uncertainties introduced by traditional handheld methods.(There are other uncontrolled day-to-day variables that persist even when using this apparatus.) We describe the software algorithms developed to handle unreliable wireless packet transmission and relative clock drift between the devices. We also report on the performance of the system under test using a mechanical walk simulator.

## FA-CHILD – A 6 Month-Interval Natural History Study in Children with Friedreich’s ataxia

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 80

***Dr. David Lynch*<sup>1</sup>, *Dr. Christian Rummey*<sup>2</sup>, *Ms. Jennifer Farmer*<sup>3</sup>, *Dr. Alexandra Clay*<sup>1</sup>, *Prof. S H Subramony*<sup>4</sup>, *Prof. Susan Perlman*<sup>5</sup>**

*1. Children’s Hospital of Philadelphia, 2. Clinical Data Science GmbH, 3. FARA, 4. University of Florida, 5. University of California, Los Angeles*

**Introduction** – Two large natural history studies in Friedreich ataxia have advanced the speed of clinical research and clinical trials in Friedreich ataxia (FRDA). These studies, called the Friedreich ataxia Clinical outcome measure study (FACOMS) and the European Friedreich ataxia Consortium for Clinical and translational studies (EFACTS), follow almost 2000 subjects with yearly evaluations. However, the common design of these studies has at least two features that limit their applicability to clinical trials. First, the studies contain a focus on all individuals with FRDA, with a smaller focus on children, who may be the most likely target of novel therapies. Secondly, the yearly visit structure of both FACOMS and EFACTS is not frequent enough to match the evaluation pattern of clinical trials. The present ongoing study is assessing the natural history of FRDA on a cohort of children with more frequent evaluations. The baseline data are reported here.

**Methods** – One hundred children with FRDA were recruited to participate in a natural history study examining medical and neurological features of FRDA. Visits occurred every six months at one of three sites: The Children’s Hospital of Philadelphia, the University of Florida, or the University of California Los Angeles. Subjects underwent a series of evaluations: FARS, 9-hole peg test, timed 25-foot walk, timed one-minute walk, Berg balance scale, and medical history review. At the primary site (CHOP), subgroups also participated in motor evoked potential testing and Creatine-CREST. Frataxin levels in blood were also determined by mass spectrometry.

**Results** – Enrollment finished on 7/1/2019, and evaluations will continue for 3 years. Of the first 85 subjects, the majority enrolled at CHOP (76.5%) with smaller numbers at UCLA and UF (10.6 and 12.9 % respectively). Mean age was 13.4 + 2.7 years, with a disease duration of 6.5 years (+ 3.3). Shorter and longer GAA repeat lengths were 824 + 189 and 966 + 308. Nine subjects (11%) carried point mutations. Mean mFARS score was 40.4 + 15.3, and 61% of subjects had disability scores of 1 or 2, 15% having disability scores of five or greater. 35 individuals (41%) could not perform a 1-minute walk, and 46 (54%) could not perform a 6-minute walk test.

**Conclusion** – The cohort identified here provides a representative group for investigation of the change over time in clinical measures and biomarkers in FRDA. Its ongoing characterization will be useful in planning of future therapeutic interventions.

# Health Related Quality of Life in Children with Friedreich's Ataxia

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Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 81

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***Dr. Christian Rummey<sup>1</sup>, Dr. Manuela Corti<sup>2</sup>, Ms. Jennifer Farmer<sup>3</sup>, Dr. David Lynch<sup>4</sup>, Prof. S H Subramony<sup>2</sup>***

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**Introduction** – Only Little has been reported on the association between clinical outcome measures and patient health-related quality of life in FRDA, specifically in affected children. The Pediatric Quality of Life (PedsQL) Instrument has been used e.g. in Duchenne Muscular Dystrophy with mixed results (Davis et al 2010; Messina et al. 2016), especially regarding sensitivity to detect change. We compared the PedsQL Generic Core Scales (including Physical and Psychosocial Domains) with the FARS Activities of Daily living scores, as well as the neurological mFARS scale (Patel et al. 2016) and FARS Functional Disease Staging (FDS) in children enrolled in the FA-COMS Natural History Study.

**Methods** – Extensive correlation analysis were conducted between functional measures and all available QoL scales/domains. Change by age and disease duration was evaluated descriptively and with the help of random coefficient regression models. Sensitivity to change was assessed using standard response means. Overall scores were compared qualitatively with PedsQL results from children affected by other diseases.

