
# International Congress for Ataxia Research

ATAXIA

FARA Friedreich's Ataxia Research Alliance

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# Abstract Book

**November 1-4, 2022**
**Renaissance Dallas Addison Hotel**
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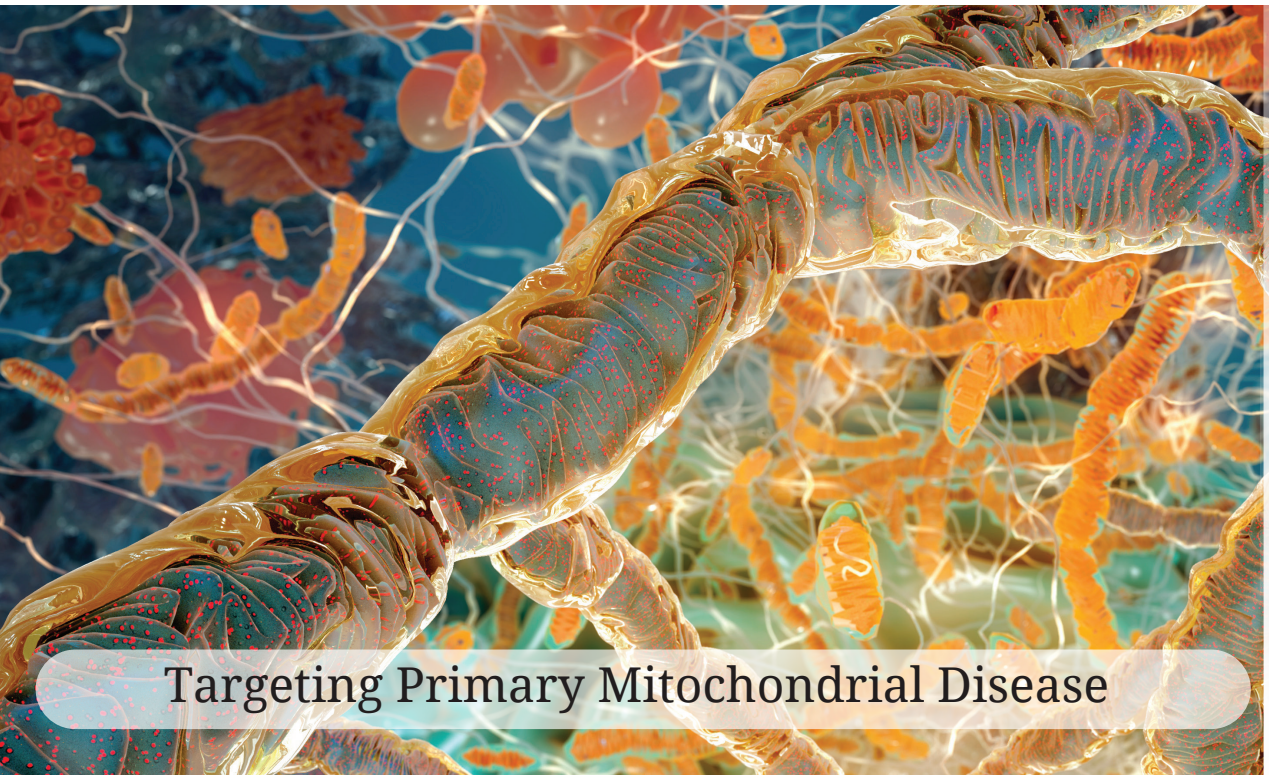


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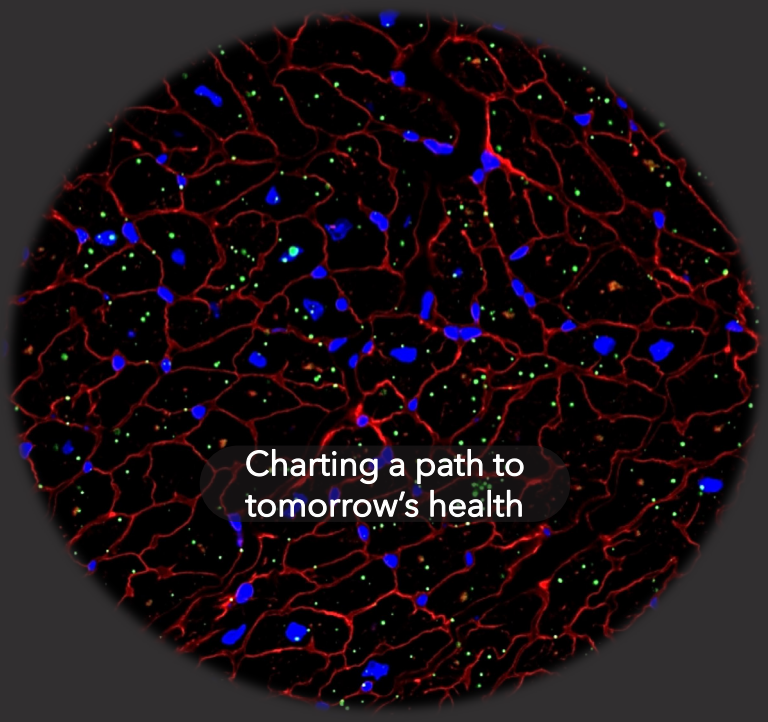


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# Keynote

# Keynote: The Future of Ataxia Clinical Trials: Strategies for Successful Academic Industry Partnerships

Tuesday, 1st November - 14:15: Keynote (Crystal Ballroom) - Invited Speaker - Abstract ID: 519

***Dr. Nina Schor*** [1]

***1.*** *National Institutes of Health*

The successful ataxia clinical trials of the future will be team partnerships likely involving academia, industry, patient and family advocates, and government. Each of these brings a different skill set and prioritization scheme to the table that makes both process and outcome success more likely. Particularly for rare diseases like the ataxias, clinical trials are best multinational and incorporate mechanisms to ensure inclusivity and accessibility to all patients. Both science and economics demand innovation in clinical trial design and rigor and transparency are critical. Relevance of any clinical trial and its findings to the lives of patients and families affected by ataxia depends critically on involvement of patients, families, and their advocates in trial design and definition of outcome success. Finally, the successful ataxia clinical trials will optimally serve as an international recruitment and training ground for the next generation of physicians and scientists dedicated to the conquest of the ataxias and the well-being of patients and families affected by them.

# Breakout: Disease Mechanisms I

# Uncovering the Regional and Cell-Type Specific Contributions Underlying Selective Cerebellar Degeneration in Spinocerebellar Ataxia Type 2

Tuesday, 1st November - 16:00: Breakout: Disease Mechanisms I (Crystal Ballroom) - Oral - Abstract ID: 323

***Ms. Ashley Robbins*** [1]***, Dr. Paul Ranum*** [2]***, Dr. Beverly Davidson*** [3]

***1.*** *1. Raymond G. Perelman Center for Cellular and Molecular Therapeutics, The Children's Hospital of Philadelphia 2. Neuroscience Graduate Program, University of Pennsylvania Perelman School of Medicine,* ***2.*** *1. Raymond G. Perelman Center for Cellular and Molecular Therapeutics, The Children's Hospital of Philadelphia,* ***3.*** *1. Raymond G. Perelman Center for Cellular and Molecular Therapeutics, The Children's Hospital of Philadelphia 3.Department of Pathology and Laboratory Medicine, University of Pennsylvania*

Spinocerebellar Ataxia Type 2 (SCA2) is a progressive, autosomal dominant neurodegenerative disorder caused by expansion of the CAG nucleotide repeat region in the *ATXN2* gene. While *ATXN2* is widely expressed in the central nervous system, the cerebellum is most vulnerable to gross atrophy and cell loss. Clinical case studies of human SCA2 cerebellar neuropathology have identified a rostral to caudal atrophy pattern. Furthermore, recent single-cell transcriptional profiling of the murine cerebellum has uncovered regional variations in neuronal and glial subtypes that may contribute to differential vulnerability to neurodegeneration. While SCA2 transgenic mouse models display early and progressive transcriptional alterations in the cerebellum, previous studies have not assessed affected subregions and cell types at fine spatiotemporal resolution. Here we identify differential expression of disease-relevant pathways that may contribute to regional and cell-type specific cerebellar vulnerability in the context of SCA2 and normal aging.

Utilizing a previously characterized the BAC-SCA2 transgenic mouse model, we profile the cerebellar transcriptome via next-generation sequencing. Tissues for whole and single nuclei transcriptomics were isolated via microdissection to retain spatial information across the rostral to caudal cerebellar vermis. Pre-symptomatic, early, and late-stage disease time points were collected to capture transcriptional alterations across the spatiotemporal landscape of SCA2 pathogenesis.

Our initial results indicate intrinsic, differential expression of gene pathways involved in ion transport, calcium signaling and neurotransmission between the anterior and posterior wild-type adult mouse cerebellum, which are further altered in SCA2. We are currently investigating contributing cell-types and the temporal progression of these transcriptional alterations via high-throughput single-nuclei transcriptome sequencing. This study is the first to characterize SCA2 degeneration and disease progression at fine spatiotemporal and single cell resolution. Our results offer new insights into the cell-type-specific contributions to regional disease vulnerability and the spatial regulation of genes and pathways for future validation and therapeutic targeting.

# (#158) Axonal swellings in a mouse model of ARSACS

Tuesday, 1st November - 16:15: Breakout: Disease Mechanisms I (Crystal Ballroom) - Oral - Abstract ID: 158

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 158

***Dr. Amy Smith-Dijak*** [1], ***Ms. Bruna Soares de Souza*** [1], ***Ms. Chloe Stewart*** [1], ***Ms. RuYi Louisa Shen*** [1], ***Dr. Alanna Watt*** [1]

***1.*** *McGill University*

**Background and Objective:** An elevated numbers of focal swellings on cerebellar Purkinje cell axons, or "torpedoes" have been observed across many neurodegenerative diseases, from essential tremor to Alzheimer's disease, and modeling suggests that they contribute to the pathophysiology of neurodegeneration. However, our recent findings show that axonal swellings in young, healthy mice enhance axonal propagation in cerebellar Purkinje cells, leading to improved performance on motor learning tasks. We wanted to determine what role axonal swellings play in disease, and whether they counteract or contribute to disease pathophysiology.

**Methods:** We studied a mouse model of Autosomal Recessive Spastic Ataxia of the Charlevoix-Saguenay (ARSACS), an early-onset form of ataxia that displays elevated numbers of axonal swellings at disease onset. We examined torpedo morphology and myelination in Sacs$^{-/-}$ and wildtype (WT) litter-matched control mice. We measured axonal propagation in cells with and without torpedoes at ~P40 using two-photon-guided dual recordings of Purkinje cell soma and axons.

**Results:** Axonal swellings in Sacs$^{-/-}$ mice show significant morphological differences from those in healthy mice. These morphological differences appear early during postnatal development. Remarkably, Purkinje cells from Sacs$^{-/-}$ mice show significant impairment of axonal propagation at an age when motor coordination deficits are just detectable. While axonal torpedoes do not worsen propagation deficits, they are unable to restore propagation to WT levels.

**Discussion and Conclusion:** Our data suggest that axonal swellings observed in Sacs$^{-/-}$ are distinct from those found in healthy mice. While axonal swellings do not appear to contribute to pathophysiology in neurodegeneration, they are also unable to restore axonal function to WT levels.

# Examining the basis for age-dependent neuronal dysfunction in spinocerebellar ataxia type 6 (SCA6)

Tuesday, 1st November - 16:30: Breakout: Disease Mechanisms I (Crystal Ballroom) - Oral - Abstract ID: 90

***Ms. Haoran Huang*** [1], ***Dr. Vikram G. Shakkottai*** [1], ***Mrs. Min Fu*** [2], ***Dr. Wei-Chih Chang*** [3], ***Ms. Miranda Dunn*** [1]

***1.*** *UT Southwestern Medical Center,* ***2.*** *University of Texas Southwestern Medical Center, Dallas, TX,* ***3.*** *University of Michigan Medical School*

**Background and Objective:** SCA6 is caused by a glutamine-encoding CAG repeat expansion in the Cav2.1 channel encoded by *CACNA1A*. SCA6 patients develop symptoms only in mid-late life, despite Cav2.1 expression beginning early in development. We sought to explore whether premature age-associated calcium dyshomeostasis occurs in SCA6.

**Methods:** We used SCA6 knockin mice, harboring an expanded 84-CAG repeat in *CACNA1A*. Heterozygous ($SCA6^{84Q/+}$) mice develop Purkinje neuron degeneration and motor deficits at 19 months. Age-associated calcium dyshomeostasis was examined by measuring longitudinal changes in ion channel transcripts, and examining Purkinje neuron firing properties using patch-clamp physiology at 6- and 12 months.

**Results:** We detected evidence for activation of the unfolded protein response (UPR) in 6-month $SCA6^{84Q/+}$ cerebella. Thapsigargin, an agent that induces calcium dyshomeostasis, impairs the regularity of Purkinje neuron firing in wild-type but not 6-month $SCA6^{84Q/+}$ mice. Also, at 6-months, transcripts of calcium channels Cav3.1, Cav2.1, Stim1 (a sensor of calcium concentration in the ER), and its associated calcium-release-activated calcium channel, Orai2, are increased. In 12-month $SCA6^{84Q/+}$ mice, UPR activation is no longer evident, concurrent with increased transcripts of not only the previously identified increase in expression of Cav3.1, and Cav2.1, but also increased expression of the calcium-activated potassium channel, BK.

**Discussion and Conclusions:** The abnormal UPR resulting from calcium dyshomeostasis is likely compensated by increased expression of calcium-handling related ion channel genes in 6-month $SCA6^{84Q/+}$ mice. At 12-months, the UPR response in $SCA6^{84Q/+}$ mice is normalized, presumably because the early ER calcium dyshomeostasis in 6-month $SCA6^{84Q/+}$ mice is now fully rescued by additional increases in BK channel expression. We hypothesize that at 19 months, when $SCA6^{84Q/+}$ mice exhibit irregular Purkinje neuron spiking and motor impairment, changes in ion channels expression are no longer able to compensate for the increasing calcium dyshomeostasis, which is now compounded by advancing age.

# (#22) Reactive Bergmann glia play a central role in spinocerebellar ataxia inflammation via the JNK pathway

Tuesday, 1st November - 16:45: Breakout: Disease Mechanisms I (Crystal Ballroom) - Oral - Abstract ID: 22

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 22

***Dr. Chandrakanth Edamakanti*** [1], ***Dr. Puneet Opal*** [1], ***Dr. Vishwa Mohan*** [1]

***1.*** *Northwestern University*

**Background and Objective:** The spinocerebellar ataxias (SCAs) are devastating neurological diseases characterized by progressive cerebellar incoordination. While neurons bear the brunt of the pathology, a growing body of evidence suggests that glial cells are also affected. However, it has been difficult to understand the role of glia given the diversity of subtypes, each with their individual contributions to neuronal health. Here we report the specific role of Bergmann glia inflammation by focusing on a signaling pathway defined by c-Jun phosphorylation that specifically is activated in this glial population.

**Results:** Using human SCA autopsy samples we have discovered that Bergmann glia—the radial glia of the cerebellum, which form intimate functional connections with cerebellar Purkinje neurons—display inflammatory JNK-dependent c-Jun phosphorylation. This phosphorylation defines a signaling pathway not observed in other activated glial populations, providing an opportunity to specifically isolate the role of Bergmann glia in SCA inflammation. Turning to an SCA1 mouse model as a paradigmatic SCA, we demonstrate that activated Bergmann glia release cytokines such as Interleukin 1 beta (IL-1β). Critically, targeting the JNK pathway pharmacologically reduces Bergmann glia inflammation accompanied by improvements in the SCA1 phenotype both behaviorally and pathologically.

**Conclusion:** This is the first study to highlight the contribution of Bergmann glia-specific inflammation in cerebellar degeneration. Moreover, these findings demonstrate the causal role for Bergmann glia inflammation in SCA1 and point to a novel therapeutic strategy that could span several ataxic syndromes where Bergmann glia inflammation is a major feature.

# (#171) Cotranslational degradation of mutant sacsin explains lack of genotype-phenotype correlation and defines molecular diagnosis in ARSACS patients

Tuesday, 1st November - 17:00: Breakout: Disease Mechanisms I (Crystal Ballroom) - Flash talk - Abstract ID: 171

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 171

***Dr. Fabiana Longo [1], Dr. Daniele De Ritis [1], Dr. Annarita Miluzio [2], Dr. Davide Fraticelli [1], Dr. Jonathan Baets [3], Dr. Marina Scarlato [1], Dr. Filippo M. Santorelli [4], Dr. Stefano Biffo [5], Dr. Francesca Maltecca [1]***

***1.*** *San Raffaele Scientific Institute,* ***2.*** *Istituto Nazionale di Genetica Molecolare, INGM, "Romeo ed Enrica Invernizzi",* ***3.*** *Antwerp University Hospital, Antwerpen,* ***4.*** *IRCCS Fondazione Stella Maris, Pisa,* ***5.*** *Istituto Nazionale di Genetica Molecolare, INGM, "Romeo ed Enrica Invernizzi", Milan*

**Background and Objective.** Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is caused by mutations in *SACS* gene encoding sacsin, a huge multimodular protein of unknown function. More than 200 *SACS* mutations have been described worldwide. Because ARSACS presents phenotypic variability, previous empirical studies attempted to correlate the nature and position of *SACS* mutations with age at onset or disease severity, although not considering the effect of each mutation on protein stability. In this work, we studied genotype-phenotype correlation in ARSACS at a functional level.

**Methods.** We analyzed a large set of skin fibroblasts derived from ARSACS patients carrying mutations of different types affecting diverse domains of the protein. We analyzed sacsin protein levels upon blockade of cellular degradative systems. We analyzed sacsin mRNA levels and translation by RT-qPCR both from total RNA and RNA from polysomal fractions. We immunoprecipitated sacsin co-translational degradation products with a newly generated antibody against its N-terminal region. We developed a method to efficiently detect sacsin in peripheral blood mononuclear cells.

**Results.** We found that sacsin is almost absent in patients with ARSACS, regardless of the nature of the mutation. As expected, we did not detect sacsin in patients with truncating mutations. We found it strikingly reduced or absent also in compound heterozygotes carrying diverse missense mutations. In this case, we excluded *SACS* mRNA decay, defective translation, or faster post-translational degradation as possible causes of protein reduction. Conversely, our results demonstrate that nascent mutant sacsin protein undergoes cotranslational ubiquitination and degradation.

**Discussion and Conclusion.** We identified preemptive degradation of a mutant protein as a novel cause of a human disease. Our results provide a mechanistic explanation for the lack of genotype-phenotype correlation in ARSACS and define a fast and unambiguous criterion for ARSACS diagnosis that is based on the evaluation of sacsin level in peripheral blood.

# (#133) Premature transcription termination induced by expanded GAAs leads to frataxin deficit in Friedreich's ataxia.

Tuesday, 1st November - 17:07: Breakout: Disease Mechanisms I (Crystal Ballroom) - Flash talk - Abstract ID: 133

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 133

***Dr. Yanjie Li [1], Dr. Jixue Li [2], Dr. Jun Wang [1], Dr. Siyuan Zhang [1], Dr. Keith Giles [1], Dr. Jill Napierala [2], Dr. Marek Napierala [3]***

***1.*** *University of Alabama at Birmingham, School of Medicine, Department of Biochemistry and Molecular Genetics,* ***2.*** *Department of Neurology, University of Texas Southwestern Medical Center, Dallas, TX,* ***3.*** *Peter O'Donnell Jr. Brain Institute, Department of Neurology, University of Texas Southwestern Medical Center, Dallas, TX*

Background and objective:
Frataxin deficiency in Friedreich's ataxia results from transcriptional downregulation of the *FXN* gene caused by expansion of intronic trinucleotide GAA repeats. We aimed to define the exact mechanism of the transcriptional defect imposed by long GAAs.
Methods:
We used multiple transcriptomic methods including ChIP-seq, PRO-seq and RNA-seq to determine the molecular mechanism of transcription inhibition caused by long GAAs. All studies were conducted using patients' cells, fibroblasts and induced pluripotent stem cells. Both proliferating as well as differentiated neurons and cardiac cells were utilized. Controls included cells from unaffected individuals as well as isogenic, CRISPR/Cas9 corrected FRDA cells.
Results:
We uncovered that transcription of *FXN* in patients' cells is prematurely terminated upstream of the expanded repeats leading to formation of a novel, truncated and stable RNA. This FXN early terminated transcript, FXN-*ett*, does not contribute to the synthesis of functional frataxin. Surprisingly, the level FXN-*ett* RNA directly correlates with the length of the longer of the two expanded GAA tracts. Targeting GAAs with antisense oligonucleotides or excision of the repeats eliminates the transcription impediment, diminishes expression of the aberrant FXN-*ett*, while increasing levels of *FXN* mRNA and frataxin.
Discussion and Conclusion:
Non-productive transcription may represent a common phenomenon and attractive therapeutic target in diseases caused by repeat-mediated transcription aberrations.

# (#485) Targeting ATAXIN-2 modulates p53-dependent apoptosis

Tuesday, 1st November - 17:14: Breakout: Disease Mechanisms I (Crystal Ballroom) - Flash talk - Abstract ID: 485

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 485

***Dr. Mandi Gandelman*** [1], ***Dr. Sharan Paul*** [1], ***Dr. Warunee Dansithong*** [1], ***Dr. Karla P. Figueroa*** [2], ***Dr. Daniel Scoles*** [1], ***Dr. Stefan M. Pulst*** [3]

***1.*** *University of Utah,* ***2.*** *Department of Neurology, University of Utah, Salt Lake City,* ***3.*** *Department of Neurology, University of Utah, Salt Lake City,*

The transcription factor p53 is activates complex integrated stress responses, including neuronal death. p53 increase and activation are common in neurodegenerative diseases, with ample evidence in animal models and human patients, however the triggers and modulators of p53-mediated cell death in neurodegeneration remain poorly understood. Expansions in the polyglutamine tract of Ataxin-2 (ATXN2) cause spinocerebellar ataxia type 2 (SCA2), and intermediate ATXN2 expansions increase risk of amyotrophic lateral sclerosis (ALS) up to 10-fold. Given the incipient success in targeting *ATXN2* for treating SCA2 and ALS, we seek to understand the related pathogenic mechanisms. We characterized the transcriptomic landscape resulting from *ATXN2* knockdown (KD) in HEK293 cells using an siRNA targeting *ATXN2* (siATXN2). We found 2187 differentially expressed genes at cutoffs of 0.5-log2fold change and a 5% FDR. Hallmark gene set enrichment analysis showed that the p53 pathway was negatively enriched after *ATXN2* KD. Ingenuity pathway analysis (IPA, Qiagen) showed that *ATXN2* KD modified the abundance of 90 out of the 98 transcripts annotated in the p53 pathway, with a highly significant negative z-score, indicating its activation would be inhibited. Because *ATXN2* KD is protective in multiple neurodegeneration models, we tested whether siATXN2 could prevent p53-dependent apoptosis triggered by etoposide, a chemotherapy drug that induces DNA damage and causes p53-dependent cell death. Treatment of HEK293 cells with etoposide predictably triggered p53 phosphorylation and an increase in cleaved caspase 3, which were prevented by ATXN2 KD. Similarly, in HEK293 cells modified by CRISPR-Cas9 to express one expanded *ATXN2* allele (ATXN2-Q58) and one wildtype allele, *ATXN2* KD also prevented p53 apoptosis caused by etoposide. These experiments indicate that targeting either WT or mutated ATXN2 decreases p53-dependent apoptosis, likely at the transcriptomic level, and constitute a novel neuroprotective mechanism harnessed by ATXN2 therapies.

# (#182) The bittersweet interrelation between O-GlcNAc transferase and the Machado Joseph disease protein ataxin-3

Tuesday, 1st November - 17:21: Breakout: Disease Mechanisms I (Crystal Ballroom) - Flash talk - Abstract ID: 182

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 182

***Ms. Priscila Pereira Sena*** [1], ***Dr. Jonasz J. Weber*** [1], ***Dr. Maxinne Watchon*** [2], ***Ms. Katherine Robinson*** [2], ***Dr. Zinah Wassouf*** [3], ***Dr. Stefan Hauser*** [4], ***Mr. Jacob Helm*** [4], ***Ms. Mahkameh Abeditashi*** [1], ***Dr. Jana Schmidt*** [1], ***Dr. Jeannette Hübener-Schmid*** [1], ***Prof. Ludger Schöls*** [4], ***Dr. Angela Laird*** [2], ***Prof. Olaf Riess*** [5], ***Prof. Thorsten Schmidt*** [1]

***1.*** *Institute of Medical Genetics and Applied Genomics, University of Tuebingen, Tuebingen,* ***2.*** *Centre for Motor Neuron Disease Research, Macquarie Medical School, Faculty of Medicine, Health and Human Sciences, Macquarie University, Sydney,* ***3.*** *Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, Cambridge,* ***4.*** *German Center for Neurodegenerative Diseases (DZNE), Tuebingen,* ***5.*** *Institute for Medical Genetics and Applied Genomics, Tübingen*

**Background and Objective:** *O*-GlcNAcylation is a nutrient sensor protein posttranslational modification (PTM) defined by the attachment of an O-linked-N-acetylglucosamine (*O*-GlcNAc) moiety to target proteins, mediated by the enzyme *O*-GlcNAc transferase (OGT). Although defective *O*-GlcNAcylation is implicated in neurodegeneration, this PTM has not been extensively investigated in polyglutamine (polyQ) disorders. We therefore aimed to evaluate OGT and *O*-GlcNAcylation in Machado-Joseph disease (MJD), a neurodegenerative condition characterized by ataxia and caused by an abnormal polyQ stretch within the deubiquitinase ataxin-3, resulting in increased propensity of this protein to aggregate.

