

This file contains the complete agenda and abstracts from the 3<sup>rd</sup> International Friedreich's Ataxia Conference, November 10 – 12, 2006. A few abstracts are not included, as the authors did not give FARA permission to publish. The program was organized by sessions and within each session there were talks and poster presentations. The abstracts appear in order at the end of each session listing.

We hope that sharing this information gives you additional knowledge about the active research in FA. We anticipate that much of the research presented at the meeting will be published in scientific journals in the near future. In addition, the conference organizers are preparing a scientific summary that will be submitted for publication in early 2007.

### **Program Agenda and Abstracts**

**Friday November 10, 2006**

**8:30 – 12:00 Session I – The Friedreich's Ataxia Disease Gene**  
**Session Chair: Robert D. Wells, PhD**

**8:30- 8:35** - Introduction

**8:35- 8:45 Title: FRDA, Sticky DNA, Transcription Inhibition, and the Polyamide Reversal of this Inhibition**

**Authors:** Dr. Robert D. Wells

**8:45- 9:00 Title: The Distribution of Polypurine•Polypyrimidine Sequences in the Human Genome**

**Authors:** Bacolla A<sup>1</sup>, Collins JR<sup>2</sup>, Gold B<sup>3</sup>, Chuzhanova N<sup>4</sup>, Ming Y<sup>2</sup>, Stephens RM<sup>2</sup>, Stefanov S<sup>3</sup>, Olsh A<sup>3</sup>, Jakupciak JP<sup>5</sup>, Dean M<sup>3</sup>, Lempicki RA<sup>6</sup>, Cooper DN<sup>4</sup>, and Wells RD<sup>1</sup>

**9:00 – 9:15 Title: Replication through GAA repeats in Cos-1 Cells**

**Authors:** Maria M. Krasilnikova, Sergei M. Mirkin

**9:15 - 9:30 Title: DNA sequence-specific polyamides alleviate transcription inhibition associated with long GAA•TTC repeats in Friedreich's ataxia**

**Authors:** Daniel A. Harki\*, Ryan Burnett<sup>†</sup>, Christian Melander<sup>†</sup>, James W. Puckett\*, Leslie S. Son<sup>‡</sup>, Robert D. Wells<sup>‡</sup>, Peter B. Dervan\*, and Joel M. Gottesfeld<sup>†</sup>

**9:30 - 9:45 Title: Overcoming the transcription defect in Friedreich ataxia (FA) with designed DNA ligands**

**Authors:** M. Rai<sup>(1)</sup>, J. Gottesfeld<sup>(2)</sup>, P. Dervan<sup>(3)</sup>, M. Pandolfo<sup>(1)</sup>

**9:45- 10:00 Title: "Influence of sequence specific polyamides on GAA•TTC repeat instability"**

**Authors:** Marek Napierala<sup>1</sup>, Jacquelynn E. Larson<sup>1</sup>, Joel M. Gottesfeld<sup>2</sup>, Peter B. Dervan<sup>3</sup> and Robert D. Wells<sup>1</sup>

**10:00 – 10:15 Title:** **Histone Deacetylase Inhibitors Reverse Gene Silencing in Friedreich's Ataxia**

**Authors:** David Herman,<sup>1</sup> Kai Jenssen,<sup>1</sup> Ryan Burnett,<sup>1</sup> Elisabetta Soragni,<sup>1</sup> Susan L. Perlman,<sup>2</sup> and Joel M. Gottesfeld<sup>1</sup>

**10:15 - 10:30 Title:** **Histone deacetylase inhibitors effect on gene silencing and frataxin levels in frataxin knockin/knockout mice**

**Authors:** M. Rai<sup>(1)</sup>, E. Soragni<sup>(2)</sup>, J. Gottesfeld<sup>(2)</sup>, M. Pandolfo<sup>(1)</sup>

**10:30 - 10:45 Brief break**

**10:45- 11:00 Title:** **Breaking the Silence in Friedreich's Ataxia**

**Authors:** Richard Festenstein, Nadine Rothe

**11:00 – 11:15 Title:** **The role of intron 1 in the normal and pathological regulation of the frataxin gene.**

**Authors:** Eriko Greene, Lata Mahishi, Ali Entezam, Daman Kumari and Karen Usdin

**11:15 - 11:30 Title:** **Integrated Dual Reporters to Measure Repression of Transcription Elongation by FRDA Repeats in Human Cells**

**Authors:** Mimi Sammarco, Ayan Banerjee, Scott Ditch, Miriam Mancuso and Ed Grabczyk

**11:30- 11:45 Title:** **The potential role of somatic instability of the GAA triplet-repeat in phenotypic expression of Friedreich ataxia**

**Authors:** Sanjay I. Bidichandani<sup>1</sup>, Irene De Biase<sup>1</sup>, Astrid Rasmussen<sup>1</sup>, Sahar Al-Mahdawi<sup>2</sup>, Antonella Monticelli<sup>3</sup>, Sergio Coccozza<sup>3</sup>, Mark Pook<sup>2</sup>

**11:45- 12:00 Title:** **Infectious delivery and expression of a 135 kb human *FRDA* genomic DNA locus complements Friedreich's ataxia deficiency in human cells**

**Authors:** Silvia Gomez-Sebastian<sup>1</sup>, Alfredo Gimenez-Cassina<sup>2</sup>, Javier Diaz-Nido<sup>2</sup>, Filip Lim<sup>2</sup> and Richard Wade-Martins<sup>1\*</sup>

**12:00 – 12:30 - Roundtable discussion**

**Poster Presentations**

**Title:** **Novel, complex interruptions of the GAA repeats in the small expanded alleles of two affected siblings with a mild, late onset form of Friedreich ataxia.**

**Authors:** D. R. Lynch, E.C. Frackelton, J. McCallum, J.M Farmer, A. Tsou, A. Santani, C.M. Mulcahy, and C.A. Stolle

**Title: Molecular Characterization of a Novel Case of Friedreich Ataxia**

**Authors:** Novita Puspasari,<sup>1,2</sup> Lingli Li,<sup>1</sup> Timothy P. Holloway,<sup>1</sup> Louise Corben,<sup>1</sup> Michael Fahey,<sup>1,2</sup> Martin B. Delatycki,<sup>1,2</sup> and Joseph P. Sarsero<sup>1</sup>

## **The Friedreich's Ataxia Disease Gene – Platform presentations abstracts**

**Title:** FRDA, Sticky DNA, Transcription Inhibition, and the Polyamide Reversal of this Inhibition

**Authors:** Dr. Robert D. Wells

**Institutions:** Institute of Biosciences and Technology, Center for Genome Research, 2121 W. Holcombe Blvd., Texas A&M University Health Science Center, Houston, TX 77030

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### **Abstract:**

Friedreich's ataxia is caused by the massive expansion of the GAA·TTC repeats in intron 1 of the frataxin gene. Long GAA·TTC repeats form very stable triplex and/or sticky DNA structures which cause the two repeat tracks to adhere to each other. These structures inhibit the transcription of the frataxin gene thus diminishing the production of the mitochondrial protein, frataxin. Recent studies revealed that sticky DNA, which is unique to FRDA, forms inside living cells and the *in vitro* association of the long GAA·TTC tracks generates two independent supercoiled domains. Interestingly, DNA sequence-specific polyamides alleviate transcription inhibition associated with these long GAA·TTC repeats. Hence, these initial investigations aimed at gene targeted therapies for FRDA appear promising.

Sticky DNA: *in vivo* Formation in *E. coli* and *in vitro* Association of Two Long GAA·TTC Sequences Related to Friedreich's Ataxia. Leslie S. Son, Albino Bacolla, and Robert D. Wells. *J. Mol. Biol.* 360, 267-284 (2006).

DNA Sequence-Specific Polyamides Alleviate Transcription Inhibition Associated with Long GAA·TTC Repeats in Friedreich's Ataxia. Ryan Burnett, Christian Melander, James W. Puckett, Leslie S. Son, Robert D. Wells, Peter B. Dervan, and Joel M. Gottesfeld. *Proc. Natl. Acad. Sci. U.S.A.* 103, 11497-11502 (2006).

Structure-Dependent Recombination Hot Spot Activity of GAA·TTC Sequences from Intron 1 of the Friedreich's Ataxia Gene. Marek Napierala, Ruhee Dere, Alexandre A. Vetcher, and Robert D. Wells. *J. Biol. Chem.* 279, 6444-6454 (2004).

**Title: The Distribution of Polypurine•Polypyrimidine Sequences in the Human Genome**

**Authors:** Bacolla A<sup>1</sup>, Collins JR<sup>2</sup>, Gold B<sup>3</sup>, Chuzhanova N<sup>4</sup>, Ming Y<sup>2</sup>, Stephens RM<sup>2</sup>, Stefanov S<sup>3</sup>, Olsh A<sup>3</sup>, Jakupciak JP<sup>5</sup>, Dean M<sup>3</sup>, Lempicki RA<sup>6</sup>, Cooper DN<sup>4</sup>, and Wells RD<sup>1</sup>

**Institutions:** <sup>1</sup> Institute of Biosciences and Technology, Houston TX; <sup>2</sup> Advanced Biomedical Computing Center, NCI-Frederick, MD; <sup>3</sup> Laboratory of Genomic Diversity, NCI-Frederick, MD; <sup>4</sup> Institute of Medical Genetics, Cardiff University, Cardiff, UK; <sup>5</sup> DNA Technology Group, NIST, Gaithersburg, MD; <sup>6</sup> Laboratory of Immunopathogenesis and Bioinformatics, SAIC-Frederick, Inc., Frederick, MD.

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**Abstract:** Expansion of a polymorphic GAA•TTC repeat in the first intron of the *FXN* gene severely compromises gene expression, leading to recessive Friedreich's Ataxia (FA). The mechanism is believed to involve non-B DNA structural intermediates either specific for the expanded sequence or common to other polypurine•polypyrimidine (R•Y) tracts. We searched the human genome for long, uninterrupted, R•Y tracts and found 814 that equaled or exceeded 250 bases, the longest totaling 1,303 bases in the *CENTA1* gene. Comparative searches in other mammalian and avian genomes also revealed large numbers of tracts. With respect to location, the 814 tracts populated all chromosomes but were significantly clustered in the pseudoautosomal region of sex chromosomes, which plays an indispensable role in male meiosis and chromosome pairing. Of the repetitive motifs with >30 nt, runs of A•T were the most common with a total of 16,679 copies, followed by GAAA•TTTC motifs with 3,217 copies. The FA-related GAA•TTC motif was only present in <400 copies. Comparisons with the chimpanzee genome revealed that the long R•Y tracts evolved at rates at least 30 times faster than genome average. These results strongly implicate the R•Y tracts in the generation of double-strand breaks, which in turn promote high recombination rates, and therefore mutation. We conclude that R•Y tracts with lengths in the range of the FA-expanded GAA•TTC repeats are not uncommon in the human genome; however, none matched the monotonous repetition of identical motifs that characterize the pathological FA-expanded sequence.

Bacolla A., *et al.* (2006). Long homopurine•homopyrimidine sequences are characteristic of genes expressed in brain and the pseudoautosomal region. *Nucleic Acids Res.* **34**, 2663-2675.

**Title: Replication through GAA repeats in Cos-1 Cells**

**Authors:** Maria M. Krasilnikova, Sergei M. Mirkin

**Institutions:** Pennsylvania State University, University of Illinois at Chicago

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**Abstract:**

Long GAA repeat in the intron of frataxin gene is responsible for severe neurological disorder Friedreich's ataxia. The propensity of GAA repeat to impede replication and transcription observed in a number of model systems was proposed as a mechanism of the disease. The detailed mechanisms of how replication and transcription are affected in humans are unknown. It was shown that GAA repeat can adopt triplex structure conformation in supercoiled plasmids. This peculiar DNA conformation is believed to cause problems for the replication and transcription machineries. However, it still has to be proven that this structure is the main cause of Friedreich's ataxia. We have previously shown that replication through GAA repeats, located within the plasmid, is slowed down in yeast. We also pointed out the correlation between replication stalling and instability of the repeat. Now we studied the effects of GAA repeat on replication in mammalian cells. We observed a very strong effect of two (GAA)<sub>57</sub> stretches located within the same plasmid on replication in mammalian cells. The orientation of two stretches turned out to be crucial for replication blockage: they should be in head to tail orientation in order to be able to block replication progression. We believe that the complex between two GAA stretches, so-called "sticky DNA" causes replication blockage. Since an extremely long GAA stretch can be viewed as two separate stretches joined together in head to tail orientation, this complex formation can potentially form within a single stretch, provided that it is long enough. This structure can cause a replication stalling and subsequent instability for the long GAA stretches, characteristic for the full-scale disease.

**Title: DNA sequence-specific polyamides alleviate transcription inhibition associated with long GAA•TTC repeats in Friedreich's ataxia**

**Authors:** Daniel A. Harki\*, Ryan Burnett†, Christian Melander†, James W. Puckett\*, Leslie S. Son‡, Robert D. Wells‡, Peter B. Dervan\*, and Joel M. Gottesfeld†

**Institutions:** \*Division of Chemistry and Chemical Engineering, California Institute of Technology; †Department of Molecular Biology, The Scripps Research Institute; ‡Center for Genome Research, Institute for Biosciences and Technology, Texas A&M University System Health Science Center

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**Abstract:** The hyperexpansion of GAA•TTC triplet repeats in the first intron of the frataxin gene is the hallmark DNA abnormality of Friedreich's ataxia (FRDA). Decreased transcription and diminished levels of frataxin protein result in FRDA-affected individuals, with disease severity correlating to the abundance of (GAA•TTC)<sub>n</sub> repeats. Such (GAA•TTC)<sub>n</sub> expansions have been shown to adopt unusual non-B DNA structures and block transcriptional elongation by RNA polymerase II. Recently, we demonstrated that β-alanine-linked pyrrole-imidazole polyamides bind GAA•TTC DNA tracts with high affinity and disrupt the intramolecular DNA•DNA-associated regions of sticky-DNA conformations. Increased transcription of the frataxin gene and elevated levels of frataxin protein were observed following polyamide treatment in the GM15850 FRDA cell line. Additionally, fluorescently-labeled, GAA-specific polyamides were shown to localize to the nucleus of GM15850 cells and significantly affect only a limited number of other genes by DNA microarray transcript analysis. These results, as well as ongoing studies with related GAA-specific polyamides, will be presented.

**Title: Overcoming the transcription defect in Friedreich ataxia (FA) with designed DNA ligands**

**Authors:** M. Rai<sup>(1)</sup>, J. Gottesfeld<sup>(2)</sup>, P. Dervan<sup>(3)</sup>, M. Pandolfo<sup>(1)</sup>

**Institutions:** (1) Department of Neurology, Université Libre de Bruxelles, Erasme Hospital, 808 Route de Lennik, 1070 Brussels, BELGIUM

(2) Department of Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

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**Abstract:**

FA is caused by insufficient levels of frataxin; the causative genetic defect involving an intronic GAA triplet-repeat expansion that interferes with frataxin transcription.  *$\beta$ -Alanine-linked pyrrole-imidazole* polyamides have been demonstrated to bind GAA\_TTC tracts with high affinity and disrupt the sticky-DNA conformation formed by long GAA\_TTC repeats. In our laboratory, we have isolated adult brain-derived neural precursor cells (NPCs) from the subventricular zone of frataxin knockin/knockout (KiKo) mice and confirmed (in vitro) that they have the capacity to self-renew and differentiate into all major CNS cell types. We have shown that fluorescent polyamide-Bodipy conjugates localize in the nucleus of our differentiated KiKo NPCs. We incubated the KiKo and control NPCs during differentiation with these designed DNA ligands and found that the level of frataxin mRNA in the treated differentiated KiKo NPCs was increased 1.9-fold. The effect on frataxin protein levels will be checked in additional cellular models that allow a higher yield of proteins.

**Title: “Influence of sequence specific polyamides on GAA•TTC repeat instability”**

**Authors:** Marek Napierala<sup>1</sup>, Jacquelynn E. Larson<sup>1</sup>, Joel M. Gottesfeld<sup>2</sup>, Peter B. Dervan<sup>3</sup> and Robert D. Wells<sup>1</sup>

**Institutions:** <sup>1</sup> Institute of Biosciences and Technology, Texas A&M University Health Science Center, Houston, TX; <sup>2</sup> The Scripps Research Institute, La Jolla, CA; <sup>3</sup> California Institute of Technology, Pasadena, CA

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**Abstract:**

Expanded GAA•TTC repeats and the processes associated with their instability are the very cause of Friedreich’s ataxia and present the first line target for therapeutic intervention. Suppression of the somatic expansion and stimulation of the already existing contraction bias observed for the long GAA•TTC alleles in somatic cells may provide therapeutic benefits either by reducing the severity of the symptoms or delaying disease onset. Several chemical modifiers were demonstrated to influence repeats instability (reviewed in 1); however, none of these agents was targeted towards a specific trinucleotide sequence.

We have analyzed the influence of the GAA•TTC specific pyrrole-imidazole polyamides on the instability of long GAA•TTC tracts in human lymphoblastoid cell lines. These compounds have recently been demonstrated to alleviate FRDA transcription inhibition (2). Extensive treatment of the lymphoblastoid cell lines with GAA•TTC specific polyamides revealed no significant changes in the length of the repeat tracts as determined by small-pool PCR analysis. We are currently conducting experiments with the GAA•TTC specific chlorambucil-conjugated polyamides (ChI-PA). Chlorambucil-conjugated polyamides combine both the excellent DNA targeting activities of polyamides and the DNA damaging (alkylation) property of chlorambucil. Sequence specific DNA damage induced by ChI-PA should stimulate cellular repair mechanisms leading to the GAA•TTC instability.

Preliminary data using quantitative RT-PCR demonstrated that ChI-PA at nanomolar concentrations can increase the frataxin mRNA expression. Their effect on the repeat instability is currently being evaluated.

1. Gomes-Pereira M. and Monckton D.G. Chemical modifiers of unstable expanded simple sequence repeats: what goes up, could come down. *Mutat. Res.* 25;598(1-2):15-34 (2006).
2. Burnett R., Melander C., Puckett J.W., Son L.S., Wells R.D., Dervan P.B., Gottesfeld J.M. DNA sequence-specific polyamides alleviate transcription inhibition associated with long GAA•TTC repeats in Friedreich's ataxia. *Proc. Natl. Acad. Sci. USA* 103(31):11497-502 (2006).

**Title: Histone Deacetylase Inhibitors Reverse Gene Silencing in Friedreich's Ataxia**

**Authors:** David Herman,<sup>1</sup> Kai Jenssen,<sup>1</sup> Ryan Burnett,<sup>1</sup> Elisabetta Soragni,<sup>1</sup> Susan L. Periman,<sup>2</sup> and Joel M. Gottesfeld<sup>1</sup>

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**Abstract:**

Friedreich's ataxia (FRDA) is caused by gene silencing through expansion of GAA•TTC triplet repeats in the first intron of the nuclear *FXN* gene, which encodes the essential mitochondrial protein frataxin. Two models have been proposed to account for gene silencing by expanded repeats, unusual DNA structures and repressive heterochromatin. We examined the chromatin structure of the *FXN* gene in normal and FRDA cell lines using antibodies to the various modification states of the core histones and chromatin immunoprecipitation methods. We find that gene silencing at expanded *FXN* alleles is accompanied by hypoacetylation of histones H3 and H4, and methylation of histone H3 at lysine 9, consistent with a heterochromatin-mediated repression mechanism. These findings suggest that histone deacetylase (HDAC) inhibitors, compounds that reverse heterochromatin, might activate the *FXN* gene. We identified one commercial HDAC inhibitor, BML-210, that partially reverses silencing in the FRDA cell line. Based on the structure of this compound, we synthesized and assayed a series of derivatives of BML-210 and identified HDAC inhibitors that reverse *FXN* silencing in primary lymphocytes from Friedreich's patients. These molecules act directly on the histones associated with the *FXN* gene, increasing acetylation at particular lysine residues on histones H3 and H4 (H3-K14, H4-K5 and H4-K12). Unlike many triplet-repeat diseases (for example, the polyglutamine expansion diseases such as Huntington's disease and the spinocerebellar ataxias), expanded GAA•TTC triplets do not alter the coding potential of the *FXN* gene; thus, gene activation would be of therapeutic benefit. Animal studies are currently underway to explore the bioavailability and efficacy of these molecules.

**Title: Histone deacetylase inhibitors effect on gene silencing and frataxin levels in frataxin knockin/knockout mice**

**Authors: M. Rai<sup>(1)</sup>, E. Soragni<sup>(2)</sup>, J. Gottesfeld<sup>(2)</sup>, M. Pandolfo<sup>(1)</sup>**

**Institutions:** (1) Department of Neurology, Université Libre de Bruxelles, Erasme Hospital, 808 Route de Lennik, 1070 Brussels, BELGIUM

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**Abstract:**

A class of histone deacetylase (HDAC) inhibitors have been recently shown to reverse FXN (the gene encoding frataxin) silencing in primary lymphocytes from individuals with Friedreich's ataxia. We have designed an animal study, using two HDAC inhibitors of this class in frataxin knockin/knockout mice.

**Title:** Breaking the Silence in Friedreich's Ataxia

**Authors:** Richard Festenstein, Nadine Rothe

**Institutions:** Imperial College

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[nadine.rothe@imperial.ac.uk](mailto:nadine.rothe@imperial.ac.uk)

**Abstract:**

We have established a link between triplet-repeat expansion diseases and heterochromatin-mediated silencing using a murine transgenic model for the archetypal epigenetic phenomenon of position effect variegation (PEV) (Saveliev, 2003). We found that the pathological GAA-repeat expansion which represses the *frataxin* gene leading to Friedreich's ataxia (FRDA) can induce epigenetic silencing *in vivo* and that this silencing is exquisitely sensitive to the dosage of modifiers of PEV such as heterochromatin protein 1 (HP1) (Saveliev, 2003). This provides a potential novel therapeutic avenue for the treatment of FRDA. We therefore went on to examine the expression at normal and FRDA disease loci at the single cell level and found that variegation, a characteristic of heterochromatin-mediated silencing, indeed occurs at those loci with expanded GAA repeats suggestive of PEV. We have tested the ability of several histone deacetylases (HDAC) to influence silencing in primary resting cells – our data suggests that we can overcome silencing using particular HDAC inhibitors (including a new HDAC inhibitor developed by Gottesfeld et al. 2006) but not others and therefore provides a potential means to reverse silencing in this disease. Moreover, we have embarked on a detailed analysis of the chromatin structure at the FRDA locus in mouse and man in order to more precisely define the molecular pathogenesis of gene silencing at this locus.

Saveliev, A., C. Everett, et al. (2003). "DNA triplet repeats mediate heterochromatin-protein-1-sensitive variegated gene silencing." Nature **422**(6934): 909-13.

Festenstein, R (2006). 'Breaking the Silence in Friedreich's ataxia' Nature Chemical Biology **2** (10) in the press.

Herman et al. (2006). 'Histone deacetylase inhibitors reverse gene silencing in Friedreich's ataxia' Nature Chemical Biology in the press, published online: 20th August 2006 | doi:10.1038/nchembio815

**Title:** The role of intron 1 in the normal and pathological regulation of the frataxin gene.

**Authors:** Eriko Greene, Lata Mahishi, Ali Entezam, Daman Kumari and Karen Usdin

**Institutions:** NIDDK, National Institutes of Health

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**Abstract:**

Expansion of a GAA•TTC-repeat tract in the first intron of the frataxin gene is responsible for most cases of Friedreich ataxia. Expansion results in a frataxin mRNA deficit. Previously proposed models suggested a role for a transcription elongation problem in the etiology of the mRNA deficit. However our analysis of intron 1 of the frataxin gene in a number of primary cells and cell lines from different individuals with Friedreich ataxia demonstrates that this region is associated with epigenetic modifications typical of transcriptionally silent chromatin. This supports recently published findings by Herman et. al. (2006). In addition, our data reveals a direct consequence of these epigenetic modifications that may be the proximal cause of a significant amount of the frataxin deficit.