**Results** – PedsQL and ADL results were available for 286 Children and Teenagers (age 5 to 18y), with the large majority (94%) being ambulatory at the initial visit. Correlations of PedsQL scales with the mFARS scale were modest at best, with the best associations achieved in the physical domain. The FARS ADL showed clearly better correlations with mFARS than the PedsQL Total and individual scales. Correlations of Parent/Proxy- with direct Children/Teen-reports were good, but not outstanding, ranging from  $r=0.72$  (TOTAL PedsQL) to 0.56 in the Social Functioning Domain.

When PedsQL scales were plotted over Age, disease duration and FDS, decline over time (disease duration and age) was also most evident in the Physical functioning domain (Figure 1). Emotional, social and school functioning domains showed only slight deterioration over disease duration.

137 patients had follow-up data available. Changes in the PedsQL scales however correlated only weakly with functional scores.

Although such a comparison might be difficult, overall PedsQL scores, on a qualitative level, were low and when compared with children affected by other diseases (e.g. diabetes, asthma, cancer) (Varni et al. 2007). They are however comparable with results from children with Duchenne Muscular Dystrophy (Henricson et al. 2013; Messina et al. 2016).

**In conclusion** – For children/teens affected by FRDA, we found the PedsQL GCS correlating only modestly with the level of impairment at cross-sectional level, most evident in the physical functioning domain. Although further studies comparing different QoL tools are needed to better elucidate the complexity of the relationship between the PedsQL and functional assessments, sensitivity to change in comparison to FRDA efficacy measures were not promising.

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## Use of Antidepressants in the FA-COMs Natural History Study

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 30

**Dr. Christian Rummey<sup>1</sup>, Ms. Jennifer Farmer<sup>2</sup>, Dr. Louise Corben<sup>3</sup>, Dr. David Lynch<sup>4</sup>**

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**Introduction** – While the traditional perspective on Friedreich ataxia (FRDA) emphasizes physical signs and symptoms, depression and anxiety may be common comorbid conditions (Flood & Perlman 1987; Neito, et al 2018, Reetz et al. 2018). The prevalence of antidepressant or anxiolytic medication use in FRDA has so far not been reported, and little is known on how the use of such compounds might impact outcome measures in FRDA, specifically the results of neurological rating scales. Understanding such effects may be important as some studies have suggestive clinical benefit with antidepressant therapy (Rohr et al. 1999). The goal of this study was to define the frequency of antidepressant use in FRDA, and the subsets of patients who take them.

**Methods** – Concomitant Medications Logs from the Friedreich's Ataxia Clinical Outcome Measures Study were manually coded, categorized and analyzed descriptively based on age and disease duration. The number of cases of depression and anxiety were taken from current medical conditions log. Analyses were stratified by disease severity (onset groups).

**Results** – The most commonly used antidepressants or anxiolytics were selective serotonin reuptake inhibitors (SSRIs) (e.g. sertraline, fluoxetine, citalopram, escitalopram, paroxetine), followed by anxiolytic benzodiazepines (clonazepam, diazepam, alprazolam, amitriptyline, etc.), and opioids (hydrocodone, tramadol, oxycodone).

We found that in children the use of antidepressants was generally low, with few individuals taking any psychoactive medicines. However, during late teenage years and early twenties, the percentage of patients using SSRIs and anxiolytics steadily increases (Figure 1).

Use of such agents was similar across multiple strata of age of onset. In the early onset group (onset <15y of age), the percentage of patients using antidepressants reaches 30% and higher in the 3<sup>d</sup> decade of life, corresponding to a disease duration of around 20y. Later onset groups show similar results, with fewer absolute number of patients. Depression was reported in 187 individuals (20%) and anxiety in 85 patients (9.3%).

The use of atypical antipsychotics (e.g. quetiapine, risperidone) was limited to very few individuals (<5), likely not exceeding prevalence in the general population.

**In conclusion** – This preliminary study suggests that in specific age groups up to 35% of FRDA patients use antidepressants, anxiolytics or occasionally antipsychotic agents. This finding justifies a more thorough analysis including a correlation with underlying diagnosis, with the goal of establishing the impact on clinical outcome measures and defining methods for prospective identification and treatment of psychiatric issues in FRDA.