**Methods:** We analyzed transiently transfected cells with wild-type and/or polyQ-expanded ataxin-3, induced pluripotent stem cell (iPSC)-derived cortical neurons from MJD patients, as well as MJD mouse and zebrafish models. MJD pathogenesis was assessed by the phenotypes and MJD molecular hallmarks. Genetic and pharmacological approaches were employed for modulating *O*-GlcNAcylation in the context of MJD.

**Results:** We provide evidence that OGT is dysregulated in MJD, therefore compromising protein *O*-GlcNAcylation. We further demonstrate that wild-type ataxin-3 modulates OGT protein levels, presenting OGT as a novel substrate for ataxin-3. Targeting OGT levels and activity impacted ataxin-3 aggregates, protein clearance and cell viability, and alleviated the motor impairment that resembles patient ataxia in an MJD animal model.

**Discussion and Conclusion:** The discovery of *O*-GlcNAcylation as an important PTM in the molecular pathogenesis of neurodegenerative disorders highlights this pathway as a promising target for those yet incurable conditions. We demonstrate that ataxin-3 has a physiological role in regulating OGT protein levels, and this mechanism is impaired in MJD. Altering OGT levels or activity in MJD models provided beneficial effects *in cellulo* and *in vivo*, thus reassuring that OGT is a disease-relevant enzyme and an auspicious candidate for the development of therapeutics for MJD.

# Plenary Session: Disease Mechanisms

# Invited Talk: Autoimmune cerebellar ataxia: What is new in 2022?

Wednesday, 2nd November - 08:30: Plenary: Disease Mechanisms (Crystal Ballroom) - Invited Speaker - Abstract ID: 520

***Prof. Jérôme Honnorat*** [1]

***1.*** *CHU de Lyon HCL*

Major advances in our knowledge concerning autoimmune and paraneoplastic cerebellar ataxias have been made in the last years. First, the discovery of several new biomarkers represents an undeniable contribution to this field. Some may serve as good biomarkers of paraneoplastic origin and others may play a direct role in the pathophysiology. Yet, many patients still lack detectable or known biomarkers, and others have only been reported in few patients leading to only partial characterization of these syndromes. A notable progress has additionally been made in the clinical characterization of patients with the well-known autoantibodies used as biomarkers. Some patients present with a subacute pancerebellar syndrome, but others may show either hyperacute or chronic onsets that complicate the differential diagnoses as well as the association with other neurological syndromes. Important advances in our understanding of the pathogenesis of cerebellar ataxias include the description of antibody effects, especially those targeting cell-surface antigens. Genetic predisposition seems also relevant in some patients and particularly some specific HLA haplotypes. Finally, immune checkpoint inhibitors used as cancer immunotherapy may rarely induce cerebellar ataxias, but even this undesirable effect may in turn serve to shed some light on their physiopathology. We will review the principal novelties of the last 20 years regarding autoimmune and paraneoplastic cerebellar ataxias.

# Specific cerebellar spike signatures determine the presentation of cerebellar movement disorders

Wednesday, 2nd November - 09:00: Plenary: Disease Mechanisms (Crystal Ballroom) - Oral - Abstract ID: 437

***Dr. Meike Van Der Heijden*** [1], ***Dr. Amanda Brown*** [1], ***Dr. Roy Sillitoe*** [1]

***1.*** *Baylor College of Medicine*

**Background and Objective**

Ataxia arises from dysfunctional cerebellar circuits. Yet, cerebellar dysfunction is also known to produce other movement disorders that can be comorbid with ataxia, namely dystonia, and tremor. However, how altering the same cerebellar circuit can produce distinct movement defects remains unknown. We therefore set out to examine whether signals generated in the cerebellum can be used to distinguish unique predictive signatures that induce abnormal movements.

**Methods**

We performed *in vivo* awake head-fixed recordings of cerebellar output neurons, known as the nuclei neurons, in healthy control mice and mouse models of ataxia, dystonia, and tremor. We comprehensively defined the spiking activity of each neuron using over twenty measurements. We trained an unsupervised classifier model on the spike activity measurements to distinguish neural signatures between ataxia, dystonia, tremor, and control mice. We tested whether different mouse models, but with similar phenotypes, displayed similar neural activity. We then used optogenetics to mimic the neural activity signatures associated with each disease phenotype.

**Results**

The classifier network found differences in spiking activity between ataxic, dystonic, and tremoring mice. More than half the neurons in mice with abnormal phenotypes had a spiking signature corresponding to the phenotypic presentation (ataxia, dystonia, tremor), irrespective of the mouse model used. Optogenetic stimulation of Purkinje cell terminals in the interposed cerebellar nucleus mimicked distinct neural activity signatures suggested by the classifier: a constant pattern (ataxia), a regularly oscillating pattern (tremor), or an irregularly bursting pattern (dystonia). Optogenetic stimulation caused abnormal motor phenotypes in freely moving mice.

**Discussion and Conclusions**

We show that alterations in cerebellar nuclei spiking activity predict the presentation of cerebellar movement disorders. We find that cerebellar models have distinct spiking signatures that are shared across mouse models with different etiologies and are sufficient to induce motor impairments in otherwise healthy mice.

# Altered calcium signaling in Bergmann glia contributes to decreased firing rate of Purkinje cells and motor deficits in a mouse model of Spinocerebellar ataxia type 1 (SCA1)

Wednesday, 2nd November - 09:15: Plenary: Disease Mechanisms (Crystal Ballroom) - Oral - Abstract ID: 156

***Dr. Marija Cvetanovic*** [1], ***Mrs. Ella Borgenheimer*** [2], ***Dr. Carmen Perez de Nanclares Fernandez*** [1], ***Dr. Jose Noriega*** [1], ***Mr. Juao-Guilherme Rosa*** [3], ***Dr. Alfonso Araque*** [1]

***1.*** *University of Minnesota,* ***2.*** *Baylor College of Medicine,* ***3.*** *Boston University*

SCA1 is a progressive neurodegenerative disease caused by an abnormal expansion of glutamine (Q) encoding CAG repeats in the *ATAXIN1* (*ATXN1*) gene. SCA1 is characterized by severe degeneration of cerebellar Purkinje cells (PCs) and reactive gliosis of Bergmann glia (BG), cerebellar astrocytes closely associated with PCs. It is likely that cerebellar microenvironment and in particular Bergmann glia contribute to the Purkinje cell specific vulnerability in SCA1. Thus, we aimed to examine changes in Bergmann glia and how they could contribute to SCA1 pathogenesis. Using single nuclei RNA sequencing (snRNA seq) we investigated changes in Bergmann glia gene expression and molecular pathways in cerebella of *Pcp2-ATXN1[82Q]* mice, a transgenic SCA1 mouse model expressing mutant ATXN1 only in PCs. We then used electrophysiology and calcium imaging of cerebellar slices to examine functional changes in Bergmann glia and how they contribute to PCs dysfunction. Using mouse genetics and behavioral analysis, we examined how BG calcium signaling changes contribute to SCA1 pathogenesis.

Single nuclei RNA sequencing identified 876 differentially expressed genes (p.adj <= 0.05) in Bergmann glia (BG), a majority of which (~73%) were upregulated in *Pcp2-ATXN1[82Q]* mice. Among the altered genes and pathways were regulators of intracellular calcium. Expressing genetically encoded calcium indicator GCaMP6 selectively in BG we have found increase in frequency of spontaneous calcium events in BG in cerebellar slices of *Pcp2-ATXN1[82Q]* mice. We found that decreasing intracellular BG calcium via delivery of calcium chelator BAPTA to Bergmann glia ameliorated decreased PCs firing rate seen in

*Pcp2-ATXN1[82Q]* mice. Contribution of BG calcium alterations to SCA1 pathogenesis was indicated by ameliorated motor deficits in *Pcp2-ATXN1[82Q]; IP3R2 KO* mice in which calcium responses are decreased in astrocytes.

These results indicate that altered calcium signaling in Bergmann glia contributes to PC dysfunction and pathogenesis in mouse model of SCA1.

# (#119) CGG repeat-elicited neurodegeneration in Fragile X Tremor-Ataxia Syndrome

Wednesday, 2nd November - 09:30: Plenary: Disease Mechanisms (Crystal Ballroom) - Oral - Abstract ID: 119

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 119

***Dr. Peter Todd*** [1]

***1.*** *University of Michigan-Ann Arbor*

**Background and Objective:**
Fragile X-associated tremor ataxia syndrome (FXTAS) is caused by a transcribed trinucleotide CGG repeat expansion in the 5' UTR of *FMR1*. CGG repeats drive neurodegeneration through formation of aberrant repeat RNA - RNA binding protein complexes and by triggering repeat-associated non-AUG initiated ("RAN") translation of toxic proteins. Despite recent advances providing insights into these pathogenic mechanisms, no effective therapies exist for this progressive and fatal condition.
**Methods:**
We used reporter assay systems in cell lines and primary neurons to identify novel *in-cell* CGG repeat RNA-protein interactors and both *cis* and *trans* modulators of CGG RAN translation. Validated hits from these modifier and interactor screens were then evaluated in *Drosophila,* rodent and human neuronal models.
**Results:**
CGG repeat RNA interacting proteins, including SRSF proteins and the kinases (e.g. SRPK1) that regulate their function, modulate RAN translation and suppress repeat toxicity in *Drosophila* and rodent neuronal model systems. Initiation both within CGG repeats in multiple reading frames and at native conserved near-cognate codons upstream of the repeat are responsive to altered expression of initiation factors that modulate start codon fidelity, ribosomal quality control, and RNA secondary structure. CGG repeats also elicit translational frameshifts to generate chimeric proteins with enhanced toxicity and altered biophysical properties. Antisense Oligonucleotides that selectively target RAN initiation sites suppress repeat associated toxicity in rodent and human neurons.
**Discussion and Conclusion**:
CGG repeat binding proteins and CGG RAN translational modifiers represent viable therapeutic strategies worthy of further development in preclinical models of FXTAS and related repeat expansion disorders.

# (#271) Sense and antisense RAN proteins in the CAG•CTG polyglutamine spinocerebellar ataxias

Wednesday, 2nd November - 09:45: Plenary: Disease Mechanisms (Crystal Ballroom) - Oral - Abstract ID: 271

***Dr. Monica Banez Coronel*** [1], ***Ms. Madeline Denton*** [1], ***Dr. Tao Zu*** [1], ***Dr. Hannah Shorrock*** [1], ***Dr. Shu Guo*** [1], ***Prof. Laura P.W. Ranum*** [2]

***1.*** *Center for NeuroGenetics, Department of Molecular Genetics and Microbiology, College of Medicine, University of Florida College of Medicine. University of Florida. Gainesville, FL.,* ***2.*** *Center for NeuroGenetics, Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL*

BACKGROUND AND OBJECTIVE. Repeat associated non-AUG (RAN) proteins have been reported in disease-relevant tissues in eleven repeat expansion disorders. In *C9orf72* ALS/FTD, recent studies demonstrate that RAN proteins are key drivers of disease and an effective therapeutic target. In spinocerebellar ataxia type 8 (SCA8) and Huntington disease (HD), which are caused by CAG·CTG repeat expansion mutations, RAN protein levels increase with disease severity. Our hypothesis is that RAN protein pathology is a common feature across the polyglutamine encoding CAG•CTG expansion disorders including many of the SCAs.

METHODS. To examine sense and antisense RAN protein accumulation across CAG·CTG expansion diseases, we developed polyclonal antibodies against polySer (AGC frame) and polyLeu (CTG frame) repeat motifs. The specificity of these novel antibodies was validated in transfected cells and HD brain tissue previously characterized for polySer and polyLeu accumulation. Using these novel antibody detection tools, we performed immunostaining in SCA1, SCA2, SCA3, SCA6 and SCA7 human postmortem tissue and transfected cells.

RESULTS. Our data show novel sense polySer and antisense polyLeu RAN proteins accumulate in brain regions most affected in SCA1,2,3,6&7, including cerebellum and pons. Regions with intense RAN-protein but little polyGln staining show neuroinflammation and demyelination. In contrast, RAN protein accumulation in cortex, which is less affected by the disease, was infrequent and not associated with markers of pathology. Cell-toxicity studies show SCA1,2,3,6&7 RAN proteins selectively decrease the survival of neuronal and glial but not HEK293T cells. In glial cells, RAN proteins colocalize with ubiquitin and cause autophagic dysfunction, and inhibiting SCA3 RAN protein accumulation with metformin reduces cell toxicity.

CONCLUSION. These data demonstrate sense and antisense RAN protein pathology is a common feature of the polyglutamine SCAs and highlights the need to understand their role in disease and develop therapeutic strategies aimed at targeting both sense and antisense transcripts.

# Breakout: Disease Mechanisms II

# Disruption of the CoQ10 biosynthetic Complex Q causes mitochondrial dysfunction and Ca2+ imbalance in Purkinje neurons in COQ8A-ataxia.

Wednesday, 2nd November - 10:30: Breakout: Disease Mechanisms II (Crystal Ballroom) - Oral - Abstract ID: 227

***Dr. Ioannis Manolaras [1], Ms. Laurence Reutenauer [2], Dr. Nadia Messaddeq [1], Dr. Bianca Habermann [3], Dr. Helene Puccio [4]***

***1.*** *Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), INSERM U1258, CNRS UMR7104, Université de Strasbourg, France.,* ***2.*** *Institut NeuroMyoGene, UMR5310, INSERM U1217, Université Claude Bernard Lyon I Faculté de médecine, Lyon, France,* ***3.*** *Institut de Biologie du Développement de Marseille (IBDM), CNRS, UMR7288, Aix-Marseille Université, Marseille, France.,* ***4.*** *Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), INSERM U1258, CNRS UMR7104, Université de Strasbourg, France. Institut NeuroMyoGene, UMR5310, INSERM U1217, Université Claude Bernard Lyon 1, Lyon, France*

COQ8A-Ataxia (previously named ARCA2) is a rare form of neurodegenerative disorder due to mutations in the *COQ8A* gene. The encoded protein is involved in the regulation of the mitochondrial biosynthesis of Coenzyme $Q_{10}$ ($CoQ_{10}$). From previous studies, histological and functional analysis on the cerebellar Purkinje neurons (PNs) of the ataxic *Coq8a*$^{-/-}$ mice indicated specific alterations involving dark cell degeneration and altered electrophysiological function. In the present study, we showed that COQ8A is highly expressed in PNs neurons whereas its closest paralog COQ8B is expressed in cerebellar granule cells (GCs). Moreover, we demonstrated that deletion of COQ8A specifically in PNs is sufficient to cause cerebellar ataxia and motor deficits in PN-specific conditional *Coq8a*$^{-/-}$ mouse model. By employing a laser capture microdissection (LCM) coupled to RNA-sequencing, we demonstrated that the underlying mechanism of the *Coq8a*$^{-/-}$ PNs - specific degeneration involves mitochondrial dysfunction that precedes the ataxic phenotype. Furthermore, *Coq8a*$^{-/-}$ PNs present abnormal dendritic arborizations, oxidative stress and altered Ca2+ homeostasis. Finally, application of 10 micromolar $CoQ_{10}$ rescued the calcium dysregulation as well as the mitochondrial and morphological phenotype of *Coq8a*$^{-/-}$ primary cerebellar PNs, suggesting that $CoQ_{10}$ could be a beneficial treatment for ARCA2.

# (#128) Hyperexcitability and hypertrophy in the inferior olivary nucleus of the spinocerebellar ataxia type 1 brainstem

Wednesday, 2nd November - 10:45: Breakout: Disease Mechanisms II (Crystal Ballroom) - Oral - Abstract ID: 128

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 128

***Mr. Logan Morrison*** [1], ***Dr. Hillary Handler*** [2], ***Ms. Haoran Huang*** [3], ***Mrs. Min Fu*** [3], ***Dr. Samuel Pappas*** [3], ***Dr. Harry Orr*** [2], ***Dr. Vikram G. Shakkottai*** [3]

***1.*** *University of Texas Southwestern Medical Center, Dallas, TX and University of Michigan, Ann Arbor, MI,* ***2.*** *University of Minnesota,* ***3.*** *University of Texas Southwestern Medical Center, Dallas, TX*

**Suggested Theme:** Disease mechanisms

**Background and Objective:** Though it is clear that brainstem dysfunction is responsible for premature death in SCA1 patients[1], little else is known about this pathology. The objective of this work is to investigate this critically understudied aspect of SCA1. Specifically, we focus on the inferior olive (IO), a nucleus in the medulla that is particularly vulnerable to SCA1-associated neurodegeneration[2, 3].

**Methods:** Due to recent technical innovations[4], we can now obtain patch-clamp recordings from IO neurons in mature (i.e., symptomatic) SCA1-knock-in (SCA1-KI) mice. As such, these studies represent the first investigation of IO function in a neurodegenerative disease context. Recordings are also paired with cell-filling, allowing us to analyze IO neuron morphology. Finally, using unbiased RNA-sequencing in the SCA1-KI medulla, we identify a subset of ion channels whose dysregulation may cause neuronal dysfunction.

**Results:** These studies reveal that SCA1-KI IO neurons are hyperexcitable. Furthermore, we identify that this is likely due to changes in afterhyperpolarization (AHP), an area of the action potential that is also abnormal in SCA1-KI Purkinje neurons[5]. Morphological reconstructions demonstrate that this hyperexcitability is associated with a more complex dendritic arbor in IO neurons. Interestingly, similar increases in IO neuron complexity have been observed following specific brainstem lesions (described as hypertrophic olivary degeneration, or HOD)[6]. RNA-seq in the medulla showed reduced transcripts for several potassium channels in SCA1-KI mice, which likely explains the observed impairment in IO neuron AHP.

**Discussion and Conclusion:** These results demonstrate that early neuronal dysfunction is shared between the SCA1 cerebellum and brainstem, and suggest that potassium channels may be a compelling therapeutic target to counter brainstem dysfunction in SCA1. In addition, this study reveals similarities in the cause and consequence of HOD and SCA1 on the IO, indicating a potential convergence of treatment strategies for the two disorders.

# (#468) Exploring the missing heritability in SPG7 heterozygous carriers with Whole Genome Sequencing

Wednesday, 2nd November - 11:00: Breakout: Disease Mechanisms II (Crystal Ballroom) - Oral - Abstract ID: 468

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 468

***Dr. Marie Coutelier*** [1], ***Dr. Jean-Loup Méreaux*** [2], ***Dr. Marine Guillaud-Bataille*** [3], ***Mrs. Lena Guillot Noel*** [2], ***Mrs. Claire-Sophie Davoine*** [2], ***Prof. Alexis Brice*** [2], ***Prof. Alexandra Durr*** [2]

***1.*** *Sorbonne Université, Paris Brain Institute (ICM Institut du Cerveau), AP-HP, INSERM, CNRS, University Hospital Pitié-Salpêtrière, France,* ***2.*** *Sorbonne Université, Paris Brain Institute - ICM, Inserm, CNRS, APHP, Hôpital de la Pitié Salpêtrière, Paris,* ***3.*** *APHP, Sorbonne Université, Department of Medical Genetics, 75013 Paris, France*

**Background and Objective:** *SPG7* biallelic mutations are the most frequent cause of autosomal recessive spastic paraplegia. The associated clinical picture is either a pure spastic paraplegia, or a complex phenotype encompassing mitochondrial features, optic atrophy, and cerebellar signs. In the recent years, the phenotype has been widened to cerebellar ataxia more generally, with or without pyramidal signs; and to clinical presentations associating extrapyramidal features, mimicking Parkinson's disease or Multisystemic Atrophy of the cerebellar type in some cases. We aim at describing the full mutational spectrum of SPG7, including non-coding mutations, and delineate the associated phenotypes.

**Methods and Results:** In 731 patients with cerebellar ataxia, we sequenced known ataxia genes, either with amplicon-based panel sequencing (n=412) or whole exome sequencing (n=319). We found biallelic coding mutations in 23 patients (3.1%), often associating spastic or mitochondrial presentations. We also identified 16 heterozygous carriers of loss of function or previously described missense variants in *SPG7*, without a second mutation, and a phenotype characteristic of *SPG7*-related cerebellar ataxia, associating either spasticity or parkinsonism. We performed short-read Whole Genome Sequencing in those patients and their relatives, when available, and report our results on missing heritability identification.

**Discussion and Conclusion:** Dominant transmission of *SPG7* mutations has been discussed in the literature. While it is suggested in some patients, a recent report described a deep intronic change responsible for an alteration of *SPG7* expression, in trans with a missense mutation. Our results and others advise genetic reexamination of heterozygous carriers of mutations in known recessive genes, especially when the phenotype is characteristic.

# A shared mechanism for SCA35 and gluten ataxia

Wednesday, 2nd November - 11:15: Breakout: Disease Mechanisms II (Crystal Ballroom) - Oral - Abstract ID: 497

***Dr. Chih-Chun Lin*** [1], ***Dr. Chun-Lun Ni*** [1], ***Mr. Christopher Driscoll*** [1], ***Dr. Sheng-Han Kuo*** [2]

***1.*** *Columbia University Irving Medical Center,* ***2.*** *Columbia Univeristy Medical Center*

Background and Objective:
Spinocerebellar ataxias (SCAs) are a group of autosomal dominant cerebellar diseases with no disease-modifying treatments. We became interested in SCA type 35 (SCA35) after we encountered a patient carrying a mutation of transglutaminase 6 (*TGM6*), the gene responsible for SCA35. Interestingly, autoantibodies against TG6, the protein of *TGM6*, are found in ataxia associated with gluten sensitivity (gluten ataxia). This suggested that TG6 is the key to both SCA35 and gluten ataxia. We thus decided to investigate the role of TG6 in cerebellar ataxia with mouse models and anti-TG6 antibodies.
Methods:
We generated a straight knockout and a Purkinje-cell-specific knockout of *Tgm6*. We then studied these mice with gait analysis and Rotarod to characterize the phenotype. In addition, we also examined the electrophysiological properties of the wild-type Purkinje cells with brain slice recording in the presence and absence of anti-TG6 antibodies.
Results:
The straight and Purkinje-cell-specific knockout of *Tgm6* both showed ataxia phenotype, suggesting that the absence of TG6 in Purkinje cell is important in the pathogenesis. Furthermore, in wild-type mice, single-channel recording of a potassium channel, BK, from the Purkinje cells showed a reduced opening probability in the presence of anti-TG6 antibodies.
Discussion and Conclusion:
Our preliminary findings demonstrated that eliminating the expression of TG6 in Purkinje cells is sufficient to generate an ataxia phenotype. In addition, in the presence of anti-TG6 antibodies, the opening probability of BK channel from Purkinje cells is reduced.
These findings suggested that mutant TG6 possibly causes ataxia by regulating the activities of BK channel in Purkinje cells. We plan to study the activities of BK channel in Purkinje cells from the *Tgm6* knockout mice. If the connection between TG6 and BK channel is established, BK channel may serve as a therapeutic target for both SCA35 and gluten ataxia.

# (#490) Molecular Mechanisms of SCA48

Wednesday, 2nd November - 11:30: Breakout: Disease Mechanisms II (Crystal Ballroom) - Flash talk - Abstract ID: 490

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 490

***Dr. Matt Scaglione*** [1], ***Ms. Ran Ming*** [1], ***Dr. Jamie Scaglione*** [1], ***Ms. Anna Umano*** [2]

***1.*** *Duke University,* ***2.*** *Duk*

**Background and Objective**

Spinocerebellar ataxia type 48 (SCA48) is a recently discovered form of SCA that is caused by mutations in the neuroprotective E3 ligase C-terminus of Hsc70 Interacting Protein (CHIP). The objective of this study was to define how mutations in CHIP cause SCA48 and to use these findings to identify novel biological roles for CHIP.

**Methods and Results**

To date, eight separate point mutations in the tetratricopeptide repeat (TPR) domain of CHIP have been identified as a cause of SCA48. Interestingly, we noticed that all these mutations mapped to a single interface in the TPR domain of CHIP suggesting that they may cause SCA48 through a single mechanism. To assess how these mutations alter CHIP function we performed fluorescence microscopy and determined that mutations in the TPR domain of CHIP that cause SCA48 result in aberrant localization of CHIP to the nucleus. Interestingly, CHIP normally localizes to the nucleus under conditions of cellular stress, however, the function of nuclear CHIP is unknown. To determine the function of nuclear CHIP we performed a proximity labeling experiment and found that SCA48 mutant CHIP associates with proteins involved in pre-mRNA processing and splicing. To validate that CHIP plays a role in pre-mRNA processing and splicing we performed RNAseq and analyzed splice variants. Strikingly, we found that CHIP plays a critical role in regulating stress-induced alternative splicing. Finally, to gain structural insight into how SCA48 mutations alter CHIP function we next utilized AlphaFold2 to generate a predicted structure of SCA48 mutant CHIP and validated this model using partial proteolysis and NMR spectroscopy.

**Discussion and Conclusion**

In conclusion, we have identified the aberrant structure and localization of CHIP as a potential cause of SCA48. Using this information, we identified a previously unknown role for CHIP in mediating pre-mRNA processing and stress-induced alternative splicing.