**Title: Integrated Dual Reporters to Measure Repression of Transcription Elongation by FRDA Repeats in Human Cells**

**Authors:** Mimi Sammarco, Ayan Banerjee, Scott Ditch, Miriam Mancuso and Ed Grabczyk

**Institutions:** LSU Health Sciences Center, New Orleans

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**Abstract:**

An unstable GAA•TTC repeat expansion in the first intron of the *FXN* gene reduces frataxin expression causing FRDA. The degree of repression correlates with the length of the repeat, but it remains unclear just how transcription is actually reduced. We engineered human cell lines with unique dual luciferase constructs incorporating defined GAA•TTC repeats to analyze the mechanism underlying the defect. To control for chromatin effects, all of our constructs are inserted into the same chromosomal location in each cell line by a site-specific recombinase. Each construct has an inducible promoter. In one series, novel tandem arrangements of firefly luciferase and sea pansy luciferase reporters flank the repeats. The ratio of the upstream and downstream reporters measures transcription elongation through the repeat. A second series features a splicing FRDA minigene construct with the dual luciferase reporters divergently transcribed by a bidirectional promoter. Defined GAA•TTC repeats are inserted into the shortened *FXN* first intron on one side of the bidirectional promoter. As in the tandem lines, the luciferase activity ratio measures repression by the repeats. The inducible promoters allow us to determine whether the rate of transcription initiation affects the degree of successful transcription elongation, which is pertinent to therapeutic strategies aiming to increase *FXN* promoter output. Different sequences flank the repeats in some reporters to determine if effects such as changes in chromatin structure are transferable. We hope to use our dual reporter cell lines as a high-throughput drug discovery platform for FRDA transcription elongation therapeutics.

**Title: The potential role of somatic instability of the GAA triplet-repeat in phenotypic expression of Friedreich ataxia**

**Authors:** Sanjay I. Bidichandani<sup>1</sup>, Irene De Biase<sup>1</sup>, Astrid Rasmussen<sup>1</sup>, Sahar Al-Mahdawi<sup>2</sup>, Antonella Monticelli<sup>3</sup>, Sergio Coccozza<sup>3</sup>, Mark Pook<sup>2</sup>

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**Abstract:**

Friedreich ataxia (FRDA) patients have expanded GAA repeats in the *FXN* gene. Primary neurodegeneration of the dorsal root ganglia (DRG), caused by their hypersensitivity to frataxin deficiency, leads to progressive ataxia. The progressive nature of the disease remains unexplained. Using a sensitive and quantitative assay for somatic instability *in vivo*, we present several lines of evidence that support the role of postnatal, progressive, tissue-specific somatic instability of the expanded GAA triplet repeat in determining the (FRDA) phenotype. (A) Small pool PCR analysis of individual *FXN* genes from autopsy tissue of six patients revealed that DRG had a high prevalence of large GAA expansions, the frequency of which correlated significantly with patient age. (B) Small pool PCR analysis of multiple tissues of an 18-week fetus, homozygous for expanded GAA repeat alleles, revealed very low levels of instability compared with adult-derived tissues. (C) The mutation load in blood samples from multiple patients increased significantly with age. (D) Somatic instability was required for the development of the FRDA phenotype in compound heterozygotes for one typical expanded GAA allele and one borderline allele. (E) Transgenic mice containing expanded GAA repeats in the context of the human *FXN* locus showed age-dependent expansions specifically in the DRG and cerebellum. Progressive pathology in FRDA is therefore likely due to age-dependent accumulation of further large expansions in specific tissues. Progressive, tissue-specific DNA instability is a common theme in the pathogenesis of triplet-repeat diseases.

**Title: Infectious delivery and expression of a 135 kb human *FRDA* genomic DNA locus complements Friedreich's ataxia deficiency in human cells**

**Authors:** Silvia Gomez-Sebastian<sup>1</sup>, Alfredo Gimenez-Cassina<sup>2</sup>, Javier Diaz-Nido<sup>2</sup>, Filip Lim<sup>2</sup> and Richard Wade-Martins<sup>1\*</sup>

**Institutions:** <sup>1</sup>The Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford, OX3 7BN United Kingdom <sup>2</sup>Centro de Biología Molecular Severo Ochoa, Universidad Autónoma de Madrid, Madrid, 28049 Spain

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**Abstract:**

Friedreich's ataxia (FA) is the most common recessive ataxia, affecting 1-2 in 50,000 Caucasians, and there is currently no effective cure or treatment. FA results from a deficiency of the mitochondrial protein frataxin brought about by a repeat expansion in intron 1 of the *FRDA* gene. The main areas affected are the central nervous system (particularly the spinocerebellar system) and cardiac tissue. Therapies aimed at alleviating the neurological degeneration have proved unsuccessful to date. We have used the recently-described infectious bacterial artificial chromosome (iBAC) system to develop iBAC-*FRDA* vectors. The iBAC system uses the high capacity (~150 kb) and high efficiency of the herpes simplex virus type 1 (HSV-1) amplicon to deliver and express complete genomic DNA loci. We have generated several iBAC-*FRDA* vectors which express the whole 80 kb *FRDA* genomic locus, driven by the endogenous *FRDA* promoter and including all introns and flanking regulatory sequences within a 135 kb genomic DNA insert.

First, we built iBAC-*FRDA* vectors in which the luciferase or Beta-galactosidase gene is embedded within the *FRDA* locus and driven by the endogenous promoter. We used reporter gene assays to demonstrate prolonged luciferase or Beta-galactosidase gene expression in primary mouse cortical neuronal cultures and differentiated SHSY5Y human neuronal cells. Such vectors will prove extremely useful to investigate physiological control of *FRDA* expression.

Second, we made iBAC-*FRDA* vectors to express the complete wild-type human *FRDA* gene. FA patient primary fibroblasts deficient in frataxin protein and exhibiting sensitivity to oxidative stress were transduced at high efficiency by iBAC-*FRDA* vectors. Following vector transduction, expression of *FRDA* protein by immunofluorescence was shown. Finally, functional complementation studies demonstrated restoration of the wild type cellular phenotype in response to oxidative stress in transduced FA patient cells. These results suggest the potential of the iBAC-*FRDA* vectors for gene therapy of Friedreich's ataxia.

## **The Friedreich's Ataxia Disease Gene – Poster presentations**

**Title:** Novel, complex interruptions of the GAA repeats in the small expanded alleles of two affected siblings with a mild, late onset form of Friedreich ataxia.

**Authors:** D. R. Lynch, E.C. Frackelton, J. McCallum, J.M Farmer, A. Tsou, A. Santani, C.M. Mulcahy, and C.A. Stolle

**Institutions:** University of Pennsylvania, Children's Hospital of Philadelphia

Corresponding author email address:lynch@pharm.med.upenn.edu

### **Abstract:**

Friedreich Ataxia (FA) is a progressive neurodegenerative disorder associated with an expanded GAA triplet repeat in both alleles of the *FRDA* gene in 97% of people with FA. This triplet repeat is located within an intron, leading to decreased RNA transcription and decreased levels of frataxin. The pathologic GAA repeat is sometimes interrupted by short stretches of other sequences. These interruptions have only been identified at present in normal alleles or in alleles carrying relatively short (<200 GAA repeat or its equivalent) expansions for technical and/or biological reasons. We recently identified a family in which two members carry a typical long (>500 GAA repeats) expanded allele and a smaller (120-130 GAAs) expanded but interrupted GAA repeat in the other allele of the *FRDA* gene. These patients presented with an extremely late adult onset form of FA, 25-35 years later than expected based on the overall length of the shorter expansion. When we sequenced the shorter GAA repeat, we found complex interruptions of the following sequence: 5' (GAA)>72 + GAGAAGAAAA + (GAA)x20 + GAAAAGAA + [GAGGAA]x4 + (GAA)x10 + to 3' that were identical in both siblings. One of these interruptions [GAGGAA] has been reported previously, but two others (GAGAAGAAAA) and (GAAAAGAA) are novel. The longest stretch of uninterrupted GAA repeats is >72, an allele size more consistent with their age of onset and mild disease course. As a second family with similar clinical features and an interrupted allele to our family has been reported, this provides strong evidence that interruption of the GAA repeat decreases the clinical severity of FA. We then sequenced 4 further individuals with FA and shorter GAA lengths less than 250 to assess whether such interruptions are more common than expected. In each of the patients, interruptions were found, with the most common one being GAGGAA rather than the GAAGAA sequence expected. This frequency suggests that interrupted repeats are more common than expected, and could readily explain some of the variability between GAA repeat length and clinical severity.

**Title: Molecular Characterization of a Novel Case of Friedreich Ataxia**

**Authors:** Novita Puspasari,<sup>1,2</sup> Lingli Li,<sup>1</sup> Timothy P. Holloway,<sup>1</sup> Louise Corben,<sup>1</sup> Michael Fahey,<sup>1,2</sup> Martin B. Delatycki,<sup>1,2</sup> and Joseph P. Sarsero<sup>1</sup>

**Institutions:** <sup>1</sup>Genetic Health Research (Bruce Lefroy Centre), Murdoch Childrens Research Institute, Royal Children's Hospital, Parkville, Victoria 3052, Australia. <sup>2</sup>Department of Paediatrics, The University of Melbourne, Royal Children's Hospital, Parkville, Victoria 3052, Australia.

Corresponding author email address: joe.sarsero@mcri.edu.au

**Abstract:**

Over 96% of Friedreich ataxia (FA) patients are homozygous for an expansion of a GAA trinucleotide repeat sequence within the first intron of the *FXN* gene. The remaining patients are compound heterozygotes for a point mutation and a GAA expansion. Genetic testing for FA routinely involves PCR screening for the presence of a GAA expansion. Heterozygous carriers exhibit a normal *FXN* allele on one chromosome and another allele containing a GAA expansion. A patient presented to us with FA and a routine PCR diagnosis using primers flanking the GAA repeat region was performed both on the patient and her mother (an obligate heterozygous carrier). The mother exhibited only a single band corresponding to a normal allele without an expansion, while the patient DNA sample did not produce a PCR product. Western blot analysis revealed decreased frataxin levels in the mother and more so in the patient. Cytogenetic analysis using G-banding and dual-probe FISH did not detect any gross chromosomal abnormalities. PCR analysis revealed the presence of *FXN* exons. We hypothesized that the lack of a PCR product from an expanded allele could either be explained by the presence of a deletion or point mutation on one side of the GAA repeat that prevents a PCR primer annealing at that particular region, or alternatively by a very long GAA repeat expansion that cannot be revealed by normal PCR conditions. Extensive short- and long-range PCR analysis has currently not been able to identify a GAA expansion region or localize the presence of a secondary point mutation or deletion. Southern blot analysis has however revealed one expanded allele in the mother and two in the patient.

Friday November 10, 2006

2:00 – 4:00pm **Session II – The Friedreich's Ataxia Protein**  
**Session Chair: Grazia Isaya, MD, PhD**

2:00- 2:05 - Introduction

**2:05- 2:20 Title: A Structural Basis for Understanding Frataxin's Function**

**Authors:** T.L. Stemmler, A Dancis, K.Z. Bencze, K.C. Kondapalli, J.D. Cook

**2:20- 2:35 Title: The structures of frataxin oligomers reveal a novel mechanism for the delivery and detoxification of iron**

**Authors:** T. Karlberg<sup>1</sup>, U. Schagerlof<sup>1</sup>, O. Gakh<sup>2</sup>, S. Park<sup>2</sup>, U. Ryde<sup>3</sup>, M. Lindahl<sup>1</sup>, K. Leath<sup>4</sup>, E. Garman<sup>4</sup>, G. Isaya<sup>2</sup> & S. Al-Karadaghi<sup>1</sup>

**2:35- 2:50 Title: Frataxin analogue in respiratory complex I**

**Authors:** Leonid A. Sazanov and Philip HInchliffe

**2:50- 3:05 Title: Functional links between frataxin and the Isc operon (abstract not published)**

**Authors:** Adinolfi S<sup>1</sup>., Pastore C<sup>1</sup>., Mayer C<sup>1</sup>., Temussi P.A<sup>2</sup>. and Pastore A<sup>1</sup>.

**3:05- 3:20 Title: Frataxin – A Complex Multi-tasking Protein. Iron binding, self-processing and interactions with physiological partners. (abstract not published)**

**Authors:** J A Cowan, T Yoon, E Dizin, J Huang,

**3:20- 3:35 Title: Frataxin is related with the mitochondrial electron transport chain**

**Authors:** Pilar González-Cabo<sup>1</sup>, Sheila Ros<sup>1</sup>, Rafael P. Vázquez-Manrique<sup>2</sup>, M. Adelaida Garcia-Gimeno<sup>1</sup>, Homera Asiz<sup>2</sup>, Howard A. Baylis<sup>2</sup>, Pascual Sanz<sup>1</sup>, Francesc Palau<sup>1</sup>

**3:35- 3:50 Title: A nematode model for Friedreich's ataxia.**

Authors: **Thomas E. Johnson, Roberto Testi and Natascia Ventura**

**3:50- 4:05 Title: A functional pool of extramitochondrial frataxin**

**Authors:** Ivano Condò, Natascia Ventura, Florence Malisan, Barbara Tomassini and Roberto Testi

4:05 – 4:30 Roundtable Discussion

**Poster Presentations**

**Title: Dissecting the two functions of frataxin by mutational analysis and yeast studies**

**Authors:** O. Gakh<sup>1</sup>, O. Li<sup>1</sup>, R. Vaubel<sup>1</sup>, S.F. Duncan<sup>1</sup>, G.C. Ferreira<sup>2</sup>, S. Al-Karadaghi<sup>3</sup>  
and G. Isaya<sup>1</sup>

## **The Friedreich's Ataxia Protein - Platform Presentations Abstracts**

**Title:** A Structural Basis for Understanding Frataxin's Function

**Authors:** T.L. Stemmler, A Dancis, K.Z. Bencze, K.C. Kondapalli, J.D. Cook

**Institutions:** Department of Biochemistry and Molecular Biology, Wayne State University School of Medicine; Department of Medicine, Division of Hematology-Oncology, University of Pennsylvania, Philadelphia, Penn

Corresponding author email address: [tstemmle@med.wayne.edu](mailto:tstemmle@med.wayne.edu)

### **Abstract:**

Mounting evidence suggests Frataxin plays a direct role in both the heme and iron-sulfur cluster pathways by acting as an iron chaperone. Structural studies confirm frataxin is a compact planer molecule and a member of the  $\alpha\alpha$  sandwich motif family. While the iron-induced assembly properties of yeast frataxin (Yfh1) have been well established, our laboratory has shown that the monomeric form of Yfh1 binds iron in a stable manner, and stabilizes a portion of the protein against oxidation in an oxidative rich environment. Others and we have also shown that frataxin's acidic residues that participate in iron binding are localized in the protein's helix-1/strand-1 interface. Furthermore, it appears that frataxin may utilize distinct molecular surfaces in addition to the protein's metal binding domain to form stable protein-protein complexes with enzymes that direct both heme and Fe-S cluster assembly.

We have prepared a series of iron binding Yfh1 mutants and tested their activity both *in vivo* and *in vitro* to provide a better understanding of how frataxin binds iron. In addition, we have tested the biochemical factors that drive frataxin's association with ferrochelatase, the enzyme that drives heme production, and with the iron-sulfur apparatus ISU proteins, in the Fe-S cluster assembly pathways. Protein complex studies directed towards providing a structural understanding of how frataxin forms a protein complex with its enzyme partners have also been completed.

**Title: The structures of frataxin oligomers reveal a novel mechanism for the delivery and detoxification of iron**

**Authors:** T. Karlberg<sup>1</sup>, U. Schagerlof<sup>1</sup>, O. Gakh<sup>2</sup>, S. Park<sup>2</sup>, U. Ryde<sup>3</sup>, M. Lindahl<sup>1</sup>, K. Leath<sup>4</sup>, E. Garman<sup>4</sup>, G. Isaya<sup>2</sup> & S. Al-Karadaghi<sup>1</sup>

**Institutions:** 1, 3 - *Depst. of Molecular Biophysics & Theoretical Chemistry, Lund University, Sweden*; 2 - *Depts of Pediatric & Adolescent Medicine and Biochemistry & Molecular Biology, Mayo Clinic College of Medicine, Rochester, USA*; 4 - *Dept. of Biochemistry, University of Oxford, UK*

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**Abstract:**

Frataxin performs key functions in iron delivery and detoxification via a currently unknown mechanism. The crystal structure of the iron-free and iron-loaded frataxin trimer and a single particle electron microscopic reconstruction of a 24-subunit iron-free and iron-loaded oligomers provide a basis for understanding the mechanisms of frataxin self-assembly in oligomeric particles, iron acquisition, detoxification and storage. They also provide an insight into the interplay between frataxin and other proteins, to which iron is delivered.

The structure of the trimer suggests that a gated mechanism controls iron delivery to different targets and iron storage, a combination of critical biological roles not found in other known iron-binding proteins. Since the trimer seems to exhibit structural details consistent with both such roles, we propose that it represents the primary functional unit of frataxin. Moreover, the trimer structure suggests that mutations found in patients with Friedreich's ataxia may destabilize trimer formation. Thus, compounds that would stabilize the frataxin trimer may provide a means to maximize the frataxin function in individuals affected by Friedreich's ataxia.

An additional remarkable feature of frataxin oligomers to be discussed in this presentation is the striking functional similarities of frataxin particles to the evolutionary unrelated ferritin superfamily of iron storage proteins.

**Title: Frataxin analogue in respiratory complex I**

**Authors: Leonid A. Sazanov and Philip HInchliffe**

**Institutions: Medical Research Council Dunn Human Nutrition Unit, Cambridge, UK**

Corresponding author email address: sazanov@mrc-dunn.cam.ac.uk

**Abstract:**

Complex I is the first and largest membrane protein complex of respiratory chains in mitochondria and bacteria. It consists of two large domains of similar size – membrane-embedded and hydrophilic. We have recently determined the structure of the hydrophilic domain of complex I from *Thermus thermophilus* (1). This subcomplex consists of eight subunits and contains all the redox centers of the enzyme, including nine iron-sulfur clusters. Unexpectedly, the novel subunit Nqo15 was found to have a similar fold to the mitochondrial iron chaperone frataxin. We identified a likely iron-binding site at the interface between Nqo15 and the rest of the complex. It may be involved in the regeneration of nearby iron-sulfur clusters. This structure may illustrate a general mode of interaction between the frataxin and its protein partners.

1. Sazanov, L.A. and Hinchliffe, P. (2006) Structure of the hydrophilic domain of respiratory complex I from *Thermus thermophilus*. *Science* **311**, 1430-1436.

**Title: Frataxin is related with the mitochondrial electron transport chain**

**Authors:** Pilar González-Cabo<sup>1</sup>, Sheila Ros<sup>1</sup>, Rafael P. Vázquez-Manrique<sup>2</sup>, M. Adelaida Garcia-Gimeno<sup>1</sup>, Homera Asiz<sup>2</sup>, Howard A. Baylis<sup>2</sup>, Pascual Sanz<sup>1</sup>, Francesc Palau<sup>1</sup>

**Institutions:** <sup>1</sup> Laboratory of Genetics and Molecular Medicine, Instituto de Biomedicina, CSIC, Valencia, Spain, and <sup>2</sup> Department of Zoology, University of Cambridge, Cambridge, U.K.

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**Abstract:** Frataxin deficiency causes Friedreich ataxia, a neurodegenerative disorder affecting sensitive neurons of dorsal root ganglia and spinocerebellar tracts. Physiological function of frataxin in mitochondria has not been established yet, although several hypotheses have been postulated including mitochondrial iron homeostasis, iron-sulphur cluster biogenesis, response to oxidative stress, iron storing, modulation of mitochondrial aconitase activity and a role in oxidative phosphorylation. Here we show that frataxin and its *Saccharomyces cerevisiae* orthologue Yfh1p interact with proteins from the mitochondrial electron transfer chain. We demonstrate that Yfh1p co-immunoprecipitates with yeast succinate dehydrogenase complex subunits Sdh1p and Sdh2p and with yeast putative orthologues of the electron transfer flavoprotein complex subunits Etf $\alpha$  and Etf $\beta$ . Synthetic interaction experiments confirmed a functional relationship between *YFH1* and succinate dehydrogenase genes. We also demonstrate synthetic genetic interaction in *Caenorhabditis elegans* between *frh-1* and *mev-1*, the nematode gene encoding the succinate dehydrogenase cytochrome b subunit of complex II in mitochondria. We suggest that both yeast frataxin, Yfh1p, and worm frataxin, FRH-1, might regulate the delivery of electrons via complex II towards ubiquinone.

We also demonstrate a physical interaction between human frataxin and human succinate dehydrogenase complex subunits SDHA and SDHB, suggesting also a key role of frataxin in the mitochondrial electron transport chain in humans. Consequently, we postulate a direct participation of the respiratory chain in the pathogenesis of the Friedreich ataxia.

Frataxin deficiency models in lower organisms may be useful for screening or evaluate alternative drugs. Sdh1p and SDHA are flavoproteins that bind covalently flavin adenine dinucleotide (FAD). Thus, we argue that FAD may be a candidate drug to be tested in our models. We have investigated the effect of riboflavin (vitamin B<sub>2</sub>) and its two derivatives, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) in both *S. cerevisiae* and *C. elegans* models. Riboflavin derivatives, FAD and FMN, rescue the growth phenotype of Yfh1p-deficient yeast strain and increase lifespan and improve physiology of frataxin knock-down (RNAi) worms. Then, we propose riboflavin and riboflavin derivatives as candidates' drugs for treatment of Friedreich ataxia patients.

**Title: A nematode model for Friedreich's ataxia.**

Authors: **Thomas E. Johnson, Roberto Testi and Natascia Ventura**

**Institutions: University of Colorado at Boulder, Laboratory of Signal Transduction, Department of Experimental Medicine and Biochemical Sciences, University of Rome "Tor Vergata"**

Corresponding author email address: johnsont@ibg.colorado.edu

Abstract:

We have identified the homolog of human frataxin in the nematode *C. elegans*, which we have termed *frh-1*. We have developed a knock-down model using RNAi to the *frh-1* gene as well as used a genetic mutant which knocks out the gene. The absence of *frh-1* is a recessive lethal with second/third stage larval lethality for the homozygote but no effect in the heterozygote. The RNAi knock-down has a life-enhancing phenotype, similar to the "Mit" phenotype that has been seen in numerous other mitochondrial knock-down and genetic mutants. The RNAi model shows differential sensitivity to oxidants and we have been studying interactions and epistatic effects of other pathways that are responsible for life-span prolongation in *C. elegans*. These models will provide insight into the cellular pathways at work in the presymptomatic phase of the disease, when new and more effective interventions may be possible.

**Title: A functional pool of extramitochondrial frataxin**

**Authors:** Ivano Condò, Natascia Ventura, Florence Malisan, Barbara Tomassini and Roberto Testi

**Institutions:** Laboratory of Signal Transduction, Department of Experimental Medicine and Biochemical Sciences, University of Rome “Tor Vergata”

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**Abstract:**

Frataxin is critical for cell survival, yet the molecular mechanisms underlying frataxin-mediated cellular protection are still largely obscure. We reported that frataxin-deficient cells are more prone to undergo stress-induced mitochondrial damage and apoptosis, induced by a variety of agents, while the overexpression of frataxin confers protection. We have found evidence for the existence of an extramitochondrial pool of mature frataxin in different cell types. The overexpression of extramitochondrial frataxin can efficiently prevent mitochondrial damage and apoptosis in all systems investigated and actually may confer significant proliferative advantage. Remarkably, extramitochondrial frataxin can fully replace mitochondrial frataxin in promoting survival of frataxin-deficient cells derived from Friedreich’s Ataxia patients.

We also recently observed that the major form of human mature frataxin might be smaller than previously recognized, suggesting alternative *in vivo* processing of the human frataxin precursors.

## **The Friedreich's Ataxia Protein - Poster Presentations Abstract**

**Title:** Dissecting the two functions of frataxin by mutational analysis and yeast studies

**Authors:** O. Gakh<sup>1</sup>, O. Li<sup>1</sup>, R. Vaubel<sup>1</sup>, S.F. Duncan<sup>1</sup>, G.C. Ferreira<sup>2</sup>, S. Al-Karadaghi<sup>3</sup> and G. Isaya<sup>1</sup>

**Institutions:** <sup>1</sup>Departments of Pediatric & Adolescent Medicine and Biochemistry & Molecular Biology, Mayo Clinic College of Medicine, Rochester, MN 55905, USA; <sup>2</sup>Department of Biochemistry & Molecular Biology, College of Medicine and H. Lee Moffitt Cancer Center and Research Institute, University of South Florida, Tampa, FL 33612, USA; <sup>3</sup>Department of Molecular Biophysics, Center for Chemistry and Chemical Engineering, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden.