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Nieto A, Hernández-Torres A, Pérez-Flores J, & Montón F. 2018. Int J Clin Health Psychol. 2018 18(1):18-26.  
Figure 1 - Use of Antidepressant Medication by Disease Duration; bar height depicts % of patients, absolute numbers are given on top of the bars

# Brain white matter degeneration in Friedreich ataxia depends on disease severity: The IMAGE-FRDA Study

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 35

**Ms. Louisa Selvadurai<sup>1</sup>, Dr. Ian Harding<sup>1</sup>, Prof. Elsdon Storey<sup>2</sup>, Prof. Gary Egan<sup>1</sup>, Dr. Rosita Shishegar<sup>1</sup>, Prof. Martin Delatycki<sup>3</sup>, Prof. Nellie Georgiou-Karistianis<sup>4</sup>, Dr. Louise Corben<sup>3</sup>**

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## Background:

Quantitative *in vivo* imaging of the brain in Friedreich ataxia (FA) demonstrates that disease-related changes manifest in both the cerebellum and the cerebrum (Selvadurai, Harding, Corben, & Georgiou-Karistianis, 2018). These include deficits in measures of white matter volume and integrity (França et al., 2009; Rizzo et al., 2011). Correlations observed between imaging-derived measures of white matter and disease severity or duration (Della Nave et al., 2008; Rezende et al., 2016) indicate a potential relationship of these measures to disease progression. However, there has been limited longitudinal investigation of these measures, which is required to evaluate whether these deficits progress over time and whether any progression is reflected in clinically-measured change. Furthermore, while heterogeneous disease trajectories are observed amongst cohorts of individuals with FA, these have not been clearly demonstrated in neuroimaging studies. Therefore, this study aimed to track white matter volume and integrity over two years in individuals with FA, and to correlate changes in these measures against variables related to disease trajectory.

## Methods:

Data was collected as part of IMAGE-FRDA, a large-scale single-site longitudinal neuroimaging study conducted at Monash University, Melbourne, Australia. Neuroimaging measurements were obtained at two time-points, two years apart from 28 individuals with FA and 29 age- and gender-matched controls. T1-weighted structural images were used to extract rates of white matter volume change using voxel-based morphometry. Diffusion weighted and magnetisation transfer imaging were used to extract rates of change in white matter microstructure (fractional anisotropy, mean diffusivity, axial diffusivity, radial diffusivity, and magnetisation transfer ratio). Rates of change were corrected for the effect of ageing in the Control group, and then compared between the FA and Control groups at the whole-brain level and in seven cerebellar and cerebral regions-of-interest. Furthermore, changes in white-matter metrics in the FA cohort were correlated against clinical parameters, including: Time 1 disease severity (measured by the Friedreich Ataxia Rating Scale; FARS), change in FARS score, and age of disease onset. Cluster-level permutation and Bonferroni corrections were applied to adjust statistical significance thresholds for multiple comparisons ( $p_{FWE} < 0.05$ ) in the whole-brain and region-of-interest analyses, respectively.

## Results:

Individuals with FA showed a significantly greater average rate of white matter volume loss compared to controls in the right peri-thalamic region at the whole-brain level (Figure 1), and in the superior cerebellar peduncle at the region-of-interest level. Furthermore, rate of white matter volume loss was related to clinically-measured disease severity in a region-specific manner, where individuals with lower Time 1 severity showed greater loss in cerebellar and brainstem regions, while those with higher Time 1 severity showed greater loss in cerebral areas (Figure 2). Significant negative correlations were also observed between age of disease onset and radial diffusivity change in right cerebellar white matter at the whole-brain level, and between Time 1 severity and axial diffusivity change

within a corticospinal tract region-of-interest.

**Conclusions:**

These findings indicate that white-matter volume loss is a more sensitive marker of disease progression in individuals with FA than loss of white matter organisation. Furthermore, this degeneration is heterogeneous with respect to brain region and clinical parameters. In particular, greater degeneration in cerebellar and brainstem regions is observed in individuals with less severe clinical symptoms, and greater degeneration in cerebral regions is observed in those with more severe symptoms. This could point to a model of cerebellar degeneration earlier in the disease process and cerebral degeneration later in the disease process. These findings have important implications for the selection of neuroimaging biomarkers of FA, as a single brain region may not show optimal sensitivity to longitudinal change across all individuals. Further longitudinal neuroimaging work in FA should attempt to compare sub-groups of individuals with FA in order to better understand variability in disease trajectory at the neurobiological level and how this may map on to known heterogeneity in the progression of clinical symptoms.