# (#445) Genotype-phenotype correlation in RFC1 repeat expansion disease

Wednesday, 2nd November - 11:37: Breakout: Disease Mechanisms II (Crystal Ballroom) - Flash talk - Abstract ID: 445

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 445

***Dr. Andrea Cortese*** [1], ***Dr. Riccardo Curro'*** [1], ***Dr. Natalia Dominik*** [1], ***Dr. A Nazli Basak*** [2], ***Prof. Chiara Briani*** [3], ***Dr. Alfredo Brusco*** [4], ***Dr. Gianni Maria Fabrizi*** [5], ***Prof. Paola Giunti*** [6], ***Dr. David Gosal*** [7], ***Prof. Marios Hadjivassiliou*** [8], ***Dr. Annette Hartmann*** [9], ***Dr. Jon Infante*** [10], ***Dr. Manu Jokela*** [11], ***Prof. Marina Kennerson*** [12], ***Dr. Paola Mandich*** [13], ***Prof. Fiore Manganelli*** [14], ***Dr. Sinead Murphy*** [15], ***Dr. Davide Pareyson*** [16], ***Dr. Gianina Ravenscroft*** [17], ***Prof. Mary M Reilly*** [1], ***Prof. Richard Roxburgh*** [18], ***Dr. Filippo M. Santorelli*** [19], ***Prof. Gabriella Silvestri*** [20], ***Dr. Michael Strupp*** [21], ***Dr. Franco Taroni*** [22], ***Dr. Tanya Stojkovic*** [23], ***Prof. Matthis Synofzik*** [24], ***Dr. Elisa Vegezzi*** [25], ***Prof. Stephan Zuchner*** [26], ***Prof. Henry Houlden*** [27]

***1.*** *UCL Institute of Neurology,* ***2.*** *24Koç University, School of Medicine, Suna and İnan Kıraç Foundation, Neurodegeneration Research Laboratory (NDAL), Research Center for Translational Medicine, Istanbul,* ***3.*** *University of Padova,* ***4.*** *Department of Medical Sciences, University of Turin, Turin,* ***5.*** *University of Verona,* ***6.*** *Ataxia Centre, Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, London,* ***7.*** *Manchester Centre for Clinical Neurosciences, Salford Royal Hospital, Northern Care Alliance NHS Foundation Trust, Manchester,* ***8.*** *Academic Department of Neurosciences, Sheffield Teaching Hospitals NHS Trust and University of Sheffield,* ***9.*** *Biomedical Center Munich Department of Physiological Chemistry Ludwig-Maximilians-Universität,* ***10.*** *University Hospital Marqués de Valdecilla-IDIVAL, University of Cantabria, Santander,* ***11.*** *Neuromuscular Research Center, Department of Neurology, Tampere University and University Hospital, Tampere,* ***12.*** *University of Sydne,* ***13.*** *University of Genova,* ***14.*** *University of Naples,* ***15.*** *Department of Neurology, Tallaght University Hospital, Dublin,* ***16.*** *Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy,* ***17.*** *Neurogenetic Diseases Group, Centre for Medical Research, QEII Medical Centre, University of Western Australia,* ***18.*** *The University of Auckland,* ***19.*** *IRCCS Fondazione Stella Maris, Pisa,* ***20.*** *Catholic University of the Sacred Heart, Rome,* ***21.*** *Department of Neurology, University Hospital, Ludwig Maximilians University, Munich,* ***22.*** *Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan,* ***23.*** *Nord/Est/Ile-de-France Neuromuscular Reference Center, Institute of Myology, Pitié-Salpêtrière Hospital, APHP, Sorbonne University, Paris,* ***24.*** *University of Tübingen,* ***25.*** *C Mondino Foundation,* ***26.*** *Department of Neurology, University of Miami Miller School of Medicine, Miami, FL 33136, USA.,* ***27.*** *Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London*

**Background**

Biallelic repeat expansions in RFC1 have been associated with cerebellar ataxia, neuropathy, vestibular areflexia syndrome (CANVAS). RFC1 disease is clinically heterogeneous in terms of age of onset, disease progression and phenotype, however the factors contributing to this variability are still unknown. We investigated the role of the repeat size in influencing clinical variables in RFC1 disease. We also assessed the presence and role of the meiotic and somatic stability of the repeat.

**Methods**

We screened 1800 patients affected by sensory neuropathy, late onset ataxia or CANVAS. Southern blotting was performed to measure the expansion size in positive cases. We analysed the correlation between repeat size and age of onset and clinical phenotype. Meiotic stability was assessed by Southern blotting on first-degree relatives of 20 patients and somatic instability was investigated by genome optical mapping on cerebellar and frontal cortex and unaffected peripheral tissue from six post-mortem cases.

**Results**

We identified a biallelic RFC1 expansion in 464 patients and measured the expansion size in 365 cases. An inverse correlation was observed between repeat size and age of neurological onset(r=-0.23), age at onset of unsteadiness(r=-0.26), cerebellar symptoms (r=-0.41) and need for walking aids (r=-0.38). Patients with isolated neuropathy carried smaller expansions (769 +/-258) compared to patients with complex neuropathy(1011 +/- 308) or CANVAS (994 +/-294). A larger expansion was associated with a higher risk of developing disabling symptoms after a shorter disease duration. The repeat was stable during vertical transmission (less than +/-10% of variations in 75% of meiotic events) and across different tissues and brain regions.

**Discussion and Conclusions**

Repeat size is one of the determinants of variability in RFC1 disorder and can have a role in predicting disease severity. Meiotic and somatic instability do not seem to contribute to the clinical heterogeneity or tissue specificity of this condition.

# (#515) Reduction of BACE1 expression attenuates motor deficits and neuropathology in spinocerebellar ataxia type 1 mice

Wednesday, 2nd November - 11:44: Breakout: Disease Mechanisms II (Crystal Ballroom) - Flash talk - Abstract ID: 515

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 515

***Dr. Alan Fowler [1], Ms. Britt DiMarzio [1], Dr. Rudolph Tanzi [1], Dr. Jaehong Suh [1]***

***1.*** *Massachusetts General Hospital / Harvard Medical School*

Spinocerebellar ataxia type 1 (SCA1) is a neurodegenerative disease that impairs motor coordination and cognitive function, leading to early lethality. Expansion of CAG trinucleotides repeat that encodes a polyglutamine (polyQ) track in ataxin-1 gene (*ATXN1*) is the genetic determinant of the disease that has no effective therapy. In a prior study (Suh et al., 2019 *Cell*), we demonstrated that loss of ataxin-1 increases the transcription of BACE1 selectively in Alzheimer's disease (AD)-vulnerable cerebrum (~100%) whereas SCA1-causing polyQ-expanded ataxin-1 leads to BACE1 increase post-transcriptionally both in the cerebrum and cerebellum (~40%) in a disease progression-dependent manner. BACE1 has been a highly invested therapeutic target for AD as it cleaves amyloid precursor protein (APP) and generates amyloid β (Aβ), the main culprit of senile plaques in AD brains. Here, we hypothesize increased BACE1 expression exacerbates SCA1 pathogenesis and test if reduction of BACE1 ameliorates the disease phenotypes. Behavioral examinations of $Atxn1^{154Q/+}$; $Bace1^{+/-}$ mice, generated from crossing of $Atxn1^{154Q/+}$ SCA1 mice with $Bace1^{+/-}$, showed that they perform significantly better than $Atxn1^{154Q/+}$ littermates in tests measuring locomotive activity (open field) and coordinated movement (rotarod and balance beam). Biochemical and immunohistological analysis revealed that BACE1 reduction attenuates the degenerations of cerebellar Purkinje and hippocampal CA2 neurons and rescues impaired hippocampal neurogenesis of SCA1 mice. Furthermore, the cleavages of Sez6 and Sez6L1, BACE1 substrates that are associated with locomotive activity, were significantly decreased in $Atxn1^{154Q/+}$; $Bace1^{+/-}$ mice. Together, these findings suggest that BACE1 reduction lessens SCA1 motor deficits and brain pathology, positioning BACE1 as a potential therapeutic target for SCA1.

# (#250) Abnormalities and sex-difference in muscle histomorphology in Autosomal recessive spastic ataxia of Charlevoix-Saguenay

Wednesday, 2nd November - 11:51: Breakout: Disease Mechanisms II (Crystal Ballroom) - Flash talk - Abstract ID: 250

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 250

***Prof. Elise Duchesne*** [1], ***Prof. Cynthia Gagnon*** [2], ***Prof. Luc J. Hébert*** [3], ***Ms. Marie-Pier Roussel*** [4], ***Dr. Bernard Brais*** [5]

***1.*** *Département des Sciences de la santé, Université du Québec à Chicoutimi, Chicoutimi, Québec, Canada.,* ***2.*** *Faculté de médecine et des sciences de la santé, Université de Sherbrooke, Sherbrooke, Québec, Canada,* ***3.*** *Départements de réadaptation et de Radiologie et médecine nucléaire, Faculté de médecine, Université Laval, Québec city, Québec, Canada,* ***4.*** *Département des Sciences fondamentales, Université du Québec à Chicoutimi, Chicoutimi, Québec, Canada,* ***5.*** *Montreal Neurological Institute, McGill University, Montreal, QC, Canada*

**Background and Objectives.** Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is characterized by cerebellar, pyramidal and neuropathic deficiencies. Motor impairments arising from these deficiencies include muscle wasting, and their progression, lead to significant mobility limitations. Muscle histomorphology has been studied in Sacs$^{-/-}$ mice but is not documented in individuals with ARSACS. The objective of this study was to compare some histomorphological characteristics of the *vastus lateralis* muscle fibers between individuals with ARSACS and healthy people.

**Methods.** Twelve subjects with ARSACS (4 men), all homozygous for the 8844deIT mutation except one woman, and 12 healthy participants (6 men) were recruited. Muscle biopsies were collected from each participant, frozen in tissue freezing medium in isopentane cooled in liquid nitrogen and stored at -80°C. Cross-sectional cuts of the muscle samples were stained using primary (rabbit anti-laminin and anti-human myosin heavy chain I) and fluorescent secondary antibodies. Immunofluorescence pictures of the sections were taken. The minimal Feret's diameter (MFD) was calculated and subsequently used to calculate the variability coefficient and the atrophy and hypertrophy factors (AF and HF). Statistical analyses were carried out using Mann-Whitney U tests.

**Results.** When compared to healthy women, the type 1 myofiber's MDF of women with ARSACS was significantly decreased (p=0.028). Type 1 myofibers of women with ARSACS present abnormal atrophy factor (p=0.007) and type 2 myofibers present abnormal atrophy factor (p=0.02) and variability coefficient (p=0.007) in comparison to healthy women. When compared to healthy men, the type 2 myofiber's MDF of men with ARSACS was significantly decreased (p=0.011). Type 2 myofibers of men with ARSACS present abnormal atrophy factor (p=0.011) and variability coefficient (p=0.011) in comparison to healthy men.

**Discussion and conclusion.** Taken together, these results suggest a neurogenic muscle atrophy in ARSACS with a different presentation between men and women.

# Breakout: Biomarkers

# (#186) The glucocorticoid receptor as a biomarker and neuronal therapeutic target of a disease-improving bile acid in SCA3/MJD

Wednesday, 2nd November - 10:30: Breakout: Biomarkers (Lalique Ballroom) - Oral - Abstract ID: 186

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 186

***Dr. Jorge Diogo Da Silva [1], Dr. Sara Duarte-Silva [1], Dr. Marta Daniela Costa [1], Dr. Andreia Neves-Carvalho [1], Dr. Mafalda Raposo [2], Dr. Carina Soares-Cunha [1], Ms. Joana S. Correia [1], Dr. Henrique Fernandes [3], Ms. Stéphanie Oliveira [1], Ms. Daniela Monteiro-Fernandes [1], Ms. Liliana Meireles-Costa [1], Prof. Cecília Rodrigues [4], Dr. Sérgio Sousa [3], Dr. Andreia Teixeira-Castro [1], Prof. Manuela Lima [2], Prof. Patrícia Maciel [1]***

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Neurodegenerative diseases generally lack appropriate biomarkers, treatments and mechanistic insight on their mode-of-action. Spinocerebellar ataxia type 3 (SCA3) is an adult-onset cerebellar ataxia caused by a polyglutamine expansion in ATXN3. Previous work pinpointed the bile acid tauroursodeoxycholic acid (TUDCA) as highly efficient in improving the phenotype of SCA3 models. We aimed to understand the mechanism of TUDCA in neurons, its potential as a specific drug for SCA3, and determine associated biomarkers of target engagement.

The nematode AT3Q130 and mouse CMVMJD135 models were used to study the effects of TUDCA, using behavioral, pharmacogenomic, anatomopathological and transcriptomic assays. Brain tissue and peripheral blood from patients was used in translatability and biomarker studies.

We observed the effect of TUDCA in SCA3 nematodes was fully dependent on the glucocorticoid receptor (GR), requiring its expression in neurons, and independent on its canonical receptor, the farnesoid X receptor (FXR). Consistently with a (previously unknown) role for this receptor in SCA3 pathogenesis, we identified close molecular interactions between GR and ATXN3, and observed GR protein was decreased in the brainstem of SCA3 mice, likely as a consequence of reduced deubiquitination by mutant ATXN3. GR levels were fully normalized by TUDCA, which is predicted to bind to GR *in silico*. Transcription of canonical GR targets in the mouse brainstem was also induced by TUDCA, with no effect on FXR targets. A similar reduction of GR protein levels was observed in SCA3 patient brains. Lastly, we propose that peripheral expression of GR and its co-chaperone FKBP5 can be used as biomarkers of clinical conversion and disease progression, respectively.

In conclusion, TUDCA targets a dysfunctional GR in neurons, decreasing its degradation and increasing its transcriptional activity, culminating in improvement of SCA3 phenotypes. While being a therapeutic target, the GR is also a potential biomarker of disease and of pharmacodynamic engagement.

# (#266) Data-derived wearable digital biomarkers predict Frataxin gene expression levels and longitudinal disease progression in Friedreich's Ataxia

Wednesday, 2nd November - 10:45: Breakout: Biomarkers (Lalique Ballroom) - Oral - Abstract ID: 266

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 266

***Dr. Balasundaram Kadirvelu [1], Dr. Constantinos Gavriel [2], Dr. Sathiji Nageshwaran [3], Dr. Jackson Chan [4], Dr. Stavros Athanasopoulos [5], Prof. Paola Giunti [6], Dr. Valeria Ricotti [7], Dr. Thomas Voit [8], Prof. Richard Festenstein [9], Prof. Aldo A Faisal [10]***

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**Background**

Friedreich's ataxia (FA) is a neurodegenerative disease caused by the epigenetic repression of the *Frataxin* gene essential for mitochondrial activity in the brain, which has a diffuse phenotypic impact on patients' motor behavior. Therefore, with current gold-standard clinical scales, it requires 18-24 month-long clinical trials to determine if disease-modifying therapies are at all beneficial.

**Methods and Results**

Our high-performance monitoring approach captures the full-movement kinematics from human subjects using wearable body sensor networks from a cohort of FA patients during their regular clinical visits. We then use artificial intelligence to convert these movement data using universal behavior fingerprints into a digital biomarker of disease state. This enables us to predict two different 'gold-standard' clinical scores (SCAFI, SARA) that serve as primary clinical endpoints. Crucially, by performing gene expression analysis on each patient their personal *Frataxin* gene expression levels were poorly, if at all, correlated with their clinical scores – fundamentally failing to establish a link between disease mechanism (dysregulated gene expression) and measures to quantify it in the behavioral phenotype. In contrast, our wearable digital biomarker can accurately predict for each patient their personal *FXN* gene expression levels, demonstrating the sensitivity of our approach and the importance of *FXN* levels in FA.

**Conclusions and Discussion**

Our data-derived biomarker approach can not only cross-sectionally predict disease and their gene expression levels but also their longitudinal disease trajectory: it is sensitive and accurate enough to detect disease progression with much fewer subjects or shorter time scales than existing primary endpoints. Our work demonstrates that data-derived wearable biomarkers have the potential to substantially reduce clinical trial durations and a first in-human demonstration of reconstructing *FXN* gene expression levels from behavioral data alone.

# (#462) Multi-omics analysis reveals very long chain ceramides as potential biomarkers and therapeutic target in Friedreich's ataxia

Wednesday, 2nd November - 11:00: Breakout: Biomarkers (Lalique Ballroom) - Oral - Abstract ID: 462

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 462

***Dr. Dezhen Wang [1], Dr. Cotticelli Maria [2], Dr. David Lynch [3], Dr. CLEMENTINA Mesaros [1]***

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

Friedreich's Ataxia (FRDA) is an autosomal neurodegenerative disease caused by the deficiency of protein frataxin. Frataxin functions in the assembly of iron-sulfur clusters that are important for iron homeostasis and metabolic functions. To identify metabolic features that can be used for potential biomarkers in FRDA plasma, we performed a targeted multi-omics (metabolomics, lipidomics, and proteomics) analysis using a discovery-validation cohort design. Muti-omics analysis revealed that FRDA patients had dysregulated sphingolipid metabolism, phospholipid metabolism, citric acid cycle, amino acid metabolism, and apolipoprotein metabolism. Sphingolipid dysfunctions were revealed by decreased very long chain ceramides but unchanged long chain ceramides in FRDA plasma, which resulted in the increased ratio of long chain ceramides to very long chain ceramides. Decreased very long chain ceramides distinguished FRDA patients from healthy controls and showed good predictive capacities with AUC values from 0.75 to 0.85. Furthermore, by performing lipidomic and stable isotope tracing experiments in induced pluripotent stem cell differentiated cardiomyocytes (iPSC-CMs) , we demonstrated that frataxin deficiency disrupted ceramide synthesis, and preferentially enriched long chain ceramides and depleted very long chain ceramides. Finally, machine learning model increased the prediction of FRDA using the combination of three metabolites. In conclusion, decreased very long chain ceramides are potential biomarkers and therapeutic target in FRDA patients.

# Sensory and corticospinal signs before ataxia onset in SCA1 and SCA3: the READISCA study

Wednesday, 2nd November - 11:15: Breakout: Biomarkers (Lalique Ballroom) - Oral - Abstract ID: 219

***Dr. Sophie Tezenas du Montcel*** [1], ***Dr. Emilien Petit*** [2], ***Mr. Titilayo Olubajo*** [3], ***Dr. Jennifer Faber*** [4], ***Mrs. Pauline Lallemant-Dudek*** [5], ***Dr. Khalaf O. Bushara*** [6], ***Prof. Susan L. Perlman, MD*** [7], ***Dr. Sub H. Subramony*** [8], ***Dr. David Morgan*** [9], ***Mrs. Brianna Jackman*** [9], ***Dr. Henry Paulson*** [10], ***Dr. Gulin Oz*** [11], ***Prof. Thomas Klockgether*** [12], ***Prof. Alexandra Durr*** [13], ***Dr. Tetsuo Ashizawa*** [14]

***1.*** *Sorbonne University,* ***2.*** *Paris Brain Institute (ICM), Sorbonne Université,* ***3.*** *The Houston Methodist Research Institute, Houston, TX, USA.,* ***4.*** *German Center for Neurodegenerative Diseases (DZNE), Bonn,* ***5.*** *Sorbonne Universite, Paris Brain Institute, Inserm, INRIA, CNRS, APHP, Paris, France,* ***6.*** *Department of Neurology, University of Minnesota, Minneapolis,* ***7.*** *University of California, Los Angeles, CA, USA,* ***8.*** *Norman Fixel Center for Neurological Disorders, College of Medicine, University of Florida, Gainesville, FL 32611, USA,* ***9.*** *Michigan State University, East Lansing, MI, USA,* ***10.*** *University of Michigan,* ***11.*** *Center for Magnetic Resonance Research, Department of Radiology, University of Minnesota, Minneapolis, Minnesota, United States,* ***12.*** *German Center for Neurodegenerative Diseases, Bonn,* ***13.*** *ICM,* ***14.*** *The Houston Methodist Research Institute*

Background and Objective: READISCA is a prospective, longitudinal observational study of spinocerebellar ataxias type 1 and 3 to provide essential markers for therapeutic interventions. We assessed clinical outcome assessment, imaging and blood biomarker scores during preataxic and early ataxic stages in SCA1 or SCA3 mutation carriers and matched controls.

Methods: We enrolled 200 participants from 18 US and two European ataxia referral centers. READISCA uses a large battery of clinical, cognitive, quantitative motor, neuropsychological measures and plasma neurofilament light chain (NfL) measurements. We calculated estimated time from onset as the difference between the age at the baseline visit and the predicted age at ataxia onset.

Results: There were 45 carriers of a pathological *ATXN1* expansion including 31 ataxic subjects (median SARA: 9 [7;10]) and 14 preataxic individuals (1 [0;2]); 116 carriers of a pathological *ATXN3* expansion, including 80 ataxic subjects (7 [6;9]) and 36 preataxic individuals (1 [0;2]). In addition, we enrolled 39 controls. Plasma NfL levels were significantly higher in preataxic individuals than controls, despite similar mean age (controls: 5.7 pg/mL, SCA1: 18.0 pg/mL (P <0.0001), SCA3: 19.8 pg/mL (P<0.0001). Preataxic individuals differed from controls by significantly more upper motor signs (SCA1 P=0.0003, SCA3 P=0.003) and by the presence of sensor impairment and diplopia in SCA3 (P=0.0448, and 0.0445 respectively). Ataxic participants were worse than preataxic individuals for most of the clinical outcomes.

Discussion and Conclusion: READISCA showed the feasibility of harmonized data acquisition in a multi-national network of 200 baseline SCA1, SCA3 and control individuals. NfL alterations, early sensory ataxia and corticospinal signs were quantifiable between preataxic participants and controls. Ataxic patients differed in many parameters from controls and preataxic individuals, with a graded increase of abnormal measures from control to preataxic to ataxic cohorts.

Acknowledgments: NIH-U01-NS104326

# Cerebrospinal fluid proteomic analysis in Friedreich ataxia

Wednesday, 2nd November - 11:30: Breakout: Biomarkers (Lalique Ballroom) - Flash talk - Abstract ID: 313

***Prof. Massimo Pandolfo*** [1], ***Dr. Chiara Dionisi*** [2], ***Ms. Virginie Imbault*** [2], ***Dr. David Communi*** [2]

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**Background.** Several treatments for Friedreich ataxia (FA) will be in clinical trials in the next few years. Regulatory authorities have specific pathways for "orphan" drugs allowing the use of a validated biomarker for initial approval. We aimed to identify changes in cerebrospinal fluid (CSF) proteins of FA patients as potential biomarkers in therapeutic trials.

**Methods.** CSF was obtained from 5 FA patients (4 females, 1 male). Median age of onset was 18 (7-23); median disease duration was 15 years (8-45); median GAA1 was 450 (445-780). Two patients were ambulatory (SARA 11 and 16), three used a wheelchair (SARA 26.5, 27.5, 33).

Nineteen residual CSF samples were used as controls. All CSF samples had normal cells, protein and glucose.

Proteins were identified by label-free data-dependent acquisition mass spectrometry (MS) coupled to micro-high performance liquid chromatography. Samples were re-analyzed for protein quantification using SWATH acquisition MS. Statistical analysis was performed using Protein Pilot, Paragorn and Peak Analyst ®. Differentially expressed proteins (DEPs) ontology and involved pathways were analyzed using Panther and Reactome.

**Results.** We found 172 DEPs (92 up, 80 down) between FA patients and controls at $P<0.05$, 34 DEPs (28 up, 6 down) at $P<0.0001$. Remarkably, there was no overlap between FA patients and controls for seven upregulated and six downregulated DEPs. Represented pathways included extracellular matrix organization, signalling, the complement cascade, adhesion molecules, synaptic proteins, neurexins and neuroligins.

**Discussion.** This study supports the hypothesis that the quantitative analysis CSF proteins may provide robust biomarkers for clinical trials as well as shed light on pathogenic mechanisms. Interestingly, DEPs in FA patients CSF point to neurodegeneration and neuroinflammation processes that may respond to treatment. Results need validation with specific protein assays and in a larger cohort of FA patients, which may also allow to correlate DEPs with clinical and genetic data.

# (#177) Gait biomarker allow to capture robust longitudinal change in Spinocerebellar ataxia type 3 (SCA3) within one year

Wednesday, 2nd November - 11:37: Breakout: Biomarkers (Lalique Ballroom) - Flash talk - Abstract ID: 177

***Dr. Winfried Ilg*** [1], ***Mr. Bjoern Mueller*** [1], ***Dr. Jennifer Faber*** [2], ***Dr. Judith van Gaalen*** [3], ***Prof. Bart van de Warrenburg*** [3], ***Prof. Thomas Klockgether*** [4], ***Prof. Ludger Schöls*** [1], ***Prof. Matthis Synofzik*** [5]

***1.*** *Hertie Institute for Clinical Brain Research, Tübingen,* ***2.*** *Neurology Univ. Hospital Bonn, DZNE,* ***3.*** *Department of Neurology, Donders Institute for Brain, Cognition, and Behaviour, Radboud University Medical Center, Nijmegen,* ***4.*** *German Center for Neurodegenerative Diseases, Bonn,* ***5.*** *University of Tübingen*

Background and Objective

Measures of gait variability assessed by different recording modalities have shown sensitivity to ataxia severity in cross-sectional studies by correlating with clinical ataxia scores. However, to serve as a valid biomarker in upcoming intervention studies for SCAs, these gait measures have to prove their sensitivity to individual, short-term longitudinal change. Here, we present the first longitudinal gait analysis study in SCAs, selecting SCA3 as the paradigmatic example.