Corresponding author email address: isaya@mayo.edu

### **Abstract:**

Friedreich ataxia is characterized by neurodegeneration, cardiomyopathy, and diabetes, which result from reduced synthesis of the mitochondrial protein frataxin. Although frataxin is ubiquitously expressed, frataxin deficiency leads to a selective loss of specific neuronal cells, cardiomyocytes, and pancreatic beta cells. How frataxin normally promotes survival of these particular cell types is as yet unknown. A large body of evidence indicates that frataxin sustains mitochondrial energy production and other cellular functions by providing iron for heme synthesis and iron-sulfur cluster assembly and repair. We have shown that frataxin is a bi-functional protein, which not only promotes the biogenesis of iron-containing enzymes but also detoxifies surplus iron thereby affording a critical antioxidant mechanism. Our current work aims at understanding how frataxin carries out both iron delivery and iron detoxification, and how each of these two functions contributes to the maintenance of mitochondrial iron balance and cell survival. Based on the recently solved structures of iron-free and iron-loaded frataxin oligomers we are investigating the effects of specific point mutations *in vitro* and in yeast. We find that mutations that impair the ferroxidation or mineralization activity of yeast frataxin are necessary for iron detoxification but do not affect the iron chaperone function of the protein. These mutations increase the sensitivity of yeast cells to oxidative stress, shortening chronological life span and precluding survival in the absence of the anti-oxidant enzyme superoxide dismutase. Mutations predicted to interfere with iron delivery from frataxin to other proteins are currently under study. [Funded by grants from NIH/NIA (AG15709) and FARA].

Friday November 10, 2006

5:00 – 6:30pm **Session III – Iron Metabolism**  
**Session Chair: Tracey Rouault, M.D.**

5:00- 5:05 - Introduction

**5:05- 5:20 Title: Mammalian iron-sulfur cluster assembly and the role of frataxin**

**Authors:** Tracey Rouault, Wing Hang Tong, Kuanyu Li, Helge Uhrigshardt, Hong Ye, Yanbo Shi

**5:20- 5:35 Title: Iron in the cardiomyopathy of Friedreich's ataxia**

**Authors:** Arnulf H. Koeppen, Susan C. Michael, Jacques B. Lamarche, and Jiang Qian

**5:35- 5:50 Title: Frataxin in FeS and heme synthesis in mitochondria**

**Authors:** Yan Zhang<sup>1</sup>, Elise Lyver<sup>1</sup>, Emmanuel Lesuisse<sup>2</sup>, Jean-Michel Camadro<sup>2</sup>, Debkumar Pain<sup>3</sup>, Timothy L. Stemmler<sup>4</sup>, Andrew Dancis<sup>1</sup>

**5:50- 6:05 Title: Iron and the dentate nucleus in Friedreich's ataxia**

**Authors:** Arnulf H. Koeppen, Susan C. Michael, Jacques B. Lamarche, and Jiang Qian

**6:05- 6:20 Title: Ferrochelatase-induced porphyrin distortion as a mechanism for metal ion selectivity**

**Authors:** Gloria C. Ferreira<sup>1</sup>, Salam Al-Karadaghi<sup>2</sup>, Ricardo Franco<sup>3</sup>, Mats Hansson<sup>2</sup>, John A. Shelnut<sup>4,5</sup> and Grazia Isaya<sup>6</sup>

**6:20- 6:35 Title: Silencing of ABCB7 in HeLa cells causes an iron deficient phenotype and mitochondrial iron overload.**

**Authors:** Patrizia Cavadini, Giorgio Biasiotto, Isabella Zanella, Marcella Corrado, Maura Poli, Rosaria Ingrassia and Paolo Arosio.

### **Poster Presentations**

**Title: Mitochondrial ferritin protects fibroblasts from Friedreich's Ataxia patients from oxidative damage.**

**Authors:** A. Campanella<sup>1</sup>, P. Santambrogio<sup>1</sup>, A. Cozzi<sup>1</sup>, F. Taroni<sup>3</sup> and S. Levi<sup>1,2</sup>

**Title: Inactivation of manganese superoxide dismutase by mitochondrial iron: a new aspect of iron toxicity**

**Authors:** Amornrat Naranuntarat, Mei Yang, and Valeria Cizewski Culotta

## **Platform presentations abstracts**

**Title: Mammalian iron-sulfur cluster assembly and the role of frataxin**

**Authors:** Tracey Rouault, Wing Hang Tong, Kuanyu Li, Helge Uhrigshardt, Hong Ye, Yanbo Shi

**Institutions:** National Institute of Child Health and Human Development

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### **Abstract:**

In mammalian cells, iron-sulfur clusters are synthesized by homologues of genes identified in bacteria and yeast. Alternative splicing of ISCU and NFU, two scaffold proteins, generates both mitochondrial and cytosolic isoforms of each protein and cytosolic ISCU is necessary for assembly of the iron-sulfur cluster of cytosolic aconitase (also known as Iron regulatory protein 1). We are interested in elucidating the role of frataxin in iron-sulfur cluster assembly. Using over-expressed fusion proteins and yeast 2 hybrid systems, we find interactions between frataxin and ISCU, ISCS, and ferrochelatase. We are working to verify these interactions by studying endogenous proteins that coprecipitate with frataxin. Our results are consistent with the hypothesis that frataxin delivers iron to ISCU and to ferrochelatase, and its role may be to maintain iron in a soluble accessible state for delivery.

### **References**

- Tong, W. H. and Rouault, T.A. (2006) Functions of mitochondrial ISCU and cytosolic ISCU in Fe-S cluster biogenesis and iron homeostasis, *Cell Metabolism*, 3, 199-210.
- Li, K., Tong, W. H., Hughes, R. M., and Rouault, T. A. (2006). Roles of the Mammalian Cytosolic Cysteine Desulfurase, ISCS, and Scaffold Protein, ISCU, in Iron-Sulfur Cluster Assembly. *J Biol Chem* 281, 12344-12351.
- Rouault, T.A. and Tong, WH, Iron-sulphur cluster biogenesis and iron homeostasis in mammals, *Nature Reviews: Molecular Cell Biology*, 2005:6, 345-351.

**Title: Iron in the cardiomyopathy of Friedreich's ataxia**

**Authors:** Arnulf H. Koeppen, Susan C. Michael, Jacques B. Lamarche, and Jiang Qian

**Institutions:** Stratton VA Medical Center and Albany Medical College, Albany, N.Y., USA; Centre hospitalier universitaire de Sherbrooke, Sherbrooke, QC, Canada

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**Abstract** (1): Hypertrophic cardiomyopathy is a common non-neural complication of Friedreich's ataxia (FRDA). Histological sections reveal abnormal cardiomyocytes, frank muscle fiber necrosis, reactive inflammation, and increased endomysial connective tissue. Scattered muscle fibers display perinuclear collections of minute iron-positive granules that lie in rows between myofibrils. In addition, clusters of much larger iron-reactive deposits are occasionally present in the endomysium, often near or within necrotic muscle fibers. We studied total iron and the iron-related proteins ferritin, mitochondrial ferritin, divalent metal transporter 1 (DMT1), and ferroportin in FRDA hearts by biochemical and histological techniques. Total iron in the left ventricular wall of 18 FRDA patients ( $30.7 \pm 19.3$  mg/100 g dry weight [mean  $\pm$  standard deviation]) was not significantly higher than the mean in 11 normal controls ( $31.3 \pm 24.1$  mg/100 g dry weight). Similarly, cytosolic holoferritin levels in 9 FRDA hearts ( $230 \pm 172$   $\mu$ g/g wet weight) were not significantly elevated above the mean in 5 normal controls ( $148 \pm 86$   $\mu$ g/g wet weight). Nevertheless, Western blots showed a distinct increase of the light ferritin subunits in FRDA myocardium when compared to normal heart. The perinuclear iron-positive granules exhibited immunoreactivity for cytosolic ferritin, mitochondrial ferritin, and ferroportin. Electron microscopy showed enhanced electron density of mitochondrial deposits after treatment with bismuth subnitrate supporting ferritin accumulation. The coarser endomysial iron deposits were strongly immunoreactive with antisera to ferritin, CD68 (a marker for monocytes and macrophages), and the DMT1 isoform(s) translated from messenger ribonucleic acids containing iron-responsive elements (DMT1+). They showed no immunoreactivity with anti-ferroportin. The described observations distinguish the progressive cardiomyopathy in FRDA from iron-storage diseases with cardiac involvement but support iron-catalyzed mitochondrial damage followed by muscle fiber necrosis and a chronic inflammatory reaction. (Supported by the Friedreich's Ataxia Research Alliance, the National Ataxia Foundation, and the laboratory facilities at the Stratton VA Medical Center, the Albany Medical College, and the Centre hospitalier universitaire de Sherbrooke).

**Title: Frataxin in FeS and heme synthesis in mitochondria**

**Authors:** Yan Zhang<sup>1</sup>, Elise Lyver<sup>1</sup>, Emmanuel Lesuisse<sup>2</sup>, Jean-Michel Camadro<sup>2</sup>, Debkumar Pain<sup>3</sup>, Timothy L. Stemmler<sup>4</sup>, Andrew Dancis<sup>1</sup>

**Institutions:** <sup>1</sup> Department of Medicine, Division of Hematology-Oncology, University of Pennsylvania, Philadelphia, PA 19104; <sup>2</sup> Laboratoire d'Ingenierie des Proteines et Controle Metabolique, Departement de Biologie des Genomes, Institut Jacques Monod, Unite Mixte de Recherche 7592 CNRS-Univesite Paris 6 and 7, France; <sup>3</sup> Department of Pharmacology and Physiology, UMDNJ, New Jersey Medical School, Newark, New Jersey 07101. <sup>4</sup> Department of Biochemistry and Molecular Biology, Wayne State University School of Medicine, Detroit MI 48201.

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**Abstract:**

Yeast frataxin (Yfh1p), the homolog of the human protein implicated in Friedreich's ataxia, is involved in iron homeostasis. However, its precise functions are complex, and pleiotropic phenotypes of the loss of function mutant have made it difficult to separate direct from indirect effects. We have been characterizing frataxin minus mitochondria from yeast. These mitochondria lack protein import capacity in vitro due to an extremely low membrane potential. The membrane potential was partially restored by introduction of additional mutations that deplete cellular or mitochondrial iron (yfh1/fet3, yfh1/aft1, yfh1/mrs3/mrs4). Curiously growth in high concentrations of iron for a brief period also restored membrane potential and import capacity.

In isolated mitochondria, Fe-S clusters were detected on newly imported yeast ferredoxin precursor and on endogenous aconitase by means of <sup>35</sup>S-cysteine labeling and native gel separation. In the frataxin minus mitochondria, a kinetic defect in new FeS cluster synthesis was noted. Import of frataxin into frataxin minus mitochondria promptly corrected the FeS cluster assembly defect without the further addition of iron. By contrast, a defect in heme synthesis observed in these same mitochondria, was not corrected by frataxin import. The roles of frataxin in FeS and heme synthesis thus may be different, and we are endeavoring to identify alleles that separate these functions.

**Title: Iron and the dentate nucleus in Friedreich's ataxia**

**Authors:** Arnulf H. Koeppen, Susan C. Michael, Jacques B. Lamarche, and Jiang Qian

**Institutions:** Stratton VA Medical Center and Albany Medical College, Albany, N.Y., USA; Centre hospitalier universitaire de Sherbrooke, Sherbrooke, QC, Canada

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**Abstract** (2): Friedreich's ataxia (FRDA) almost invariably affects the dentate nucleus, and the lesion contributes to the clinical phenotype. Histological sections reveal neuronal loss and peculiar clusters of synaptic terminals called "grumose" degeneration. This degenerative process is also known from progressive supranuclear palsy, a sporadic disease, and spinocerebellar ataxia type 3. It has not been studied heretofore in FRDA. We investigated the possibility that mitochondrial iron dysmetabolism in FRDA contributes to the pathogenesis of this unusual lesion in the iron-rich dentate nucleus. Iron levels in frozen autopsy specimens from 10 patients with FRDA ( $1.45 \pm 0.59$   $\mu\text{mol/g}$  wet weight [mean  $\pm$  standard deviation]) did not differ from those in 8 normal controls ( $1.78 \pm 0.88$   $\mu\text{mol/g}$ ) or from data in the literature ( $1.85 \pm 0.09$   $\mu\text{mol/g}$  [45 unselected autopsies]). Ferritin levels in FRDA and normal dentate nuclei were also identical at  $195.6 \pm 56.5$   $\mu\text{g/g}$  and  $210.9 \pm 9.0$   $\mu\text{g/g}$  wet weight, respectively. Nevertheless, the ratio of heavy to light ferritin subunits in FRDA was significantly lower at  $2.9 \pm 0.9$  when compared to the normal ratio of  $10.5 \pm 6.5$ . Slide techniques showed neuronal loss in the dentate nucleus and commensurate disappearance of juxtaneuronal ferritin-reactive oligodendroglia. Ferritin reaction product shifted to microglia and astrocytes while the oligodendroglia of the adjacent white matter were not affected. The regions of grumose degeneration were immunoreactive for ferroportin, an iron-carrying transmembrane protein. The cytoplasm of Purkinje cells and their axons were also strongly ferroportin-reactive. Ferroportin in grumose degeneration co-localized with a protein of presynaptic membranes, SNAP-25. Ferroportin messenger ribonucleic acid contains an iron-responsive element in its 5'-untranslated region, and increased biosynthesis of this protein may be an indicator of iron excess in synaptic terminals abutting against the nerve cells of the dentate nucleus. The observations also suggest that axons giving rise to grumose degeneration derive from Purkinje cells though these neurons are only occasionally affected by FRDA. (Supported by the Friedreich's Ataxia Research Alliance, the National Ataxia Foundation, and the laboratory facilities at the Stratton VA Medical Center, the Albany Medical College, and the Centre hospitalier universitaire de Sherbrooke).

**Title: Ferrochelatase-induced porphyrin distortion as a mechanism for metal ion selectivity**

**Authors:** Gloria C. Ferreira<sup>1</sup>, Salam Al-Karadaghi<sup>2</sup>, Ricardo Franco<sup>3</sup>, Mats Hansson<sup>2</sup>, John A. Shelnut<sup>4,5</sup> and Grazia Isaya<sup>6</sup>

**Institutions:** <sup>1</sup>University of South Florida, Tampa, FL, USA, <sup>2</sup>Lund University, Lund, Sweden, <sup>3</sup> Universidade Nova de Lisboa, Caparica, Portugal, <sup>4</sup>Sandia National Laboratories, Albuquerque, NM, USA, <sup>5</sup> University of Georgia, Athens, GA, USA, <sup>6</sup> Mayo Clinic, Rochester, MN, USA.

Corresponding author email address: [gferreir@health.usf.edu](mailto:gferreir@health.usf.edu)

**Abstract:**

Ferrochelatase catalyzes the terminal step of the heme biosynthetic pathway by inserting ferrous iron into protoporphyrin IX. Using resonance Raman (RR) spectroscopy and time-resolved crystallography, the structural properties of ferrochelatase-bound porphyrins and porphyrin metalation were examined, especially with respect to the porphyrin deformation occurring in the active site. We propose that the ferrochelatase-induced distortion of the porphyrin substrate not only enhances the reaction rate but also modulates which divalent metal ion is incorporated into the porphyrin ring. In addition, we hypothesize that the ferrochelatase-frataxin interaction controls the type of metal ion delivered to ferrochelatase and consequently regulates iron and heme metabolisms.

[This work was supported by the American Cancer Society, RSG-96-05106-TBE and American Heart Association, 0655091B (to G.C.F), Swedish Research Council (to S. A.-K. and M. H.), Muscular Dystrophy Association, Friedreich's Ataxia Research Alliance and National Institutes of Health, AG15709 (to G. I.). Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy under Contract DE-AC04-94AL85000 (J.A.S.).]

**Title: Silencing of ABCB7 in HeLa cells causes an iron deficient phenotype and mitochondrial iron overload.**

**Authors:** Patrizia Cavadini, Giorgio Biasiotto, Isabella Zanella, Marcella Corrado, Maura Poli, Rosaria Ingrassia and Paolo Arosio.

**Institutions:** Department Materno Infantile e Tecnologie Biomediche, University of Brescia, Brescia, Italy

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**Abstract:**

Mitochondria have a central role in iron metabolism, since they are the sites of the synthesis of heme and of iron/sulphur complexes (Fe/S), and alteration of mitochondrial iron is associated with some diseases such as Friedreich ataxia, sideroblastic anemia, and the rare sideroblastic anemia with ataxia (ASAT). This is a genetic diseases caused by defects of the mitochondrial transporter ABCB7. The yeast homologue of this protein was found to have a role in the transport of Fe/S complexes from mitochondrion to cytosol, and its deletion caused mitochondrial iron overload, inhibition of cytosolic Fe/S enzymes and oxidative damage. In mouse models, the deletion of the gene either systemic or in most tissues was found to be embryonically lethal, with the exception of liver and endothelial cells. In the mouse liver, ABCB7 deficiency caused inhibition of the Fe/S containing cytosolic aconitase, and major alteration of iron metabolism, without mitochondrial iron loading and mitochondrial damage (Pondarre et al, 2006). To study the function of this protein in mammalian cells and the role of mitochondria in cellular iron homeostasis, we initially identified the conditions to silence its mRNA in HeLa cells performing sequential transfections with siRNAs. The phenotype of the ABCB7-deficient cells was characterized by a strong reduction of cellular proliferation, which was not affected by iron supplementation, by evident signs of cellular iron deficiency and by a large increase of mitochondrial iron deposition. In addition, the cells showed a higher sensitivity to H<sub>2</sub>O<sub>2</sub> toxicity. The activity of mitochondrial enzymes, such as citrate synthase or succinate dehydrogenase, was not modified, while ATP content increased, probably because of the ATPase activity of ABCB7. In contrast, the aconitase activity, particularly that of the cytosolic/IRP1 enzyme, was reduced. A similar inhibition of aconitase was obtained also with treatments that abolish mitochondrial membrane potential. ABCB7-deficiency caused also an inhibition of the mitochondrial Mn-SOD (SOD2), probably for competition with Mn, as reported in yeast cells (Yang et al, 2006). To distinguish between the effects of different types of mitochondrial iron overload, we analyzed HeLa cells expressing mitochondrial ferritin (MtF). The level of mitochondrial iron load was analogous to that obtained after ABCB7 silencing, but it did not reduce cellular proliferation and resistance to oxidative damage by H<sub>2</sub>O<sub>2</sub>. More interestingly, ABCB7 silencing of the MtF expressing cells did not induce further increase of mitochondrial iron accumulation, and the iron excess was poorly available to MtF. The results support the hypothesis that ABCB7 has a role in the transport of Fe from mitochondria to cytosol, and in the maturation of cytosolic Fe/S enzymes. They also indicate mitochondrial iron excess may be caused by different mechanisms and that the one associated with ABCB7-deficiency facilitates oxidative damage and is poorly available to MtF and possibly also to ferrochelatase. These data contribute to explain the phenotype of ASAT.

## **Iron Metabolism – Poster presentations abstracts**

**Title: Mitochondrial ferritin protects fibroblasts from Friedreich's Ataxia patients from oxidative damage.**

**Authors:** A. Campanella<sup>1</sup>, P. Santambrogio<sup>1</sup>, A. Cozzi<sup>1</sup>, F. Taroni<sup>3</sup> and S. Levi<sup>1,2</sup>

**Institutions:** (1) IRCCS H. San Raffaele, (2) Vita-Salute San Raffaele University, Via Olgettina 58 Milano, 20132 Italy, [levi.sonia@hsr.it](mailto:levi.sonia@hsr.it), tel:+3926434755, fax:+390226434844; (3) Istituto Nazionale Neurologico "C. Besta", Milano, Italy.

Corresponding author email address: [levi.sonia@hsr.it](mailto:levi.sonia@hsr.it)

### **Abstract:**

Human mitochondrial ferritin (MtFt) is an iron storage protein, encoded by an intronless gene on chromosome 5q23.1 and specifically targeted and processed in the mitochondria. Its structure and function are similar to those of cytosolic H-ferritin, but it lacks of a functional IRE and its expression is tissue specific, rather than ubiquitous. Previous studies on Friedreich's Ataxia (FRDA) yeast model indicated that MtFt rescued the respiratory defect typical of the frataxin-deficient strains. It protected the mitochondrial Fe/S enzymes activity, preserved the integrity of mitochondrial DNA, increased the resistance to oxidative damage and reduced the development of mitochondrial iron loading. To verify if this MtFt protective role is maintained also in mammalian cells, we overexpressed MtFt in fibroblasts from FRDA patients. We tested two different FRDA and one WT fibroblast cell lines, setting up transfection condition yield to about 40% efficiency. MtFt appeared accumulated specifically into mitochondria and it was correctly assembled in its native functional form. We founded that MtFt expression protected the viability of cultured FRDA fibroblasts from treatment with H<sub>2</sub>O<sub>2</sub>. Then we investigated the level of mtROS in WT or FRDA fibroblasts by florescent probes. The mtROS resulted higher in FRDA than in WT cells and the H<sub>2</sub>O<sub>2</sub> insult improved this difference. When we transfected the FRDA fibroblasts with MtFt, they showed a significant reduction of mtROS formation respect the non transfected cells. Thus also FRDA fibroblasts appeared to take advantage of mitochondrial ferritin protection against damages due to oxidative stress.

**Title: Inactivation of manganese superoxide dismutase by mitochondrial iron: a new aspect of iron toxicity**

**Authors:** Amornrat Naranuntarat, Mei Yang, and Valeria Cizewski Culotta

**Institutions:** Department of Environmental Health Sciences, The Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD 21205, USA

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**Abstract:**

Activation of manganese-containing superoxide dismutase (Sod2p) requires yeast Mtm1p, a member of the mitochondrial carrier family. While the precise role of Mtm1p is still unclear yeast lacking *MTM1* have dramatically reduced Sod2p activity as well as elevated concentrations of mitochondrial iron. In *mtm1* mutants iron becomes mis-incorporated into the Sod2p protein resulting in the inactivation of the enzyme. Our results indicate that the elevated mitochondrial iron in *mtm1* mutants directly competes with manganese for occupancy of the Sod2p active site. To investigate whether loss of Sod2p activity was a common phenotype associated with mitochondrial iron overload, we monitor Sod2p activity in other mutants that display high mitochondrial iron. Iron accumulated in Fe-S cluster assembly mutants, *grx5* and *ssq1*, is reactive with Sod2p causing a reduction in Sod2 activity similar to what was observed in *mtm1* mutants. Surprisingly, mutation of *YFH1*, the yeast homologue of human frataxin, while accumulating elevated mitochondrial iron did not show reduced Sod2p activity. We also found that deletion of *YFH1* in *mtm1* mutants can reverse the loss of Sod2p activity seen in this strain. This result suggests that Yfh1p may contribute to iron that is bioavailable to Sod2p. Differential bioavailability of mitochondrial metals also affects the activity of *E. coli* SODs targeted to yeast mitochondria allowing another means to monitor bioavailability of Sod2p-reactive iron. Our evidence suggests the existence of at least two pools of iron in the mitochondria: A “Sod2p-inert” pool that predominates under normal conditions and a “Sod2p-reactive” pool that accumulates under certain cases of mitochondrial iron overload. In addition, there is a small pool of Sod2p-reactive iron present even without disruptions in iron homeostasis which can be seen upon manganese starvation. It is possible that the oxidative damage associated with human disorders of mitochondrial iron overload may be due to the loss of the anti-oxidant activity of Sod2p in addition to an increased production of reactive oxygen species via Fenton chemistry.