# **Topic: Clinical Trials and Clinical Trial Design**



# Speech, Posture, and Gait Results of a Phase 2, Double-Blind Study to Evaluate TAK-831 in Adults with Friedreich Ataxia

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 244

**Dr. Dmitri Volfson<sup>1</sup>, Dr. Greg Hather<sup>1</sup>, Dr. Jonathan Norton<sup>1</sup>, Prof. Fay Horak<sup>2</sup>, Mr. Matthew Johnson<sup>3</sup>,  
Dr. Elena Izmailova<sup>4</sup>, Ms. Jennifer Farmer<sup>5</sup>, Dr. David Lynch<sup>6</sup>, Dr. Hao Wang<sup>1</sup>**

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## Background

TAK-831 is an inhibitor of D-amino acid oxidase (DAAO) enzyme that prevents D-serine metabolism. D-serine is an N-methyl-D-aspartate (NMDA)-type glutamate receptor co-agonist and a GluR delta2 agonist. Recently, efficacy, safety, pharmacodynamic effects, and pharmacokinetics were evaluated in a Phase 2, randomized, double-blind, placebo-controlled, parallel-arm study. The results for traditional clinical endpoints are summarized in a separate abstract. Digital speech assessments and digital assessments of gait, balance, and upper limb coordination are reported here. We tested the hypotheses that digital measures would be feasible for a multisite trial as well as sensitive to change and related to traditional, clinical measures.

## Methods

This study included two dose levels of oral TAK-831 in adult subjects with Friedreich ataxia. Sixty-seven subjects were randomized in approximately a 2:1:2 ratio to placebo, TAK-831 75 mg BID, or TAK-831 300 mg BID. Treatment lasted for 12 weeks. Digital endpoints were measured at weeks 2, 7, and 12, relative to the baseline.

For speech measurements, subjects completed a battery of tasks suitable for evaluating components of speech such as voice quality, voice control, timing, and breath support, all implicated in ataxia. These included sustained vowel, passage reading, monologue, automated task (days of the week), and syllable repetition tasks. The data were analyzed objectively by Redenlab using custom acoustic analysis software.

For movement measurements, each subject was tested using APDM's Mobility Lab system while performing the Upper Limb Coordination section of the Friedreich's Ataxia Rating Scale (FARS) examination and sitting balance tasks. Patients able to stand and/or walk unsupported wore APDM Opal sensors during additional tasks of standing balance with eyes open/eyes closed and two 25-foot walks at a natural speed. The data were analyzed objectively by APDM's Mobility Lab software.

Linear mixed-effect models were used to assess the relationship between the digital (speech, posture, and gait) and traditional measures including the 9-Hole Peg Test (9-HPT-1) and FARS Activities of Daily Living (ADLs). The sensitivity of both types of measure to inter-subject differences was assessed using similar models. Finally, the effect of treatment with TAK-831 on digital measurements was similarly assessed.

## Results

Data analysis is ongoing, and further results will be presented.

## Conclusion

The study showed that it is feasible to collect speech and posture/gait data in a multicenter trial of Friedreich ataxia or a similar condition that is treated in specialized centers.

## Improving clinical trial design using digital technology for symptom assessment in Friedreich ataxia (FA) and spinocerebellar ataxias (SCAs) 1, 2, 3, and 6

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 215

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Friedreich ataxia (FA) and spinocerebellar ataxias (SCAs) are familial neurodegenerative disorders that are characterized by progressive deterioration of gait, balance, and motor coordination. Studies for novel treatments in these slowly progressive conditions require large sample sizes and extended follow-up times due to high variability in measurements of clinical symptomology. We hypothesize that digital assessment of symptoms with instrumented versions of standard clinical assessments offers better endpoints than traditional clinical rating scales due to higher sampling frequency and less inter-rater variability, potentially allowing improved interventional study design with smaller samples and shorter trial duration. Therefore, as a part of an academic and industry partnership, we designed the Instrumented Data Exchange for Ataxia Study (IDEA Study), a longitudinal observational study in which we plan to enroll a total of 144 participants (48 individuals with FA, 48 individuals with either SCA1, SCA2, SCA3, or SCA6, and 48 healthy controls) and follow these participants for a period of up to 24 months.