Methods

We performed a multi-centric (Bonn, Nijmegen, Tübingen), combined cross-sectional and longitudinal (1-year interval) analysis in 28 SCA3 subjects (including 8 pre-ataxic mutation carriers). Gait movements were assessed at each site by a multi-kinect recording system with six cameras. Gait was analyzed with preferred and slow speed, and analysis focused on measures of spatio-temporal variability.

Results

Cross-sectional analysis showed increased variability in SCA3 patients compared to healthy subjects (step length variability, lateral sway, p=0.01), with high correlations to clinical ataxia severity (SARA score, lateral sway p=0.0057, effect size ρ=0.45; step length variability p=0.0032, ρ=0.48).

Longitudinal analysis revealed significance changes in gait measures between baseline and one-year follow-up, with high effect sizes (step length variability: p=0.03, effect size $r_{prb}$=0.74; lateral sway: p=0.01, $r_{prb}$=0.855). Sample size estimation for lateral sway shows a required cohort size of n=57 for detecting a 50% reduction of natural progression by a hypothetical intervention. In contrast, the SARA score (baseline mean: 5.57, follow-up mean: 6.18, p=0.35, effect size $r_{prb}$=0.18) failed to detect longitudinal changes (required sample size SARA n=197).

Discussion

Measures of gait variability capture ataxic-specific gait changes with high sensitivity to longitudinal change, even within one year. They thus present promising motor biomarkers in upcoming intervention studies.

# (#253) Quantitative motor assessment of upper limb ataxia with Q-motor: a cross-sectional validation study including novel ataxia tasks

Wednesday, 2nd November - 11:44: Breakout: Biomarkers (Lalique Ballroom) - Flash talk - Abstract ID: 253

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 253

***Dr. Andreas Traschütz*** [1], ***Mr. Dominik Hermle*** [1], ***Mr. Robin Schubert*** [2], ***Mr. Pascal Barallon*** [2], ***Dr. Rebecca Schuele*** [1], ***Dr. Ralf Reilmann*** [2], ***Prof. Matthis Synofzik*** [3]

***1.*** *Hertie Institute for Clinical Brain Research, Tübingen,* ***2.*** *George-Huntington-Institute, Münster,* ***3.*** *University of Tübingen*

**Background and Objective:** With its relevance for everyday function**,** upper limb coordination is a promising, yet insufficiently explored domain for digital-motor outcome assessment in clinical ataxia trials, especially when ambulation is lost. Leveraging the trial-ready quantitative motor (Q-Motor) system, we here developed and validated a comprehensive task battery to capture upper limb ataxia.

**Methods:** In this cross-sectional single-center study, we recruited 46 patients with predominantly degenerative cross-genotype cerebellar ataxias (mean age: 50 years, range: 18-80; mean SARA: 12 points, range: 2-28), and 48 age- and sex-matched controls. Q-Motor assessments with a force transducer and an electromagnetic position sensor comprised both existing bilateral tasks particularly promising for ataxia (foot tapping, finger tapping, diadochokinesia, grip lift) (47 parameters/side), and tasks newly designed to capture ataxia (multi-directional two-dimensional target pointing, spiral drawing) with the dominant hand (450 parameters, including spatial, temporal, spatiotemporal, and texture measures).

**Results:** Target hits per second (AUC: 0.97) and frequency of foot tapping, finger tapping or diadochokinesia (AUC: 0.91-0.94) were excellent discriminators between ataxia and controls. Target hits per second and finger tapping frequency correlated strongly with the SARA (Spearman rho: -0.87 and -0.81), and even specifically with the sum of its upper limb items (-0.85 and -0.72). For diadochokinesia, temporal variability showed the strongest correlation with the SARA (0.71) and its upper limb composite (0.67, all $p<0.001$). Parameters in upper limb tasks had moderate to strong correlations with activities of daily living function (FARS ADL, $|rho|$: 0.5-0.6), and dexterity as assessed by the 9-hole peg test (0.53-0.78, all $p<0.001$).

**Discussion and Conclusion:** Severity of upper limb ataxia can be captured by the Q-Motor system with physiologically interpretable and functionally relevant measures of a finger tapping, diadochokinesia, and a novel two-dimensional target pointing task. Longitudinal assessments are ongoing to explore their responsiveness to change in a clinical trial setting.

# (#159) A natural history study to track brain and spinal cord changes in individuals with Friedreich's ataxia: The TRACK-FA protocol.

Wednesday, 2nd November - 11:51: Breakout: Biomarkers (Lalique Ballroom) - Flash talk - Abstract ID: 159

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 159

***Prof. Nellie Georgiou-Karistianis [1], Dr. Louise A Corben [2], Dr. Kathrin Reetz [3], Dr. Helena Bujalka [1], Dr. Isaac Adanyeguh [4], Dr. Manuela Corti [5], Dr. Dinesh Deelchand [4], Prof. Martin B Delatycki [2], Dr. Imis Dogan [3], Dr. Rebecca Evans [6], Ms. Jennifer Farmer [7], Dr. Marcondes Franca [8], Dr. William Gaetz [9], Dr. Ian Harding [10], Dr. Karen Harris [11], Dr. Steven Hersch [12], Dr. Richard Joules [13], Dr. james joers [4], Dr. Michelle Krishnan [14], Dr. Michelle Lax [13], Prof. Eric Lock [15], Dr. David Lynch [16], Dr. Thomas Mareci [17], Mr. Sahan Muthuhetti Gamage [11], Prof. Massimo Pandolfo [18], Dr. Marina Papoutsi [13], Dr. Gabriele Proetzel [6], Dr. Thiago Rezende [8], Prof. Timothy Roberts [9], Dr. Sandro Romanzetti [3], Prof. Jörg Schulz [3], Dr. Traci Schilling [19], Dr. Adam Schwarz [6], Dr. Sub H. Subramony [20], Dr. Bert Yao [19], Dr. Stephen Zicha [6], Dr. Christophe Lenglet [4], Prof. Pierre-Gilles Henry [4]***

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**Background and objective**

Drug development for neurodegenerative diseases such as Friedreich ataxia (FRDA) is limited by a lack of validated, sensitive biomarkers of disease progression. TRACK-FA aims to assess the potential value of neuroimaging biomarkers and provide a scientific basis for instituting them in clinical trials.

**Methods**

200 individuals with FRDA and 107 control participants, with a minimum age of five years will be recruited across seven international study sites.

Inclusion criteria for participants with genetically confirmed FRDA involve, age of disease onset ≤ 25 years, disease duration ≤ 25 years, Friedreich Ataxia Rating Scale (FARS) functional staging score of ≤ 5 and a total modified FARS score of ≤ 65, on enrolment into the study. Control participants will be matched for handedness, age, years of education and gender.

Participants will be evaluated at three study visits over two years. Each visit comprises of a harmonized Magnetic Resonance Imaging (MRI) and Spectroscopy (MRS) scan of the brain and spinal cord, clinical, cognitive, mood and speech assessments, and collection of a blood sample.

**Results**

Primary outcomes, previously informed by neuroimaging studies, will include measures of spinal cord and brain morphometry, spinal cord and brain microstructure (using diffusion MRI), brain iron accumulation (using Quantitative Susceptibility Mapping) and spinal cord biochemistry (using MRS).

Secondary and exploratory outcome measures include clinical/cognitive assessments and blood biomarkers used for internal validation of primary outcome measures, and to investigate their relationship with the underlying neuropathology.

The study commenced in February 2021, with a total of 69 FRDA and 29 control participants enrolled as of the submission of this abstract.

**Discussion and conclusion**

Prioritising areas of immediate need, TRACK-FA aims to deliver a set of clinical trial ready neuroimaging biomarkers. Once validated, these biomarkers can be used to measure the efficacy of new therapeutics in forestalling disease progression.

# Plenary Session: Cerebellar non-motor circuits and functions

# Invited Talk: Cerebellum and non-motor behaviors - cerebellar contributions to autism

Wednesday, 2nd November - 14:00: Plenary: Cerebellar Non-motor Circuits and Functions (Crystal Ballroom) - Invited Speaker - Abstract ID: 521

***Dr. Peter Tsai*** [1]

**1.** *Peter O'Donnell Jr. Brain Institute, Department of Neurology & Neurotherapeutics, UT Southwestern Medical Center, Dallas, TX*

The cerebellum has traditionally been thought to have exclusive roles in regulation of motor-centric functions. Examination of the connectivity of the cerebellum, however, reveals prominent connections to brain regions that aren't primary motor in their function. Moreover, cerebellar processes - especially during development - appear to result in robust non-motor sequelae, and data from many neuropsychiatric and neurodevelopmental conditions increasingly implicate the cerebellum in their pathogenesis. Our lab has focused on these non-motor roles for the cerebellum with a focus on cerebellar roles in neurodevelopmental conditions: identifying critical roles for the cerebellum in non-motor behaviors, developing a regional topography for these cerebellar-regulated non motor behaviors, examining the cerebellar-regulated brain-wide networks contributing to these behaviors, and examining whether it is possible to leverage these discoveries for clinical benefit.

# (#496) Cerebello-cerebral functional connectivity in SCA3 prior to ataxia onset: Resting state fMRI findings from READISCA

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 496

Wednesday, 2nd November - 14:30: Plenary: Cerebellar Non-motor Circuits and Functions (Crystal Ballroom) - Oral - Abstract ID: 496

***Dr. Sheeba Anteraper*** [1], ***Dr. Ying Zhang*** [2], ***Dr. Michal Povazan*** [3], ***Dr. Guita Banan*** [4], ***Dr. Philipp Ehses*** [5], ***Dr. Jennifer Faber*** [5], ***Dr. james joers*** [6], ***Dr. Romain Valabrègue*** [7], ***Dr. Chiadi Onyike*** [8], ***Dr. Peter Barker*** [9], ***Dr. Jeremy D. Schmahmann*** [10], ***Dr. Eva-Maria Ratai*** [11], ***Dr. Sub H. Subramony*** [12], ***Dr. Thomas Mareci*** [13], ***Dr. Khalaf O. Bushara*** [14], ***Dr. Henry Paulson*** [15], ***Prof. Alexandra Durr*** [16], ***Dr. Thomas Klockgether*** [5], ***Dr. Tetsuo Ashizawa*** [17], ***Dr. Christophe Lenglet*** [6], ***Dr. Gulin Oz*** [18]

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**Background and Objective**

Spinocerebellar Ataxias (SCAs) are rare, autosomal dominant diseases that result in progressive degeneration of the cerebellum and its connections. There is a strong need to improve our mechanistic understanding of the changes in cerebello-cerebral circuitry prior to ataxia onset so that novel therapeutic strategies can be developed. Analyzing high quality magnetic resonance imaging (MRI) data with superior spatio-temporal resolution from the NIH-funded project, “Clinical Trial Readiness for SCA1 and SCA3 (READISCA),” will guide such efforts precipitating clinical translation.

**Methods**

We performed seed-based resting-state functional connectivity (RsFc) analysis on preataxic SCA3 individuals (N=24, SARA<3) and matched healthy controls (N=15) using the CONN toolbox. We employed cerebellar regions of interest (ROIs) generated from functional gradient values (a continuous measure useful to establish clusters of voxels based on functional similarity) derived from high spatio-temporal resolution resting state fMRI datasets from the Human Connectome Project (N=1003). We used the top 5% values of gradient 1 (default-mode network [DMN] and language) and gradient 2 (working memory and frontoparietal/ventral-dorsal attention), and the lowest 5% values of gradient 1 (motor).

**Results**

Contrasting cerebellar ROIs corresponding to DMN, significant cerebello-cerebral RsFc differences were found in regions corresponding to cerebral cortical DMN such as medial pre-frontal and posterior cingulate cortices (height-threshold of $p<0.005$ and FDR cluster-threshold of $p<0.05$). Other cerebellar ROI, e.g. motor, did not reveal any between-group differences, speaking to the non-motor nature of functional differences prior to ataxia manifestation.

**Discussion and Conclusion**

Over the past decade non-motor functions of the cerebellum and its involvement in cognitive and affective processing have been substantiated. Our preliminary results highlight the sensitivity of resting-state fMRI to detect preataxic changes in SCA3 gene carriers. Elucidating the role of cerebello-cerebral networks at this early disease stage is expected to facilitate the development of interventions such as non-invasive cerebellar stimulation.

# The Cerebellar Cognitive Affective Syndrome Scale in Spinocerebellar Ataxias: A CRC-SCA/READISCA Study

Wednesday, 2nd November - 14:45: Plenary: Cerebellar Non-motor Circuits and Functions (Crystal Ballroom) - Oral - Abstract ID: 124

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**Background:** The Cerebellar Cognitive Affective/Schmahmann Syndrome (CCAS) is documented in the spinocerebellar ataxias (SCAs). The 10-domain CCAS Scale (CCAS-S), designed to detect the CCAS, provides a total raw score, total domains meeting fail criteria (total fail score), and CCAS category (Possible=1 fail, Probable=2 fails, Definite=3 fails). Using the CCAS-S, we investigated CCAS prevalence in the most common SCAs; the ability of the CCAS-S to discriminate between Symptomatic, Pre-symptomatic, and Control individuals; and CCAS-S correlates.

**Methods:** Using CRC-SCA/READISCA natural history data, we analyzed CCAS-S performance in 309 individuals Symptomatic for SCA1, SCA2, SCA3, SCA6, SCA7, or SCA8, 26 individuals Pre-symptomatic for SCA1 or SCA3, and 37 non-carrier Controls. We evaluated the impact of age and education on performance, and compared total raw, fail, and domain scores between Symptomatic, Pre-symptomatic, and Control groups, and between SCA types. We calculated sensitivity and selectivity amongst Symptomatic individuals and Controls, respectively, and correlated CCAS-S performance of Symptomatic individuals against genetic repeat length, onset age, disease duration, motor ataxia severity, depression, and fatigue.

**Results:** Definite CCAS was identified in 46% of the Symptomatic group, matching the original scale validation results, with a 5.4% false positive rate amongst Controls. Symptomatic individuals had poorer global performance than Controls after accounting for age and education, which significantly influenced performance. Verbal fluency tasks most consistently discriminated Symptomatic individuals from Controls. Performance did not differ between Controls and Pre-symptomatic individuals (unknown nearness to symptom onset). Amongst clinical parameters, CCAS-S correlated most consistently with motor ataxia severity.

**Discussion:** The high CCAS prevalence in a large SCA cohort, revealed using the CCAS-S, underscores the CCAS as the

third cornerstone of clinical ataxiology, accompanying motor and vestibular deficits. These results have important implications for SCA patient care, emphasizing the clinical relevance of incorporating the cerebellum into the neural circuits subserving cognition and emotion.

# (#419) Impulsivity Trait in Cerebellar Ataxia and Parkinson Disease

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 419

Wednesday, 2nd November - 15:00: Plenary: Cerebellar Non-motor Circuits and Functions (Crystal Ballroom) - Oral - Abstract ID: 419

***Dr. Sheng-Han Kuo*** [1], ***Ms. Tiffany Chen*** [1], ***Dr. Chi-Ying Lin*** [2], ***Ms. Megan Auman*** [3], ***Ms. Nadia Amokrane*** [1], ***Ms. Natasha Desai*** [1], ***Mr. Hakmook Kang*** [3], ***Dr. Daniel Claassen*** [3]

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**Background and objectives:** Individuals with cerebellar ataxia (CA) can develop impulsive behavioral symptoms, often resulting in negative interpersonal consequences, detrimentally impacting their quality of life. Limited evidence exists concerning impulsivity in CA and its associated behavioral changes. We assessed impulsive traits in CA using the Barratt impulsivity scale (BIS-11) and compared them with those of Parkinson disease (PD), to investigate the differences in the impulsive trait profiles between CA and PD.

**Methods:** We conducted a dual-center cross-sectional study with CA and PD subjects enrolled through consecutive sampling from movement disorders clinics at Columbia University and Vanderbilt University, respectively. Age-matched controls were recruited at the respective institutions. Participants were excluded if they had prior or comorbid neurological and psychiatric diseases known to be associated with impulsivity. All subjects completed the BIS-11 questionnaire as a measure of impulsive traits. We used a general linear model and a least absolute shrinkage and selection operation regression to compare the total, subscale, and individual items of the BIS-11 scores between groups. Subgroup analyses were performed to isolate cerebellar contributions to impulsivity from potential effects of extracerebellar pathology and dopaminergic dysfunction or medications.

**Results:** A total of 190 participants: 90 age-matched controls, 50 CA, and 50 PD participants completed the assessments. Persons with CA reported 9.7% greater BIS-11 scores than controls ($p < 0.001$), while persons with PD participants reported 24.9% higher than controls ($p < 0.001$). In CA, the most impacted domain of impulsivity was non-planning. In contrast, persons with PD noted greater impulsivity across the non-planning, attentional, and motor domains.

**Discussion:** Impulsivity in CA is uniquely driven by the non-planning trait, unlike in PD. This suggests that the cerebellum and basal ganglia may differentially govern impulsive behaviors with the cerebellum contributing to the brain circuitry of impulsivity in a domain-specific manner.

# (#191) Cognitive-affective manifestations in ataxic and pre-ataxic phases of Spinocerebellar Ataxia type 3/Machado-Joseph Disease

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 191

Wednesday, 2nd November - 15:15: Plenary: Cerebellar Non-motor Circuits and Functions (Crystal Ballroom) - Oral - Abstract ID: 191

***Dr. Gabriela Bolzan*** [1], ***Ms. Maria Eduarda Müller Eyng*** [1], ***Prof. Vanessa Bielefeldt Leotti*** [1], ***Prof. Maria Luiza Saraiva-Pereira*** [1], ***Prof. Laura Bannach Jardim*** [2]

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**Background:** Cognitive deficits have been related to Machado-Joseph Disease (SCA3/MJD), but the Cerebellar Cognitive Affective/Schmahmann Syndrome (CCAS) needs further investigation. The present study aimed to characterize cognitive-affective deficits and their natural history in ataxic and pre-ataxic SCA3/MJD carriers.

**Methods:** Subjects at 50% risk, ataxic carriers and unrelated controls were evaluated in-person or in virtual settings with a structured interview, CCAS Scale (CCAS-S), Stroop Color-Word Test (SCWT), Reading the Mind in the Eyes Test (RMET), and Hamilton Anxiety and Depression Scales. SARA >2.5 or FARS-ADL >4 divided carriers into ataxic and pre-ataxic. Time after onset or time left to gait ataxia onset (TimeToAfterOnset) were estimated. Differences between groups and correlations with TimeToAfterOnset, SARA and FARS-ADL were checked.

**Results:** After random selection, 116 individuals were included: 23 ataxic, 35 pre-ataxic, and 58 controls. CCAS-S, semantic fluency, phonemic fluency, category switching, affect, SCWT and RMET showed significant differences between ataxic and controls, while pre-ataxic seemed to display intermediate values that mostly did not differ from both controls and ataxic. These variables correlated with TimeToAfterOnset and SARA scores in the carriers' group. Correlations with SARA were stronger in the pre-ataxic group. CCAS-S had the strongest correlations with time and SARA.

**Discussion:** Cognitive-affective deficits in SCA3/MJD involved executive function, language, affect, and social cognition, which seem to be altered prior to the ataxia onset, follow disease progression, and progress with a different pace in the ataxic stage. CCAS-S was the most promising biomarker and should be evaluated in longitudinal studies.

# Breakout: Emerging Therapies (Preclinical)

# (#334) CRISPR gene-editing rescues molecular and motor phenotypes in Fmr1 CGG knock-in mice

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 334

Wednesday, 2nd November - 16:00: Breakout: Emerging Therapies (Preclinical) (Crystal Ballroom) - Oral - Abstract ID: 334

***Dr. Carolyn Yrigollen*** [1], ***Mr. Bryan Simpson*** [1], ***Mr. Euyn Lim*** [1], ***Dr. Yong Hong Chen*** [1], ***Dr. Beverly Davidson*** [2]

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Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) is one of several disorders caused by an expanded CGG repeat in the 5' untranslated region of the Fragile X gene (*FMR1*). FXTAS occurs in individuals with a premutation allele (55-200 CGGs). The *Fmr1* CGG knock-in (KI) mouse model with approximately 150 CGG repeats recapitulates features of FXTAS and was used to evaluate a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) Cas9 based therapy that targets the mutation *in vivo*.

Cas9 and guideRNAs were delivered by adeno associated viral (AAV) vectors bilaterally into the striatum of adult mice to target the CGG repeats for deletion. Three weeks post injection, treated striatal tissue of KI mice had decreased *Fmr1* mRNA expression, rescuing the three-fold upregulation seen in untreated KI mice. The Fmr1 protein was still synthesized at normal levels. DNA was sequenced and both partial and complete deletion of the CGG repeats were observed.

To study phenotypic rescue, the CRISPR viral vectors were delivered by intracerebroventricular injection of neonatal mice. Motor performance on an accelerating rotarod was rescued in KI mice treated with the CRISPR therapy but not those treated with a sham therapy at 28-30 weeks of age. Importantly, treated mice performed similarly to their WT littermates. Robust expression of SpCas9 and GFP were observed in the cortex, hippocampus, striatum, and cerebellum, of treated KI animals by qRT-PCR a year post injection. These regions did not have reduced *Fmr1* levels, likely because of incomplete transduction and editing of the cell population with this delivery strategy.

These results are the first *in vivo* editing of *Fmr1* CGG repeats using CRISPR. The therapeutic benefits, including prevention of motor deficits and rescue of molecular dysregulation in KI mice, are important indicators that CRISPR mediated gene-editing is worth further development for treatment of FXTAS and other Fragile X-associated disorders.

# (#481) Progress towards a viral gene therapy for Christianson syndrome

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 481

Wednesday, 2nd November - 16:15: Breakout: Emerging Therapies (Preclinical) (Crystal Ballroom) - Oral - Abstract ID: 481

***Dr. Collin Anderson*** [1], ***Dr. Karla P. Figueroa*** [2], ***Dr. Sharan Paul*** [1], ***Dr. Warunee Dansithong*** [1], ***Dr. Mandi Gandelman*** [1], ***Dr. Daniel Scoles*** [1], ***Dr. Stefan M. Pulst*** [3]

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**Background and Objective**

The *shaker* rat carries a spontaneous loss-of-function mutation in solute carrier family 9, member A6 (*Slc9a6*, encoding NHE6), leading to Purkinje cell (PC) degeneration and progressive ataxia. Human *SLC9A6* loss-of-function mutations cause Christianson syndrome, an epileptic and ataxic encephalopathy with cognitive and behavioral deficits. We sought to study whether viral-mediated functional complementation in the *shaker* rat might yield symptomatic improvements.

**Methods**

We generated an adeno-associated virus (AAV) using the PhP.eB capsid expressing the rat *Slc9a6* gene, tagged with GFP, and targeted to PCs using a mouse L7-6 (L7) promoter. We administered AAVs through intracerebroventricular injection at 5 weeks of age, prior to PC death. We tracked motor ataxia through 25 weeks of age, and following motor experiments, we harvested cerebella for histology and molecular analyses, quantifying *Slc9a6* mRNA and NHE6 protein expression, as well as several cerebellar health markers. Next, we generated a second AAV using the AAV9 capsid, targeting human Slc9a6 ubiquitously using a CAG promoter and performed a dose response study to evaluate safety.

**Results**

Administration of PHP.eB AAV targeting expression of rat *Slc9a6* to PCs prior to symptom onset reduced the *shaker* motor, molecular, and cellular phenotypes. Functional complementation restoring an average of ~10% wildtype NHE6 not only reduced motor ataxia, but also significantly increased both mRNA and protein expression of several key cerebellar markers, CALB1, PCP2, and RGS8. Safety-focused dose response studies using the CAG promoter and the human *SLC9A6* gene revealed that *shaker* rats tolerated high titers approaching 10^12 vg copies.

**Discussion and Conclusion**

Viral gene therapy with AAV9 may be a viable therapeutic strategy for Christianson syndrome. High doses may be tolerated, although relatively minimal cerebellar NHE6 restoration appears necessary to substantially improve cerebellar health and prevent motor ataxia. Future studies will quantify the relationship between CAG-h*SLC9A6* AAV and phenotypic severity.

# (#256) Intranasal delivery of Extracellular Vesicles carrying silencing sequences alleviates Spinocerebellar Ataxia Type 3 (SCA3)

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 256

Wednesday, 2nd November - 16:30: Breakout: Emerging Therapies (Preclinical) (Crystal Ballroom) - Oral - Abstract ID: 256

***Mr. David Rufino-Ramos*** [1], ***Mr. Kevin Leandro*** [1], ***Dr. Vítor Carmona*** [1], ***Mrs. Inês Morgado Martins*** [1], ***Ms. Patrícia Rosado*** [1], ***Mrs. Rosário Faro*** [1], ***Dr. Rita Perfeito*** [1], ***Dr. Rui Jorge Nobre*** [1], ***Prof. Luís Pereira de Almeida*** [1]

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**Background and Objective**: Extracellular vesicles (EVs) are membrane-based structures produced by cells, with capacity to carry miRNAs, non-coding RNAs with around 22 nucleotides. MiRNA enrichment into EVs is promoted by specific motifs, designated as ExoMotifs.