**Saturday, November 11, 2006**

7:30 - 9:45 AM

**Session IV - Cellular and Mitochondrial Pathophysiology of Friedreich's Ataxia**

**Session Chairs: Massimo Pandolfo, MD and Michio Hirano, MD**

7:30 – 7:45 AM

**Introduction**

7:45 – 8:00 AM

**Title: Consequences of changes in mitochondrial metabolism in mouse models of altered frataxin expression**

**Authors: Tim J. Schulz, Doreen Pomplun, Kim Zarse, Anja Voigt, René Thierbach, Marc Birringer, Michael Ristow**

8:00 – 8:15 AM

**Title: Early consequences of frataxin deficiency affect cytoplasmic iron-sulfur proteins and lead to oxidative damage and UPR, late responses include induction of the heme pathway**

**Authors: Chunye Lu, Robert Schoenfeld, Yuxi Shan and Gino Cortopassi**

8:15 – 8:30 AM

**Title: Gene expression studies in heart and skeletal muscle of frataxin deficient mice.**

**Authors: Giovanni Coppola, Maren Engelhardt, Matthew Suberlak, Eric Wexler, Manuela Santos, Massimo Pandolfo, Daniel Geschwind**

8:30 – 8:45 AM

**Title: Proteomic analysis of the yeast model of Friedreich ataxia reveals manganese deficiency and oxidative damage to magnesium-binding proteins.**

**Authors: Jordi Tamarit, Veronica Irazusta, Armando Moreno-Cermeño, Joaquim Ros.**

8:45 – 9:00 AM

**Title: Up-regulation of glucose metabolism after chronic inhibition of GSK-3 protects neurons against death triggered by a mitochondrial complex I inhibitor.**

**Authors: Alfredo Gimenez-Cassina, Filip Lim and Javier Diaz-Nido**

9:00 – 9:15 AM

**Title: Identification of a mitochondrial proteolytic activity that degrades human frataxin**

**Authors: Ngolela Esther Babady, Yuan-Ping Pang, Orly Elpeleg and Grazia Isaya**

9:15 – 9:30 AM

**Title: Reduction in human mitochondrial frataxin levels increases ROS associated stress: implications for Friedreich's ataxia and "normal" cells**

**Authors: Halweg, C., Menendez, D., Karthikeyan G., Geiger K., and Resnick, M. A.**

9:30 – 9:45 AM

**Title: Selective expression of catalase in the peripheral nervous system ameliorates the symptoms of frataxin deficiency in a Drosophila model of FRDA**

**Authors:** Peter R. Anderson, Kim Kirby, Arthur J. Hilliker, and John P. Phillips

9:45 – 10:15 AM      Roundtable Discussion

**Poster presentations**

**Title** :Glutathione-dependent redox status of frataxin-deficient cells in a yeast model of Friedreich Ataxia. (abstract not published)

**Authors:** Auchère F., Santos R., Lesuisse E., Camadro JM

**Title: Consequences of changes in mitochondrial metabolism in mouse models of altered frataxin expression**

**Authors:** Tim J. Schulz, Doreen Pomplun, Kim Zarse, Anja Voigt, René Thierbach, Marc Birringer, Michael Ristow

**Institutions:** University of Jena, University of Potsdam

Corresponding author email address: fara@mristow.org

**Abstract:**

Employing cre-recombinase mediated excision techniques, we have completely disrupted or partly reduced frataxin expression in pancreatic beta-cells (fully published) liver (partly published), and adipose and brain tissues (unpublished). Furthermore we have inadvertently created a line with a non-tissue-specific reduction of frataxin expression by approx. 70 percent (unpublished). Lastly we have overexpressed frataxin in mice employing different techniques. For the sake of time, presentation of data will be cumulative and will summarize multiple but synergistic changes in mitochondrial metabolism and oxidative phosphorylation, alterations in signal transduction, and lastly consequences for energy dissipation, including potential roles in disease like obesity, diabetes and cancer.

**Title: Early consequences of frataxin deficiency affect cytoplasmic iron-sulfur proteins and lead to oxidative damage and UPR, late responses include induction of the heme pathway**

**Authors:** Chunye Lu, Robert Schoenfeld, Yuxi Shan and Gino Cortopassi

**Institutions:** University of California, Davis

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**Abstract:**

Deficiency of frataxin protein expression causes Friedreich's ataxia, resulting in neurodegeneration, cardiodegeneration and insulin resistance. We have investigated the consequences of frataxin deficiency using tet-inducible siRNA against frataxin in HEK294 cells. We confirmed the existence of an extramitochondrial frataxin pool, and demonstrate that the number of mature frataxin molecules in the cytoplasm is equivalent or greater than mitochondrial frataxin molecules. Using tetracycline to inhibit frataxin expression, cytoplasmic frataxin was half-depleted three-fold faster than mitochondrial frataxin. In parallel with cytoplasmic frataxin depletion, the activity and amount of the cytoplasmic iron-sulfur cluster proteins aconitase and ISU1 declined, respectively, at 2 days. By co-immunoprecipitation we demonstrate that frataxin binds the ISU protein isd11. From day 2 onward the antioxidant cytoplasmic CuZnSOD protein was induced, and increased protein oxidative damage was observed from day 4 onward, and the unfolded protein response transcription factors ATF4 and CHOP were also induced. By contrast, mitochondrial aconitase activity declined much later, at 7 days, and the heme-dependent transcripts ALAS1 and MAOA were induced only after day 8, coincident with the decrease in heme-containing cytochrome c protein, and consistent with our previous results in humans and mice demonstrating defects in mitochondrial hemes a and c. Overall, these results suggest that the earliest consequences of frataxin deficiency occur in cytoplasmic iron-sulfur proteins and result in oxidative damage and stress, and trigger the unfolded protein response, which has been linked with demyelination, and that depletion of mitochondrial cytochromes a and c and induction of ALAS1 occur only after mitochondrial frataxin depletion. These data suggest that the consequences of extra-mitochondrial frataxin deficiency could be important in the Friedreich's ataxia pathophysiological mechanism, and might provide novel avenues for therapeutic strategies.

**Title:** Gene expression studies in heart and skeletal muscle of frataxin deficient mice.

**Authors:** Giovanni Coppola, Maren Engelhardt, Matthew Suberlak, Eric Wexler, Manuela Santos, Massimo Pandolfo, Daniel Geschwind.

**Institutions:** Department of Neurology, University of California at Los Angeles

Corresponding author email address: dhg@ucla.edu

**Abstract:**

Cardiomyopathy and diabetes impact survival and quality of life in FRDA patients. Even though they have both been linked to mitochondrial dysfunction, their pathogenesis is not clear. We studied gene expression patterns in heart and skeletal muscle from a mouse model of frataxin deficiency using microarrays, in order to identify tissue-specific, frataxin-related gene expression changes.

RNA was extracted from heart and skeletal muscle of three knock-in/knock-out mice (Miranda et al 2002) and co-hybridized with RNA from control mice on Agilent microarrays. Each experiment was performed in duplicate, for a total of 12 slides. Data analysis was performed using the limma Bioconductor package, and significance threshold was set at  $p < 0.005$ . Eighty-four and 167 genes showed differential expression (mostly downregulation) in frataxin deficient heart and skeletal muscle, respectively. From a functional standpoint, expression changes detected in both tissues included mitochondrial, HSP40 and hemoglobin-related transcripts. Heart-specific changes included coordinated down-regulation of contractile proteins. Skeletal muscle-specific changes involved lipid metabolism and oxidation of fatty acids, both believed to play a role in insulin resistance. In addition, a subset of genes showed changes in opposing directions in the two tissues, suggesting a role in the tissue-specificity of the disease. We are confirming and expanding these findings in cellular models of frataxin deficiency, as well as on fibroblasts from patients.

These studies can further our understanding of tissue-specific changes related to frataxin deficiency. In addition, using gene expression changes observed across models, we are building a panel of genes consistently changing in response to frataxin deficiency, a potentially useful tool in evaluating new therapeutic strategies, as normalization of these patterns may be a shorter and more concrete initial endpoint.

**Title: Proteomic analysis of the yeast model of Friedreich ataxia reveals manganese deficiency and oxidative damage to magnesium-binding proteins.**

**Authors:** Jordi Tamarit, Veronica Irazusta, Armando Moreno-Cermeño, Joaquim Ros.

**Institutions:** Facultat de Medicina, Universitat de Lleida, Catalonia, Spain

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**Abstract:**

We have performed a proteomic analysis of the yeast model of Friedreich ataxia. We have found that yeast cells lacking yfh1 (yeast frataxin homolog) display increased amounts of proteins involved in antioxidant defenses, including manganese-superoxide dismutase. However, this enzyme shows lower activity than that found in wild type cells. Our results indicate that this paradox is due to decreased manganese acquisition by yfh1 null mutants. This cellular manganese deficiency could be a side-effect of iron accumulation. Interestingly, the activities of three iron/sulfur-containing enzymes were recovered by manganese treatment. These data indicate that generalized deficiency of iron-sulfur protein activity (one of the hallmarks of Friedreich ataxia) could be a consequence of manganese superoxide dismutase deficiency. We have also identified several oxidatively modified proteins in the proteome of null yfh1 yeast cells. Oxidative damage to proteins was evaluated in 2D-gels by the titration of carbonyl groups. These groups are generated in amino acid side chains during stress conditions. Our results indicate that iron-induced oxidative stress in this model of Friedreich ataxia targets magnesium-binding proteins and results in loss of function of several ATP-binding enzymes.

**Title: Up-regulation of glucose metabolism after chronic inhibition of GSK-3 protects neurons against death triggered by a mitochondrial complex I inhibitor.**

**Authors:** Alfredo Gimenez-Cassina, Filip Lim and Javier Diaz-Nido

**Institutions:** Centro de Biología Molecular, Universidad Autónoma de Madrid

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**Abstract:**

Alterations in mitochondria are present in most neurodegenerative disorders and have been suspected to be major contributors to neuronal cell dysfunction and demise. Rotenone inhibits mitochondrial complex I and triggers apoptosis in cultured mammalian neurons, which constitutes an interesting experimental model system to monitor the influence of mitochondrial dysfunction on neurodegeneration.

As Glycogen synthase kinase-3 (GSK-3) is a multifunctional kinase thought to promote neurodegeneration, we have studied the effects of modulating GSK-3 activity on rotenone neurotoxicity. Using this system, we have demonstrated that overexpression of a dominant-negative mutant (K85R) form of GSK-3 or treatment with chemical inhibitors of this kinase protect both mouse brainstem primary neurons and human neuron-like cells against rotenone-induced apoptosis.

Neuroprotection induced by GSK-3 inhibition is abolished by blockade of glycolysis. Interestingly, chronic inhibition of GSK-3 elicited changes in subcellular localisation of some glycolytic-related proteins. Whereas Hexokinase II (HKII) localizes to mitochondria, Glucose Transporter-3 (GLUT-3) translocates to the plasma membrane. An enzymatic assay based on lactate production confirmed that inhibition of GSK-3 results in a rise in glycolytic rate.

Thus chronic inhibition of GSK-3 appears to protect neurons against mitochondrial dysfunction by eliciting changes in cell metabolism which include an increase in glycolytic rate to overcome the energetic depletion. Moreover, the translocation of HKII to mitochondria might additionally have an anti-apoptotic role by modulating the permeability of the mitochondrial membrane. These results emphasize the potential of enhancing glycolysis to compensate for mitochondrial dysfunction in neurodegenerative diseases.

**Title:** Identification of a mitochondrial proteolytic activity that degrades human frataxin

**Authors:** Ngolela Esther Babady\*<sup>†</sup> , Yuan-Ping Pang<sup>‡</sup>, Orly Elpeleg<sup>§</sup> and Grazia Isaya\*<sup>†¶</sup>

**Institutions:** \*Departments of Pediatric & Adolescent Medicine and <sup>†</sup>Biochemistry & Molecular Biology and <sup>‡</sup>Computer-Aided Molecular Design Laboratory, Mayo Clinic College of Medicine, Rochester, Minnesota 55905, USA; <sup>§</sup>Shaare Zedek Medical Center, Metabolic Disease Unit, Hebrew University School of Medicine, Jerusalem 91031, Israel.

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**Abstract:**

Frataxin is a conserved mitochondrial protein whose deficiency causes mitochondrial iron imbalance, energy deficit, and oxidative stress, leading to the progressive neurological and cardiac disease Friedreich ataxia (FRDA). We reported previously that the mature form of human frataxin is degraded to shorter products by an unknown protease, designated frataxin degrading peptidase (FDP). We hypothesized that FDP might represent a new mechanism to modulate mitochondrial iron balance via regulation of frataxin turnover. We have now purified FDP from different sources. Interestingly, the enzyme corresponds to a homodimeric mitochondrial oxidoreductase central to energy metabolism. The presence of proteolytic activity against human frataxin has been confirmed with a recombinant form of the human oxidoreductase produced in *E. coli*. The 3D structure of the enzyme suggests the presence of a Ser-His-Glu catalytic triad in the homodimer interface. Indeed, the proteolytic activity is stimulated by conditions that destabilize the homodimer, while serine protease inhibitors or point mutations in the catalytic triad abolish the activity. Proteolytically active oxidoreductase removes a functionally critical domain from the N-terminus of frataxin, rendering it unable to assemble and therefore unable to store iron. Combining oxidoreductase and proteolytic functions in the same protein may be a mechanism to integrate changes in energy metabolism with mitochondrial iron balance. Further studies will determine if the moonlighting proteolytic activity of this enzyme contributes to the phenotypic manifestations of FRDA and whether its inhibition could represent a target for therapies of the disease.

[Supported by grants for the Muscular Dystrophy Association and American Heart Association (to GI) and a pre-doctoral fellowship from the American Heart Association (to NEB)].

**Title: Reduction in human mitochondrial frataxin levels increases ROS associated stress: implications for Friedreich's ataxia and “normal” cells**

**Authors:** Halweg, C., Menendez, D., Karthikeyan G., Geiger K., and Resnick, M. A.

**Institutions:** Chromosome Stability Section, Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, NIH, DHHS, Research Triangle Park, NC 27709

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**Abstract:**

Friedreich's ataxia (FRDA) is an autosomal recessive neurodegenerative disease resulting from a deficiency of frataxin, a highly conserved nuclear-encoded protein localized in mitochondria. The defect in most FRDA individuals is due to a triplet repeat expansion in intron I of the FXN gene resulting in reduced levels of transcription. FRDA patients exhibit multiple symptoms including gait and limb ataxia, dysarthria, cardiomyopathy, high incidence of diabetes, and premature death. Interestingly, FRDA is a quantitative disease in that the level of reduction of frataxin appears to correlate with severity. While the function of frataxin remains largely unknown, it appears to be involved in mitochondrial iron homeostasis. A pathogenic consequence of reduced levels of frataxin could be the accumulation of iron leading to the appearance of reactive oxygen species (ROS) including highly reactive hydroxyl radicals.

We have employed siRNAs targeted to frataxin RNA to reduce frataxin levels by 80% in order i) to address the role of this protein in iron dependent response to environmental agents and ii) to possibly understand why a 70-90% reduction has a dramatic impact on human health, unlike the situation in heterozygotes (i.e., ~50%). We observed that an 80% reduction in frataxin levels resulted in a synergistic increase in the sensitivity of U2OS cells to ROS stress resulting from a combination of excess iron and hydrogen peroxide. Since iron accumulation has been observed in cardiac tissue from FRDA patients, our results support the view that FRDA patients may be at risk to oxidative stress. Indeed using a yeast model we previously showed that reduced expression of the yeast homologue coded by *YFH1* caused an increase in oxidative stress in addition to nuclear and mitochondrial DNA damage. Using lymphoblastoid cell lines derived from FRDA patients as well as our siRNA model system, we are currently extending our findings to determine if reduced levels of frataxin in human cells lead to an increase in susceptibility to DNA damage. The role of the tumor suppressor protein p53 in the pathogenesis of Friedreich's ataxia is also being examined since it is a crucial mediator of cellular responses to stresses such as DNA damage.

**Title:** Selective expression of catalase in the peripheral nervous system ameliorates the symptoms of frataxin deficiency in a *Drosophila* model of FRDA

**Authors:** Peter R. Anderson<sup>1</sup>, Kim Kirby<sup>1</sup>, Arthur J. Hilliker<sup>2</sup>, and John P. Phillips<sup>1</sup>

**Institutions:** <sup>1</sup>University of Guelph, Guelph, ON, Canada; <sup>2</sup>York University, Toronto, ON, Canada

**Corresponding author email address:** John P. Phillips (jphillip@uoguelph.ca)

**Abstract:**

We have utilized RNAi-mediated suppression of the *Drosophila frataxin homolog (Dfh)* to develop a *Drosophila* model of FRDA (Anderson et. al. 2005 HMG 14:1497). *Dfh* suppression recapitulates the principal hallmarks of FRDA, including diminished activities of heme- and iron-sulfur-containing enzymes, loss of intracellular iron homeostasis and increased susceptibility to iron toxicity. Ubiquitous suppression of *dfh* produces striking phenotypes: giant, extremely long-lived larvae and conditional short-lived adults exhibiting impaired mobility. Selective reduction of DFH in the peripheral nervous system (PNS), a neuronal focus of FRDA pathology, permits normal pre-adult development but imposes a marked reduction in adult lifespan. To determine if oxidative stress predicted to arise from altered iron homeostasis contributes to deleterious phenotypes in tissues susceptible to DFH depletion, we analyzed the effects of overexpression of the primary antioxidant enzymes SOD1, SOD2, or Catalase, in DFH-depleted *Drosophila* PNS. We show that overexpression of SOD1 or SOD2 in the PNS exacerbates DFH depletion phenotypes, while overexpression of Catalase ameliorates the symptoms arising from Frataxin deficiency. These data highlight the potential importance of reactive oxygen species-specific therapy in the treatment of FRDA and other related disorders. We hypothesize that pathological outcomes of Frataxin deficiency in target tissues like the PNS are based on the metabolic/biochemical profile of those tissues and that therapeutic intervention may therefore need to be tailored to each target tissue. Accordingly, we are currently targeting *dfh*-knockdown and ROS intervention to other FRDA focal tissues, including the heart and skeletal musculature.

**Saturday November 11, 2006**

**10:30am – 12:45pm Session V – Friedreich's Ataxia Models**

**Session Chair: Hélène Puccio, PhD**

**10:30 – 10:35** - Introduction

**10:35 – 10:50 Title: A yeast model of Friedreich's Ataxia: genotoxicity of mitochondrial iron accumulation**

**Authors:** Astrid C. Haugen<sup>1</sup>, Jennifer B. Collins<sup>2</sup>, Joel Parker<sup>3</sup>, Charles J. Tucker<sup>2</sup>, G. Karthikeyan<sup>1</sup>, Michael A. Resnick<sup>1</sup>, and Bennett Van Houten<sup>1</sup>.

**10:50 – 11:05 Title: Caenorhabditis elegans models of Friedreich Ataxia**

**Authors:** Kim Zarse, Tim J. Schulz, Marc Birringer, Michael Ristow

**11:05 – 11:20 Title: Drosophila frataxin prevents oxidative-stress inactivation of mitochondrial aconitase.**

**Authors:** José V. Llorens<sup>1</sup>, Juan A. Navarro<sup>1,2</sup>, José A. Botella<sup>2</sup>, M. José Martínez-Sebastián, Stephan Schneuwly<sup>2</sup>, Maria D. Moltó<sup>1</sup>.

**11:20 – 11:35 Title: Down-regulation of frataxin gene expression by lentivector-mediated RNA interference causes death of cultured human neuron-like cells.**

**Authors:** Gloria Palomo, Alfredo Gimenez-Cassina, Filip Lim, Christina Mauritz and Javier Diaz-Nido

**11:35 – 11:50 Title: GAA repeat expansion mouse models of Friedreich ataxia**

**Authors:** Sahar Al-Mahdawi, Ricardo Mouro Pinto, Dhaval Varshney, Lorraine Lawrence<sup>1</sup>, Margaret B. Lowrie<sup>1</sup>, Rosalind King<sup>2</sup>, J. Mark Cooper<sup>2</sup>, Julian Blake<sup>3</sup>, Sian Hughes<sup>4</sup>, Zoe Webster<sup>5</sup> and Mark A. Pook

**11:50 – 12:05 Title: Frataxin deficiency in skeletal muscle leads to a mitochondrial myopathy in the mouse.**

**Authors:** Marie WATTENHOFER-DONZE, Nadège CARELLE, Laurence REUTENAUER, Anne GANSMULLER, Pierre RUSTIN\*, Michel KOENIG and Hélène PUCCIO.

**12:05 – 1:00 Additional Presentations and Roundtable Discussion**

**Poster Presentations**

**Title: The E. coli frataxin homologue, CyaY, maintains iron balance in yeast**

**Authors:** Tibor Bedekovics and Grazia Isaya

**Title: Iron-independent oxidative stress in the yeast model of Friedreich's ataxia**

**Authors:** Anne-Laure Bulteau<sup>1</sup>, Renata Santos<sup>2</sup>, Andrew Dancis<sup>3</sup>, Monique Gareil<sup>1</sup>, Jean-Jacques Montagne<sup>2</sup>, Jean-Michel Camadro<sup>2</sup> and Emmanuel Lesuisse<sup>2</sup>

**Title:** *Drosophila* frataxin overexpression impairs the correct development and function of muscular and nervous systems in the fruit fly

**Authors:** Juan A. Navarro<sup>1</sup>, José V. Llorens<sup>1</sup>, José A. Botella<sup>2</sup>, María J. Martínez-Sebastián<sup>1</sup> and María D. Moltó<sup>1</sup>

**Title:** *FXN-EGFP* Genomic Reporter Transgenic Mice

**Authors:** Joseph P. Sarsero,<sup>1</sup> Timothy P. Holloway,<sup>1</sup> Lingli Li,<sup>1</sup> Samuel McLenachan,<sup>2</sup> Kerry J. Fowler,<sup>3</sup> Ivan Bertoncello,<sup>4</sup> Lucille Voullaire,<sup>2</sup> Sophie Gazeas,<sup>3</sup> and Panos A. Ioannou<sup>2</sup>

## **FA models – Platform presentations abstracts**

**Title: A yeast model of Friedreich's Ataxia: genotoxicity of mitochondrial iron accumulation**

**Authors:** Astrid C. Haugen<sup>1</sup>, Jennifer B. Collins<sup>2</sup>, Joel Parker<sup>3</sup>, Charles J. Tucker<sup>2</sup>, G. Karthikeyan<sup>1</sup>, Michael A. Resnick<sup>1</sup>, and Bennett Van Houten<sup>1</sup>.

**Institutions:** <sup>1</sup>Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC 27709, U.S.A. <sup>2</sup>Laboratory of Molecular Toxicology, Microarray Center, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC 27709, U.S.A. <sup>3</sup>Constella Group, Research Triangle Park, NC, 27709, USA.

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### **Abstract:**

The *YFH1* gene is the *Saccharomyces cerevisiae* homologue of the human FRDA gene encoding the frataxin protein. Cells lacking *YFH1* exhibit 1) accumulation of iron, which cannot be exported from the mitochondria; 2) oxidation of proteins; 3) oxidative DNA damage, which leads to petite colony formation with defects or loss of mitochondrial DNA and 4) nuclear chromosomal damage (*Human Mol. Gen.* 12:3331-3342, 2003). The cellular impact of mitochondrial iron overload in yeast was determined by global gene expression profiling in a *yfh1Δ* deletion mutant with defective mitochondrial function and no mitochondrial DNA (i.e., rho<sup>0</sup>). In order to replicate human disease, we also conducted transcription profiling on a yeast strain with a rheostatable system that is capable of a progressive shutdown of the *YFH1* gene. The expression data for frataxin, which was reduced threefold to eightfold in generations 3 through 24, resulted in nearly identical events as those occurring in the knockout experiment. Expression data mapped onto the yeast regulatory network of 22,605 protein-protein/protein-DNA interactions revealed the following proteins as important centers of activity: EHD3, a member of a family of enoyl-CoA hydratase/isomerases; Rcs1/Aft1, transcriptional activator of the iron regulon; mitochondrial ribosomal proteins Mrps5 and Mrp4; Hap4, a heme activator protein involved in respiration regulation; Cox9, cytochrome c oxidase subunit VIIa; and Cad1, an AP-1-like bZIP transcriptional activator involved in multiple stress responses, iron metabolism, and pleiotropic drug resistance. We are currently systematically knocking out candidate yeast genes in the frataxin knockdown strain that apparently control the mostly downregulated cytochrome, aconitase, heme, mitochondrial protein synthesis, and iron/sulfur cluster assembly pathways. Gene expression profiling in these strains is currently underway. In conclusion, our attempt to mimic human disease, not only has shown that the loss of frataxin and subsequent iron overload in yeast leads to severe impairment of all mitochondrial function, but that it begins with the very early stages of protein reduction, before any mitochondrial DNA lesions are observed. This result implies that damage by reactive oxygen species is a secondary effect of altered mitochondrial physiology due to loss of iron-sulfur centers.

**Title: Caenorhabditis elegans models of Friedreich Ataxia**

**Authors: Kim Zarse, Tim J. Schulz, Marc Birringer, Michael Ristow**

**Institutions: University of Jena**

Corresponding author email address: fara@mristow.org

**Abstract:**

Impaired expression of mitochondrial genes causes alterations in life span of the nematode *C.elegans*. Intriguingly, while some of these genes have been shown to extend life expectancy and reduce aging processes, others are known to shorten life span in the same model organism. Reduced expression of frataxin causes decreased life span in humans. Surprisingly, reduced expression of the *C.elegans* frataxin homologue *frh-1* has been associated with both increased as well as decreased life span by different laboratories. To further elucidate these conflicting findings, we here show that different RNAi-constructs directed against *frh-1* reduce *C.elegans* life span. Moreover, we show that *frh-1*-inhibiting RNAi impairs oxygen consumption, and that respiratory rate is positively correlated with life span in this multicellular eukaryote ( $r=0.8566$ ), suggesting that more than 73 percent of life span variance in *C.elegans* is explained by changes in respiratory rate. Taken together, impaired mitochondrial metabolism due to RNAi-based inhibition of the frataxin homologue *frh-1* causes both impaired respiration as well as decreased life span in *C.elegans*.