Participants will be recruited from ataxia clinics at the University of Chicago, University of California Los Angeles, Johns Hopkins University, and Massachusetts General Hospital. In-clinic assessments of gait, balance, and motor coordination will be performed using APDM's Mobility Lab system (<http://www.apdm.com/mobility/>) every six months and scores compared to traditional un-instrumented assessments. Passive monitoring of gait and turns in daily life will be assessed for 14 days with APDM's Instrumented Smart-Socks and an Opal inertial sensor on a lumbar belt in participants enrolled at the University of Chicago site. An Apple Watch and iPhone with a custom-built app (<https://www.digitalartefacts.com/>) will be deployed in FA patients and matched healthy controls with instructions to complete a series of motor and speech tests and patient reported outcomes (PROs) every two weeks for the duration of the study.

We aim to (1) Determine the cross-sectional reliability, sensitivity, and validity of an in-clinic instrumented Scale for the Assessment and Rating of Ataxia (iSARA); (2) Determine feasibility of home monitoring of mobility in a subset of patients and controls and test whether these at-home outcomes are more sensitive than both the traditional clinical rating scales and the in-clinic iSARA; (3) Determine the longitudinal change and variability of quantitative assessment of gait, balance, and motor coordination; and (4) Use digital mobile technology to measure walking, balance, speech and upper-limb tasks at home for the duration of the study in FA patients and matched healthy

controls. Study enrollment is expected to start mid-2019. We will present the study outline, participant inclusion and exclusion criteria, statistical analysis plan, and a detailed description of the digital technology used.

# The Effects of Friedreich's Ataxia on Patient Lives: A Disease Consequence Model

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Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 123

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Friedreich's Ataxia (FA) is a multi-system disease, which has multiple serious symptoms and thus affects many aspects of patients' lives. A Disease Consequence Model is a way of depicting and therefore understanding the different facets of disease, the experience of the affected individual living with the disease and therefore the impact that potential therapies might be expected to have on quality of life.

A Patient Focused Drug Development Meeting was held on FA in June 2017. Individuals with FA provided testimony on their experience with the disease both verbally during the meeting and in written testimony submitted to the meeting organizers. Polls were also taken during the meeting, and responses were collected from both in-person and virtual attendees. Taken together, this represents a significant body of data about the individual experience with FA. After the meeting, the Friedreich's Ataxia Research Alliance (FARA) formed a working group to use this data to inform endpoint development for FA. This working group included FARA, affected individuals and families, drug developers, clinicians and experts in outcome measure development. The group collated the data from the meeting, integrated it with clinical experience, and used it to develop a Disease Consequence Model. This visual representation of the disease, is structured according to bodily systems (nervous, cardiac, musculoskeletal), and documents the symptoms, impairments and subsequent impact on participation in daily living activities. This model has provided an excellent reference point to distill what is important to individuals with FRDA and importantly, the gaps in patient reported outcomes related to daily living endpoints.

The model exemplifies the complexity of disease, both in terms of the many systems involved, the many symptoms, and how they affect every aspect of a patient's life. Several symptoms, such as fatigue and sleep issues, could not be directly related to specific systems and are likely to have multiple causes. Almost every group of symptoms was thought to independently affect personal, domestic and community activities of daily living in some way, resulting in the significant burdens of disease reported by patients.

The FA Disease Consequence Model has been and will be used by researchers, drug development companies, regulators and payers to help understand the many aspects of disease and how they affect patient life, as well as how proposed therapeutics might change the patient experience.

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## A Randomized, Double-Blind, Controlled, Study of RT001 in Subjects with Friedreich's Ataxia

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Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 73

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**Background:** Friedreich's ataxia (FA) is an inherited, neurodegenerative disease (ND) caused by a critical level of intron expansion of the gene that codes for frataxin (FXN). FXN activity level < 30% leads to mitochondrial dysfunction and lipid peroxidation (LPO). RT001 is a di-deuterated linoleic acid that reinforces cell membranes leading to decreased LPO and decelerates toxic cellular cascades.

**Prior Study:** In a placebo-controlled study of RT001 in FA (RT001-002), subjects receiving RT001 had increased cardiac workload capability after one month. The present trial is a randomized, double-blind, placebo-controlled phase 2/3 study of RT001 to assess the efficacy, and safety, and tolerability of RT001 in subjects with FA over a 9-month exposure period.