Therefore, the aim of this work was to investigate whether ExoMotifs would promote packaging of engineered miRNA-based silencing sequences into EVs to use them as therapeutic vehicles to treat Spinocerebellar Ataxia Type 3 (SCA3). SCA3 is a neurodegenerative disorder caused by abnormal over-repetition of a CAG tract within the ataxin-3 (ATXN3) gene, conferring toxic properties to the ATXN3 protein.

**Methods**: An ExoMotif signal was associated with silencing sequences targeting mutant ATXN3 (mutATXN3) to promote its packaging into EVs, which was evaluated by qRT-PCR. Additionally, neuronal targeting proteins were expressed at EVs surface, and their neuronal targeting efficiency was evaluated in vitro and in vivo by immunocytochemistry and flow cytometry. To evaluate target engagement, engineered EVs were administered by daily intranasal administration to mice expressing a mutATXN3 dual luciferase reporter system.

**Results**: We found that silencing sequences with the ExoMotif retained the capacity to silence mutant ataxin-3 and were effectively incorporated into EVs. Furthermore, the bioengineered EVs significantly decreased mutATXN3 mRNA levels in neuronal cells. Importantly, continuous intranasal administration of therapeutic EVs significantly decreased the brain luminescence associated with mutATXN3 dual luciferase reporter system.

**Discussion and Conclusion**: MutATXN3 silencing sequences enriched in EVs reach the brain via intranasal route and are therapeutically active reducing *in vivo* the disease-causing ataxin-3.

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# Inhibiting Hsp90 as a therapeutic approach for ARSACS

Wednesday, 2nd November - 16:45: Breakout: Emerging Therapies (Preclinical) (Crystal Ballroom) - Oral - Abstract ID: 275

***Dr. Suran Nethisinghe*** [1], ***Dr. Rosella Abeti*** [1], ***Mr. Maheswaran Kesavan*** [1], ***Dr. W. Christian Wigley*** [2], ***Prof. Paola Giunti*** [1]

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**Background and Objective:** Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a neurodegenerative disease caused by mutations in the *SACS* gene, encoding sacsin, a large modular protein with conserved domains that suggest a role either as a scaffold in protein folding or in proteostasis. Cells from patients with ARSACS display an altered organisation of the intermediate filament (IF) cytoskeleton and impaired mitochondrial health. Hsp90 is the master regulator of the heat-shock response (HSR). Inhibiting Hsp90 activates the HSR leading to the induction of molecular chaperones which can reduce protein aggregation. Here, we used vimentin bundling as a biomarker of sacsin function to test the therapeutic potential of Hsp90 inhibition by the C-terminal-domain-targeted compound KU-32. We also examined whether KU-32 treatment could improve mitochondrial health.

**Methods:** Dermal fibroblasts from controls, carriers, and patients with ARSACS were incubated with DMSO (vehicle) or KU-32 prior to analysis. For intermediate filament analysis, vimentin was detected by immunofluorescence. Mitochondrial membrane potential ($\Delta\Psi_m$) was measured, and the ability for the electron transport chain (ETC) to maintain $\Delta\Psi_m$ was assessed by challenging with oligomycin. Mitochondrial mass was assessed by measuring mitochondrial and total volume, cell by cell.

**Results:** Fibroblasts from patients with ARSACS have significantly increased vimentin bundling compared to controls, and this was also present in carriers despite them being asymptomatic. KU-32 treatment significantly reduced vimentin bundling in carrier and patient cells. We found that cells from patients with ARSACS were unable to maintain $\Delta\Psi_m$ upon challenge with mitotoxins and KU-32 treatment could restore ETC function. Mitochondrial mass was significantly increased in cells from a patient with ARSACS but was restored to control levels upon treatment with KU-32.

**Discussion and Conclusion:** Our findings suggest that targeting the HSR by Hsp90 inhibition alleviates vimentin bundling and may represent a promising area for development of therapeutics for ARSACS.

# (#96) Development of an ATXN3-targeted siRNA therapy for Spinocerebellar ataxia type 3

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 96

Wednesday, 2nd November - 17:00: Breakout: Emerging Therapies (Preclinical) (Crystal Ballroom) - Flash talk - Abstract ID: 96

***Ms. Anna J. Barget [1], Mr. Jason A. Gilbert [2], Ms. Madison R. Salvato [1], Dr. Elane Fishilevich [2], Dr. Maria do Carmo Costa [1]***

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Spinocerebellar ataxia type 3 (SCA3) is caused by a dominantly inherited expanded CAG repeat in the *ATXN3* gene. Our goal was to develop a non-allele-selective siRNA targeting *ATXN3* effective to broadly decrease levels of human mutant *ATXN3* gene products throughout the brain of SCA3-YACMJD84.2 (Q84) transgenic mice.

We subsequently screened anti-*ATXN3* siRNAs for efficacy to decrease levels of *ATXN3* transcripts (qPCR) in Be(2)c cells, AAV-ATXN3-injected mouse livers, and Q84 mouse livers. Next, we intracerebral ventricle (ICV)-injected two siRNAs at two doses (150 and 300mg) in 7-week-old hemizygous Q84 mice. Q84 and non-transgenic mice injected with vehicle were also included in the study. We collected brains from treated mice two- and four-weeks post-injection and evaluated: a) siRNA distribution by immunohistochemistry (IHC); b) levels of human mutant (hm) *ATXN3* and mouse *Atxn3* transcripts (qPCR) and proteins (Western blot (WB) and IHC); and c) signs of potential inflammation by assessment of *Gfap* and *Iba1* levels (qPCR, WB and IHC).

siRNA was broadly distributed throughout the brain showing uptake by neurons and non-neuronal cells, with both treatment durations displaying similar results. While both anti-*ATXN3* siRNAs were effective to decreasing *ATXN3* transcripts and proteins, in a dose-dependent way, in the four separate brain regions (brainstem, cerebellum, spinal cord and forebrain) of Q84 mice, siRNA-2 showed the highest efficacy. Across the four brain regions, siRNA-2-treated Q84 mice (300mg) displayed 29-49% of hm*ATXN3* transcripts, 34-46% of hmATXN3 soluble protein, 12-49% of ATXN3 high-molecular-weight species, 32-52% of mouse *Atxn3* transcripts, and 23-31% of mouse Atxn3 protein of vehicle-treated Q84 mice. Intranuclear neuronal accumulation of ATXN3 was highly reduced with no signs of astrogliosis or neuroinflammation in siRNA-treated mice.

Anti-*ATXN3* siRNAs showed high potency to broadly decreasing hm*ATXN3* gene products in SCA3 transgenic mouse brains with no signs of inflammation and, thus could represent a possible therapy for SCA3.

# (#493) Metformin decreases RAN proteins and improves behavioral phenotypes in SCA8 BAC mice

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 493

Wednesday, 2nd November - 17:07: Breakout: Emerging Therapies (Preclinical) (Crystal Ballroom) - Flash talk - Abstract ID: 493

***Dr. Setsuki Tsukagoshi*** [1], ***Dr. Lisa E.L. Romano*** [1], ***Ms. S. Elaine Ames*** [1], ***Dr. Monica Banez Coronel*** [1], ***Dr. Tao Zu*** [1], ***Prof. Laura P.W. Ranum*** [1]

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**Background and Objective:** Spinocerebellar ataxia type 8 (SCA8) is caused by a CTG•CAG repeat expansion mutation in the *ATXN8/ATX8OS genes*. Repeat-associated non-AUG (RAN) translation, which was first reported in SCA8, has now been reported in 11 repeat expansion diseases. RAN translation is highly regulated by the double-stranded RNA-dependent protein kinase (PKR) pathway and RAN proteins are dramatically reduced by metformin, an FDA-approved drug and novel PKR inhibitor. Recently, metformin was shown to decrease RAN protein levels, improve behavior, and increase motor neuron survival in transgenic mouse model of a related repeat expansion disorder, *C9orf72* amyotrophic lateral sclerosis. Here we test the hypothesis that metformin will decrease RAN protein toxicity and improve disease in SCA8 mice.

**Methods:** SCA8 BAC transgenic mice, which express *ATXN8* and *ATXN8OS* using the endogenous human promoters, were used to test the effects of metformin on RAN protein levels and behavioral phenotypes. SCA8 and non-transgenic (NT) mice were treated with or without metformin, and DigiGait (16 weeks), rotarod (32 weeks) and immunohistochemistry (IHC) (20 weeks) were used to evaluate the effects of metformin.

**Results:** Metformin-treated SCA8 mice showed improved rotarod (n>15/group, p=0.0017) and DigiGait (e.g. brake, n>15/group, p=0.002) performance compared to untreated SCA8 mice, which was comparable to NT animals. Immunohistochemistry studies showed a dramatic reduction of poly-Serine (n>7 mice/group, p=0.0003) and poly-Glutamine (n>7 mice/group, p=0.0088) RAN proteins in the brainstem of metformin treated compared to untreated SCA8 mice.

**Conclusion and Discussion:** These data show metformin reduces RAN protein load and improves behavior in SCA8 mice. We are currently analyzing additional behavioral, molecular and histopathological phenotypes. Since metformin is an FDA approved drug, if effective in mice it could be rapidly moved into clinical trials to test its efficacy as a safe and affordable treatment for patients with this devastating disorder.

# (#289) Dentatorubral-pallidoluysian atrophy (DRPLA): ASO therapeutic development and understanding the impact of ATN1 CAG expansion

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 289

Wednesday, 2nd November - 17:14: Breakout: Emerging Therapies (Preclinical) (Crystal Ballroom) - Flash talk - Abstract ID: 289

***Dr. Joanna Korecka*** [1], ***Dr. Tojo Nakayama*** [2], ***Dr. Lisseth Burbano*** [2], ***Dr. Boxun Zhao*** [3], ***Ms. Charlotte Oettgen*** [1], ***Ms. Renata DiDonato*** [2], ***Ms. Aleksandra Krzywanska*** [4], ***Ms. Aliza Ben-Varon*** [5], ***Dr. Claudia Lentucci*** [2], ***Ms. Victoria Suslovitch*** [2], ***Ms. Sophie Stroeks*** [2], ***Ms. Diana Chin*** [2], ***Ms. Aubrie Soucy*** [2], ***Dr. Jeffrey Carroll*** [6], ***Dr. Vikram Khurana*** [1], ***Dr. Timothy Yu*** [2]

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**Background:** Dentatorubral-pallidoluysian atrophy (DRPLA) is a rare, progressive brain disorder caused by a CAG trinucleotide expansion within the atrophin-1 gene, *ATN1*. There is currently no treatment for DRPLA and little is known about the consequence of *ATN1* polyQ expansion on neuronal function. Antisense oligonucleotide (ASO)-based therapy is a promising strategy for this disorder.

**Methods:** We developed 2'MOE ASOs to induce RNaseH1-mediated knockdown of *ATN1* as potential gene-targeted therapeutics for DRPLA. We undertook high throughput screening of 392 ASOs designed against 27 regions of *ATN1* conserved between human and mouse, using BE(2)M-17 neuroblastoma cells, followed by microwalking secondary screens of 51 ASOs targeting three promising *ATN1* regions in wild-type and DRPLA patient-derived iPSC neurons. Mouse *in vivo* studies of ASO safety and target engagement are currently ongoing. In parallel, we are using DRPLA patient iPSC-derived neuronal cultures to evaluate the consequences of pathogenic *ATN1* CAG expansion and *ATN1* dose reduction (via ASO-mediated knockdown and CRISPR/Cas9 knockout) on stem cell survival, proliferation, capacity for neuronal differentiation and neuronal function, to **1.** improve our understanding of *ATN1* function, and **2.** infer the safety of *ATN1* knockdown as a clinical strategy.

**Results:** We identified 6 lead gapmer ASOs targeting *ATN1* exonic sequences that demonstrate efficient *ATN1* mRNA knockdown with IC50s between 300-500nM by free-uptake (gymnosis) in iPSC-derived neurons. Preliminary data shows that the increased polyQ expansion in DRPLA patient-derived iPSCs results in accumulation of intracellular p62 aggregates, a pathology amendable by 2 ASO candidates.

**Conclusions & Discussion:** Future studies are aimed at identification of DRPLA-specific neuronal pathology both *in vitro* and *in vivo*, and rescue of the observed phenotypes with ASO-mediated *ATN1* mRNA knockdown. More generally, in this study we will establish a paradigm through which ASO therapeutics for neurodegenerative diseases can be de-risked for clinical trial through patient-matched iPSC modeling.

# (#260) A CAG expansion-selective chemical screen identifies compounds that selectively reduce CAG-expansion transcript levels across spinocerebellar ataxias

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 260

Wednesday, 2nd November - 17:21: Breakout: Emerging Therapies (Preclinical) (Crystal Ballroom) - Flash talk - Abstract ID: 260

***Dr. Hannah Shorrock*** [1], ***Ms. Asmer Aliyeva*** [1], ***Ms. Jesus Frias*** [1], ***Ms. Emily Davey*** [1], ***Ms. Victoria DeMeo*** [1], ***Ms. Claudia Lennon*** [2], ***Ms. Amy Mascorro*** [1], ***Ms. Sharon Shaughnessy*** [1], ***Dr. Kaalak Reddy*** [1], ***Dr. John Cleary*** [1], ***Prof. Andy Berglund*** [1]

***1.*** *RNA Institute, University at Albany-SUNY,* ***2.*** *The RNA Institute*

Background

The spinocerebellar ataxias (SCAs) are a group of dominantly inherited neurodegenerative diseases. A large subgroup of SCAs (1, 2, 3, 6, 7 and 12) are caused by CAG-expansion mutations and involve expression of expansion RNAs, which generally leads to the production of proteins containing expanded polyglutamine tracts that are thought to be toxic. There are currently no approved treatments for these diseases and preclinical therapy development mainly focuses on disease-specific approaches. The objective of this study was to develop a CAG expansion-selective chemical screen to identify compounds with therapeutic efficacy across multiple CAG-expansion SCAs.

Method

We generated a reporter cell line that expresses both a tagged $(CAG)_{60}$ expansion and a no-repeat control using the piggyBac transposon system. We used this cell line to perform a chemical screen of 1584 compounds and selected candidate small molecules that selectively reduce levels of CAG-expansion transcripts and associated polyglutamine expansion proteins for further studies.

Results

Detailed characterization of the lead candidate CAG-expansion reporter cell line shows that it recapitulates transcriptional hallmarks of CAG-expansion SCAs. By performing chemical screens in this cell line, we identified multiple structurally diverse candidate compounds that selectively reduce levels of CAG-expansion transcripts and polyglutamine proteins compared to the zero-repeat control. Lead candidate compounds were able to selectively reduce levels of disease associated expansion transcripts across multiple CAG SCA patient derived fibroblast lines without inducing toxicity or affecting non-expanded alleles in control fibroblasts.

Conclusion

This approach, using a reporter cell line with a CAG repeat expansion that is not in the genetic context of any specific SCA, has enabled the identification of small molecules capable of reducing CAG-expansion transcript levels across multiple SCAs. Together this work has the potential to identify small molecules with therapeutic potential across SCAs and will pave the way for shared therapeutics for CAG-expansion spinocerebellar ataxias.

# Breakout: Imaging

# Longitudinal multimodal MRI in SCA3: Evaluation of imaging biomarker candidates from the early pre-ataxic to the late ataxic stage

Wednesday, 2nd November - 16:00: Breakout: Imaging (Lalique Ballroom) - Oral - Abstract ID: 179

***Dr. Jennifer Faber*** [1], ***Dr. Tamara Schaprian*** [1], ***Prof. Bart van de Warrenburg*** [2], ***Dr. Judith van Gaalen*** [2], ***Prof. Dagmar Timmann*** [3], ***Dr. Katherina Steiner*** [3], ***Dr. Andreas Thieme*** [3], ***Prof. Paola Giunti*** [4], ***Dr. Hector Garcia-Moreno*** [4], ***Dr. Gulin Oz*** [5], ***Dr. james joers*** [5], ***Dr. Khalaf O. Bushara*** [6], ***Dr. Jeroen de Vries*** [7], ***Dr. Heike Jacobi*** [8], ***Dr. Kathrin Reetz*** [9], ***Dr. Janna Krahe*** [10], ***Dr. Micheal Povazan*** [11], ***Dr. Chiadi U. Onyike*** [11], ***Prof. Peter Barker*** [12], ***Dr. Jeannette Hübener-Schmid*** [13], ***Dr. Magda Santana*** [14], ***Dr. Thomas Klockgether*** [1]

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*Background and Objective:* Spinocerebellar ataxia type 3 (SCA3) is the most common inherited ataxia worldwide. Clinical trials with gene silencing approaches will start in the near future. To treat mutation carriers before the clinical onset of the disease is an intriguing option. Disease biomarkers will be important outcome measures for such approaches. Longitudinal measures are a pre-requisite to evaluate their potential.
*Methods:* Within the European spinocerebellar ataxia type / Machado-Joseph disease initiative (ESMI), we assessed standardized longitudinal multimodal magnetic resonance imaging (MRI) (T1-weighted, T2-weighted, diffusion-weighted imaging (DWI) in pre-ataxic and ataxic SCA3 mutation carriers (Baseline=101; 1-year-follow-up=36) and healthy controls. We analyzed the data with voxel-based morphometry (VBM) and volumetry. DWI was analyzed with whole brain tract-based-spatial-statistics (TBSS) and tracking approaches.
*Results:* VBM and volumetric approaches showed atrophy of the anterior lobe, cerebellar white matter and upper cervical spinal cord already in the pre-ataxic stage, but volumetric changes in the 1-year follow up did not differ significantly from healthy controls. TBSS evaluation of fractional anisotropy (FA) showed FA reduction in the cerebellum and brainstem, but not cerebral tracts. We found no significant longitudinal changes. Tracking of the main cerebral tracts did not show significant group differences between SCA3 and healthy controls.
*Discussion and conclusion:* Volumetry of the anterior lobe, the cerebellar white matter, the brainstem, especially the pons, and upper cervical spinal cord are potential biomarkers for assessing disease progression in the pre-ataxic stage. The 1-year follow up may not have been long enough to assess longitudinal changes. Increasing the group size is needed to finally evaluate the various parameters with regard to their usefulness as biomarkers, especially for the early pre-ataxic stage of the disease. A combination of imaging and fluid biomarker such as neurofilament

light (NfL) should be considered.

# Pons and middle cerebellar peduncle diameters are diagnostic of Multiple System Atrophy of the cerebellar type (MSA-C)

Wednesday, 2nd November - 16:15: Breakout: Imaging (Lalique Ballroom) - Oral - Abstract ID: 381

***Dr. Christopher Stephen*** [1], ***Dr. Mark Vangel*** [2], ***Dr. Anoopum Gupta*** [1], ***Mr. Jason MacMore*** [1], ***Dr. Jeremy D. Schmahmann*** [1]

***1.*** *Ataxia Center, Cognitive Behavioral Neurology Unit and Laboratory for Neuroanatomy and Cerebellar Neurobiology, Department of Neurology, Massachusetts General Hospital and Harvard Medical School,* ***2.*** *Biostatistics Center and Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital and Harvard Medical School*

**Background and Objective:**
Multiple System Atrophy of the cerebellar type (MSA-C) is a sporadic, adult-onset synucleinopathy with autonomic neuropathy and ataxia and may have overlap with the parkinsonian form (MSA-P). New diagnostic criteria involves combined clinical, and ill-defined radiological features. We sought to develop an MRI biomarker for the diagnosis of MSA-C.
**Methods:**
In an exploratory cohort (88 MSA-C, 44 spinocerebellar ataxia, 13 Friedreich's ataxia, 15 idiopathic ataxia), we performed baseline/longitudinal measurements (2002-2015) of anteroposterior (AP) mid-pons and transverse middle cerebellar peduncle (MCP) diameters on conventional MRI. 73 Human Connectome Project subjects were sampled to derive normative data and pons diameter-volume correlations. In a validation cohort (49 MSA-C, 13 MSA-P, 99 other ataxias, 79 Parkinson's disease [PD], 275 other movement disorders), we similarly performed baseline/longitudinal measurements (2015-2021).
**Results:**
Normative data (n=73) for pons AP diameter was 23.9±1.6 mm, MCP diameter 16.7.9±1.5 mm. Pons diameter-volume correlation was r=0.94, p<0.0001.
Exploratory cohort:
In MSA-C vs. other ataxias at first scan, pons AP diameter was 19.3±2.6 mm vs. 20.7±2.6 mm, p=0.0006 and mean MCP diameters 12.0±2.6 mm vs. 14.3±2.1 mm, p<0.0001.
Rate of change of axial pons diameter was -1.08±0.45 mm/year in MSA-C vs. -0.16±0.17 mm/year in other ataxias, and MCP diameter -1.37±0.67 mm/year in MSA-C vs. -0.19±0.22 mm/year in other ataxias, both p<0.0001.
Validation cohort:
The findings in MSA-C were similar.
In a large movement disorders cohort excluding MSA-C/P, other ataxias/atypical parkinsonism, AP pons diameter at first scan mean of 22.5±1.5 and MCPs 17.1±1.2 mm differed significantly from MSA/other ataxias.
Pons/MCP measures differed significantly between MSA-C vs. MSA-P, and MSA-P vs. PD but were indistinguishable between Possible, Probable, and Definite MSA-C.
**Discussion and Conclusion:**
Pons and MCP diameters are key biomarkers in MSA-C. A rate of change of ~1 mm/year in each measure is sensitive and specific for the diagnosis of MSA-C.

# Dorsal Root Ganglia Imaging is a Potential Biomarker in Friedreich's Ataxia

Wednesday, 2nd November - 16:30: Breakout: Imaging (Lalique Ballroom) - Oral - Abstract ID: 472

***Dr. Rafaella Tacla*** [1]***, Dr. Thiago Rezende*** [1]***, Dr. Valdir Fialkowski*** [2]***, Dr. Alberto Martinez*** [1]***, Prof. Marcondes C. França Jr*** [1]

***1.*** *Department of Neurology, School of Medical Sciences – University of Campinas (UNICAMP), Campinas, SP, Brazil,* ***2.*** *MR Research Manager Philips Latin America*

Background and Objective: Dorsal root ganglia (DRG) damage is an early and universal feature in Friedreich's ataxia (FA). However, it is not yet clear whether it is due to atrophy, hypoplasia or both. Despite that, no study attempted to evaluate this structure *in vivo* in affected patients. Hence, we developed a high resolution MRI protocol to image lumbar DRG and to quantify their volumes. In the current abstract, we present the initial results of the study showing the comparison between patients vs controls.

Methods: All scans were obtained in a Philips 3T scanner using a surface 16-channel coil. We employed the following sequences: PROSET T2 isotropic, DTI isotropic, and T2-FFE. Eleven healthy controls (5 men, mean age 31.4 years) and 13 FA patients (3 men, mean age 29.6 years) were selected. Segmentation was performed by one of the investigators using the *MRIcron* software to calculate the DRG volume. Data were analyzed using the T test taking BMI, age, and sex as covariates.

Results: We obtained high resolution DRG images using a relatively short acquisition time (17 minutes on average). Acquired data enabled easy segmentation of DRG in both sides from L2 to L5. In an exploratory analysis, we found significant between-group differences from L3 to L5 relative to DRG volumes (controls vs patients) L3: 201.1±51.4 x 157.7±44.8mm3; L4: 216.5 ±41.1 x 174.2 ±53.4mm3; L5: 304.6±72.1 x 227.3±53.2mm3].

Discussion and conclusion: Although preliminary, these results indicate that DRG imaging is feasible in a reasonable acquisition time. This novel MRI-derived parameter looks very promising as a potential biomarker for FA. Further multicentric longitudinal studies enriched with young patients are now needed to validate the clinical relevance of DRG volumetry in FA.

# (#149) Tract-specific spinal cord diffusion tensor imaging in Friedreich's ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 149

Wednesday, 2nd November - 16:45: Breakout: Imaging (Lalique Ballroom) - Oral - Abstract ID: 149

***Dr. Ana Luisa Hernández [1], Dr. Thiago Rezende [1], Dr. Mariana Brito [2], Dr. ALberto Martinez [2], Prof. Marcondes C. França Jr [2]***

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Background and objectives: Spinal cord (SC) damage is a hallmark in Friedreich's Ataxia (FRDA). Neuroimaging has been able to capture some SC macroscopic changes, but no study has evaluated microstructural SC white matter (WM) damage in vivo. We designed a cross-sectional study to evaluate microstructural integrity in SC WM tracts of FRDA patients using diffusion tensor imaging (DTI) with an automated analysis pipeline.

Materials and Methods: We recruited thirty patients and matched healthy controls who underwent 3T MRI. Cervical SC T2 and DWI acquisitions were obtained. Images were processed using the Spinal Cord Toolbox v.4.3.0. We measured cross-sectional area (CSA) and WM DTI parameters (axial diffusivity - AD, fractional anisotropy - FA, radial diffusivity - RD, and mean diffusivity - MD) from levels C2 through C5. Age, duration, and FARS scores were also obtained.

Results: Mean age and disease duration of patients were 31±10 and 11±9 years, respectively. CSA reduction in the FRDA group was present amongst all levels. We also found between-group differences in FA, MD, and RD in total white matter (TWM), dorsal columns (DC), fasciculus gracilis (FG), fasciculus cuneatus (FC), and corticospinal tracts (CST) in all levels. FA and RD from TWM, DC, FC, and CST correlated with FARS scores, and in CST they also correlated with disease duration.