**Title:** *Drosophila* frataxin prevents oxidative-stress inactivation of mitochondrial aconitase.

**Authors:** José V. Llorens<sup>1</sup>, Juan A. Navarro<sup>1,2</sup>, José A. Botella<sup>2</sup>, M. José Martínez-Sebastián, Stephan Schneuwly<sup>2</sup>, Maria D. Moltó<sup>1</sup>.

**Institutions:**

1 Departament de Genètica, Universitat de València, 46100-Burjassot, Valencia, Spain

2 Institute of Zoology, University of Regensburg, D-93040 Regensburg, Germany

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**Abstract:**

In order to gain insight into the function of frataxin, we have generated an RNAi model silencing the *Drosophila frataxin homolog* gene (*fh*) by means of the UAS-GAL4 system. An analysis was performed using *Drosophila* GAL4 lines expressing in a ubiquitous pattern, nervous system, muscles and heart because patients show a progressive polyneuropathy, myopathy and hypertrophic cardiomyopathy. Full lethality was achieved when *fh* was widely silenced and interfered in the mesoderm and in the embryonic heart. Interestingly, the interference of *fh* with neural GAL4 drivers was compatible with normal embryonic development but inducing climbing deficits and shortened life span when *fh* was specifically reduced in the sensory organs.

We present an experimental paradigm that resulted in a ubiquitous reduction of *fh* expression together with the presence of a 30% *fh* expression level that circumvented the preadult lethality paralleling the situation in FA patients. The interference of *fh* using this condition also induced shortened life span and reduced climbing abilities.

Since oxidative stress has been suggested to be a key factor in the pathophysiology of FA, we exposed our RNAi model to a hyperoxia atmosphere to assess the role of oxidative insult. Under hyperoxia, interfered flies showed a severely reduced life span, indicating an enhanced sensitivity to oxidative stress. As previously reported from *in vitro* experiments (4), a dramatic reduction of aconitase activity which seriously impaired the mitochondrial respiration, while the activities of succinate dehydrogenase, respiratory complex I and II (and indirectly complexes III and IV) were normal. These results clearly indicate a role for frataxin in protecting aconitase activity from oxidative stress-dependent inactivation, supporting its function as an aconitase chaperone (1).

This work shows the first evidences about essential function of frataxin in protecting aconitase from oxidative stress-dependent inactivation in a multicellular organism and supports an important role of oxidative stress in the progression of FA. We suggest that in FA the regular oxidative mediated inactivation of aconitase occurring during the normal aging process is enhanced due to the lack of frataxin.

1. Bulteau, A.L., O'Neill, H.A., Kennedy, M.C., Ikeda-Saito, M., Isaya, G. and Szweda, L.I. (2004) Frataxin acts as an iron chaperone protein to modulate mitochondrial aconitase activity. *Science* **305**, 242-245.

**Title: Down-regulation of frataxin gene expression by lentivector-mediated RNA interference causes death of cultured human neuron-like cells.**

**Authors:** Gloria Palomo, Alfredo Gimenez-Cassina, Filip Lim, Christina Mauritz and Javier Diaz-Nido

**Institutions:** Centro de Biología Molecular, Universidad Autónoma de Madrid

Corresponding author email address: [javier.diaznido@uam.es](mailto:javier.diaznido@uam.es)

**Abstract:**

We have recently described an optimized and reproducible procedure to differentiate human SH-SY5Y neuroblastoma cells into a homogeneous population of neuron-like cells (*J. Neurosci. Res.* 84 (4) 755-767; 2006). The fully differentiated cells are postmitotic and are very similar to primary cultured neurons in terms of their morphology and marker protein expression. Indeed, the cells appear to develop healthy and well-established axonal and dendritic networks.

In an attempt to generate a novel cellular model of Friedreich's ataxia, these human neuron-like cells were transduced with lentiviruses encoding for short hairpin RNAs complementary to the frataxin gene. We found that two of three lentivectors encoding for shRNAs were very efficient at down-regulating frataxin gene expression to almost negligible levels. Interestingly, most frataxin-depleted neuron-like cells underwent neurite retraction, atrophy and cell death. No deleterious effects were observed in cells transduced with lentivectors encoding for either a scrambled shRNA or green fluorescent protein.

As a control, non-differentiated proliferating SH-SY5Y neuroblastoma cells were also transduced with the lentiviruses encoding for short hairpin RNAs complementary to the frataxin gene. Curiously, frataxin gene down-regulation only caused a small, though significant, increase in death of these proliferating cells. Instead, frataxin gene down-regulation led to a decreased proliferation ability of these non-differentiated neuroblastoma cells.

The greater sensitivity of differentiated neuron-like cells to frataxin deficiency correlates well with the predominantly neurological deficits in Friedreich's ataxia. Thus, the availability of large numbers of a homogeneous population of well differentiated human neuron-like cells undergoing degeneration after frataxin gene down-regulation may be an excellent model system to study the molecular consequences of frataxin depletion within the context of mature neuronal cells.

**Title: GAA repeat expansion mouse models of Friedreich ataxia**

**Authors:** Sahar Al-Mahdawi, Ricardo Mouro Pinto, Dhaval Varshney, Lorraine Lawrence<sup>1</sup>, Margaret B. Lowrie<sup>1</sup>, Rosalind King<sup>2</sup>, J. Mark Cooper<sup>2</sup>, Julian Blake<sup>3</sup>, Sian Hughes<sup>4</sup>, Zoe Webster<sup>5</sup> and Mark A. Pook

**Institutions:** Biosciences, School of Health Sciences & Social Care, Brunel University, Uxbridge, UB8 3PH, <sup>1</sup>Imperial College London, Exhibition Road, London SW7 2AZ, <sup>2</sup>Dept. of Clinical Neurosciences, Royal Free & University College Medical School, Rowland Hill Street, London NW3 2PF, <sup>3</sup>Norfolk and Norwich University Hospital, <sup>4</sup>Rockerfeller Building, University College London, <sup>5</sup>Embryonic Stem Cell Facility, MRC Clinical Sciences Centre, Hammersmith Hospital, DuCane Road, London W12 0NN, UK

Corresponding author email address: Mark.Pook@brunel.ac.uk

**Abstract:**

Friedreich ataxia (FRDA) is a neurodegenerative disorder caused by an unstable GAA repeat expansion mutation within intron 1 of the *FXN* gene. However, the origins of the GAA repeat expansion, its unstable dynamics within different cells and tissues and its effects on frataxin expression are not yet completely understood. Therefore, we have chosen to generate an FRDA mouse model by using the human *FXN* GAA repeat expansion itself as the genetically modified mutation. We have previously reported the establishment of two lines of human *FXN* YAC transgenic mice that contain unstable GAA repeat expansions within the appropriate genomic context. We now describe the generation of FRDA mouse models by cross breeding both lines of human *FXN* YAC transgenic mice with heterozygous *Fxn* knockout mice. The resultant FRDA mice that express only human-derived frataxin show comparatively reduced levels of frataxin mRNA and protein, decreased aconitase activity and oxidative stress, leading to progressive neurodegenerative and cardiac pathological phenotypes. Co-ordination deficits are present, as measured by accelerating rotarod analysis, together with a progressive decrease in locomotor activity and increase in weight. Large vacuoles are detected within neurons of the dorsal root ganglia (DRG), predominantly within the lumbar regions in six month-old mice, but spreading to the cervical regions after one year of age. Secondary demyelination of large axons is also detected within the lumbar roots of older mice. Lipofuscin deposition is increased in both DRG neurons and cardiomyocytes, and iron deposition is detected in cardiomyocytes after one year of age. These GAA repeat expansion-based mouse models that exhibit progressive FRDA-like pathology will be of great use in testing potential therapeutic strategies, particularly GAA repeat-based strategies.

**Title: Frataxin deficiency in skeletal muscle leads to a mitochondrial myopathy in the mouse.**

**Authors:** Marie WATTENHOFER-DONZE, Nadège CARELLE, Laurence REUTENAUER, Anne GANSMULLER, Pierre RUSTIN\*, Michel KOENIG and Hélène PUCCIO.

**Institutions:** IGBMC, Molecular pathology department, Illkirch, France  
\* Hopital Robert Debré, Paris, France

Corresponding author email address: hpuccio@titus.u-strasbg.fr

**Abstract:**

In order to understand both the physiopathology of the Friedreich ataxia and the function of frataxin, and to circumvent the embryonic lethality of the ubiquitous murine frataxin knock out, we have established several tissue specific murine knock out lines via the Cre/Lox system. Although skeletal muscle is not primarily affected in FRDA, several reports demonstrate mitochondrial dysfunction in skeletal muscle of FRDA patients. Skeletal muscle involvement in FRDA as a mitochondrial disorder is not surprising since this tissue is rich in mitochondria and depends on efficient oxidative energy metabolism. We will present the initial characterization of a novel mouse model deleted for frataxin specifically in skeletal muscle (the recombinase is under the control of the human skeletal actin promoter: HSA mutant). Our results indicate that deletion of frataxin in skeletal muscle leads to a mitochondrial myopathy with a biochemical profile specific to FRDA. The HSA mutant mice present no obvious phenotype at birth. The first clinical signs are visible at around 5-8 weeks as a progressive loss of weight. Muscle weakness measured by grip test develops with variability among mice at approximately 9-11 weeks. Death occurs at  $16,6 \pm 6$  weeks. Histological analysis showed muscle fibers of variable size with central nuclei, numerous ragged-red fibers (RRF) which are indicative of mitochondrial accumulation. Consistently, EM analysis allowed to visualize an abnormal accumulation of mitochondria, central nuclei, enlarged endoplasmic reticulum and degenerating muscle fibers. No lipid accumulation was detected so far. Activities of the Fe-S enzymes of the respiratory chain are significantly reduced in the mutant mice, a biochemical hallmark of FRDA. The further characterization of this model may allow to better understand the function of frataxin in the skeletal muscle and could be useful in testing novel therapeutic strategies.

FA models – Poster presentations

**Title: The *E. coli* frataxin homologue, CyaY, maintains iron balance in yeast**

**Authors:** Tibor Bedekovics and Grazia Isaya

**Institutions:** Department of Pediatric and Adolescent Medicine, Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN, 55905

Corresponding author email address: isaya@mayo.edu

**Abstract:**

Frataxin is structurally conserved from bacteria to humans. Eukaryotic frataxins are known to be involved in the maintenance of mitochondrial iron balance via roles in iron delivery and iron detoxification. The *E. coli* frataxin homolog, CyaY, has been shown to bind and donate iron for the assembly of [2Fe-2S] clusters. However, deletion of the CyaY gene does not cause any sensible phenotypic changes, which is in contrast with the severe phenotypes associated with partial or complete loss of frataxin in humans and other eukaryotes. We have expressed a mitochondria-targeted form of CyaY in a *S. cerevisiae* strain depleted of the endogenous yeast frataxin protein (yfh1 $\Delta$ ). We have found that CyaY can complement to a large extent, although not completely, the typical phenotypic changes of yfh1 $\Delta$  yeast cells, including the loss of Fe-S enzyme activities, the inability to grow on non-fermentable carbon source, and the increased susceptibility to oxidative stress. We have also found that CyaY is able donate iron to yeast ferrochelatase *in vitro* and to partially restore heme synthesis in yeast. Our findings suggest that the function of frataxin/CyaY is conserved across species; however, CyaY may be functionally redundant in *E. coli*. The existence of proteins that can functionally replace frataxin could help to explain why only certain tissues are affected by frataxin deficiency in FRDA. Genetic screens to identify these proteins are underway. [Supported by grants from NIH/NIA (AG15709) and FARA].

**Title: Iron-independent oxidative stress in the yeast model of Friedreich's ataxia**

**Authors:** Anne-Laure Bulteau<sup>1</sup>, Renata Santos<sup>2</sup>, Andrew Dancis<sup>3</sup>, Monique Gareil<sup>1</sup>, Jean-Jacques Montagne<sup>2</sup>, Jean-Michel Camadro<sup>2</sup> and Emmanuel Lesuisse<sup>2</sup>

**Institutions:** 1. Laboratoire de Biologie et Biochimie Cellulaire du Vieillissement, Université Paris 7, Paris, France.

2. Laboratoire d'Ingénierie des Protéines et Contrôle Métabolique, Département de Biologie des Génomes, Institut Jacques Monod, Unité Mixte de Recherche 7592 CNRS-Universités Paris 6 and 7, France.

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**Abstract:** *Saccharomyces cerevisiae* cells lacking frataxin ( $\Delta yfh1$ ) showed no growth defect when cultured anaerobically. In this condition, a significant part of aconitase was functional, with intact 4 Fe/4 S cluster. When shifted to aerobic conditions, aconitase was rapidly degraded, and oxidatively modified proteins (carbonylated and HNE-modified proteins) accumulated in both the cytosol and the mitochondria. The ATP-dependent mitochondrial protease Pim1 (Lon) was strongly activated, although its expression level remained unchanged, and the cytosolic 20S proteasome activity was strongly inhibited. These features indicate that the cells were subjected to major oxidative stress conditions in aerobiosis. Accumulation of oxidatively modified proteins, Pim1 activation and proteasome inhibition did not depend on the mitochondrial iron content, since these phenotypes remained unchanged when the cells were grown in iron-limiting conditions. Moreover, these phenotypes were not observed in another mutant ( $\Delta ggc1$ ) that overaccumulates iron in the mitochondrial compartment to the same extent than  $\Delta yfh1$  cells. We conclude that oxygen is primarily involved in the deleterious phenotypes that are observed in frataxin-deficient yeast cells. We are currently studying the effect of an aerobic shift on the whole transcriptome and the mitochondrial proteome of frataxin-deficient cells compared to wild-type cells.

**Title:** *Drosophila* frataxin overexpression impairs the correct development and function of muscular and nervous systems in the fruit fly

**Authors:** Juan A. Navarro<sup>1</sup>, José V. Llorens<sup>1</sup>, José A. Botella<sup>2</sup>, María J. Martínez-Sebastián<sup>1</sup> and María D. Moltó<sup>1</sup>

**Institutions:** <sup>1</sup> Departament de Genètica, Universitat de València, 46100-Burjassot, Valencia, Spain; <sup>2</sup> Institute of Zoology, University of Regensburg, D-93040 Regensburg, Germany

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**Abstract:**

Here, we report the consequences of frataxin overexpression in *Drosophila melanogaster*, an animal easy to manipulate at different levels, from DNA to behaviour. For this purpose, we generated several lines of transgenic flies with general and tissue-specific overexpression of the *Drosophila frataxin homolog* gene (*fh*) using the UAS-GAL4 system.

Early and ubiquitous overexpression of *fh* provoked the death of all individuals during late larval and early pupae stages. Full lethality was also detected for *fh* overexpression in the early development of muscle, heart and peripheral nervous system. Immunohistochemistry revealed in embryos with systemic 9-fold increase of frataxin level, defects in specific muscles, aberrant axonal tracts, axonal pathfinding defects and increase in the number of sensory ventral neurons. Lack of some pericardial cells all along the tubular structure of the developing heart, was detected in embryos overexpressing *fh* in this tissue. On the other hand, viable offspring was always obtained when *fh* overexpresses in several neural patterns. We have detected no abnormalities if the overexpression was limited to the dopaminergic neurons, precursor cells of chordotonal organs or larval brain. However, if the overexpression was restricted to sensory and motor neurons, the life span of the flies was shortened and the climbing abilities were impaired, features often related to increase sensitivity to oxidative damage in *Drosophila*.

Strikingly, overexpression of fly frataxin also enhanced the oxidative-mediated inactivation of mitochondrial aconitase, which showed a 40% reduction in its activity under a high oxidative atmosphere (99.5% O<sub>2</sub>). An excess of frataxin function could induce defects in *Drosophila* likely in an aconitase-related manner as occurs when frataxin expression is reduced (1). All these data indicate that the key tissues on Friedreich's ataxia are also sensitive to frataxin increase in *Drosophila*, and suggest that the same process is being disrupted by either an excess or defect of *fh* expression.

(1) J.V. Llorens, J. A. Navarro, J. A. Botella, M. J. Martínez-Sebastián, S. Schneuwly, M. D. Moltó. *Drosophila* frataxin prevents oxidative-stress inactivation of mitochondrial aconitase. 3<sup>rd</sup> International FA Scientific Meeting.

**Title: *FXN-EGFP* Genomic Reporter Transgenic Mice**

**Authors:** Joseph P. Sarsero,<sup>1</sup> Timothy P. Holloway,<sup>1</sup> Lingli Li,<sup>1</sup> Samuel McLenachan,<sup>2</sup> Kerry J. Fowler,<sup>3</sup> Ivan Bertoncello,<sup>4</sup> Lucille Voullaire,<sup>2</sup> Sophie Gazeas,<sup>3</sup> and Panos A. Ioannou<sup>2</sup>

**Institutions:** <sup>1</sup>Genetic Health Research (Bruce Lefroy Centre), <sup>2</sup>Cell and Gene Therapy, <sup>3</sup>Disease Model Unit, Murdoch Childrens Research Institute, Royal Children's Hospital, Parkville, Victoria 3052, Australia. <sup>4</sup>Stem Cell Research Laboratory, Peter MacCallum Cancer Centre, East Melbourne, Victoria 3002, Australia.

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**Abstract:**

To elucidate the mechanisms regulating *FXN* expression and to develop an *in vivo* assay for agents that might upregulate *FXN* expression in a therapeutically relevant manner we have generated transgenic mice with a BAC genomic reporter construct consisting of an in-frame fusion between the normal human *FXN* gene and the *EGFP* gene. Production of full-length frataxin-EGFP fusion protein was demonstrated by immunoblotting. EGFP expression was observed as early as day E3.5 of development. Most tissues of adult transgenic mice were fluorescent. The level of *FXN-EGFP* expression in peripheral blood, bone marrow and in cells obtained from enzymatically disaggregated tissues was quantitated by flow cytometry. There was a two-fold increase in EGFP expression in mice homozygous for the transgene when compared to hemizygous mice. *FXN-EGFP* transgenic mice were mated with mice heterozygous for a knockout mutation of the murine *Fxn* gene, to generate mice homozygous for the *Fxn* knockout mutation and hemizygous or homozygous for the human transgene. The *FXN-EGFP* transgene was able to rescue the embryonic-lethality of the homozygous *Fxn* KO mice with no signs of any abnormality as determined by behavioural and histological tests. The *FXN-EGFP* transgene thus appears to produce a fully active bifunctional hybrid protein. These transgenic mice are a valuable tool for the examination of spatial and temporal aspects of *FXN* gene expression, and for the preclinical evaluation of pharmacological inducers of *FXN* expression in a whole animal model. In addition, tissues from these mice should also be valuable for stem cell transplantation studies.

**Saturday November 11, 2006**

**2:30 – 6:30pm Session VI – Therapeutic Approaches**  
**Session Chair: Robert Wilson, MD, PhD**

**2:30- 2:45 Title: Prevalence of Left Ventricular Hypertrophy and Cardiac Functional Associations in a Young Cohort with Friedreich's Ataxia**

**Authors:** Plehn J, Horton K, Ernst I, DiProspero N

**2:45- 3:00 Title: High Doses of SNT-MC17/idebenone well tolerated, a characterization of the pharmacokinetic, safety and tolerability of a new formulation**

**Authors:** Klaus Kutz, MD, Julian Gray MD, Ph. D , Thomas Meier, Ph.D, Pierre Vankan, PhD

**3:00- 3:15 Title: A Six month double-blind, placebo-controlled Phase 2 Clinical Trial to Determine the safety and efficacy of Idebenone Administered to patients with Friedreich's ataxia**

**Authors:** Di Prospero NA, Baker AE, and Fischbeck KF

**3:15 - 3:30 Title: Phase II Clinical Trial of MitoQ for the Treatment of People with Friedreich Ataxia**

**Authors:** Susan Perlman, M.D.

**3:30- 3:45 Title: Development of CoQ<sub>10</sub> Analogs for the Treatment of Respiratory Chain Diseases.**

**Authors:** Guy Miller, MD, PhD, William D. Shrader, PhD, Patrice Rioux, MD, PhD

**3:45- 4:00 Title: Trialling Pioglitazone to counteract Friedreich ataxia**

**Authors:** Vincent Paupe, Emmanuel Dassa, Sergio Goncalves, Isabelle Husson & Pierre Rustin

**4:00 – 4:15 Break**

**4:15 - 4:30 Title: Ongoing large scale molecular screening on a frataxin deficient cell line**

**Authors:** Carelle N, Seznec H, Villa P\*, Haiech J\*, Reutenauer L, Koenig M, Puccio H.

**4:30 - 4:45 Title: Development of Genomic Reporter Assays and Mouse Models for the Pharmacological Therapy of Friedreich Ataxia**

**Authors:** Joseph P. Sarsero,<sup>1</sup> Lingli Li,<sup>1</sup> Timothy P. Holloway,<sup>1</sup> Lucille Voullaire,<sup>2</sup> Sanne van den Hengel,<sup>2</sup> Marion Zanese,<sup>2</sup> and Martin B. Delatycki<sup>1,3</sup>

**4:45 - 5:00 Title: Rational selection of Friedreich's ataxia therapeutics**

**Authors:** LaKechia Grant, Jun Sun, Hongzhi Xu, S.H. Subramony, Jonathan B. Chaires and Michael D. Hebert

**5:00 – 5:15 Title: From the bench to the clinic: *Proof of concept*-study with recombinant human erythropoietin in patients with Friedreich's ataxia**

**Authors:** Barbara Scheiber-Mojdehkar<sup>1</sup>, Brigitte Sturm<sup>1</sup>, Sascha Hering<sup>2</sup>, Sylvia Boesch<sup>2</sup>

**5:15 - 5:30 Title: A Mitochondrial Permeable Iron Chelator Prevent the Cardiac Hypertrophy Observed in the Frda/MCK Knockout Mouse: A Novel Therapeutic Strategy.**

**Authors:** Whitnall, M.\* , Puccio, H.<sup>o</sup>, Koenig, M.<sup>o</sup> Ponka, P.<sup>o</sup>, and Richardson, D.R.\*

**5:30 - 5:45 Title: Deferiprone reduces ataxia and cerebellar iron accumulation in Friedreich's Ataxia (abstract not published)**

**Authors:** Arnold Munnich\* and Ioav Cabantchik\*\*

**5:45 - 6:00 Title: Functional recuperation in a Friedreich's ataxia mouse model with a HSV-1 amplicon vector**

**Authors:** Filip Lim, Gloria Palomo, Christina Mauritz, Alfredo Gimenez, Belen Illana, Francisco Wandosell and Javier Diaz-Nido

**6:00 – 6:15 Break**

**6:15-7:00pm Roundtable Discussion**

**Poster Presentations**

**Title: Follow-up study of the cardiac involvement in 55 FRDA Patients.**

**Authors:** Giuseppe De Michele, MD, Anna Giunta, MD, Alessandro Filla, MD, Francesco Manguso, MD, Valentina Scarano, MD, Francesco Cacciatore, MD, and Stefania Maione, MD.

**Title: Functional Outcomes of Six Month Treatment of Friedreich's Ataxia with Idebenone**

**Author:** Kathleen Farrell, OTR/L

**Title: Antioxidant Use in Friedreich Ataxia**

**Authors:** David R. Lynch, Jennifer M. Farmer, Robert B. Wilson, Amy Tsou, Susan Perlman, S. H. Subramony, Christopher M. Gomez, Katherine Mathews Tetsuo Ashizawa, George R. Wilmot, and Laura J. Balcer

**Title: Left ventricular structural and functional abnormalities in early Friedreich Ataxia cardiomyopathy: assessment by quantitative echocardiography.**

**Authors:** Philip M Mottram<sup>\*1</sup>, Lesley Donelan<sup>1</sup>, John S Gelman<sup>1</sup>, Martin B Delatycki<sup>2</sup>, Louise Corben<sup>2</sup>, Roger E Peverill<sup>1</sup>.