**Trial Design:** Sixty ambulatory subjects with genetic and clinical evidence of FA will undergo baseline cardiopulmonary exercise testing (CPET). They will also undergo multiple tests of function including FARS-Neuro, FARS-ADL, fatigue Global Clinical Impression, visual analogue scale, and a timed 1-minute walk test. They will then be randomized in equal proportions to receive either RT001 5.7g/d or an inactive comparator for 9 months. CPET and the other functional tests will be repeated at 4 months, and 9 months after study initiation. Patient reported outcomes will be collected monthly. All tests will be repeated after a 3-month washout period. After the randomized period, an open-label study extension will be offered to any eligible patients in either arm of the study.

**Analysis:** The primary efficacy analysis will be maximum workload performed during CPET. If a significant improvement in CPET is demonstrated in the RT001 group vs placebo, the other functional tests will be used to confirm the clinical significance of the CPET findings.

**Conclusion:** The prior pilot study (CLN-RT001-002) demonstrating improvement in cardiac workload in FA subjects treated with RT001 was used to design the current randomized, double-blind, placebo-controlled study of 60 subjects over a 9-month exposure period.

## **Review of results of randomized clinical trials, 1 year long, in Friedreich ataxia: including results from MICONOS**

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Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 29

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Friedreich ataxia is a rare, slowly progressive disorder. Researchers have not yet defined a “perfect” clinical marker or a bio-marker to assess disease progression.

Clinical assessment, using this ataxia rating scale is the current clinical marker. However the accuracy of the ataxia rating scales, is questionable when the patient is wheelchair-dependent. In view of this and the slowly progressive nature of Friedreich ataxia, large, randomized controlled trials (RCT) of at least 12 months’ duration are needed to evaluate the effects of pharmacological treatments in Friedreich ataxia.

There have been five pharmacological treatments used in such RCT in Friedreich’s ataxia over the last 15 years. To date, results for 4 of them have been published (results of the pioglitazine RCT, finished in March 2013, are still awaited) and below is a meta-analysis of the results. More detailed results will be presented at the conference.

# Safety and efficacy of Interferon Y in Friedreich ataxia, using clinical and paraclinical disease indicators

Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 40

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**Importance.** Friedreich ataxia (FRDA) is a devastating neurodegenerative disease that still lacks an effective treatment. To find a cure is an urgent unmet medical need.

**Objective.** To assess the safety and efficacy of IFNy in FRDA patients.

**Design.** 6 months, open-label clinical trial. Sets of measures were acquired 6 months before the start of the treatment, at the start and after 3 and 6 months of treatment, and 6 months after treatment discontinuation.

**Setting.** The study was conducted in a referral center.

**Participants.** Twelve patients with a genetic diagnosis of FRDA were recruited. Eleven patients completed the trial and 1 patient withdrew from the trial.

**Intervention.** 11 FRDA patients were treated with IFNy administered subcutaneously at 200 µg x 3 times/week.

**Main Outcomes and Measures.** Primary efficacy outcome was the Scale for Assessment and Rating of Ataxia (SARA) score. Secondary outcomes were disability and quality of life measured by WHO-DAS and SF-36 questionnaires, PBMC frataxin levels by immunoblotting, cardiac structure and function by echoCG and ECG, the structure of the retina by OCT, and the structure and function of the brain by MRI.

**Results.** IFNy treatment was generally well tolerated. IFNy completely stopped the progression of the SARA score during the 6-months treatment period, compared to the 6 months preceding the treatment ( $p < 0.009$ ). IFNy reduced the thickness of the cardiac interventricular septum and reduced the Sokolow-Lyon index, with a rebound after treatment discontinuation. It enhanced the activation of the left motor cortex during dominant hand movements and affected the activity of three resting state brain networks. Also, the enhanced activation of the right motor cortex, during bilateral hand movements, correlated with SARA score changes. Other measures did not show significant changes.

**Conclusions and Relevance.** The results point toward the efficacy of IFNy in affecting the progression of the disease. Placebo-controlled clinical studies are necessary to definitively assess the efficacy of IFNy in FRDA.