Conclusion: DTI was able to capture abnormalities in SC WM tracts, which correlated with clinical features in FRDA. CSA and CST FA in C2 correlated best with disease severity, whereas DC FA showed the largest effect size to differentiate patients and healthy controls. SC WM microstructure is a potential neuroimaging biomarker to be explored in the disease.

# (#452) In vivo evaluation of dentato-thalamo-cortical tract integrity in Friedreich ataxia using diffusion MRI

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 452

Wednesday, 2nd November - 17:00: Breakout: Imaging (Lalique Ballroom) - Flash talk - Abstract ID: 452

***Dr. Mario Tranfa*** [1], ***Dr. Giuseppe Pontillo*** [1], ***Mrs. Sara Bosticardo*** [2], ***Dr. Chiara Pane*** [3], ***Dr. Simona Schiavi*** [2], ***Ms. Giulia Biolo*** [2], ***Dr. Alessandro Daducci*** [2], ***Prof. Francesco Saccà*** [3], ***Dr. Arturo Brunetti*** [1], ***Prof. Nellie Georgiou-Karistianis*** [4], ***Dr. Ian Harding*** [5], ***Dr. Sirio Cocozza*** [1]

***1.*** *Department of Advanced Biomedical Sciences, University "Federico II", Naples, Italy,* ***2.*** *Diffusion Imaging and Connectivity Estimation (DICE)Lab, Verona,* ***3.*** *Department of Neurosciences and Reproductive and Odontostomatological Sciences, University of Naples "Federico II", Naples, Italy,* ***4.*** *School of Psychological Sciences and Turner Institute for Brain and Mental Health, Monash University, Melbourne, Australia,* ***5.*** *Monash Biomedical Imaging, Monash University, Melbourne, Australia*

**Background and Objective**

Brain involvement in Friedreich Ataxia (FRDA) is characterized by widespread microstructural alterations, extending beyond brainstem and cerebellum. Nevertheless, no information about the degree of involvement of the dentato-thalamo-cortical tract (DTT), the cerebellar motor system main efference, is available. Aim of this study was to explore the microstructural integrity of this tract in FRDA using diffusion MRI (dMRI).

**Methods**

Scans of 57 FRDA and 52 healthy-controls (HCs) from three different sites were evaluated. In all subjects a volumetric T1-weighted sequences, for brain parcellation purposes, and a high resolution dMRI sequence, for the quantification of DTT bundles, were obtained. Tracts computation was obtained as follows: fibers connecting each dentate nucleus (DN) to the contralateral thalamus, encompassing ipsilateral red nucleus and ending in the primary motor cortex were calculated for each HC. A study specific template was calculated as the average of all tracts, and then applied to each patient's space to extract microstructural indices of bundle integrity (fractional anisotropy -FA-, radial -RD- and mean diffusivity -MD-).

**Results**

After excluding subjects with poor image quality, data of 50 FRDA patients (mean age 34.8±13.9;M/F=29/21) and 38 HCs (mean age 36.1±12.7;M/F=18/20) were compared. A significant decrease in FA in FRDA, compared to HCs, emerged on both sides (0.38±0.03vs0.42±0.02, on the left; 0,39±0.03vs0.43±0.02, on the right, p-values<0.001), coupled to a significant increase in MD and RD (all p-values<0.001).

**Discussion and Conclusion**

Our analysis further expands the current knowledge about brain involvement in FRDA, by showing the presence of significant microstructural abnormalities at the level of the main cerebellar efference in these patients. This finding is in line with the hypothesis of an anterograde secondary degeneration arising from the DN to the primary motor cortex and corroborate the possibility of employing dMRI to longitudinally evaluate damage spread and possibly treatment response in FRDA.

# (#72) Long-term Cardiac magnetic resonance imaging study in Friedreich's Ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 72

Wednesday, 2nd November - 17:07: Breakout: Imaging (Lalique Ballroom) - Flash talk - Abstract ID: 72

***Dr. Thiago Rezende*** [1], ***Dr. ALberto Martinez*** [1], ***Dr. Mariana Brito*** [1], ***Dr. Renan Nunes*** [1], ***Dr. Luís Miguel da Silva*** [2], ***Dr. Karen Takazaki*** [1], ***Dr. Melina Martins*** [1], ***Dr. Fernando Cendes*** [1], ***Dr. Michael Jerosch-Herold*** [3], ***Dr. Otavio Coelho-Filho*** [1], ***Prof. Marcondes C. França Jr*** [1]

***1.*** *University of campinas,* ***2.*** *Discipline of Cardiology, School of Medical Sciences, University of Campinas – UNICAMP, Campinas, SP, BRAZIL,* ***3.*** *Harvard Medical School*

**Background and Objective:** Biomarkers are urgently needed to assist in the clinical care and the design of clinical trials for Friedreich´s ataxia (FRDA). Heart failure is the main cause of death in FRDA, but reliable cardiac biomarkers are still missing. Cardiac magnetic resonance imaging (cMRI) has emerged as a promising candidate, although longitudinal cMRI data are scarce in the disease.

**Methods:** We acquired baseline cMRI of 18 healthy controls and 42 FRDA patients, 17 of which underwent 5 year-interval repeat cMRI. We quantified the left ventricle ejection fraction (LVEF), mass and volumes. In addition, we assessed late gadolinium enhancement (LGE), extracellular volume fraction (ECV) and intracellular water-lifetime ($\tau_{ic}$). Neurological decline was determined using the FRDA rating scale (FARS).

**Results:** Cross-sectional analyses revealed that the FRDA group had preserved LVEF, but increased LV volumes and thickness, ECV and $\tau_{ic}$ when compared to controls. Nine out of 41 patients had epicardial LGE. In addition, LV volumes correlated with FARS scores. For the follow-up analyses, there was no significant LVEF decline, but an increase of the LV diastolic volume and decrease of the LV volume/mass ratio were found. After 5 years, 5 additional patients presented epicardial LGE.

**Discussion and Conclusion:** The cross-sectional analyses showed that FRDA patients present a preserved LVEF, but increased LV volumes and thickness due to both an expansion of the myocardial interstitium and an increase in cardiomyocyte size. Assessing the main findings relevant to the longitudinal follow up, these findings indicate an evolving heart damage and a switch from a hypertrophic cardiomyopathy phenotype towards a dilated cardiomyopathy phenotype. To conclude, cMRI uncovered new insights about the cardiac burning in FRDA and might be a useful biomarker to track the disease progression.

# Neuroinflammation in the cerebellum and brainstem in Friedreich ataxia: an [18F]-FEMPA PET study

Wednesday, 2nd November - 17:14: Breakout: Imaging (Lalique Ballroom) - Flash talk - Abstract ID: 78

***Dr. Wasim Khan [1], Dr. Louise A Corben [2], Prof. Martin B Delatycki [3], Ms. Hiba Bilal [1], Dr. Lucy Vivash [4], Prof. Gary Egan [1], Dr. Ian Harding [1]***

***1.*** *Monash University,* ***2.*** *Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia, School of Psychological Sciences, The Turner Institute for Brain and Mental Health, Monash University, Clayton, Victoria, Australia,,* ***3.*** *Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia,,* ***4.*** *Monash*

**Background**

Neuroinflammation is proposed to accompany, or even contribute to, neuropathology in Friedreich ataxia (FRDA), with implications for disease treatment and tracking. However, *in vivo* evidence is currently lacking. In this study, we examine brain glial activation and systemic immune dysfunction in people with FRDA, and quantify their relationship with symptom severity, duration and onset age.

**Methods**

Fifteen individuals with FRDA and 13 healthy controls underwent brain positron emission tomography (PET) imaging using the translocator protein (TSPO) radioligand [$^{18}$F]-FEMPA, a marker of glial activation. [$^{18}$F]-FEMPA binding was assessed in brain regions key to FRDA neuropathology, including the cerebellar dentate nuclei, cerebellar cortex, and brainstem. Peripheral inflammatory cytokines were also quantified.

**Results**

We identified significantly increased TSPO receptor expression in the dentate nuclei ($d$=0.67), superior cerebellar peduncles ($d$=0.74), and midbrain ($d$=0.87), alongside increased blood plasma interleukin-6 (IL-6; $d$=0.73) in individuals with FRDA compared to controls. Increased [$^{18}$F]-FEMPA binding in the dentate nuclei, brainstem, and cerebellar anterior lobe correlated with earlier age of symptom onset (controlling for the genetic triplet repeat expansion length; all $r_{part}$<-0.6), and in the pons and anterior lobe with shorter disease duration (r=-0.66; -0.73).

**Conclusions**

Neuroinflammation is evident in brain regions implicated in FRDA neuropathology. Increased neuroimmune activity may be related to earlier disease onset, and attenuate over the course of the illness. Neuroinflammation may represent a novel treatment target in FRDA, with *in vivo* measures providing tools for measuring and tracking changes over time.

# (#416) Natural history of magnetic resonance imaging in the pre-ataxic stage of Machado-Joseph Disease: BIGPRO study

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 416

Wednesday, 2nd November - 17:21: Breakout: Imaging (Lalique Ballroom) - Flash talk - Abstract ID: 416

***Prof. Laura Bannach Jardim*** [1], ***Dr. Camila Oliveira*** [2], ***Prof. Vanessa Bielefeldt Leotti*** [3], ***Mr. Mauricio Anes*** [1], ***Ms. Sandra Polita*** [4], ***Dr. Amanda Cappelli*** [2], ***Dr. Anastacia Rocha*** [2], ***Dr. Gabriela Ecco*** [2], ***Dr. Gabriela Bolzan*** [2], ***Ms. Nathalia Kersting*** [2], ***Prof. Juliana Duarte*** [2], ***Prof. Maria Luiza Saraiva-Pereira*** [5], ***Prof. Marcondes França*** [6], ***Dr. Thiago Rezende*** [7]

***1.*** *Hospital de Clinicas de Porto Alegre, Porto Alegre / Brazil,* ***2.*** *Universidade Federal do Rio Grande do Sul,* ***3.*** *Departamento de Estatística, Universidade Federal do Rio Grande do Sul, Porto Alegre,* ***4.*** *Hospita,* ***5.*** *Universidade Federal do Rio Grande do Sul, Porto Alegre / Brazil,* ***6.*** *Universidade Estadual de Campinas (UNICAMP), Campinas / Brazil,* ***7.*** *University of campinas*

**Background and objectives:** Disease-modifying therapies are expected to be available soon for Spinocerebellar ataxia type 3/Machado-Joseph disease (SCA3/MJD). We report longitudinal results of magnetic resonance imaging (MRI) in the pre-ataxic disease stage, from BIGPRO study.

**Methods:** Subjects at 50% risk for SCA3/MJD mutation participated and were divided into pre-ataxic carriers (SARA<3) and controls after double-blind genotyping. The CAGexp size was used to estimate age of ataxia onset (AO) of pre-ataxic carriers, and therefore time to onset (TimeTo) of gait ataxia. MRI was performed at baseline and in median (IQR) 30 (7) months. Cerebellar volumetries (ACAPULCO), deep gray-matter (T1-Multiatlas), cortical thickness (FreeSurfer), cervical spinal cord area (SCT) and white matter (DTI-Multiatlas) were assessed. Differences between pre-ataxic carriers and controls were described at baseline. Variables that presented with $p<0.1$ after Bonferroni correction were followed longitudinally. Progression was calculated for TimeTo and study duration. Correction for confounding factors (age, sex and estimated intracranial volume) was done with Z-scores for TimeTo. Effect sizes (ES) were calculated, and significance level of 5% was adopted.

**Results:** 32 pre-ataxics and 20 controls were included; 29 completed the longitudinal assessment. Variables that distinguished pre-ataxic carriers from controls were spinal cord area (C1 level); right inferior cerebellar peduncle (ICP) and right medial lemniscus FAs; bilateral middle cerebellar peduncles (MCP), right ICP and right medial lemniscus MDs; and bilateral medial lemniscus and right ICP RDs. All correlated with CAGexp. Right ICP and right medial lemniscus FAs; left MCP MD and right ICP RD progressed significantly over TimeTo. No MRI variable showed significant progression over study duration time.

**Discussion and Interpretation:** DTI parameters were the best biomarkers for the pre-ataxic stage of SCA3/MJD. Right ICP and right medial lemniscus FAs, left MCP MD and right ICP RD, were able to distinguish pre-ataxics from controls and also showed progressive worsening in TimeTo.

# Workshop: Cerebellar Neuroanatomy and Physiology

# (#92) Intra-Cerebellar Regional Molecular Differences Confer Vulnerability in Spinocerebellar Ataxia Type 1 Pathology

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 92

Wednesday, 2nd November - 16:00: Workshop: Cerebellar Neuroanatomy and Physiology (Waterford A/B) - Oral - Abstract ID: 92

***Katie Hamel*** [1], ***Dr. Marija Cvetanovic*** [1]

***1.*** *University of Minnesota*

Spinocerebellar Ataxia type 1 (SCA1) is a dominantly inherited neurodegenerative disease caused by abnormal expansion of the polyglutamine (polyQ) repeat in the ATAXIN1 (ATXN1) protein. Patients with SCA1 suffer from progressive cognitive and motor impairments. Although mutant ATXN1 is expressed throughout the brain, the cerebellum undergoes severe degeneration – particularly the Purkinje cells (PCs) in the cerebellar cortex. The cerebellar cortex is divided into the vermis and hemispheres. The anterior vermis, posterior vermis, and hemispheres have been shown to contribute to distinct functions such as motor planning/coordination and cognitive behaviors. Previous studies of SCA1 in mice have mostly focused on the primary fissure of the vermis, but little is known about SCA1 pathogenesis across the cerebellar cortex. This is notable because studies in patients with SCA1 indicated increased pathology in hemispheres and posterior vermis. Using immunohistochemistry with 18-week-old $Atxn1^{154Q/2Q}$ mice, we found increased atrophy of PCs and exacerbated loss of climbing fiber synapses in the posterior vermis and hemispheres. To further study the molecular differences that may underlie this increased sensitivity of PCs in the posterior vermis and hemispheres, I developed a quick and reproducible dissection method to isolate three regions of the cerebellar cortex: cerebellar cortex of the anterior vermis (CCAV), cerebellar cortex of the posterior vermis (CCPV), and cerebellar cortex of the hemispheres (CCH). Utilizing this dissection method and RNAsequencing analysis, I have found region-specific differences in gene expression between cerebellar cortical regions within wild-type mice. These inherent differences in the wild-type cerebellum could contribute to the regional vulnerability seen in the posterior vermis and hemispheres in $Atxn1^{154Q/2Q}$ mice particularly in Purkinje and glial cells of these regions.

This work aims to identify the alterations in molecular pathways and PC activity underlying regional SCA1 pathogenesis in hopes of providing more efficient therapies to treat this relentless disease.

# (#295) Characterization of cerebellar astrocyte reactivity and metabolism in a new mouse model of Friedreich´s ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 295

Wednesday, 2nd November - 16:15: Workshop: Cerebellar Neuroanatomy and Physiology (Waterford A/B) - Oral - Abstract ID: 295

***Mr. Andrés Vicente-Acosta [1], Mr. Jorge Galán-Cruz [2], Dr. Saul Herranz-Martin [1], Mr. Mario Amores [3], Dr. Ruth Pazos-Rodríguez [3], Dr. Javier Diaz-Nido [1], Dr. Frida Loria [3]***

***1.*** *Universidad Autonoma de Madrid / Centro de Biología Molecular Severo Ochoa,* ***2.*** *Unversdad Autónoma de Madrid,* ***3.*** *Laboratorio de apoyo a la investigación / Hospital Universitario Fundación Alcorcón*

**Background and objective**: Friedreich´s ataxia is a rare neurodegenerative disease caused by a deficiency in the protein frataxin, which is mainly caused by an expansion of the GAA triplet in the first intron of the frataxin gene. The pathophysiological manifestations of the disease are mainly observed in neurons, but recent evidence indicates that non-neuronal cells like astrocytes are contributing to the neurodegenerative process. One of the main limitations for the study of this disease is the absence of good experimental models that fully recapitulate the human disease. Therefore, our aim was to characterize the phenotype of astrocytes derived from a new mouse model of Friedreich's ataxia called YG8JR. This human frataxin YAC knock-in mouse contain more than 800 repeats of the GAA expansion, harboring a global null allele of mouse frataxin.

**Methods:** We studied and characterized primary cerebellar astrocytes obtained from 5-day-old pups, evaluating in parallel the astroglial reactivity status of adult mice at different time points.

**Results**: When compared to control animals, cerebellar astrocytes from the YG8JR mice show significant mitochondrial and metabolic alterations that were accompanied by a shift in their reactivity profile. In addition, preliminary data from the *in vivo* study of the reactivity profile in the adult mice suggest that the YG8JR mice have higher expression levels of C3 and GFAP in different brain areas.

**Discussion and conclusion:** The characterization of cerebellar astrocytes derived from the YG8JR mouse could be useful to understand the role of glial cells in this disease, constituting as well a good model to test different treatments aiming a reducing the astrogliosis and/or neurodegeneration observed in these animals.

# (#410) Impaired reinforcement learning in patients with cerebellar ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 410

Wednesday, 2nd November - 16:30: Workshop: Cerebellar Neuroanatomy and Physiology (Waterford A/B) - Oral - Abstract ID: 410

***Dr. Jonathan Nicholas [1], Dr. Christian Johannes Amlang [1], Dr. Chi-Ying Lin [2], Ms. Natasha Desai [1], Dr. Leila Montaser Kouhsari [3], Dr. Sheng-Han Kuo [4], Dr. Daphna Shohamy [1]***

***1.*** *Columbia University Irving Medical Center,* ***2.*** *Baylor College of Medicine,* ***3.*** *Stanford University School of Medicine,* ***4.*** *Columbia Univeristy Medical Center*

Background and Objective:
Supervised learning (i.e., learning from error) and reinforcement learning (i.e., learning from reward) are two of the most fundamental learning processes in the brain. Recently, cerebellar granular cells were found to encode reward omission in mice and the cerebellar Purkinje cells were identified to track reward-related learning in monkeys. These new findings challenge the traditional concept that the cerebellum relies on supervised learning to modify motor activities and behavior while the basal ganglia is dependent on reinforcement learning.
The goal of our study was to further characterize cerebellar learning processes as well as learning impairment pattern in patients with cerebellar dysfunction.
Methods:
Nineteen patients with cerebellar ataxia and 57 age and sex-matched controls (3 per patient) completed cognitive and neuropsychological tests as well as 2 learning experiments. Experiment 1 consisted of a reinforcement learning task. Experiment 2 provided an internal control by allowing decisions to be made based on either episodic memory or reinforcement learning.
Results:
In experiment 1, patients with cerebellar ataxia were impaired in two ways compared to controls. First, patients sub-optimally adjusted their rate of learning throughout the task. Second, they made fewer decisions based on the values learned through reinforcement. These results were confirmed in experiment 2, where patients with cerebellar ataxia were again impaired at reinforcement learning but used episodic memory for decisions.
Discussion and Conclusion:
Patients with cerebellar dysfunction have deficits in reward-based learning. This study also provides further evidence that an intact cerebellum is necessary not just for supervised learning but also for reinforcement learning.

# Patterned Purkinje Cell Loss During Normal Aging in Mice

Wednesday, 2nd November - 16:45: Workshop: Cerebellar Neuroanatomy and Physiology (Waterford A/B) - Oral - Abstract ID: 450

***Ms. Sarah Donofrio*** [1], ***Ms. Lita Duraine*** [1], ***Dr. Roy Sillitoe*** [1]

***1.*** *Baylor College of Medicine*

Background and Objective:
Despite its relatively homogenous cellular composition, the cerebellar cortex is highly patterned. Cerebellar patterns are “striped,” and this modular organization is reflected in cellular birth dates, lineage, molecular expression, afferent termination, and electrophysiological properties. Here we tested whether cerebellar Purkinje cell loss follows a patterned organization during normal aging. These data may uncover a common pathological mechanism shared between typical aging and neurodegenerative diseases.
Methods:
We used wholemount immunohistochemistry (IHC) to reveal global cerebellar patterns and frozen tissue section IHC to closely examine distinct regions. We stained the tissue for calbindin, a Purkinje cell-specific marker, and zebrin II, a marker of Purkinje cell stripes. We also used transmission electron microscopy to test for subcellular changes. To test whether Purkinje cell pathology was accompanied by motor behavior defects, we measured behavioral changes in mice aged 1 to 2 years with a custom-built tremor monitor and horizontal ladder. Mice aged 2 to 3 months served as young controls.
Results:
We found that aged mice displayed a striped pattern of Purkinje cell loss. The pattern was consistent across mice and resembled the striped zebrin II expression pattern, with Purkinje cell loss occurring preferentially in zebrin II-negative stripes. At the subcellular level, the Purkinje cells of aged mice had severe myelin degeneration. Aged mice displayed higher power tremor compared to young mice but did not display ataxia.
Discussion and Conclusion:
Purkinje cell loss during normal aging follows a striped pattern that is similar, but not identical, to the zebrin II pattern. Despite the common association of Purkinje cell loss and ataxia, aged mice do not have obvious gait defects, although tremor is enhanced. These data uncover a differential vulnerability of Purkinje cell subpopulations during normal aging, providing insight into how cerebellar function deteriorates with age.

# Plenary Session: Emerging and Existing Therapies

# Invited Talk: Emerging and existing therapies in ataxias

Thursday, 3rd November - 08:30: Plenary: Emerging and Existing Therapies (Crystal Ballroom) - Invited Speaker - Abstract ID: 522

***Dr. Beverly Davidson*** [1]

***1.*** *1. Raymond G. Perelman Center for Cellular and Molecular Therapeutics, The Children's Hospital of Philadelphia 3.Department of Pathology and Laboratory Medicine, University of Pennsylvania*

Emerging and existing therapies in ataxias

# Cerebellar tDCS in Friedreich Ataxia: A randomized, double-blind, sham-controlled, crossover trial.

Thursday, 3rd November - 09:00: Plenary: Emerging and Existing Therapies (Crystal Ballroom) - Oral - Abstract ID: 388

***Prof. Gilles Naeije*** [1], ***Dr. Virginie Destrebecq*** [1], ***Dr. Camille Comet*** [1], ***Mr. Nick Alaerts*** [1], ***Prof. Antonin Rovai*** [2]

***1.*** *Department of Neurology, CUB Hôpital Erasme, Université libre de Bruxelles (ULB), Brussels,* ***2.*** *2Laboratoire de Neuroanatomie et de Neuroimagerie translationnelles (LN2T), UNI — ULB Neuroscience Institute, Université libre de Bruxelles (ULB), Brussels*

Background

Increasing cerebellar brain inhibition (CBI) by cerebellar transcranial direct current stimulation (ctDCS) mitigates cognitive and motor symptoms in ataxic patients of mixed origins.

Objective

Test if five days of ctDCS improves cerebellar cognitive affective syndrome (CCAS) and motor performances in a cohort of subjects with Friedreich Ataxia (FRDA).

Methods

24 subjects underwent both sham ctDCS and anodal ctDCS (real ctDCS) sessions in randomized order in a cross-over sham-controlled study design. Sham/Real Sessions were 12 weeks apart. ctDCS was delivered by a constant current stimulator through a pair of saline-soaked surface 3”x3” sponge electrodes. During anodal stimulation, a constant current of 0.057 mA per $cm^2$ was applied for 20 minutes over the cerebellum[1] each day for five successive days. For the sham condition, a cathodal stimulation was used in a same setting with a constant current of 0.008 mA per $cm^2$. CCAS-scale, SARA score, 9HPT and Click test (CT) were performed before and after each five days sessions.

Furthermore, to evaluate the CBI effect of ctDCS, five subjects with FRDA and five controls also underwent magnetoencephalographic Cortico-kinematic Coherence (CKC) evaluation before and after 5 days of ctDCS.

Results

Patients mean age, SARA score and GAA1 were respectively of 31±14, 23±9 and 665±277. Five days of Anodal ctDCS led to significant improvement of CCAS-scale (99.6±16.6 vs 89.8±15, p=0.0002), SARA score (23.1±9 vs 21.6±9, p=0.006) and CT (28±11.7 vs 31.1±13.7s, p= 0.02). Sham ctDCS did not modify CCAS-scale (90.7±14.1 vs 90.1±15.9, p=0.86), SARA score (22.8±8.5 vs 22.7±8.7, p=0.9) nor CT (28.7±13.6s vs 29.6±14.4s, p= 0.1).

Five days of Anodal ctDCS led to significant reduction of CKC levels (0.79±0.44 vs 1.08±0.34, p=0.016).

Conclusions

Anodal ctDCS significantly improves cognitive function in FRDA and modestly motor performances through strengthening of CBI. Further studies are needed to target which patients could benefit the most of ctDCS.

# GeneTACTM small molecules increase frataxin in a mouse model of Friedreich ataxia, restore FXN and improve mitochondrial function in patient-derived cells, and achieve sustained biodistribution in CNS and heart in rats and non-human primates

Thursday, 3rd November - 09:15: Plenary: Emerging and Existing Therapies (Crystal Ballroom) - Oral - Abstract ID: 459

***Dr. Abhijit Bhat [1], Dr. Chengzhi Zhang [1], Ms. Melanie Bell [1], Mr. Sumon Datta [1], Dr. Nancy Levin [1], Ms. Hannah Schehr [1], Dr. Andrew Powers [1], Dr. Sean Jeffries [1], Dr. Pratik Shah [1], Dr. Joao Siffert [1], Dr. Aseem Ansari [1]***

***1.*** *Design Therapeutics*

**BACKGROUND & OBJECTIVE:** Friedreich Ataxia (FA) is a multisystem degenerative disease caused by inherited GAA repeats in the frataxin (FXN) gene leading to reduced FXN protein and mitochondrial dysfunction. This study presents the safety and efficacy of FA GeneTACTM small molecules.