**Title:** Non-viral delivery of frataxin to mitochondria for Friedreich's Ataxia.

**Authors:** R Mark Payne, Wendy Tomamichel, Qiujuan Wang

## **Therapeutic Approaches – Platform presentations abstract**

**Title:** Prevalence of Left Ventricular Hypertrophy and Cardiac Functional Associations in a Young Cohort with Friedreich's Ataxia

**Authors:** Plehn J, Horton K, Ernst I, DiProspero N

**Institutions:** George Washington University School of Medicine, Medstar Research Institute, National Heart, Lung and Blood Institute, National Institute of Neurologic Diseases and Stroke

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### **Abstract:**

**Background:** The prevalence of cardiomyopathic features in young patients with Friedreich's Ataxia (FA) is not well-documented and the relationship of hypertrophy, the main feature of FA myopathy, to tissue Doppler measures of ventricular function has not been described. We, therefore, analyzed echocardiographic examinations of 48 subjects enrolled in NINDS 05-N-025, a study evaluating the efficacy of the antioxidant agent, idebenone, in FA. We report both M-mode and two-dimensionally (2D) derived LV mass and prevalence rates based on established normal age-based threshold values as well as associated pulsed and tissue Doppler parameters of LV function.

**Results:** Left ventricular (LV) wall thickening exceeding 1.1 cm (established upper limit of normal for adult patients) was found in only 39% of subjects while M mode-determined, cubed height-indexed, left ventricular hypertrophy (LVH) was present in 45% of cases. LV concentric remodeling, an early marker of hypertensive cardiomyopathy was found in 73% of subjects as indicated by sub-threshold relative wall thickness (RWT).

Biplane 2D LV ejection fraction was mildly reduced (<55%) in only 2 subjects with a group mean value of  $71 \pm 9\%$  (normal range 55-75%) while cardiac index was unimpaired at  $3.6 \pm 1.1$  liters/minute.

All measures of LV mass (LVM) including RWT, height indexed M-mode, 2D myocardial cross-sectional area and 2D biplane methods were significantly associated with prolonged isovolumic relaxation ( $r=0.41$ ,  $p \leq 0.005$ ,  $r=0.40$ ,  $p \leq 0.01$ ,  $r=0.33$ ,  $p \leq 0.05$ ,  $r=0.37$ ,  $p \leq 0.01$ , respectively) and decreased early mitral annular tissue velocity ( $r=0.61$ ,  $0.61$ ,  $0.59$  and  $0.49$ , respectively with  $p \leq 0.0001$  for all but the latter where  $p \leq 0.005$ ) both markers of early relaxation impairment. LVM did not correlate with the pulsed Doppler filling velocity ratio, a load-dependent relaxation indicator but was associated with early transmitral velocity/annular tissue Doppler ratio an indicator of elevated LV filling pressure ( $r=0.53$ ,  $0.57$ ,  $0.60$  and  $0.56$ , respectively,  $p < 0.0001$  for all). Though LVM was not associated with reduced midwall shortening fraction all measures negatively correlated with systolic annular velocity (range 0.29-0.45,  $p < 0.01$  for all) another sensitive marker of systolic impairment.

**Conclusions:** 1) The prevalence of detectable myopathy in young patients is methodology-based with RWT, a simple, rapidly-determined echo parameter, identifying disease in up to 73% of cases. 2) LV hypertrophy in FA does not correlate with global systolic dysfunction. 3) Subtle LV diastolic and systolic dysfunction as evidenced by tissue Doppler markers may be responsible for the suggested mild elevation in LV filling pressure.

**Title: High Doses of SNT-MC17/idebenone well tolerated, a characterization of the pharmacokinetic, safety and tolerability of a new formulation**

**Authors:** Klaus Kutz, MD, Julian Gray MD, Ph. D , Thomas Meier, Ph.D, Pierre Vankan, PhD

**Institutions:** Santhera Pharmaceuticals, Liestal, Switzerland

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**Abstracts:**

***Introduction:*** In recent years clinical research has demonstrated that idebenone has therapeutic effects in Friedreich's Ataxia (FRDA) patients. Published data suggest that higher doses of this drug candidate may be associated with increased efficacy. Here, a new formulation of idebenone, SNT-MC17, is investigated for pharmacokinetics, safety and tolerability at doses up to 2250 mg/day.

***Methods:*** Four Phase I studies in healthy adult males were performed to evaluate the pharmacokinetic and safety characteristics of the newly developed 150 mg SNT-MC17 tablet. In two single dose studies, oral doses of 150 mg and 7 x 150 mg SNT-MC17 were given after a continental breakfast. In a third study, a single dose of 150 mg and 5 x 150 mg SNT-MC17 were administered in a fasting state or following a high-fat meal. A fourth study was performed where 150 mg and 5 x 150 mg SNT-MC17 were given as single doses and thereafter tid at 8-hour intervals for 14 days. Free idebenone, (the non-metabolized parent drug), total idebenone (parent drug and conjugated metabolites), and the total amount of the three major metabolites, QS10, QS6, and QS4, were determined. Safety assessments included adverse events, routine hematology and biochemistry, vital signs, and ECGs.

***Results:*** The pharmacokinetic analysis showed that plasma concentrations of free idebenone are very low, exceeding the lower limit of quantification (LLOQ) only at a few measurement points, regardless of whether or not SNT MC17 was administered in a fasting or non-fasting state. Plasma concentrations above the LLOQ were found for up to six hours after administration of a single dose of 5 x 150 mg SNT-MC17 and up to 12 hours after administration of a single dose of 7 x 150 mg SNT-MC17. Plasma concentrations of free idebenone between 0.1% and 1% of total measurable idebenone in plasma, were detected, indicating a very high first-pass effect. Idebenone immediately conjugated or converted to its metabolites QS10, QS6, and QS4 by side chain reduction and subsequent conjugation. Under repeated dose conditions, pre-dose plasma concentrations of free idebenone could only be detected at the 5 x 150 mg dose level. Due to its very short half-life, idebenone did not accumulate in plasma. Food slightly increased the relative bioavailability of idebenone at the 5 x 150 mg dose level. In all studies and under all study conditions, the main metabolites in plasma were total idebenone and total QS4 and to a lesser extent total QS10 and total QS6. The analysis of the urinary data revealed that the most prominent drug derived material was total QS4, representing nearly 50% of the drug administered. SNT-MC17, given as single oral doses of up to 7 x 150 mg or as 750 mg tid ( 2250 mg daily dose ) for a period of two weeks was well tolerated. Most common adverse events were gastro-intestinal effects such as loose stools, nausea, and unspecified abdominal pain. No adverse effects were reported on hematology, liver or renal biochemistry parameters. ECG findings were normal.

***Conclusions:*** The four Phase I studies performed showed that SNT-MC17 given as single oral doses of up to 1050 mg and as repeated daily oral doses up to 750 mg tid

were well tolerated. The most frequently observed AEs were gastrointestinal and a dose dependent discoloration of the urine. No clinically relevant effects on vital signs, physical findings, or laboratory parameters were observed. These findings confirm the tolerability of SNT-MC17/idebenone at high doses and suggest that daily doses up to 2250 mg, may be appropriate for further study in clinical efficacy trials among Friedreich's Ataxia patients.

**Title:** A Six month double-blind, placebo-controlled Phase 2 Clinical Trial to Determine the safety and efficacy of Idebenone Administered to patients with Friedreich's ataxia

**Authors:** Di Prospero NA, Baker AE, and Fischbeck KF

**Institutions:** National Institute of Neurological Disorders and Stroke

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**Abstract:**

*Background:* Friedreich's ataxia (FA) is a progressive, autosomal recessive, multisystem degenerative disease for which there is currently no effective treatment. Recent studies demonstrated that idebenone treatment at 5 mg/kg/day leads to a modest reversal of cardiomyopathy in patients with FA, but its effects on neurological function are unclear. It is possible that higher doses of idebenone may prevent the progression of neurodegeneration.

*Design:* This is a 6 month phase 2 double-blind, placebo-controlled trial to assess the safety and efficacy of idebenone administered to adolescents and children with FA.

*Study Population:* 48 subjects composed of children (ages 9-11) and adolescents (ages 12-17) with FA divided evenly among 4 treatment arms (placebo, ~5 mg/kg/day, ~15 mg/kg/day, or ~45 mg/kg/day of idebenone).

*Outcome Parameters:* Our primary objective is to examine the change in the level of oxidative stress by measuring the oxidative marker 8-hydroxy-2-deoxyguanosine in plasma and urine from baseline and after 6 months of treatment with placebo or varying doses of idebenone. Secondary endpoints include types and frequency of adverse events, if any, compliance with the dosing regimen, and measurements of the following: International Cooperative Ataxia Rating Scale (ICARS), Friedreich's ataxia Rating Scale (FARS), functional measures, health related quality of life score (SF-10), and activities of daily living.

**Title: Phase II Clinical Trial of MitoQ for the Treatment of People with Friedreich Ataxia**

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**Abstract:** Antipodean Pharmaceuticals are commencing a one year multicenter, multinational, randomized, placebo controlled phase II clinical trial of MitoQ in patients with Friedreich Ataxia.

MitoQ (mitoquinone mesylate) is a mitochondria-targeted antioxidant, that has been developed to protect the mitochondria from lipid peroxidation by reactive oxygen and nitrogen species and so prevent apoptosis.

This project aims to demonstrate whether MitoQ can slow the progression of FRDA.

The trial will recruit up to 130 FRDA patients at centers in Australia, New Zealand and USA.

The primary objective of the trial is to compare the effects of MitoQ with placebo on the change in disease progression in one year, measured by the Friedreich Ataxia Rating Scale (FARS).

The secondary objectives of the trial are to compare the effects of MitoQ with placebo on components of the FARS, other indicators of the severity of FRDA, quality of life, cardiac measures and to assess the pharmacokinetics and safety of MitoQ in this population.

If this study of MitoQ is positive then Antipodean intend to file MitoQ as a treatment for FRDA.

**Title: Development of CoQ<sub>10</sub> Analogs for the Treatment of Respiratory Chain Diseases.**

**Authors:** Guy Miller, MD, PhD, William D. Shrader, PhD, Patrice Rioux, MD, PhD

**Institutions:** Edison Pharmaceuticals

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**Abstract:**

The discovery and development of new classes of drugs for any disease possess significant challenges, especially in heterogenous patient populations such as inherited mitochondrial diseases. Despite significant advances in disease diagnosis, individuals with inherited respiratory chain diseases often elude a definitive molecular diagnosis. This is further compounded by the *syndrome-like* nature of these conditions, owing to heteroplasmy, differential organ system involvement, and epigenetic events.

Recent data suggests that compounds of the CoQ<sub>10</sub> *para*-benzoquinone class may yield benefit in FRDA, however, significant developmental challenges have been witnessed with the CoQ<sub>10</sub> family of compounds including: i) optimization of the pharmacokinetics and bioavailability, and ii) selection of biomarker endpoints enabling mechanistic verification of compound action and concomitant indicators of clinical benefit.

Herein we will present: i) a rationale strategy for the design and synthesis of analogs of the *para*-benzoquinone class through systematic alteration of their redox encryption (molecular warhead) head and lipophilic tail; and ii) a translational strategy about *in vitro* and *in vivo* profiling of serum-derived metabolic markers enabling compound selection and clinical translational initiatives.

**Title: Trialling Pioglitazone to counteract Friedreich ataxia**

**Authors:** Vincent Paupe, Emmanuel Dassa, Sergio Goncalves, Isabelle Husson & Pierre Rustin

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**Abstract:**

So far the only drug which has shown some efficacy to counteract at least one aspect of Friedreich ataxia, *i.e.* the cardiac hypertrophy, is idebenone, identified nearly 10 years ago [1]. Its limited impact (if any) on the neurological condition [2] makes it urgent to identify and to test other molecules. Several promising molecules are now developed targeting the impaired transcription of the Frataxin gene [3, 4] , but it might take some times before we will be in position to test these in patients. In addition, a successful fight against the disease might finally result from the concurrent action of different molecules targeting different molecular events associated with Friedreich ataxia.

According to our present knowledge, the molecular mechanism of the disease can be resumed as an impairment of the iron-sulfur cluster assembly which leads to reduced energy production in the cells and a hyper-sensitivity to oxidative stress linked to improper handling of mitochondrial iron [5]. Based on this assumption, any molecule that could improve the energy production by raising the level of some mitochondrial energy producing enzymes or could improve anti-oxidant defences of the cells appears a good candidate to be tested [6, 7]. If in addition such molecule would already been known to cross the blood-brain barrier and to possibly act on some patho-physiological processes in the brain of mice and humans, it would become an even better candidate [8, 9]. Finally, if this molecule would already be on the market without showing strong side effects, we might wonder why it has not been already tested. Pioglitazone indeed has all these different virtues. We therefore tested if it had any particular toxicity in human cells with low frataxin *in vitro* and since we did not observed any toxicity we settled a clinical trial to be started in France this year...

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**Title: Ongoing large scale molecular screening on a frataxin deficient cell line**

**Authors: Carelle N, Seznec H, Villa P\*, Haiech J\*, Reutenauer L, Koenig M, Puccio H.**

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**Abstract:**

Although mice models have brought great contributions in understanding the physiopathology of Friedreich ataxia (FRDA) and are necessary for preclinical trials, they are unfortunately not suitable for large-scale drug screening. Therefore, we have developed a deficient murine cellular model based on an antisense strategy using a ribozyme targeted against frataxin mRNA. These frataxin deficient cells (frataxin mRNA residual rate:  $16 \pm 5$  to  $29 \pm 7\%$ ) exhibit a proliferation defect associated with a decrease in ATP production compared to the control cell line.

In collaboration with the IFR 85 screening platform, we miniaturized the output assay of the model in order to be able to work in 96-well microplates format on a robotized platform. An initial screen was performed using more than one thousand drugs derived from a chemical library delivered by Prestwick Chemical Inc. This library consists of a collection of 1120 drugs, all off patent, selected for their structural diversity and their broad therapeutical spectrum.

Results of the screen will be shown. Putative active drugs will be analyzed in more details to validate their efficiency first on cellular and in the long term on animal models in order to try to identify novel pharmacological compounds that may potentially work in combating FRDA.

**Title: Development of Genomic Reporter Assays and Mouse Models for the Pharmacological Therapy of Friedreich Ataxia**

**Authors:** Joseph P. Sarsero,<sup>1</sup> Lingli Li,<sup>1</sup> Timothy P. Holloway,<sup>1</sup> Lucille Voullaire,<sup>2</sup> Sanne van den Hengel,<sup>2</sup> Marion Zanese,<sup>2</sup> and Martin B. Delatycki<sup>1,3</sup>

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**Abstract:**

Our efforts to develop new therapeutic strategies for Friedreich ataxia (FA) rely on using information and resources from the Human Genome Project to establish novel assays of *FXN* gene expression and to generate accurate 'humanized' mouse models of the disorder.

As the coding sequence is usually unaltered in individuals with FA and as there is a correlation between GAA expansion length, the amount of residual frataxin produced and the severity of disease, pharmacological upregulation of *FXN* gene expression may restore frataxin to therapeutic levels. To facilitate screening of compounds that modulate *FXN* expression in a physiologically relevant manner, we have established a Genomic Reporter Assay (GRA) consisting of stable human cell lines containing an *FXN-EGFP* fusion construct (in-frame fusion of the EGFP gene with the entire normal human genomic *FXN* locus on a BAC clone). *FXN* expression is analyzed by flow cytometry. We have identified a number of compounds able to modulate *FXN* gene expression using this assay. We have also optimized the assay for use in High Throughput Screening procedures by measuring EGFP levels and cell viability fluorometrically. A chemical library of FDA-approved drugs is currently being screened and analyzed.

Knockout mouse models of FA display important phenotypes of the disorder but do not accurately recapitulate the molecular basis of the disease and thus cannot be used to evaluate strategies for overcoming the molecular effects of the GAA expansion. Our strategy is to generate accurate humanized mouse models of FA, which contain the entire human *FXN* genomic locus and surrounding regulatory elements and harbor a long GAA expansion. Such mice should not only manifest the main phenotypic symptoms of the disorder, but also provide the same underlying molecular cause of the disease as found in individuals with FA. Using homologous recombination, a long GAA expansion was introduced into the appropriate location in the first intron of the human *FXN* gene present on a BAC clone. The modified BAC was used to generate transgenic mice. Real-Time RT-PCR and Western blot analyses confirmed that the presence of the introduced GAA expansion results in markedly decreased *FXN* gene expression and in lower levels of frataxin. The modified human transgene is able to rescue the embryonic lethality of homozygous *Fxn* knockout mice. Rescue mice are being assessed by a series of behavioral, neurological and histological tests for phenotypic symptoms of FA. These transgenic mice should not only facilitate analysis of the progressive pathophysiology of FA but also permit the evaluation of potential new therapies specifically targeted to overcoming the molecular effects of the GAA expansion.

**Title: Rational selection of Friedreich's ataxia therapeutics**

**Authors:** LaKechia Grant, Jun Sun, Hongzhi Xu, S.H. Subramony, Jonathan B. Chaires and Michael D. Hebert

**Institutions:** The University of Mississippi Medical Center, University of Louisville

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**Abstract:**

We have adapted the competition dialysis technique to identify small molecules that promote the duplex form of the GAA repeat found in Friedreich's ataxia. We have also developed a cell system whereby the effects of these molecules on transcription through GAA repeats can be monitored and find that several compounds increase reporter expression. We reason that such small molecules may have potential therapeutic value. Efficacy of our approach is validated by preliminary experiments that show the compound pentamidine increases endogenous frataxin levels in cultured patient tissue. We are currently screening additional compounds using our reporter system and cultured patient tissue and will present these findings.

**Title: From the bench to the clinic: *Proof of concept*-study with recombinant human erythropoietin in patients with Friedreich`s ataxia**

**Authors:** Barbara Scheiber-Mojdehkar<sup>1</sup>, Brigitte Sturm<sup>1</sup>, Sascha Hering<sup>2</sup>, Sylvia Boesch<sup>2</sup>

**Institutions:** 1Department of Medical Chemistry, Medical University of Vienna, Austria, 2Department of Neurology, Innsbruck Medical University, Austria

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**Abstract:**

Friedreich`s ataxia (FRDA) is caused by a GAA-trinucleotide expansion in the frataxin gene, resulting in a reduced expression of frataxin, which may be involved in mitochondrial iron homeostasis and/or assembly of iron-sulfur (FeS) proteins and heme synthesis. Clinically there is an intramitochondrial iron accumulation in heart, liver, nervous system and spleen of FRDA-patients, as well as a reduction of mitochondrial DNA, the FeS cluster-containing subunits of the mitochondrial electron transport chain (complex I-III) and of the enzyme aconitase and increased generation of free radicals. Frataxin is implicated to be necessary for normal heme biosynthesis, but there are no reports that FRDA is commonly associated with anemia.

We found that additionally to its reported neuro- and cardioprotective properties recombinant human erythropoietin significantly increases frataxin expression in isolated primary lymphocytes from FRDA-patients, and many other cell types such as neurons and primary cardiac cells.

To confirm the *in vitro* effects of rhuEPO on frataxin-expression, we performed an open-label pilot-study in FRDA-patients. Here we present the first clinical trial with rhuEPO: Our results reveal a stable up-regulation of frataxin in 70% of the patients during 8 weeks with rhuEPO. Additionally we found a significant reduction in DNA-damage and oxidative stress.

The results of this *proof of concept*-study justify continuation of our study and further clinical trials with recombinant human erythropoietin in FRDA.

**Title: A Mitochondrial Permeable Iron Chelator Prevent the Cardiac Hypertrophy Observed in the Frda/MCK Knockout Mouse: A Novel Therapeutic Strategy.**

**Authors:** Whitnall, M.\*, Puccio, H.<sup>δ</sup>, Koenig, M.<sup>δ</sup> Ponka, P.<sup>‡</sup>, and Richardson, D.R.\*

**Institutions:**\*Department of Pathology, University of Sydney, Sydney, New South Wales, 2006 Australia; <sup>δ</sup>Institut de Genetique et de Biologie Moleculaire et Cellulaire, CNRS/INSERM/Universite Louis Pasteur, 67404 Illkirch Cedex, CU de Strasbourg, France; <sup>‡</sup>Lady Davis Institute for Medical Research 3755 Cote Ste Catherine Rd, Montreal, Quebec, H3T 1E2, Canada.

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**Abstract:**

There is no effective treatment for the severe cardiomyopathy and neurological deficits that occur in the most common autosomal recessive disease, Friedreich's ataxia (FA). The identification of potentially toxic iron (Fe) deposits in the mitochondria of patients with FA suggests that Fe may play a role in its pathogenesis due to cytotoxic radical generation. We examined if an Fe chelator that permeates the mitochondrion can prevent the pathology observed in a mouse model of FA, namely the Frda/MCK conditional frataxin knockout mouse. This model reproduces the cardiac pathology observed in FA, including myocardial hypertrophy and mitochondrial Fe-loading. In these studies, the lipophilic and mitochondrion-permeable ligand, pyridoxal isonicotinoyl hydrazone (PIH), was used in combination with the hydrophilic chelator, desferrioxamine (DFO). Iron chelation slowed total body weight loss in Frda/MCK frataxin knockout mice. Significantly, treatment with the chelators markedly inhibited the development of myocardial hypertrophy and deposition of myocardial Fe deposits. While chelation removed excess Fe from cardiac tissue, it did not negatively impact on hematological indices. Our study shows that Fe chelation therapy prevented the cardiac hypertrophy observed in Frda/MCK mice. These results indicate that mitochondrial Fe deposition in the heart plays a significant role in the pathogenesis of FA and that iron chelation may, at least in part, be a useful therapeutic strategy.

**Title: Functional recuperation in a Friedreich's ataxia mouse model with a HSV-1 amplicon vector**

**Authors:** Filip Lim, Gloria Palomo, Christina Mauritz, Alfredo Gimenez, Belen Illana, Francisco Wandosell and Javier Diaz-Nido

**Institutions:** Centro de Biología Molecular, Universidad Autónoma de Madrid

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**Abstract:**

There is currently no effective cure or treatment for Friedreich's ataxia (FA), the most common of the hereditary ataxias. The disease is caused by drastically reduced expression levels of frataxin, as a result of mutations in the FRDA gene. One of the key difficulties in mimicking the disease in animal models is to express frataxin at the precise levels and with the appropriate tissue distribution to provoke pathology but not to result in lethality. The laboratory of Helene Puccio and Michel Koenig (Strasbourg) have generated conditional (floxed) frataxin transgenic mice to aid in circumventing these problems. We have generated CRE-expressing HSV-1 amplicon vectors and present results showing loss of frataxin protein levels in neurons from these loxP [frda] mice infected by these vectors. Using stereotactic injection, we have also achieved in vivo delivery of these CRE-expressing vectors into the olivocerebellar circuit of loxP[frda] mice to generate a localised gene knockout model. These mice develop a behavioural deficit in the rotarod assay detectable after 4 weeks, and with a stable difference with respect to lacZ vector-injected control mice up to the latest time point measured (16 weeks). We have also generated viral vectors expressing human frataxin cDNA and present results showing correction of this ataxic phenotype in our FA model. When frataxin-expressing vectors were injected into mice rendered ataxic 4 weeks previously, behavioural recovery was detectable as soon as 4 weeks after the second injection and with a stable difference up to 12 weeks with respect to mice re-injected with lacZ vector. Our neurotropic vector approach will serve to rapidly and specifically address the neurological aspects of FA, both in understanding the pathogenic mechanisms as well as developing therapies.

## **Poster Presentations**

**Title:** Follow-up study of the cardiac involvement in 55 FRDA Patients.

**Authors:** Giuseppe De Michele, MD, Anna Giunta, MD, Alessandro Filla, MD, Francesco Manguso, MD, Valentina Scarano, MD, Francesco Cacciatore, MD, and Stefania Maione, MD.

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**Abstract:** Cardiac involvement in FRDA has been noted since Friedreich's original description and is the most frequent cause of death. Left ventricular hypertrophy, usually concentric and non-obstructive, with preserved systolic function, is found in about 40% of cases.

We conducted a longitudinal study on a personal series of FRDA patients since January 1981 to December 2002, in order to better describe the natural history of FRDA cardiomyopathy and to define the correlation between GAA expansion and cardiac phenotype.

Fifty-five patients were included in the study. Their mean age at first evaluation was  $37.0 \pm 8.1$  years, with a mean disease duration of  $25.1 \pm 9.0$  years and a mean disease onset of  $11.9 \pm 4.7$  years. The mean of follow-up duration was  $10.2 \pm 5.5$  yrs (range 3-23 years).

All patients underwent a clinical examination, a 12 leads electrocardiography and a complete echoDoppler study. The presence of the cardiac involvement (CI) was defined by the occurrence of interventricular septum (IVS) or posterior wall (PW) thickening, or decreased ejection fraction (EF).