**Trial Registration.** ClinicalTrials.gov Identifier: NCT03888664.

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# Top-Line Results of a Phase 2, Double-Blind Study to Evaluate Efficacy and Tolerability of TAK-831 in Adults with Friedreich Ataxia

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Friday, 15th November - 12:30: Poster Session 2 - Abstract ID: 51

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## Background

TAK-831 is an inhibitor of D-amino acid oxidase (DAAO) enzyme that decreases D-serine metabolism. D-serine is an N-methyl-D-aspartate (NMDA)-type glutamate receptor co-agonist and a GluR delta2 agonist. DAAO is highly expressed in the cerebellum, a region responsible for motor coordination. TAK-831 increased the D-serine level in the cerebellum in a genetic mouse model (YG8sR) that has GAA repeat expansions in frataxin. Improvements in beam crossing speed were observed after both single and multiple dose administration of TAK-831 3 mg/kg in this mouse model.

## Methods

This study was a Phase 2, randomized, double-blind, placebo-controlled, parallel-arm, proof-of-concept study designed to evaluate the efficacy, safety, pharmacodynamic (PD) effects, and pharmacokinetics (PK) of two dose levels of oral TAK-831 in adult subjects with Friedreich ataxia (FA). Sixty-seven subjects were randomized in approximately a 2:1:2 ratio to placebo, TAK-831 75 mg BID, or TAK-831 300 mg BID. Treatment lasted for 12 weeks. Subjects were assessed on the 9-hole peg test (9-HPT) and other efficacy endpoints at weeks 2, 7, and 12. The primary endpoint was change from baseline on the inverse of time to complete the 9-HPT (9-HPT-1) at week 12.

Secondary outcome measures included the modified Friedreich Ataxia Rating Scale neurological examination (mFARS-neuro), of which the total score provides a functional assessment of patients, the Timed 25-Foot Walk (T25FW), and the Activities of Daily Living component of the FARS (FARS ADLs).

The doses of TAK-831 selected for this study were based on PK data from multiple Phase 1 studies including the SRD/MRD, brain target occupancy data from the PET study, CSF D-serine data, and safety data from all Phase 1 studies. The PK/PD modeling analyses showed that the higher dose regimen resulted in steady-state exposures associated with peak target occupancy of DAAO of >90%. The lower dose was chosen to provide at least a 3-fold exposure difference from the higher dose to understand the dose-response relationship and potentially identify the no-effect dose so that development could move directly into the pivotal study.

## Results

The 67 randomized patients were well balanced demographically across treatment arms. Overall, the mean age at screening was 31 years, 37 (45%) patients were female, and 66 (99%) identified their race as white. Regarding disease status, 30 (45%) subjects were ambulatory and the mean baseline plasma D-serine was 0.148 µg/mL. Retention in the study was excellent, with 63 (94%) subjects completing study treatment and follow-up.

The study did not show statistically-significant results on the primary endpoint, change from baseline on the 9-HPT-



1 at week 12. The placebo-adjusted change from baseline was -0.00054 (standard error [SE] = 0.000746) on TAK-831 75 mg BID and -0.00069 (SE = 0.000616) and TAK-831 300 mg BID. These non-significant differences are small in magnitude. Note that on the inverted (1/seconds) scale a positive change indicates higher speed. For the change in mFARS-neuro, all treatment arms showed a trend toward less impairment after 12 weeks, but the TAK-831 arms did not statistically separate from placebo. Likewise, the TAK-831 arms did not statistically separate from placebo on the T25FW test or FARS ADLs. Patients in the 75 mg BID and 300 mg BID groups achieved the targeted TAK-831 exposure and the expected D-serine elevation levels in plasma at steady-state.

TAK-831 was safe and well tolerated in the FA population. Overall, 85% of subjects on TAK-831 had at least one adverse event (AE), compared to 93% on placebo. Most AEs were mild in intensity. One subject had an AE of severe flank pain that was deemed unrelated to treatment; the other AEs were mild or moderate. The most frequently reported TEAEs were headache (30%, 29%, and 42%), fall (22%, 7%, 23%), nausea (15%, 7%, 31%), cough (7%, 7%, 19%), oropharyngeal pain (11%, 7%, 15%), fatigue (15%, 0%, 12%), and nasal congestion (4%, 7%, 15%) for the placebo, 75 BID mg, and 300 BID mg groups, respectively. Four subjects (6%) had AEs that led to discontinuation of study drug. There were no serious AEs or deaths.

### **Conclusions**

Based on the results of this study, we conclude that TAK-831 was safe and well tolerated in FA patients but was not effective as a treatment for this indication. We sincerely appreciate the contributions of the FA patients who enabled us to complete this study in an expeditious manner and reach a clear scientific conclusion.

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