**METHODS**: The pharmacodynamics of FA GeneTACTM molecules were assessed *in* vitro in FA patient-derived B-lymphoblastoid cells (LCL), induced pluripotent stem cell derived cardiomyocytes (iPS-CM) and neurons (iPS-N), peripheral blood mononuclear cells (PBMCs), and *in* vivo in the Pook800J FA mouse model. Pharmacokinetics, biodistribution and safety were evaluated in rodents and non-human primates after weekly IV administrations.

**RESULTS**: In FA LCLs, GeneTACTM molecules dose-dependently increased FXN mRNA and protein relative to vehicle controls. In contrast, 12 unrelated compounds reported to improve FXN levels or mitochondrial function had no appreciable effects in the same cell system. FA GeneTACTM molecules increased FXN mRNA by ~10-fold in PBMCs from FA patients (N=23 donors with >100 to > 1500 GAA repeats). Treatment with GeneTACTM molecules at ≥ 3 nM for up to two weeks increased FXN mRNA and protein levels in FA iPS-CMs and iPS-Ns; 10nM treatment for 14 days in FA iPS-Ns restored the FXN protein comparable levels as in non-FA iPS-Ns. FA GeneTACTM molecules also improved mitochondrial respiration in FA LCLs and iPS-CMs.

FXN levels were increased in brain and heart of Pook800J FA mice following a single dose of an FA GeneTACTM molecule. Well-tolerated doses of FA GeneTAC TM molecules in rats and monkeys were associated with tissue levels that exceed those shown to produce FXN restoration in FA patient cells *in vitro*.

**CONCLUSION**S: FA GeneTACTM molecules restore FXN and improve mitochondrial function across FA cell types *in vitro* and increase FXN *in* vivo. The data support the potential of FA GeneTACTM molecules to address the root cause of Friedreich ataxia.

# CLR01, a molecular tweezer, attenuates motor dysfunction and pathology in SCA3 in vivo models

Thursday, 3rd November - 09:30: Plenary: Emerging and Existing Therapies (Crystal Ballroom) - Oral - Abstract ID: 276

***Dr. Sara Duarte-Silva [1], Ms. Daniela Vilasboas-Campos [2], Ms. Daniela Monteiro-Fernandes [1], Dr. Joana Pereira-Sousa [3], Dr. Andreia Neves-Carvalho [4], Dr. Alexandra Silva [5], Prof. Ana Luísa Carvalho [6], Prof. Gal Bitan [7], Dr. Sandra Macedo-Ribeiro [5], Dr. Andreia Teixeira-Castro [8], Prof. Patrícia Maciel [1]***

***1.*** *Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal,* ***2.*** *1. Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal; 2. ICVS/3B's - PT Government Associate Laboratory Braga/Guimarães, Portugal,* ***3.*** *Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal ICVS/3B's - PT Government Associate Laboratory Braga/Guimarães,* ***4.*** *1- Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, 4710-057 Braga, Portugal; 2- ICVS/3Bs - PT Government Associate Laboratory, Braga/Guimarães, Portugal.,* ***5.*** *Instituto de Biologia Molecular e Celular (IBMC) and Instituto de Investigação e Inovação em Saúde (i3S), Universidade do Porto, 4200-135 Porto,* ***6.*** *Center for Neuroscience and Cell Biology & Department of Life Sciences, University of Coimbra, 3004-517 Coimbra, Portugal.,* ***7.*** *Department of Neurology, David Geffen School of Medicine, Brain Research Institute, and Molecular Biology Institute, University of California, Los Angeles, Los Angeles, CA, USA.,* ***8.*** *Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal; ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal*

**Background:** During the process of protein aggregation, folding intermediates and misfolded states of proteins likely accumulate, and the intermolecular contacts between non-native states result in the formation of various aggregate species, including oligomers, amorphous aggregates, and amyloid fibrils. Amyloid fibrils are often thermodynamically more stable than the native state, favoring their formation, ultimately leading to cell dysfunction and death. Therefore, inhibiting the formation and toxicity of these aggregated species is a very exciting therapeutic route to follow. Molecular tweezers (MTs) are inhibitors of abnormal protein self-assembly and toxicity. Among these, CLR01 has been found to inhibit the formation of toxic oligomers and aggregates of multiple disease-associated proteins by binding to positively charged lysine residues, temporarily reversing their charge from positive to negative, and disrupting hydrophobic interactions. **Objective:** Here, we aimed at testing the impact of CLR01 in *in vivo* models of SCA3 pathogenesis. **Results and discussion:** Pre-symptomatic chronic treatment of C. elegans expressing mutant ATXN3 proteins with CLR01 ameliorated their neuronal dysfunction. Importantly, when CLR01 was administered to the animals post-symptomatically, it was still able to suppress mutant ATXN3-mediated motor behavior defects, mimicking the most frequent clinical situation of symptom-driven diagnosis and treatment initiation of SCA3 patients. Importantly, CLR01 chronic and early symptomatic treatment also delayed disease installation and significantly improved motor behavior of a SCA3 transgenic mouse model, rescuing motor neuron pathology. **Conclusion:** These results indicate the MT CLR01 as a potentially effective therapeutic for SCA3, likely through the inhibition of ATXN3 self-assembly.

# Restoring calcium homeostasis in Purkinje cells arrests neurodegeneration and neuroinflammation in the ARSACS mouse model

Thursday, 3rd November - 09:45: Plenary: Emerging and Existing Therapies (Crystal Ballroom) - Oral - Abstract ID: 104

***Dr. Andrea Del Bondio*** [1], ***Dr. Fabiana Longo*** [1], ***Dr. Daniele De Ritis*** [1], ***Dr. Erica Spirito*** [1], ***Dr. Paola Podini*** [1], ***Dr. Bernard Brais*** [2], ***Dr. Angela Bachi*** [3], ***Dr. Angelo Quattrini*** [1], ***Dr. Francesca Maltecca*** [1]

***1.*** *San Raffaele Scientific Institute,* ***2.*** *McGill University,* ***3.*** *IFOM*

BACKGROUND AND OBJECTIVE- Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS) is caused by loss-of-function mutations in *SACS* gene encoding sacsin, highly expressed in Purkinje cells (PCs). ARSACS patients, as well as mouse models, display early loss of PCs as main neuropathological feature. Previous studies in *SACS*$^{-/-}$-deficient cell models indicated a role for sacsin in intermediate filaments dynamics and mitochondrial function. However, the mechanisms underlying PC degeneration in ARSACS remain unexplored, with no treatments available so far.

METHODS- We assessed calcium ($Ca^{2+}$) homeostasis, mitochondria and ER trafficking by imaging studies in cultured primary PCs from *Sacs*$^{-/-}$ mice. We applied multi-OMICS approaches to define changes in *Sacs*$^{-/-}$ cerebellar proteome and transcriptome, sacsin immunoprecipitation to identify sacsin interactors. We performed Ceftriaxone administration, followed by motor behavioral tests and histological analysis in cerebellum.

RESULTS- We found that $Ca^{2+}$ homeostasis is strongly affected in *Sacs*$^{-/-}$ PCs. This phenotype is due to defective mitochondrial and ER trafficking to distal dendrites when sacsin is absent and to strong downregulation of key $Ca^{2+}$ buffering-proteins. Faulty organellar trafficking is dependent on alteration of cytoskeletal linker proteins, that we identified as specific sacsin interactors and found deregulated in *Sacs*$^{-/-}$ cerebellum and in ARSACS patient cells. Likely secondary to $Ca^{2+}$-induced PC degeneration, we detected neuroinflammation in *Sacs*$^{-/-}$ cerebellum, as documented by striking upregulation of several inflammatory genes, especially linked to microglial and astrocytic activation. Based on this pathogenetic cascade, we treated *Sacs*$^{-/-}$ mice with Ceftriaxone, a repurposed drug which exerts neuroprotection by limiting neuronal glutamatergic stimulation, and thus $Ca^{2+}$ fluxes into PCs. Ceftriaxone treatment significantly improved motor performances of *Sacs*$^{-/-}$ mice. We correlated this effect to restored $Ca^{2+}$ homeostasis, which led to an arrest in PC degeneration thus attenuating neuroinflammation.

DISCUSSION AND CONCLUSION- These results improve the knowledge of ARSACS pathogenesis and offer encouraging perspectives for the treatment of ARSACS patients.

# Breakout: Cell and Animal Models

# (#447) Generation of mechanosensory neurons from human pluripotent stem cells

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 447

Thursday, 3rd November - 10:30: Breakout: Cell and Animal Models (Crystal Ballroom) - Oral - Abstract ID: 447

***Ms. Amy Hulme*** [1], ***Dr. Jeffrey R McArthur*** [1], ***Dr. Yang Guo*** [2], ***Dr. Marek Napierala*** [3], ***Prof. Boris Martinac*** [2], ***Prof. David J Adams*** [1], ***Dr. Rocio Finol-Urdaneta*** [1], ***Prof. Mirella Dottori*** [1]

***1.*** *1. Illawarra Health and Medical Research Institute, University of Wollongong, Wollongong, NSW, Australia. 2. Molecular Horizons, University of Wollongong, Wollongong, NSW, Australia,* ***2.*** *The Victor Chang Cardiac Research Institute, Darlinghurst, NSW, Australia,* ***3.*** *University of Alabama at Birmingham, Birmingham, Alabama, USA. University of Texas Southwestern Medical Centre, Dallas, Texas, USA*

**Background and Objective:** Mechanosensation, the transduction of mechanical stimuli into neural signals, is essential for everyday functions, such as enabling sitting, walking, holding objects, and internal organ sensation. A major challenge in studying mechanosensation and the pathophysiology of associated developmental and degenerative diseases, such as Friedreich's Ataxia (FA), is the availability of human mechanosensory neurons, which include proprioceptors (that detect movement, muscle pressure, and tension) and low threshold mechanoreceptors (LTMRs) (that detect touch, hair deflection, and vibration). This study aimed to differentiate human pluripotent stem cells (hPSCs) into different populations of mechanosensory neurons for use in cellular and disease modelling.

**Methods:** In this study, we have developed a novel and robust methodology for generating and functionally characterising mechanosensitive induced-LTMRs (iLTMRs) and induced-proprioceptors (iPNs) from hPSCs.

**Results:** The generated neurons display expression and functional characteristics akin to proprioceptors and LTMRs and exhibit distinct mechanically sensitive responses to stretch and to submicrometer (0.1 μm) probe indentation stimuli. Furthermore, knockdown of PIEZO2 ablated > 80% of the mechanosensitive currents in both induced mechanosensory neuron (iMSNs) sub-classes. Furthermore, iPNs and iLTMRs display different mechanical activation thresholds, frequency tuning, and adaptation kinetics reflective of distinct sensory specializations. Using this cellular model, we generated mixed populations of iMSNs derived from FA hPSCs and their isogenic corrected counterparts. Although FA iMSNs respond to mechanical stimulation, sustained and higher frequency stimulation lead to action potential failure in the FA-derived, but not in the corrected iMSNs, consistent with features of the disease phenotype.

**Discussion and Conclusion:** Collectively, this work describes the generation of exquisitely sensitive mechanosensory neurons to further our understanding of human mechanosensory physiology and, in the long term, will enable the development of therapies directed toward these neuronal populations following trauma and/or neurodegenerative conditions.

# (#364) Patient-specific iPSCs reveal vascular dysfunction in Friedreich's Ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 364

Thursday, 3rd November - 10:45: Breakout: Cell and Animal Models (Crystal Ballroom) - Oral - Abstract ID: 364

***Dr. Jarmon Lees*** [1], ***Mr. Alan Zhang*** [1], ***Dr. Anne M. Kong*** [1], ***Mr. Andrew Treller*** [2], ***Prof. Geraldine M. Mitchell*** [1], ***Prof. Mirella Dottori*** [3], ***Prof. Alice Pebay*** [4], ***Dr. Stephen Wilcox*** [5], ***Mr. Jeffrey M. Pullin*** [1], ***Dr. Davis McCarthy*** [1], ***Dr. Mark Chong*** [1], ***Dr. Roger Peverill*** [6], ***Prof. Martin B Delatycki*** [7], ***Dr. Marek Napierala*** [8], ***Dr. Shiang Y. Lim*** [1]

***1.*** *St Vincent's Institute of Medical Research,* ***2.*** *Australian Catholic University,* ***3.*** *University of Wollongong,* ***4.*** *The University of Melbourne,* ***5.*** *Walter and Eliza Hall Institute,* ***6.*** *Monash University and Monash Health,* ***7.*** *Murdoch Children's Research Institute,* ***8.*** *UT Southwestern Medical Center*

**Background and Objective:** Friedreich's ataxia (FRDA) is a hereditary neuromuscular disorder and heart disease is the leading cause of premature mortality in FRDA patients. Clinical reports indicate that FRDA cardiomyopathy may be associated with abnormalities of the small coronary arteries, which are primarily composed of vascular endothelial cells (ECs) and smooth muscle cells (SMCs). Here, we aim to examine the phenotype and functionality of ECs and SMCs derived from FRDA patient-specific induced pluripotent stem cells (iPSCs).

**Methods:** Cardiac biopsies of FRDA patients were stained for fibrosis and vascular markers. ECs and SMCs were differentiated from iPSCs derived from three FRDA patients and CRISPR-corrected isogenic control iPSCs for assessment of cell viability, cell proliferation, cell migration, angiogenic potential, oxidative stress, mitochondrial function, extracellular matrix (ECM) proteome, and transcriptomic changes.

**Results:** Histopathological analysis of cardiac tissue of FRDA patients revealed focal interstitial fibrosis and thickening of the EC layer with narrowed and partially occluded small vessels. *In vitro*, compared to the isogenic controls, FRDA-ECs displayed reduced angiogenic potential, reduced proliferation, increased levels of mitochondrial superoxide, and reduced levels of mitochondrial membrane potential. FRDA-SMCs displayed increased migratory potential, reduced proliferation, increased levels of mitochondrial superoxide, and mitochondrial membrane potential. Proteomic analysis of ECM secreted by FRDA-SMCs showed multiple dysregulated proteins including ITGAV, TGFB2, CAV1, and ITGA6, which are involved in angiogenesis and migration/invasion. However, cell viability of ECs and SMCs was not affected by the FRDA genotype. RNA-sequencing revealed multiple differentially expressed genes conserved across both vascular cell types, including downregulated *FXN* and upregulation of the long non-coding RNA *MEG3* in FRDA.

**Discussion and Conclusion:** Collectively, these results strongly support a role for vascular dysfunction in the FRDA pathology, which may contribute to the development and progression of FRDA cardiomyopathy and represent a promising cellular target for therapy.

# Spinocerebellar ataxia type 1 characteristics in patient-derived fibroblast and induced pluripotent stem cell-derived neuronal cultures

Thursday, 3rd November - 11:00: Breakout: Cell and Animal Models (Crystal Ballroom) - Oral - Abstract ID: 480

***Dr. Ronald Buijsen*** [1], ***Mr. Michel Hu*** [1], ***Ms. Maria Sáez-González*** [1], ***Dr. Eleni Mina*** [1], ***Dr. JP Frimat*** [1], ***Prof. Arn van den Maagdenberg*** [1], ***Dr. Spyros Petrakis*** [2], ***Prof. Willeke van Roon-Mom*** [1]

***1.*** *Leiden University Medical Center,* ***2.*** *Institute of Applied Biosciences/Centre*

**Background**

SCA1 is a neurodegenerative disease caused by a polyglutamine expansion in the ataxin-1 protein. This results in aberrant protein aggregation and neuropathology, mainly in the cerebellum. Furthermore, it has been shown that the mutant ataxin-1 protein causes aberrant neurodevelopment, bioenergetic dysfunction and metabolic alterations. The aim of our study is to identify SCA1-relevant phenotypes in patient-specific fibroblast cell lines and neuronal cultures obtained from SCA1 hiPSCs.

**Methods**

hiPSC were generated and differentiated into neuronal cells. The presence of protein aggregates was assessed using immunohistochemistry and compared to subcellular localization of aggregates in postmortem human brain material. Dendritic length and number of branching points were evaluated after transient GFP transfection. Mitochondrial respiration was measured using the Seahorse XF96 Extracellular Flux Analyzer where oxygen consumption rate and ATP production were used as outcome measures. Finally, gene expression changes were used to identify novel disease mechanisms.

**Results**

A subtle deficit in bioenergetics was shown in patient-derived fibroblasts, which was more in SCA1 hiPSC-derived neuronal cultures. There was a decreased basal and maximal respiration and ATP production in patient-derived cells compared to control cells, supporting a role for mitochondrial dysfunction in pathogenesis. In neuronally differentiated SCA1 hiPSC, ataxin-1 positive nuclear and cytoplasmic aggregates were identified, which was similar to aggregates in SCA1 postmortem patient brain material. Furthermore, SCA1 cells showed reduced cellular process length and number of branching points. RNA sequencing analysis showed 1050 differentially expressed genes. The most significantly altered genes were *DCT*, *EPHA5* and *HCNJ8*.

**Conclusions**

SCA1 patient-derived cells recapitulate key pathological features of SCA1 providing a valuable tool to investigate new SCA1-related disease processes deficits. This model can be used to screen for drugs that may prevent or rescue neurodegeneration in this devastating disease. We identified novel genes and pathway that can be used to develop new therapies.

# (#487) A preclinical behavioral assay and analytical platform to model genetic background, age, and zygosity in CHIP-dependent ataxia phenotypes (SCA48/SCAR16)

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 487

Thursday, 3rd November - 11:15: Breakout: Cell and Animal Models (Crystal Ballroom) - Oral - Abstract ID: 487

***Mr. Arjun Putcha [1], Ms. Amanda Brown [1], Mr. Alex Eaker [1], Mr. Nicholas Field [1], Prof. Jonathan Schisler [1]***

***1.*** *McAllister Heart Institute, Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina*

Background: Rodent models are important preclinical models for ataxia research. Previous methods of examining motor coordination and balance in mice include the rotarod, horizontal bar, static rods, and parallel bars. Confounding the literature is that the genetic background of laboratory mice influences behavior and disease progression. *STUB1* mutations are causal for two forms of cerebellar ataxia: SCA48 and SCAR16. Reports of phenotypes in *Stub1* knockout mice vary from lab to lab, perhaps due to differences in genetic background. **We developed a behavioral assay and analytical platform in R to measure motor function in preclinical spinocerebellar ataxia models that account for age, weight, genotype, and strain.**

Methods: We created a *Stub1*-lox-stop-lox mouse on a C57BL/6J background and a second line of mice on a mixed B6/129 mixed strain. Our wire hang and ledge tests are modified versions of the horizontal bar and static rod tests. We developed an analytical platform in R to model the effect of age, sex, strain, and genotype and used the MiniMUGA genotyping array for genetic quality control.

Results: We found that in two strains of mice, only the loss of both *Stub1* alleles, compared to one or two functional alleles, affected the four different ataxia measures (wire hang, ledge, observational, inappropriate behavior). Sex and strain did associate with inappropriate behavior – inability/refusal to balance/hang. Age and weight associated with multiple ataxia outcomes. Genotyping arrays allowed us to define and compare our study mice to other laboratory strains.

Conclusion: The ease of administration, the minimal amount of extraneous material, and the lack of expensive equipment required for our behavioral procedure pose several advantages over other techniques. Our analytical pipeline provides a visual readout of the experimental variables associated with ataxia metrics. Genotyping analysis of the strains used in ataxia studies is paramount to rigor and reproducibility.

# (#157) Activation of the type I interferon response associated with frataxin knockdown in iPSC-derived cardiomyocytes

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 157

Thursday, 3rd November - 11:30: Breakout: Cell and Animal Models (Crystal Ballroom) - Flash talk - Abstract ID: 157

***Dr. M.Grazia Cotticelli*** [1], ***Dr. Shujuan Xia*** [1], ***Dr. Rachel Truitt*** [2], ***Dr. Nicolai Doliba*** [2], ***Dr. Andrea Rozo*** [2], ***Dr. John Tobias*** [2], ***Mr. Taehee Lee*** [1], ***Mr. Justin Chen*** [3], ***Dr. Jill Napierala*** [4], ***Dr. Marek Napierala*** [4], ***Dr. Wenli Yang*** [2], ***Dr. Robert Wilson*** [2]

***1.*** *Children's Hospital of Philadelphia,* ***2.*** *University of Pennsylvania,* ***3.*** *University of Hawaii,* ***4.*** *University of Alabama at Birmingham*

Background and Objective: Friedreich ataxia, the most common hereditary ataxia, is a neuro- and cardio-degenerative disorder caused, in most cases, by decreased expression of the mitochondrial protein frataxin. Cardiomyopathy is the leading cause of premature death. Our objective was to study the changes associated with decreased frataxin in human cardiomyocytes.

Methods: We differentiated induced pluripotent stem cells (iPSCs) into cardiomyocytes and compared cells in which frataxin was knocked down to cells transfected with control siRNA post differentiation, which controlled for genetic background and variability in differentiation.

Results: Transcriptome analysis of four biological replicates identified severe mitochondrial dysfunction, calcium mishandling, and a type I interferon response as pathways most affected by frataxin knockdown. We confirmed that in iPSC-derived cardiomyocytes, loss of frataxin leads to mitochondrial dysfunction and increased cytosolic calcium concentrations. The type I interferon response was activated in multiple cell types following acute frataxin knockdown and was caused, at least in part, by release of mitochondrial DNA into the cytosol, which activated the cGAS-STING sensor pathway.

Discussion and Conclusion: Our data support the hypothesis that decreased frataxin activates a cell-intrinsic innate immune response. We observed mtDNA released into the cytosol following frataxin knockdown, as well as upregulation of type I interferon, mostly through activation of the cGAS-STING pathway. In conclusion, we developed a novel, isogenic FRDA model using iPSC-derived cardiomyocytes to study FRDA cardiomyopathy. Our data are consistent with previous results, confirm the suitability of the model, and reveal novel pathways that may underlie the pathophysiology of the disease and that suggest points of therapeutic intervention.

# (#290) Neurodegenerative synergy between RAN translation and CGG repeat RNA toxicity in rodent models of FXTAS

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 290

Thursday, 3rd November - 11:37: Breakout: Cell and Animal Models (Crystal Ballroom) - Flash talk - Abstract ID: 290

***Ms. Samantha Grudzien*** [1], ***Ms. Amy Krans*** [2], ***Dr. Hayley McLoughlin*** [1], ***Dr. Geena Skariah*** [1], ***Dr. Brittany N Flores*** [1], ***Prof. Sami Barmada*** [1], ***Prof. Peter Todd*** [3]

***1.*** *University of Michigan-Ann Arbor,* ***2.*** *University of Michigan-,* ***3.*** *University of Michigan*

Background: Fragile X-associated tremor/ataxia syndrome (FXTAS) is a relatively common neurodegenerative ataxia that arises from a trinucleotide CGG repeat expansion that ranges from 55 to 200 repeats in the 5' UTR of FMR1. CGG repeats may drive neurodegeneration through RNA mediated mechanisms or repeat-associated non-AUG (RAN) translation of a polyglycine product, FMRpolyG. Data in transgenic models indicates that FMRpolyG synthesis is required for toxicity, but whether FMRpolyG is sufficient to elicit maximal toxicity remains unclear.
Methods: In rodent neurons, we expressed vectors that generate both FMRpolyG and CGG repeat RNA from the native FMR1 5'UTR; just FMRpolyG in the absence of the repeat (through alternative codon usage and AUG initiation codon), or just the repeat RNA (CGG-RNA) but with little FMRpolyG production (through near-cognate start codon removal). In current studies, we intracerebroventricularly injected neonate mice with adeno-associated viruses (AAVs) that express these same constructs, with behavioral and pathological phenotypic assessments at 3 and 6 months post injection.
Results: Primary hippocampal rodent neurons that express 100 CGG repeats in the native FMR1 5'UTR context exhibit greater toxicity than constructs that solely express FMRpolyG, despite greater FMRpolyG production from the AUG initiated alternative codon construct. Compared to control GFP constructs, expression of the CGG repeat as RNA alone did not evoke toxicity. In AAV injected mice that express the repeats in their native 5'UTR context, we observed robust P62 and FMRpolyG inclusion formation. Currently, eight mice per group are aging out, and subsequent behavioral and histological analysis are ongoing.
Discussion: Our results in rodent cultures imply synergy between FMRpolyG production and CGG-RNA toxicity that we are evaluating in vivo. Future work will attempt to determine how this synergy is achieved, and whether its selective abrogation is feasible.