Diabetes mellitus was present in two patients at the entry. Electrocardiographic abnormalities were detected in 50 patients (91%), the most common findings being a wide range of repolarization abnormalities (85%).

Resting abnormal echocardiograms were detected in 24 patients (44%). *Concentric LV thickening* was observed in 18/24 patients (75%), all with normal left ventricle (LV) cavity dimension and preserved systolic function. *Asymmetric septal thickening* was observed in 5/24 patients (21%), in one of them associated with dilated LV and reduced EF (45%). In the last patient a *dilated LV* with mild systolic dysfunction was detected, in absence of appreciable thinning of the walls. Concerning the diastolic function, as expressed by the Doppler transmitral filling parameters, only the isovolumic relaxation period (IRP) was found significantly prolonged in the patients with CI.

At the end of the follow-up, 19 (61%) of the 31 patients without CI at baseline developed echocardiographic abnormalities. Eleven (35%) showed an increase in wall thickening, in four of them (13%) accompanied by EF decrease, and 8 (26%) a progressive EF decrease without LV anatomical remodelling.

The 19 patients who developed CI had a more severe neurological impairment at baseline and larger expansions on the shortest allele (GAA1). Cox regression analysis confirmed that neurological impairment at baseline and larger GAA1 size increase the risk of developing CI. Diabetes mellitus and ECG abnormalities at baseline were not predictive of CI development.

The 24 patients with CI at the baseline showed a trend toward a further increase in wall thickness without LV dilation. Five of them also developed a moderate LV systolic dysfunction.

In conclusion concentric or septal hypertrophy is a frequent finding in FRDA patients. A clear transmitral filling pattern of "abnormal relaxation", typical of the reactive cardiac hypertrophy, is not identifiable. The abnormal IRP appears as the only marker of an impairment in LV active relaxation.

The follow-up study showed that patients with hypertrophic cardiomyopathy progressed to LV systolic dysfunction in absence of ventricular thinning or dilatation. Furthermore, LV functional impairment is not necessarily due to a LV anatomical remodelling since patients without hypertrophy may develop it. Finally, the GAA1 size plays a role in predicting the development of CI during the course of the disease.

**Title: Functional Outcomes of Six Month Treatment of Freidreich's Ataxia with Idebenone**

**Author:** Kathleen Farrell, OTR/L

**Institution:** National Institutes of Health, Bethesda, MD

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**Abstract:**

**Objective:** To determine the functional outcomes of six months of treatment with idebenone of Freidreich's Ataxia.

**Design:** Double-blind, placebo controlled trial.

**Setting:** Biomedical research hospital

**Participants:** 48 subjects between 9 to 18 years

**Interventions:** 6 month supply of high, medium, or low dosage of drug or placebo administered 3 times/day

**Outcome Measures:** D-KEFS Trail Making Test, Part B Condition 5 – Motor Speed and the Jebsen-Taylor Hand Function Test

**Results:** At baseline, all female subjects were 3 standard deviations (SD) or greater above the mean (M) time (in seconds) to complete the Jebsen-Taylor Hand Function Test for both the dominant and non-dominant hand. All male subjects were 5 SD or greater above M for completion with the dominant hand and 6 SD or greater above M with the non-dominant hand. At the six month follow-up, both female and male subjects were 3 SD and 5 SD above M respectively for completion times with their dominant hand but the times for completion with their non-dominant hands increased to 6 SD for and 7 SD above M for female and male subjects respectively. At baseline 31.25% of the subjects completed the Motor Speed test within 1 SD of M and at the six month follow-up that number increased to 39.6%.

**Conclusions:** As a group, the subjects declined in their abilities for completing the Jebsen-Taylor Hand Function Test although some subjects actually improved their times. There was an increase though in the number of subjects who completed the Motor Speed test within 1 standard deviation of the mean. The varying level of drug dosage or receipt of a placebo may indicate that a target range of the drug is most beneficial.

**Title: Antioxidant Use in Friedreich Ataxia**

**Authors:** David R. Lynch, Jennifer M. Farmer, Robert B. Wilson, Amy Tsou, Susan Perlman, S. H. Subramony, Christopher M. Gomez, Katherine Mathews Tetsuo Ashizawa, George R. Wilmot, and Laura J. Balcer

**Institutions:** University of Pennsylvania School of Medicine, Children's Hospital of Philadelphia, University of California Los Angeles, University of Mississippi, University of Minnesota. University of Iowa, University of Texas Medical Branch, Emory University.

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**Abstract:** Friedreich ataxia (FA) is an autosomal recessive disorder characterized by progressive ataxia, dysarthria, diabetes, scoliosis and cardiomyopathy. While some antioxidants improve biomarkers of disease activity, formal clinical trials are necessary to determine the effect of antioxidants on clinical function in FA. Since many antioxidants are available without a prescription, we reviewed the data available from a large multicenter study of FA to ascertain the prevalence of antioxidant use.

**Methods:** We examined 158 patients with FA at 6 sites. Use of the antioxidants vitamin E, idebenone, coenzyme Q10, selenium and N-acetyl cysteine was recorded, as well as the dosage and duration of therapy. Logistic regression analyses were used to define contributors to the use of antioxidants, and linear regression analyses to examine the relation of FARS and performance measure scores with antioxidant use accounting simultaneously for age, GAA repeat length of the smaller *FRDA* allele, and gender.

**Results:** Antioxidant use was prevalent among this cohort. Only 36% of patients reported no antioxidant use, and 1 % took all five antioxidants queried. Vitamin E was the most commonly taken antioxidant, followed by coenzyme Q10. We then examined whether antioxidant use was associated with specific demographic features of FA patients. Idebenone use was significantly associated with younger age, longer GAA repeat size, and presence of cardiomyopathy. Younger age tended to be associated with use of vitamin E and coenzyme Q10, but these were not fully significant using a  $p < 0.01$  as a criterion. No other features were significantly associated with an increased use of any other specific antioxidant.

**Discussion:** A majority of FA patients in a large cohort use readily available antioxidants. Except in the use of idebenone, there were no predictors for use of specific antioxidants in terms of genetic severity, gender, or age. The present data illustrate the need for systematic clinical trials, but also demonstrate the degree to which present levels of antioxidant use may hinder attempts at clinical trials. Most proposed trials in FA will use a placebo control in order to obtain maximal opportunity to detect a response to therapeutic agents. However, the widespread use of antioxidants suggest that patients and caregivers already believe that such agents are useful, and thus it may be difficult to find patients who are antioxidant naive or willing to discontinue their use for clinical trials.

**Title: Left ventricular structural and functional abnormalities in early Friedreich Ataxia cardiomyopathy: assessment by quantitative echocardiography.**

**Authors:** Philip M Mottram<sup>1</sup>, Lesley Donelan<sup>1</sup>, John S Gelman<sup>1</sup>, Martin B Delatycki<sup>2</sup>, Louise Corben<sup>2</sup>, Roger E Peverill<sup>1</sup>.

**Institutions:** <sup>1</sup>*Centre for Heart and Chest Research, Monash University Department of Medicine, Monash Medical Centre, Melbourne;* <sup>2</sup>*Bruce Lefroy Centre for Genetic Health Research, Royal Children's Hospital, Melbourne.*

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**Abstract:**

**Background:** Friedreich Ataxia (FRDA) patients have a high incidence of hypertrophic cardiomyopathy. Using tissue Doppler imaging (TDI), we sought to determine myocardial functional abnormalities in FRDA patients, and relate these to structural changes and clinical characteristics.

**Methods:** 55 adult patients (23 female; mean age 30±9yrs) with normal blood pressure, sinus rhythm, and normal LV ejection fraction were studied, and compared with 59 age- and sex-matched controls. Detailed echocardiography was performed, included TDI measurement of longitudinal velocities at the septal and lateral mitral annulus.

**Results:** FRDA patients had reduced LV diastolic diameter compared with controls, but greater wall thickness ( $p<0.0001$  for all), increased relative wall thickness (RWT,  $0.48\pm0.10$  vs.  $0.33\pm0.05$ ,  $p<0.0001$ ) and LV mass index ( $97\pm27$  vs.  $78\pm20$  gm/m<sup>2</sup>,  $p<0.0001$ ), while left atrial size was similar to controls. TDI-derived early diastolic velocities (E') were significantly lower in FRDA (septal E'  $8.4\pm2.1$  vs.  $10.9\pm2.2$ cm/s, lateral E'  $12.0\pm3.7$  vs.  $17.6\pm3.4$ cm/s,  $p<0.0001$  for both). Lateral E' velocities in FRDA were negatively correlated with age ( $r=-0.57$ ,  $p<0.001$ ), blood pressure ( $r=-0.42$ ,  $p<0.01$ ), RWT ( $r=-0.41$ ,  $p<0.01$ ), and duration of neurological symptoms ( $r=-0.72$ ,  $p<0.001$ ). After adjusting for age and blood pressure in multivariate analysis, symptom duration and RWT explained 57% of the variability in lateral E' velocity.

**Conclusion:** FRDA patients with preserved LV ejection fraction have sub-clinical abnormalities of LV function and structure. TDI-derived mitral annular velocities are sensitive markers of early cardiomyopathic change in these patients and correlate with neurological symptom duration.

**Title: Non-viral delivery of frataxin to mitochondria for Friedreich's Ataxia.**

**Authors:** R Mark Payne, Wendy Tomamichel, Qiujuan Wang

**Institutions:** Department of Pediatrics, Section of Pediatric Cardiology, Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, 1044 W. Walnut, Indianapolis, IN 46202.

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**Abstract:**

Friedreich's Ataxia (FRDA) is the most common cause of human ataxia. It is caused by a deficiency of the protein, Frataxin, arising from a triplet GAA expansion in intron-1 of the FRDA gene. It results in iron accumulation, and possible oxidant stress in mitochondria. Long term iron exposure destroys mitochondrial and nuclear function leading to progressive cardiomyopathy and ataxia. Patients with FRDA may be treated with either antioxidant therapy, or iron chelation therapy. These therapies have not been substantially effective and patients die from hypertrophic cardiomyopathy at a young age.

Virally-mediated gene therapy for a few mitochondrial defects has been accomplished *in vitro*, but gene-based therapy *in vivo* has been hindered by multiple factors including: A) Mitochondria are in all tissues. B) Gene products must localize specifically to mitochondria. C) Viral delivery mechanisms are immunogenic.

We have used novel protein transduction domains (PTD), notably TAT, to deliver multiple proteins to mitochondria both for cells in culture, and *in vivo*. PTD's are short, positively charged peptides that are highly efficient at delivering protein cargos across cell membranes. This transduction into mitochondria does not require energy or receptors, and is dependent on the concentration gradient of the TAT-fusion protein. Using TAT, we have been able to localize multiple therapeutic and reporter proteins to the mitochondria, and show that they are appropriately processed, even across the placenta. We are developing a TAT-fusion protein to replace the deficient protein in Friedreich's Ataxia (Frataxin). To date, we have developed TAT-fusion protein expression and purification protocols that allow for soluble expression of TAT-Frataxin, and have shown it will target mitochondria of human Frataxin deficient fibroblasts in culture. The Frataxin remains in mitochondria in excess of 21 days (in culture), and does not induce an inflammatory response in mice when injected chronically *in vivo* over 2 months. Our goals in these experiments are threefold: 1) To provide proof of principle that a TAT-Frataxin fusion protein can rescue, or partially rescue, the phenotype of fibroblast cells in culture from FRDA patients. 2) Determine if we can rescue the phenotype of a mouse model with conditional loss of Frataxin in heart and brain that recapitulates Friedreich's Ataxia. 3) Show that TAT-Frataxin can be developed as a novel therapy for the treatment of Friedreich's Ataxia using protein transduction domains.

**Sunday November 12, 2006**

**9:00 – 12.00 Session VII - Phase III Efficacy Measures**

**Session Chairs: Martin Delatycki, MD and David R. Lynch, MD, PhD**

**9:00- 9:05** - Introduction

**9:05- 9:20 Title: Sensitivity of clinical outcome measures in Friedreich ataxia**

**Authors:** D. R. Lynch, J. M. Farmer, S. Perlman, S. H. Subramony, C. M. Gomez, K. Mathews, T. Ashizawa, G. R. Wilmot, R. B. Wilson, L. J. Balcer

**9:20- 9:35 Title: The Friedreich Ataxia Rating Scale is the most sensitive neurological scale to measure disease progress in Friedreich ataxia**

**Authors:** M. C. Fahey, L.A. Corben, V. Collins, A. J. Churchyard, M. B. Delatycki

**9:35- 9:50 Title: Neurological, cardiological and oculomotor progression in 104 Friedreich's ataxia patients during long-term follow-up (abstract not published)**

**Authors:** Ribai P., MD<sup>1,2</sup>; Pousset F., MD<sup>3</sup>; Tanguy ML., MD<sup>4</sup>; Rivaud-Pechoux S., PhD<sup>2</sup>; Leber I., MD<sup>2,5</sup>; Gasparini F., MD<sup>5</sup>; Charles P., MD, PhD<sup>1</sup>; Béraud A.S, MD<sup>3</sup>; Schmitt M, PhD<sup>6</sup>; Koenig M, PhD<sup>6</sup>; Mallet A, MD<sup>4</sup>; Brice A., MD<sup>1,2,5,7</sup>; Durr A., MD, PhD<sup>1,2</sup>

**9:50- 10:05 Title: Assessment of Motor Processing in Friedreich Ataxia suggests effects of the disease on the function of the dorsolateral prefrontal cortex.**

**Authors:** Corben LA<sup>1</sup>, Georgiou-Karistianis N<sup>2</sup>, Fahey MC<sup>1</sup>, Bradshaw JL<sup>2</sup>, Collins V<sup>1</sup>, Churchyard, A<sup>3</sup>, Delatycki MB<sup>1</sup>.

**10:05- 10:20 Title: Vestibular, saccadic and fixation abnormalities in Friedreich ataxia**

**Authors:** M. C. Fahey, L. Millist, S. Aw, M.J. Todd, L.A. Corben, V. Collins, A. J. Churchyard, O. White, P. Cremer, M. Halmagyi, M. B. Delatycki

**10:20- 10:30-** Brief break

**10:30- 10:45 Title: Non-invasive Evaluation of Oxidative Stress and Neuronal Viability in Cerebella of Patients with Friedreich's Ataxia by <sup>1</sup>H MRS at 4 Tesla (abstract not published)**

**Authors:** Gülin Öz<sup>1</sup>, Isabelle Iltis<sup>1</sup>, Diane Hutter<sup>1</sup>, Christopher Gomez<sup>2</sup>

**10:45- 11:00 Title: Cerebellar proton MR spectroscopy and DWI provide biomarkers of disease progression in patients with Friedreich's ataxia**

**Authors:** R Lodi<sup>1</sup>, C Tonon<sup>1</sup>, G Rizzo<sup>1</sup>, E Malucelli<sup>1</sup>, D Manners<sup>1</sup>, F Fortuna<sup>2</sup>, A Pini<sup>3</sup>, V Carelli<sup>2</sup>, B Barbiroli<sup>1</sup>

**11:00- 11:15 Title: Mitochondrial DNA damage and gene expression profiling in**

**lymphoblastoid and peripheral lymphocytes from patients with Friedreich's Ataxia: effects of idebenone.**

**Authors:** Bennett Van Houten<sup>1</sup>, Astrid C. Haugen<sup>1</sup>, Jennifer B. Collins<sup>2</sup>, Charles J. Tucker<sup>2</sup>, Joel Parker<sup>2</sup>, Nicholas DiProspero<sup>3</sup> and Kenneth Fischbeck<sup>3</sup>

**11:15- 11:30 Title:** Reduced levels of the Frataxin protein can be measured in patients and carriers with a simple, rapid dipstick test.

**Authors:** Marusich, M.F., Willis, J.A., and Capaldi, R.A.

**11.30- 12.00 Roundtable discussion**

**Poster presentations**

**Title:** Gait parameters associated with disease severity and progression in patients with Friedreich Ataxia

**Authors:** Joyce Maring PT EdD<sup>1-2</sup>, Willie Ching PT NCS<sup>1</sup>, Nicholas Diprospero MD PhD<sup>1</sup>, Earllaine Croarkin MPT NCS<sup>1</sup>

**Title:** Aerobic Capacity is Reduced in Subjects with Friedreich's Ataxia and is Correlated with Clinical Rating Scales and GAA Repeat Length

**Authors:** B. Drinkard, R. Arena, S. Paul, A. Baker, N. Di Prospero

**Title:** Quality of life in Friedreich ataxia: a study of the SF-36 in 63 individuals

**Authors:** M.C. Fahey, C.L. Wilson, L.A. Corben, V. Collins, A. J. Churchyard, M. B. Delatycki

**Title:** The 25 foot walk velocity accurately reflects real world correlations of ambulation in Friedreich ataxia

**Authors:** M. C. Fahey, L.A. Corben, V. Collins, A. J. Churchyard, M. B. Delatycki

**Title:** CONTENT AND DISCRIMINANT VALIDITY OF UPPER EXTREMITY FORCE CONTROL TESTING FOR PEOPLE WITH FRIEDREICH ATAXIA.

**Authors:** Michael Harris-Love<sup>1</sup>, Earllaine Croarkin<sup>2</sup>, Nicholas DiProspero<sup>3</sup>, Scott Paul<sup>2</sup>

**Title:** ELECTROPALATOGRAPHIC ASSESSMENT OF TONGUE-TO-PALATE CONTACTS IN DYSARTHRIA ASSOCIATED WITH FRIEDREICH'S ATAXIA

**Authors:** Joanne Mohr<sup>1</sup>, Bruce Murdoch<sup>1</sup>, Louise Cahill<sup>1</sup>, Deborah Theodoros<sup>1</sup>, Martin Delatycki<sup>2</sup>

## Platform presentation abstracts

### **Title: Sensitivity of clinical outcome measures in Friedreich ataxia**

**Authors:** D. R. Lynch, J. M. Farmer, S. Perlman, S. H. Subramony, C. M. Gomez, K. Mathews, T. Ashizawa, G. R. Wilmot, R. B. Wilson, L. J. Balcer

**Institutions:** University of Pennsylvania, Children's Hospital of Philadelphia, UCLA, University of Mississippi, University of Minnesota. University of Texas Medical Branch, University of Iowa, Emory University.

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### **Abstract:**

At present, no clinical outcome measure has been fully validated in a large, defined cohort of patients with FA. Examination based measures and composite performance measures have shown promise in cross sectional analysis as potential clinical outcome measures. In this work we examined the previously reported FA-COMS (Lynch et al, Neurology, 2006) cohort in serial analysis over 2 years.

**Methods:** We examined 155 patients with genetically confirmed FA at 6 sites: Assessments included a functional/disability rating scale, an activities of daily living (ADL) scale, a quantitative neurological exam (the FARS), and a series of performance measures: the 9HPT; the T25W; low-contrast Sloan letter chart testing (Sloan charts), a measure of low-contrast letter acuity that captures visual dysfunction in patients with FA; and the "PATA test" of speech. The ratio of the standard deviation of change (sd) to the observed change in each measure was used to provide an index on the sensitivity to change of each measure.

**Results and Discussion** At one year (124 pts), the ratio of SD of change to change for each measure (lower numbers represent greater responsiveness) was the following:  $T25W^{-1}$  (reciprocal) =5.4,  $9HPT^{-1}$  =4.2, Performance measure composite =3.3, FARS exam =2.1. All of these measures demonstrate significant change over one year in FA, but these ratios are suboptimal for use in clinical trials of a rare disease at they will necessitate sample sizes likely requiring 100 or more patients per arm. However, at 2 years (56 patients), the ratios were generally improved:  $T25W^{-1}$  =1.9,  $9HPT^{-1}$  =1.16, FARS, =1.7, Performance measure composite =1.13. Thus at 2 years, the 9HPT and performance measure composite appeared superior to the exam based FARS. Further studies will assess whether inclusion of performance measures of vision and speech in the performance measure composite improves the sensitivity to change of performance measures composites.

**Title: The Friedreich Ataxia Rating Scale is the most sensitive neurological scale to measure disease progress in Friedreich ataxia**

**Authors:** M. C. Fahey, L.A. Corben, V. Collins, A. J. Churchyard, M. B. Delatycki

**Institutions:** Bruce Lefroy Centre for Genetic Health Research, Murdoch Childrens Research Institute, Department of Paediatrics, University of Melbourne, Royal Children's Hospital, Flemington Road, Parkville, Victoria, 3052, AUSTRALIA and Monash Neurology, Monash Medical Centre, Clayton Road, Clayton, Victoria, 3168, AUSTRALIA

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**Abstract:** A number of neurological scales have been used as outcome measures in Friedreich Ataxia (FRDA). An appropriate and sensitive scale to measure disease progress is critical to detect the benefit of therapies.

**Objective:** To compare the Friedreich Ataxia Rating Scale (FARS) to other scales proposed as outcome measures for FRDA.

**Methods:** Seventy-six participants were assessed with the Friedreich Ataxia Rating Scale (FARS) and the International Cooperative Ataxia Rating Scale (ICARS) and seventy-two of these participants were also assessed with the Functional Independence Measure (FIM) and the Modified Barthel Index (MBI). Forty-three participants had repeat measures at an interval of 12 months. Sensitivity and responsiveness were assessed using the effect size for each measure and the sample size required for a placebo controlled clinical trial.

**Results:** The FARS demonstrated high correlation with the MBI and FIM (Spearman's  $\rho = 0.90$  &  $0.94$ ,  $p < 0.001$ ) and the ICARS (Spearman's  $\rho > 0.96$ ,  $p < 0.001$ ). Significant score change over 12 months was detected by the FARS, ICARS and FIM. The FARS had the greatest effect size (0.34 compared to 0.26 for the ICARS). An equivalently powered study using the ICARS would require twice the number of patients. If the FIM were used four times the patients would be required.

**Conclusions:** Of the scales assessed, the FARS is the best to use in FRDA clinical trials. This is based on effect size and power calculations that show fewer participants are required to demonstrate the same effect of an intervention. In spite of this, the effect size was moderate and further work is required to develop more sensitive and responsive instruments.

**Title: Assessment of Motor Processing in Friedreich Ataxia suggests effects of the disease on the function of the dorsolateral prefrontal cortex.**

**Authors:** Corben LA<sup>1</sup>, Georgiou-Karistianis N<sup>2</sup>, Fahey MC<sup>1</sup>, Bradshaw JL<sup>2</sup>, Collins V<sup>1</sup>, Churchyard, A<sup>3</sup>, Delatycki MB<sup>1</sup>.

**Institutions:** <sup>1</sup> Bruce Lefroy Centre for Genetic Health Research, Murdoch Childrens Research Institute, Victoria, Australia <sup>2</sup> Experimental Neuropsychology Research Unit, School of Psychology, Psychiatry and Psychological Medicine, Monash University, Victoria, Australia. <sup>3</sup> Monash Neurology, Monash Medical Centre, Clayton Road, Clayton, Victoria, 3168, Australia

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**Abstract:**

**Background:** Friedreich Ataxia (FRDA) is the most common of the genetic ataxias, affecting approximately 1 in 30,000 people. The limited literature available on the cognitive components of FRDA implicates a slowing of motor and reaction time suggestive of reduced information processing speed in the setting of intact executive function. However the exact nature of these impairments remains poorly understood.

**Method:** A tapping board was used to assess motor reprogramming in FRDA. Participants were required to perform a reciprocating movement between two designated buttons with occasional “oddball” stimuli. In these conditions the required movement varied in terms of direction, distance or both direction and distance (designated “change of direction”, “change of extent” and “change of direction and extent”). The time (ms) spent depressing the button (“down” time) and moving between the buttons (“movement” time) was compared between twelve individuals with FRDA and twelve control participants matched for age, sex, handedness and education. We examined three variables around the oddball stimulus: the movement and down time immediately preceding the change movement (“pre-change”), the movement associated with the change (“change”) and the movement immediately after the change (“post-change”). We predicted the variable associated with movement reprogramming (“change”) would reflect the greatest cognitive load. Analysis of variance was used to assess differences in mean down or movement time between patients and controls. To determine the level of association between the motor reprogramming measures and FRDA disease parameters, Pearson correlation coefficients were calculated for movement time and down time with age at disease onset, disease duration, scoring on the Friedreich Ataxia Rating Scale (FARS) and GAA repeat sizes in 16 individuals with FRDA.

**Results:** There was a statistically significant difference in the “change” movement time between individuals with FRDA and control participants in all conditions [change of direction ( $F(1,22) = 12.3, p=0.002$ ); change of extent ( $F(1,22) = 13.2, p=0.001$ ); change of direction and extent ( $F(1,22) = 18.9, p<0.001$ )]. In contrast, there was no statistically significant difference between the two groups for “change” downtime in any of the conditions.

There was a significant correlation between age of onset and “change” movement time in all three conditions examined [change of direction ( $r = -0.668, p<0.01$ ); change of extent ( $r = -0.575, p<0.05$ ); change of direction/extent ( $r = -0.678, p<0.01$ )]. There was also a significant correlation between the FARS score and the “change” movement time in the conditions of change of direction ( $r=0.521, p<0.05$ ) and change of direction and

extent ( $r=0.688$ ,  $p<0.01$ ). In contrast, the only significant correlation for down time was with age of onset in the condition of Change of Direction ( $r=-0.516$ ,  $p<0.05$ ).

**Conclusion:** Individuals with FRDA demonstrated slowed execution of movement in conditions with a greater cognitive load. This may reflect connectivity changes to the dorsolateral prefrontal cortex and associated areas. Furthermore, a correlation between age of onset and “change” movement time in all conditions, suggests a possible impact of FRDA on developing cognitive capacity and motor planning.

**Title: Vestibular, saccadic and fixation abnormalities in Friedreich ataxia**

**Authors:** M. C. Fahey, L. Millist, S. Aw, M.J. Todd, L.A. Corben, V. Collins, A. J. Churchyard, O. White, P. Cremer, M. Halmagyi, M. B. Delatycki

**Institutions:** Bruce Lefroy Centre for Genetic Health Research, Murdoch Childrens Research Institute, Department of Paediatrics, University of Melbourne, Royal Children's Hospital, Flemington Road, Parkville, Victoria, 3052, AUSTRALIA Monash Neurology, Monash Medical Centre, Clayton Road, Clayton, Victoria, 3168, AUSTRALIA, Royal Melbourne Hospital, Royal Parade, Parkville, Victoria, 3052, AUSTRALIA, Royal Prince Alfred Hospital, Missenden Road, Camperdown, NSW, 2050

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**Abstract:**

**Aim:** To comprehensively assess eye movement abnormalities in Friedreich ataxia (FRDA) and correlate these results with other clinical measures.

**Methods:** We studied 21 individuals with genetically determined FRDA. All were assessed with infrared oculography. Seventeen of these individuals were assessed with scleral coils and 15/17 underwent a full paradigm of eye movement recordings. Ten subjects were analysed using 2-dimensional equipment and five using 3-dimensional recording equipment. Additional information including visual quality of life (VF-15), Sloan low contrast letter acuity (SLCLC) and Friedreich Ataxia Rating Scale (FARS) examination was recorded.

**Results:** Fixation abnormalities consisting of square wave jerks and flutter were common and were broadly associated with disease progress. Saccadic latency was prolonged and correlated with clinical measures of disease severity, including the FARS (Pearson correlation = 0.75,  $p < 0.01$ ) and the SLCLC (Pearson correlation = 0.85,  $p < 0.01$ ). Vestibular abnormalities were evident in the group as evidenced by prolonged latency (25 ms, normal ,8ms)) and decreased gain (0.49, normal =1). The VF-15 score correlated with SLCLC (Pearson correlation = 0.73,  $p < 0.01$ )

**Conclusions:** The range of eye movement abnormalities suggest that neurological dysfunction in FRDA includes brainstem, cortical and vestibular systems. The correlation of latency with FARS score raises the possibility of its use as a biomarker for FRDA.

**Title: Cerebellar proton MR spectroscopy and DWI provide biomarkers of disease progression in patients with Friedreich's ataxia**

**Authors:** R Lodi<sup>1</sup>, C Tonon<sup>1</sup>, G Rizzo<sup>1</sup>, E Malucelli<sup>1</sup>, D Manners<sup>1</sup>, F Fortuna<sup>2</sup>, A Pini<sup>3</sup>, V Carelli<sup>2</sup>, B Barbiroli<sup>1</sup>

**Institutions:** <sup>1</sup>Dipartimento di Medicina Clinica e Biotecnologia Applicata, Policlinico S. Orsola-Malpighi, <sup>2</sup>Dipartimento di Scienze Neurologiche, University of Bologna, Bologna, Italy <sup>3</sup>U.O. Neuropediatria, Ospedale Maggiore, Bologna

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**Abstract:** Friedreich ataxia (FRDA) is the most common form of autosomal recessive spino-cerebellar ataxia caused, in the vast majority of cases, by a GAA triplet expansion in the *FRDA* gene on chromosome 9q13. The *FRDA* gene product, frataxin, is a widely expressed mitochondrial protein which is severely reduced in FRDA patients. Pathological changes are most prominent in the dorsal root ganglia and posterior columns of the spinal cord, cerebellum, cortico-spinal tract. In the cerebellum loss of dentate neurons is a prominent pathological finding. Proton MRS (<sup>1</sup>H-MRS) and diffusion weighted imaging are non invasive techniques that allow *in vivo* quantification of the neuronal marker N-acetyl-aspartate (NAA) and integrity of tissue microstructure, respectively. Aim of the study was to identify *in vivo* markers of neuro-degeneration in Friedreich's Ataxia patients.

Nine FRDA patients (7 males, 15-43 years, age range) homozygous for the GAA expansion and eight sex- and age-matched healthy volunteers were studied. Patients were assessed neurologically using the ICARS scale by the same neurologist (CT). Single voxel <sup>1</sup>H-MRS was performed using a 1.5 Tesla GE Signa Horizon LX system in the left cerebellum and including the dentate nucleus (voxel volume: 6 cm<sup>3</sup>). Absolute concentrations of NAA, creatine (Cr) and choline (Cho) were measured by acquiring spectra at 5 echo times (TE= 35, 70, 100, 144, 288ms; repetition time, TR, 4000ms) and using water as internal standard (TE from 25 to 1000ms; TR = 15000ms). Axial DW images were obtained using a single-shot echo-planar imaging sequence. Gradient strengths corresponding to b-values ranging of 0 and 900 s/mm<sup>2</sup> were applied to calculate on a pixel by pixel basis average diffusivity maps (ADC). Regions of interest (ROI) included left and right dentate nucleus.

In the patients the number of GAA triplet repeats in the smaller allele (GAA1) ranged from 270 to 768 and the total ICARS score from 13 to 65. Cerebellar [NAA] was significantly reduced in the FRDA patients compared to healthy controls (p<0.001). In the patients cerebellar [NAA] (mM) showed a negative correlation with GAA1 (r=-0.74; p<0.05) and, a more significant negative correlation, with the total ICARS score (r=-0.82; p<0.01). Similarly, dentate nucleus ADC values showed a correlation with GAA1 (r=0.73; p<0.05) and, a more significant correlation, with the total ICARS score (r=0.87; p<0.01).

In accordance with the neuropathological features, the neuronal marker N-acetyl-aspartate is reduced and ADC values increased in the cerebellum of FRDA patients. The NAA concentration was more reduced and ADC values increased in patients with the higher number of GAA repeats and, in particular, in patients with a more severe neurological impairment as assessed using the ICARS scale. These results indicate that <sup>1</sup>H-MRS and DWI can quantify *in vivo* the extent of neurodegeneration in FRDA and provide robust surrogate markers of disease progression that may be used to evaluate the effect of therapeutical interventions (Lodi et al. *Ann. Neurol.* 2001).

**Title: Mitochondrial DNA damage and gene expression profiling in lymphoblastoid and peripheral lymphocytes from patients with Friedreich's Ataxia: effects of idebenone.**

**Authors:** Bennett Van Houten<sup>1</sup>, Astrid C. Haugen<sup>1</sup>, Jennifer B. Collins<sup>2</sup>, Charles J. Tucker<sup>2</sup>, Joel Parker<sup>2</sup>, Nicholas DiProspero<sup>3</sup> and Kenneth Fischbeck<sup>3</sup>

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This NIH inter-institute collaborative project seeks to test the hypothesis that Friedreich's ataxia (FRDA) patients: 1) accumulate mitochondrial DNA (mtDNA) damage in peripheral lymphocytes; 2) share common gene expression patterns unique to the pathogenesis of the disease; and 3) show diminution of both of these effects by idebenone (IDB) treatment. Mitochondrial DNA (mtDNA) damage, an indicator of oxidative stress, was been measured using a highly sensitive quantitative PCR (QPCR) in lymphoblastoid cells from patients with FRDA. No significant level of basal DNA lesions was found, but preliminary data shows more mtDNA damage in FRDA cell lines following treatment with hydrogen peroxide than in age-matched control cell lines. We are currently testing the hypothesis that pretreatment with IDB will lead to decreased mtDNA damage in the cell lines and help prevent oxidant injury. As part of a double-blind placebo-controlled phase II study, lymphocytes from 48 FRDA patients participating in the IDB drug trial are also being assessed for mtDNA damage using the QPCR assay. Levels of damage are being measured before and after drug treatment. Preliminary data from patients lymphocyte DNA, prior to idebenone treatment, reveals a range of lesions spanning 0 to over 1 lesion per 10 kb of mtDNA. However, no positive correlation with mtDNA lesions and repeat length or severity of the disease was observed. Gene expression profiling (using a 22,000 oligonucleotide gene array Agilent chip) of lymphoblastoid cells from FRDA patients and controls as analyzed using Ingenuity Pathways analysis, revealed global transcriptional changes associated with cell death, cardiovascular disease, neurological disease, and muscular and skeletal disorders. It is hypothesized that a common set of genes will be altered in FRDA patients, and that idebenone treatment will lead to abrogation of these changes as compared to control cells. Gene expression profiles of lymphocytes from the 48 FRDA patients are currently being compared to a pool of normal individuals. Analysis of gene expression profiles before and after IDB treatment, combined with extensive clinical analysis, will help correlate the gene expression changes associated with the severity of the disease and also the effectiveness of the response. Our long term objective is to develop quantitative biomarkers for disease progression.

**Title: Reduced levels of the Frataxin protein can be measured in patients and carriers with a simple, rapid dipstick test.**

**Authors:** Marusich, M.F., Willis, J.A., and Capaldi, R.A.

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**Abstract:** Friedreich's Ataxia (FA) is a progressive neurodegenerative disorder caused by reduction in expression of the mitochondrial protein Frataxin. Reduced Frataxin levels result in dysregulated iron metabolism and increased mitochondrial free iron levels. The increased free iron, in particular  $Fe^{3+}$ , in turn are believed to cause increased levels of free radicals and reactive oxygen species. Current hypotheses regarding the molecular pathology of FA suggest that it is the accumulated damage caused by these free radicals and reactive oxygen species that accounts for the progressive degenerative course characteristic of Friedreich's Ataxia. Although the genetic basis of FA is well characterized and gene-based tests are commonly used for diagnosis of FA, simple tests to measure levels of the Frataxin protein are needed: 1) to help better characterize the genotype-phenotype relationship, and 2) to monitor the efficacy of therapies directed towards a cure, which will require up-regulating levels of the Frataxin protein. To this end, we have developed a simple immunodipstick test for Frataxin protein. The dipstick tests can be used to measure Frataxin levels accurately and rapidly in cells and tissues, and have a dynamic linear range of 10 to 1000 picograms Frataxin per test (using recombinant Frataxin as a reference standard). We show that Frataxin dipsticks can be used to measure reduced Frataxin levels in lymphoid cells taken from FA patients and FA carriers. The residual Frataxin levels measured in FA patient-derived cells correlate inversely with the number of homozygous GAA repeats in these cells. Importantly, the tests can measure Frataxin levels in samples obtained by non-invasive (cheek swabs) or minimally invasive (fingerprick whole blood samples) means. Therefore, these simple Frataxin dipsticks are suitable for use both as single diagnostic tests as well as for the repetitive "theranostic" testing that will be needed to monitor efficacy of FA therapies.

## **Poster Presentations - Abstracts**

### **Title: Aerobic Capacity is Reduced in Subjects with Friedreich's Ataxia and is Correlated with Clinical Rating Scales and GAA Repeat Length**

**Authors:** B. Drinkard, R. Arena, S. Paul, A. Baker, N. Di Prospero

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#### **Abstract:**

Friedreich's Ataxia (FA) is an autosomal recessive disorder caused by a guanine adenine adenine (GAA) repeat expansion in the gene frataxin. This mutation results in reduced expression of the frataxin protein, which is normally localized to the mitochondria. Mitochondria from FA subjects have reduced levels of electron transport chain complexes with resultant reduction in ATP formation.

**Purpose:** To examine the relationship between aerobic capacity, GAA repeat length, and indicators of disease severity as assessed by two clinical rating scales in a group of children and adolescents with FA.

**Methods:** Forty eight subjects (23 females, mean age: 13.4±2.4 years) with genetically confirmed FA participated in this study. Exercise testing was conducted with a recumbent cycle ergometer using 3 minute stepwise increases in resistance to determine peak power (W<sub>peak</sub>) with simultaneous measurements of oxygen consumption (Peak VO<sub>2</sub>), minute ventilation (VE), and carbon dioxide production (VCO<sub>2</sub>). Subjects were assessed for neurological function using the International Cooperative Ataxia Rating Scale (ICARS) and Friedreich's Ataxia Rating Scale (FARS).

**Results:** The mean number (± SD) of long and short GAA triplet repeats, Peak VO<sub>2</sub>, peak VE/VCO<sub>2</sub>, W<sub>peak</sub>, ICARS, and FARS were 733.9 ±225.3, 978.3 ±212.2, 16.1 ±6.4 mlO<sub>2</sub>•kg<sup>-1</sup>•min<sup>-1</sup> (55% of predicted), 35.5 ±6.7 (Normal: <34), 41.8 ±26.8 Watts, 39.7 ±14.3 and 57.6 ±18.4, respectively. Pearson correlation results are listed in Table 1.

	Peak VO <sub>2</sub>	Peak VE/VCO <sub>2</sub>	W <sub>peak</sub>
Short GAA triplet repeat	R=-0.31 p=0.03*	R=0.30 p=0.04*	R=-0.31 p=0.03*
Long GAA triplet repeat	R=-0.13 p=0.34	R=0.17 p=0.26	R=-0.17 p=0.25
ICARS	R=-0.44 p=0.002*	R=0.35 p=0.01*	R=-0.64 p<0.001*
FARS	R=-0.50 p<0.001*	R=0.38 p=0.008*	R=-0.69 p<0.001*

\*: Statistically significant

**Conclusions:** Aerobic exercise testing is an accepted evaluation technique in several patient populations. The results of the present study indicate that exercise capacity and ventilatory efficiency are reduced and are related to clinical rating scales and the short GAA repeat length, which may be associated with mitochondrial function, in subjects with FA. The use of aerobic exercise testing may provide clinically valuable information in patients with FA and offer a new modality to assess the efficacy of therapeutic interventions.

**Title:** Quality of life in Friedreich ataxia: a study of the SF-36 in 63 individuals

**Authors:** M.C. Fahey, C.L. Wilson, L.A. Corben, V. Collins, A. J. Churchyard, M. B. Delatycki

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**Abstract:**

**Objective:** To examine quality of life for people with Friedreich ataxia (FRDA) using the SF-36 Version 2.

**Methods:** 63 individuals with FRDA were assessed using the SF-36 V2 and clinical scales including the Friedreich Ataxia Rating Scale (FARS) and the Functional Independence Measure (FIM) and the Modified Barthel Index (MBI). Scores were generated from the SF-36 V2 using Australian population data.

**Results:** Quality of life is significantly worse in individuals with FRDA compared to Australian population norms. The Physical and Mental Component Summary Scores (PCS and MCS normed scores) are lowest in people with moderate clinical severity (PCS  $\bar{x}$  = 30.5±5.9, MCS  $\bar{x}$  = 43.8±14.8) compared to those with mild (PCS  $\bar{x}$  = 42.8±8.9, MCS  $\bar{x}$  = 45.6±9.8) and severe disease (PCS  $\bar{x}$  = 32.2±7.9, MCS  $\bar{x}$  = 53.6±12.9). Surprisingly, the domains that make up the Mental Component Summary improve with disease severity. Those with disease onset in adulthood ( $\geq 18$  years) have worse quality of life in both Physical and Mental Component scores for the equivalent disease severity compared to those with onset before 18 years ( $\geq 18$  yrs PCS  $\bar{x}$  = 33.6±9.9, MCS  $\bar{x}$  = 42.7±11.2,  $< 18$  yrs PCS  $\bar{x}$  = 35.9±9.0, MCS  $\bar{x}$  = 49.7±13.2). Multivariate analysis indicates that disease severity, age of onset and disease duration have the most influence on quality of life.

**Conclusions:** Quality of life is significantly affected in FRDA. Those with moderate disease severity perceive the lowest quality of life. Individuals with disease onset in adulthood perceive poorer quality of life than those with earlier onset disease and may therefore be at higher risk of negative psychological consequences.

**Title:** The 25 foot walk velocity accurately reflects real world correlations of ambulation in Friedreich ataxia

**Authors:** M. C. Fahey, L.A. Corben, V. Collins, A. J. Churchyard, M. B. Delatycki,

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**Abstract:**

**Background:** A variety of ambulation measures are used to assess neurological disease, including short walk and endurance tests. Recently microprocessor based accelerometers have been developed to accurately quantify physical activity in the community setting and are considered the gold standard for measuring this parameter.

**Methods:** We compared the 25 foot walk test, 50 foot walk test, timed up and go and the six minute walk test to activity in the community as measured by the Stepwatch accelerometer (Cymatech, Seattle) in 20 individuals with Friedreich ataxia (FRDA).

**Results:** The velocity over 25 feet correlated as well with daily activity as measured by maximum daily step count and average daily step count ( $r > 0.89$   $p < 0.001$ ) as the six minute walk test ( $r = >0.85$ ,  $p < 0.001$ ). Furthermore the results for 25 foot velocity follow a normal distribution and therefore are appropriate for conversion to a z-score for use in a composite scale.

**Conclusion:** The 25 foot walk velocity accurately reflects ambulation in the community setting and is an appropriate tool for assessing ambulation in FRDA intervention studies.

**Title:** CONTENT AND DISCRIMINANT VALIDITY OF UPPER EXTREMITY FORCE CONTROL TESTING FOR PEOPLE WITH FRIEDREICH ATAXIA.

**Authors:** Michael Harris-Love<sup>1</sup>, Earllaine Croarkin<sup>2</sup>, Nicholas DiProspero<sup>3</sup>, Scott Paul<sup>2</sup>

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**Abstract:**

**PURPOSE:** To determine the content and discriminant validity of an upper extremity (UE) force control (FC) task in participants with FRDA and control subjects. **SUBJECTS:** Twenty subjects obtained through a sample of convenience at a Federal hospital participated in the study (FRDA: n=10, age 14.3 ±2.3 years, 4 females; age and gender-matched controls: n=10, age 13.7 ±3.1 years, 4 females). **METHODS:** This was a prospective, observational, clinical trial with a multi-factor, within-groups and between-groups design. The isometric force control task was performed using a Biodex Dynamometer at 25% and 50% of maximal voluntary contraction (MVC) of the elbow flexors (EF) and extensors (EE) over a 30 s period. Performance was measured by calculating the mean torque accuracy ratio (target torque/mean torque) and coefficient of variation. Dominant limb UE dexterity was assessed using the Nine-Hole Peg Test (NHPT). **ANALYSIS:** Analysis of differences for FC was determined with a three-way analysis of covariance (ANCOVA with age as the covariate) and the coefficient of determination (R<sup>2</sup>) via stepwise regression was used to establish the relationship between FC and the NHPT ( $\alpha=.05$ ). **RESULTS:** Significant group interaction effects were found for FC accuracy of the EE in the 50% MVC condition (FRDA: 0.118 ±0.047 vs controls: 0.054 ±0.044, p=.003). Significant main effects were found for FC variation by group (F=11.78, p=.003). Interaction effects were found across both muscle groups and force conditions for FC variation (p=.023-.038). Using FC accuracy and variation of the EE at 50% MVC to predict the NHPT time of the FRDA group yielded a R<sup>2</sup> of .75 (p=.007). The FC accuracy of the EE at 50% MVC had a R<sup>2</sup> of .76 (p=.001) and was the only variable entered into the model to predict the NHPT time of the control group. **CONCLUSIONS:** FC testing exhibited acceptable content and discriminant validity in this pilot study. This form of assessment may provide an objective, proxy measure of ataxic movement suitable for clinical and research environments.

**Title: Gait parameters associated with disease severity and progression in patients with Friedreich Ataxia**

**Authors:** Joyce Maring PT EdD<sup>1-2</sup>, Willie Ching PT NCS<sup>1</sup>, Nicholas Diprospero MD PhD<sup>1</sup>, Earllaine Croarkin MPT NCS<sup>1</sup>

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**Abstract:**

**Purpose:** To describe gait characteristics of children diagnosed with Friedreich Ataxia (FA) and investigate relationships between gait characteristics and other measures of disease progression and severity.

**Subjects:** 39 subjects diagnosed with FA were included in this study. Ages of participants ranged from 9-17 years with a mean of age of 13.1 years. Mean number of years of disease duration of the participants was 5.2 years with a range of 1-14 years.

**Methods:** Gait parameters were measured using a Stride Analyzer, a portable gait analysis system that records foot-floor contact data from footswitches in order to calculate gait parameters. Parameters were averaged over three 10 meter trials for each participant and temporal and kinematic data summarized using the Stride Analyzer software. Scores on the International Cooperative Ataxia Rating Scale (ICARS), a composite assessment of neurological function for patients with ataxia and disease duration were used as measures of disease severity. Temporal and kinematic gait characteristics were summarized for the group using descriptive statistics. Pearson correlations were performed to determine to what extent measures of gait velocity (GV), stride length (SL) and double limb stance (DLS) time were related to the above described measures of disease severity. GV and SL measurements were adjusted for variations in the height of the participants (Hof, AL 1996).

**Results:** Compared to published values of similar aged healthy children, subjects had slower GV ( $0.873 \pm 0.30$ ), longer SL ( $1.07 \pm 0.22$ ) and a decreased gait cycle time ( $1.36 \pm 0.61$  sec.). Double limb stance (DLS) occurred during 33% of the gait cycle compared to the typical 20% in healthy children. The adjusted values for GV and SL, as well as the DLS time correlated significantly ( $p < .05$ ) with ICAR scores and disease duration. Absolute values for the correlations ranged between 0.339 and 0.685. Adjusted GV and the ICARS demonstrated the strongest association ( $r = -0.685$ ;  $p < .001$ ) in this group of participants.

**Conclusion:** Children with FA have slower GV and increased DSL compared to published norms. Gait parameters may provide valid, reliable and continuous measures of disease progression in patients with FA.

**Title: ELECTROPALATOGRAPHIC ASSESSMENT OF TONGUE-TO-PALATE CONTACTS IN DYSPHAGIA ASSOCIATED WITH FRIEDREICH'S ATAXIA**

**Authors:** Joanne Mohr<sup>1</sup>, Bruce Murdoch<sup>1</sup>, Louise Cahill<sup>1</sup>, Deborah Theodoros<sup>1</sup>, Martin Delatycki<sup>2</sup>

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**Abstract:**

**Introduction**

Friedreich's ataxia (FRDA) is one of a heterogeneous group of degenerative spinocerebellar disorders. Although not always present at the disease onset, dysarthria is a common feature of FRDA with a prevalence of >90% noted in some studies. Perceptual studies have revealed marked impairment in articulatory, respiratory and velopharyngeal function in FRDA. The aim of the present study was to use electropalatography to examine the spatial characteristics of tongue-to-palate contacts exhibited by a group of five subjects with FRDA.

**Methods and Results**

Diagnosis of FRDA was confirmed by molecular genetic assessment. Five non-neurologically impaired adults served as control subjects. Two single syllable real words consisting of CVC construction were read aloud six times by each subject while wearing an EPG palate. The word initial consonants for analysis included the alveolar stop /t/ and the velar stop /k/. Each of the target words was preceded by a neutral *schwa*. The results revealed a number of significant changes in the tongue-to-palate contacts utilised by the FRDA participants compared to controls. The FRDA group exhibited increased amount of tongue-to-palate contact for both the velar and alveolar stop. Compared to controls the FRDA participants consistently showed an increase in the centre of gravity (COG) values during the alveolar stop production, indicative of a posterior shift to the tongue to palate contact. In contrast, the FRDA participants all demonstrated a decrease in COG values for velar stop production indicative of an anterior shift in contact compared to controls.

**Discussion**

The tongue-to-palate contact patterns were deviant in several ways compared to controls that might contribute, at least in part, to their perceived articulatory deficit. In particular the major deviations included a greater amount of tongue-to-palate contact and centralisation of the contacts with respect to anterior/posterior direction during consonant production.