# (#117) An induced pluripotent stem cell-based model to investigate proprioceptive neuronal pathology in Friedreich ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 117

Thursday, 3rd November - 11:44: Breakout: Cell and Animal Models (Crystal Ballroom) - Flash talk - Abstract ID: 117

***Dr. Chiara Dionisi*** [1]***, Dr. Marine Chazalon*** [1]***, Dr. Myriam Rai*** [2]***, Ms. Céline Keime*** [3]***, Ms. Virginie Imbault*** [1]***, Prof. Serge Schiffmann*** [1]***, Dr. Hélène Puccio*** [4]***, Dr. David Communi*** [1]***, Prof. Massimo Pandolfo*** [5]

***1.*** *Université Libre de Bruxelles,* ***2.*** *Friedreich's Ataxia Research Alliance,* ***3.*** *Institut de Génétique et de Biologie Moléculaire et Cellulaire,* ***4.*** *Université Claude Bernard - Lyon I,* ***5.*** *McGill University*

The selective loss of proprioceptive neurons is a distinctive feature in Friedreich Ataxia (FA), but its link with frataxin (FXN) deficiency is still unclear. We used a pluripotent stem cell-based model to investigate the presence of specific pathological features in neurons derived from FA patients in comparison to healthy controls (CT) and we included isogenic controls (IC) to evaluate if some of these marks could be reverted by the removal of the mutation responsible for the disease.

Proprioceptive-enriched cultures differentiated from CT, FA and IC lines, were characterized by RNA Sequencing and Mass Spectrometry-based proteomics. The epigenetic state of the FXN locus was examined by Chromatin Immunoprecipitation. The analysis was implemented with the investigation of neurite outgrowth and with the assessment of the electrophysiological properties of putative proprioceptors by patch-clamp.

The analysis of differentially expressed genes and proteins indicated the dysregulation of pathways involved in cytoskeletal organization at the growth cone, in axon guidance and, at later stages of maturation, in synaptic plasticity. These data were corroborated by the observation of aberrations in neurite extension in FA cultures. Our preliminary data also suggested an alteration in the pattern of tonic firing in FA and IC neurons. Despite a partial reversal of the epigenetic state of the FXN locus and the reactivation of gene expression and FXN synthesis, only a partial recovery of the detected alterations was observed in IC neurons. Additionally, many differentially expressed non-coding RNAs were shared between FA and IC neurons.

Our study highlighted abnormalities affecting proprioceptive neurons differentiation in FA, particularly their ability to extend towards and communicate with their targets. The lack of full recovery in IC neurons suggested the need for further investigations to clarify the mechanistic link between frataxin deficiency and the observed alterations, and the contribution of other regulatory elements to FA pathogenesis.

# (#204) Novel genetic modifiers of SCA3/MJD: an EMS screening in a C. elegans model of the disease.

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 204

Thursday, 3rd November - 11:51: Breakout: Cell and Animal Models (Crystal Ballroom) - Flash talk - Abstract ID: 204

***Ms. Daniela Vilasboas-Campos [1], Mr. Jorge Fernandes [1], Ms. Cármen Vieira [1], Dr. Andreia Teixeira-Castro [1], Prof. Patrícia Maciel [1], Dr. Marta Daniela Costa [1]***

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In Spinocerebellar ataxia type 3/Machado-Joseph disease (SAC3/MJD), an abnormal CAG expansion in *ATXN3* causes late-onset cerebellar ataxia. Considering that the CAG repeat size explains about half of the disease heterogeneity, additional modifier loci must predict the remaining phenotypic variability in SCA3/MJD. The identification of novel modifier genes is therefore crucial to propose effective therapies for this disease. With that purpose, we developed an EMS-based screening in a *C. elegans* model of MJD/SCA3 to identify modifiers of the animals' motor dysfunction, a key disease hallmark.

The SCA3/MJD model was treated with EMS and mutants with a visually detectable improved motor phenotype were isolated from the F2 descendant population. Automated motor behavioural assays were used to quantify and validate the impact of EMS-induced mutations on motility. The pattern of mutant ATXN3 neuronal aggregation was also investigated in mutants with unchanged transgene expression relatively to the parental strain.

A total of 1251 mutants were isolated from the F2 generation of SCA3/MJD EMS-treated animals. Qualitative evaluations of the motility in descents revealed 90 mutants with better motor function than the original strain. The thrashing activity of these worms, quantified in the Wmicrotracker, confirmed that 54 mutants presented a significantly improved motility. A systematic study of this screening hits, including the evaluation of their motor performance using another motility assay and the quantification of mutant ATXN3 neuronal aggregation led to a final set of ten AT3q130 mutant strains in which whole genome sequencing will identify genetic modifiers of MJD/SCA3.

The identification of genetic modifiers of SCA3/MJD has the enormous potentiality to reveal novel genetic factors acting on this disorder. The relevance/validation of these modifiers can be further ascertained in other models and in patients. Importantly, such genetic modifier pathways can provide targets for new drug development, offering new therapeutic possibilities for SCA3/MJD patients.

# Breakout: Clinical Outcome Assessments and Natural History Studies

# The FA App: A Smartphone/Tablet Platform for Global Virtual Research in Friedreich Ataxia

Thursday, 3rd November - 10:30: Breakout: Clinical Outcome Assessments and Natural History Studies (Lalique Ballroom) - Oral - Abstract ID: 438

***Dr. Ian Harding*** [1], ***Mr. James Morgan*** [2], ***Dr. Louisa P. Selvadurai*** [3], ***Prof. Adam Vogel*** [4], ***Mr. Devon Borysiewicz*** [5], ***Ms. Monica Rex*** [6], ***Ms. Jennifer Farmer*** [6], ***Mr. Thomas Anthony*** [7]

***1.*** *Monash University,* ***2.*** *Department of Neuroscience, Monash University,* ***3.*** *Turner Institute for Brain and Mental Health, School of Psychological Sciences, Monash University, Clayton, VIC, Australia,* ***4.*** *The University of Melbourne,* ***5.*** *RedenLab Inc,* ***6.*** *Friedreich's Ataxia Research Alliance,* ***7.*** *EndFA*

Background: Research in rare inherited ataxias often relies on small cohort sizes, burdensome participant travel, one-off/infrequent data collection, and/or regionally siloed recruitment. Mobile digital technologies remove many of these barriers, offering opportunities to expand the scope and scale of research in diseases like Friedreich ataxia (FA). FA App is a freely available smartphone/tablet app developed in academic-advocacy-philanthropic partnership that provides a platform for virtual FA research studies.

Methods: We developed a multi-domain protocol to measure finger movement, processing speed, speech, and affect using simple touch-screen tasks, speech recordings, and patient-reported outcome measures (20-25mins total) for implementation in the FA App. An antecedent feasibility study was undertaken in a cohort of 19 people with inherited degenerative ataxias using a web-based platform. Participants independently completed the tasks monthly for 9 months.

Results: After 9 months, 15 of 19 participants remained in the study. Of these, 11 completed all 9 timepoints, 3 missed one or two timepoints, and 1 completed fewer than half. All touch-screen tasks provided moderate to excellent month-to-month within-subject reliability: simple speeded finger tapping was highly stable (ICC=0.89), simple reaction time more variable (ICC=0.76), and choice reaction time showed the greatest month-to-month fluctuation (ICC=0.59).

Conclusions: Participants were able to independently complete the task battery, compliance was high, attrition was acceptable, and task measures were relatively stable with increasing variability mirroring increasing task complexity. These results support roll-out in the FA App to assess patterns of short-term symptom variability and longitudinal progression in a larger cohort. This work also motivates further development of remote, digital functional assessments that can be undertaken without researcher/clinician oversight in order to leverage opportunities for increased research accessibility, sample sizes, and data sampling frequency. Further research collaborations and project integration in FA App are invited (Thomas@thefaapp.org).

# What matters to patients – a framework and resource for development of meaningful outcomes in ataxias

Thursday, 3rd November - 10:45: Breakout: Clinical Outcome Assessments and Natural History Studies (Lalique Ballroom) - Oral - Abstract ID: 514

***Dr. Rebecca Schuele [1], Dr. Julie Greenfield [2], Mrs. Charlotte Dubec [3], Mrs. Chantal Gobeil [4], Mrs. Lori Renna Linton [5], Mr. Andreas Nadke [6], Dr. Ruby Wallis [7], Prof. Cynthia Gagnon [8], Dr. Sophie Tezenas du Montcel [9], Prof. Matthis Synofzik [10]***

***1.*** *Hertie Institute for Clinical Brain Research, Tübingen,* ***2.*** *Ataxia UK, London, UK,* ***3.*** *Département de Biostatistique, Santé Publique et Information Médicale (BIOSPIM)- Hôpitaux Universitaires Pitié Salpêtrière - Charles Foix 47/83 boulevard de l'Hôpital 75013 PARIS,* ***4.*** *Fondation de l'Ataxie Charlevoix-Saguenay, 1000 Sherbrooke O, Montréal,QC,H3A 3G4, Canada,* ***5.*** *EURO-HSP, c/o Eurordis, Plateforme Maladies Rares 99 rue Didot, 75014 Paris,* ***6.*** *German Hereditary Ataxia Society (DHAG) & EURO-Ataxia, 70372 Stuttgart, Germany,* ***7.*** *Ataxia UK, London, N6 5JW, UK,* ***8.*** *Universite d,* ***9.*** *Sorbonne University,* ***10.*** *University of Tübingen*

*Background:* Establishing outcomes that can document disease progression and treatment response with high sensitivity is one of the central pillars of trial readiness for ataxias. While the field has made critical advances in development of a broad range of clinical and patient-reported outcome assessments, imaging markers, digital-motor measurements and molecular biomarkers, a clear operational framework to determine meaningfulness of these outcomes to patients is starkly missing. Yet, anchoring these outcomes to meaningful aspects of health impacted by ataxia is critical for their applicability in clinical trials and acceptance by regulators.

*Objectives:* To establish a comprehensive, patient-derived concept of aspects of health impacted by ataxia in a relevant way ('meaningful aspects of health') and provide a framework to develop patient-centered outcome assessments as well as anchor existing outcomes to meaningful aspects of health.

*Methods*: Two-step electronic survey to determine frequency, severity, and relevance of ataxia-related symptoms in an international cohort of > 1000 ataxia patients conducted in six languages (Dutch, English, French, German, Italian, Turkish) and across four continents.

*Results*: In a first survey (1047 respondents), a comprehensive list of symptoms present in ataxia was gathered. In a second survey (801 respondents) symptoms were evaluated based on their severity and relevance ("impact on day-to-day life physically and/or emotionally"). Top-ranking symptoms mapped to the domains ambulation, upper limb function, speech, bladder and bowel function, and social interaction. Based on the results, a comprehensive model of 'meaningful aspects of health' in ataxia was constructed and concepts of interest reflecting these aspects were deduced which are accessible to measurement.

*Discussion*: The ataxia meaningful aspects of health and the measurable concepts of interest derived from this model provide an invaluable resource that can be used by all outcome researchers in the ataxia field to anchor their outcomes to patient-centered concepts and thus demonstrate their relevance.

# (#135) The FA-HI & FACR-HI: Development and Validation of Two Novel Friedreich's Ataxia Outcome Measures

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 135

Thursday, 3rd November - 11:00: Breakout: Clinical Outcome Assessments and Natural History Studies (Lalique Ballroom) - Oral - Abstract ID: 135

***Mr. Spencer Rosero*** [1], ***Dr. Jane Larkindale*** [2], ***Ms. Susan Walther*** [3], ***Ms. Jamison Seabury*** [1], ***Ms. Anika Varma*** [1], ***Ms. Ellen Wagner*** [1], ***Mrs. Jennifer Weinstein*** [1], ***Mrs. Nuran Dilek*** [1], ***Mr. John Heatwole*** [4], ***Mr. Zachary Rose*** [1], ***Ms. Christine Zizzi*** [1], ***Dr. David Lynch*** [5], ***Ms. Courtney Park*** [5], ***Ms. Mckenzie Wells*** [5], ***Dr. Chad Heatwole*** [1]

***1.*** *University of Rochester Center for Health +Technology,* ***2.*** *Pepgen,* ***3.*** *The Friedreich's Ataxia Research Alliance,* ***4.*** *Pittsford Sutherland High School,* ***5.*** *Children's Hospital of Philadelphia*

**Background and Objective:** In preparation for upcoming clinical trials involving patients with FA, there is a clear need for reliable, sensitive, and disease-specific patient-reported outcome measures capable of detecting small, clinically relevant changes in therapeutic gain over time. According to the Food and Drug Administration (FDA), patient-measuring reported outcome measures are an effective mechanism to support drug labeling claims. In response to the need for therapeutic advancement for the FA community, we have developed and validated the FA-HI (Friedreich's Ataxia Health Index) and the FACR-HI (Friedreich's Ataxia Caregiver Reported Health Index) for use in FA therapeutic trials and clinical monitoring.

**Methods:** We conducted semi-structured qualitative interviews with FA patients and caregivers to identify symptoms of potential importance in FA. Next, we conducted a cross-sectional study to determine the FA symptoms of greatest prevalence and importance. We selected questions for the FA-HI and the FACR-HI based on their high relevance and potential responsiveness to therapeutic intervention. Instrument subscales, measuring granular areas of symptomatic health, were generated using factor analysis. We performed beta testing, known groups testing, and test-retest reliability assessments to optimize instrument clarity, usability, meaningfulness, responsiveness, and reliability.

**Results:** Thirty-nine patients and caregivers participated in the qualitative interviews and 202 participants completed our cross-sectional study. The final FA-HI and FACR-HI each contain 18 subscales that measure how a patient feels and functions. Validation testing found the FA Health-Indices and their subscales to be highly relevant, reliable, and capable of differentiating between patients with different levels of disease state.

**Discussion and Conclusion:** The development, optimization, and validation of the FA-HI and the FACR-HI provide researchers and clinicians with reliable mechanisms to measure relevant changes in how a patient feels and functions over time or in response to therapeutic intervention.

# (#412) Longitudinal observation of clinical scales and oculomotor neurophysiology since the pre-ataxic stage of Machado-Joseph disease: BIGPRO study

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 412

Thursday, 3rd November - 11:15: Breakout: Clinical Outcome Assessments and Natural History Studies (Lalique Ballroom) - Oral - Abstract ID: 412

***Prof. Laura Bannach Jardim*** [1], ***Dr. Camila Oliveira*** [2], ***Prof. Vanessa Bielefeldt Leotti*** [3], ***Dr. Amanda Cappelli*** [2], ***Dr. Anastacia Rocha*** [2], ***Dr. Gabriela Ecco*** [2], ***Dr. Gabriela Bolzan*** [2], ***Ms. Nathalia Kersting*** [2], ***Prof. Maria Luiza Saraiva-Pereira*** [4]

***1.*** *Hospital de Clinicas de Porto Alegre, Porto Alegre / Brazil,* ***2.*** *Universidade Federal do Rio Grande do Sul,* ***3.*** *Departamento de Estatística, Universidade Federal do Rio Grande do Sul, Porto Alegre,* ***4.*** *Universidade Federal do Rio Grande do Sul, Porto Alegre / Brazil*

Background and Objective: Little is known about the preclinical stage of Machado-Joseph Disease, also known as spinocerebellar ataxia type 3 (SCA3?MJD). Future disease-modifying treatments might be more effective if start early, even in preclinical disease. Therefore, we aimed to validate clinical and oculomotor variables for the pre-ataxic disease progression.

Methods: Pre-ataxic, ataxic carriers and controls were assessed at three visits with clinical scales - SARA, NESSCA, ICARS, INAScount, SCAFI and CCFS - and video-oculography - measurements of vestibulo-ocular reflex gain (VORr), saccades, pursuit and nystagmus. Pre-ataxic carriers (SARA less than 3) expected to start ataxia in 4 or less years were called “near onset” carriers. The progression of ataxic and pre-ataxic carriers, considering status at the end of the study, was described in two ways: using a dimension of time versus the start of gait ataxia for all carriers, TimeToAfterOnset,; and according to the study duration.

Results: 35 ataxic, 38 pre-ataxic carriers, and 22 controls were included. Visits 2 and 3 happened at median (IQR) 13 (0) and 27 (7) months. NESSCA (effect size 0.09), INAScount (0.07) and the vestibulo-ocular reflex gain (VORr, 0.12) significantly progressed in pre-ataxic carriers, in the “TimeToAfterOnset” timeline. In the study duration, NESSCA (1.36) and vertical pursuit gain (1.17) significantly worsened in “near onset” pre-ataxic carriers. 6/11 “near onset” pre-ataxic carriers converted to ataxia during the study. For a clinical trial with 80% power and 2-year duration, 57 “near onset” pre-ataxic carriers are needed per study arm to detect a 50% reduction in the conversion rate.

Discussion and Conclusion: There was significant deterioration of VORr, NESSCA and INAScount in the pre-ataxic phase, and “TimeToAfterOnset” was sometimes superior to the study duration, to detect these changes. For trials in pre-ataxic carriers, we recommend recruiting “near onset” subjects and using the conversion rate as the primary outcome.

# Development and validation of the dysarthria impact scale

Thursday, 3rd November - 11:30: Breakout: Clinical Outcome Assessments and Natural History Studies (Lalique Ballroom) - Flash talk - Abstract ID: 444

***Prof. Adam Vogel*** [1]***, Ms. Lisa Graf*** [2]***, Dr. Jess Chan*** [1]***, Prof. Graham Hepworth*** [1]***, Dr. Merit Bade*** [3]***, Prof. Matthis Synofzik*** [4]

***1.*** *The University of Melbourne,* ***2.*** *University Hospital Tübingen,* ***3.*** *Center for Neurology, University Hospital Tübingen,* ***4.*** *University of Tübingen*

**Background:** The loss of the ability to speak is a devastating and inevitable outcome of many neurodegenerative diseases. It results in daily disadvantage, stigmatization, social marginalization, and underemployment. There are few well designed patient reported outcomes specifically designed to measure the impact of dysarthria on quality of life.

**Objectives**: To develop and validate the Dysarthria Impact Scale (DIS) as a patient reported outcome (PRO) in neurological disease.

**Methods**: 200 plus participants with either hereditary ataxia, Huntington's disease, patients in a head and neck ward, or healthy controls recruited. The DIS was initially developed as a 22-item questionnaire. Item reduction resulted in two versions, a full 17 item version and a 6-item brief form. Participants provided speech samples, and were assessed using disease severity tools, and benchmark tests for voice related quality of life and the Short Form 36. Speech was analyzed perceptually by expert listeners for intelligibility (ability to be understood). A subset of participants completed the survey 1 month apart.

**Results**: The short and full versions of the DIS are highly correlated with benchmark tools (Pearson 0.7-0.9). Test-retest one month apart was high (>0.97). ROC analysis for separating dysarthric versus control speakers yielded an area under the curve of (>0.95). Materials have been translated from English into German, French, Polish, Czech and Turkish.

**Discussion**: We provide a brief, easy to use, validated and sensitive PRO for describing the impact of dysarthria in neurological disease.

# (#216) The S-Factor, a new measure of disease severity in spinocerebellar ataxia: Findings and implications

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 216

Thursday, 3rd November - 11:37: Breakout: Clinical Outcome Assessments and Natural History Studies (Lalique Ballroom) - Flash talk - Abstract ID: 216

***Dr. Louisa P. Selvadurai*** [1], ***Prof. Susan L. Perlman, MD*** [2], ***Dr. George R. Wilmot*** [3], ***Dr. Sub H. Subramony*** [4], ***Dr. Christopher M. Gomez*** [5], ***Dr. Tetsuo Ashizawa*** [6], ***Dr. Henry Paulson*** [7], ***Dr. Chiadi U. Onyike*** [8], ***Dr. Liana S. Rosenthal*** [9], ***Dr. Haris I. Sair*** [10], ***Dr. Sheng-Han Kuo*** [11], ***Dr. Eva-Maria Ratai*** [12], ***Dr. Theresa Zesiewicz*** [13], ***Dr. Khalaf O. Bushara*** [14], ***Dr. Gulin Oz*** [15], ***Dr. Cameron Dietiker*** [16], ***Prof. Michael D. Geschwind*** [16], ***Dr. Alexandra B. Nelson*** [16], ***Dr. Puneet Opal*** [17], ***Prof. Talene A. Yacoubian*** [18], ***Prof. Peggy C. Nopoulos*** [19], ***Dr. Vikram G. Shakkottai*** [20], ***Dr. Karla P. Figueroa*** [21], ***Dr. Stefan M. Pulst*** [22], ***Dr. Peter E. Morrison*** [23], ***Dr. Jeremy D. Schmahmann*** [1]

***1.*** *Massachusetts General Hospital,* ***2.*** *David Geffen School of Medicine at UCLA, Los Angeles, CA,* ***3.*** *Department of Neurology, Emory University School of Medicine, Atlanta, GA,* ***4.*** *McKnight Brain Institute, Department of Neurology, University of Florida, Gainesville, Florida, United States of America,* ***5.*** *University of Chicago,* ***6.*** *The Houston Methodist Research Institute,* ***7.*** *University of Michigan,* ***8.*** *Johns Hopkins University School of Medicine, Baltimore, Maryland,* ***9.*** *Neurology, Johns Hopkins University,* ***10.*** *Russell H. Morgan Department of Radiology and Radiological Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland,* ***11.*** *Columbia Univeristy Medical Center,* ***12.*** *Massachusetts General Hospital, Charlestown,* ***13.*** *Department of Neurology, University of South Florida Ataxia Research Center, Tampa, FL,* ***14.*** *Department of Neurology, University of Minnesota, Minneapolis,* ***15.*** *Center for Magnetic Resonance Research, Department of Radiology, University of Minnesota, Minneapolis, Minnesota, United States,* ***16.*** *Department of Neurology, University of California, San Francisco,* ***17.*** *Northwestern University,* ***18.*** *Department of Neurology, University of Alabama at Birmingham,* ***19.*** *Department of Psychiatry, University of Iowa Carver College of Medicine,* ***20.*** *Department of Neurology & Neurotherapeutics, University of Texas Southwestern Medical Center, Dallas, TX,* ***21.*** *Department of Neurology, University of Utah, Salt Lake City,* ***22.*** *Department of Neurology, University of Utah, Salt Lake City,,* ***23.*** *Department of Neurology, University of Rochester Medical Center, Rochester*

**Background:** Spinocerebellar ataxias (SCAs) are progressive neurodegenerative disorders, but there is no metric that predicts disease severity over time. We hypothesized that by developing a new metric, the Severity Factor (S-Factor) using immutable disease parameters, it would be possible to capture disease severity independent of clinical rating scales.

**Methods:** Extracting data from the CRC-SCA and READISCA natural history studies, we calculated the S-Factor for 438 participants with symptomatic SCA1, SCA2, SCA3, or SCA6, as follows: ((length of CAG repeat expansion – maximum normal repeat length) /maximum normal repeat length) x (current age – age at disease onset) x 10). Within each SCA type, the S-Factor at the first Scale for the Assessment and Rating of Ataxia (SARA) visit (baseline) was correlated against scores on SARA and other motor and cognitive assessments. In 281 participants with longitudinal data, the slope of the S-Factor over time was correlated against slopes of scores on SARA and other motor rating scales.

**Results:** At baseline, the S-Factor showed moderate-to-strong correlations with SARA and other motor rating scales

at the group level, but not with cognitive performance. Longitudinally the S-Factor slope showed no consistent association with the slope of performance on motor scales. Approximately 30% of SARA slopes reflected a trend of non-progression in motor symptoms.

**Conclusion**: The S-Factor is an observer-independent metric of disease burden in SCAs. It may be useful at the group level to compare cohorts at baseline in clinical studies. Derivation and examination of the S-factor highlighted challenges in the use of clinical rating scales in this population.

# (#215) FA-CHILD – A 3-year, 6 Month-Interval Natural History Study in Children with Friedreich's ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 215

Thursday, 3rd November - 11:44: Breakout: Clinical Outcome Assessments and Natural History Studies (Lalique Ballroom) - Flash talk - Abstract ID: 215

***Dr. Christian Rummey*** [1]***, Dr. Sub H. Subramony*** [2]***, Prof. Susan L. Perlman, MD*** [3]***, Dr. David Lynch*** [4]

***1.*** *Clinical data science GmbH,* ***2.*** *McKnight Brain Institute, Department of Neurology, University of Florida, Gainesville, Florida, United States of America,* ***3.*** *David Geffen School of Medicine at UCLA, Los Angeles, CA,* ***4.*** *University of Pennsylvania & Childrens Hospital of Philadelphia*

**Background** and Objective – As understanding of the natural neurological course of FRDA advances rapidly, attention is shifting to potential gaps in translating findings into clinical trial design. This is particularly important for individuals less than 18 years of age, a group that gene therapy programs will naturally focus on, and that is known to have increased variability in disease progression and, at particularly young ages, a divergent progression profile. The FA-CHILD study particularly investigates this variability under the stricter conditions of 6-month intervals. In addition, new promising outcome measures were evaluated.

**Methods** – Progression in FA-CHILD in all well-established parameters (i.e., mFARS score, Upright Stability, ADL, timed 25-foot walk and the 9-hole peg test) as well as several new or less commonly used measures (Berg Balance Scale, 6- and 1-min walk tests, timed up and go test) is reported. Using linear mixed effect modeling the complete population is evaluated, as well as ambulation, age-and and severity-based subgroups. The study attempted to minimize the impact of the covid pandemic by providing the possibility of virtual follow up visits.

**Results** – Three sites (Children's Hospital of Philadelphia, University of Florida, University of California Los Angeles) enrolled a total of 108 patients, with visits occurring between Oct 10th, 2017, and Sep 2nd, 2021. 105 subjects provided post-BL data (496 follow up visits). Subgroup results indicate differential progression rates. Both and the Berg Balance scale and the one-minute walk showed promising results as new outcome measures.

**Discussion and Conclusion** – Results confirm the mFARS- and Upright stability scores as sensitive and relevant outcome measures in this young, severely affected population. The cohort identified provides a representative group for investigation of the change over time in clinical measures and biomarkers in FRDA. Its ongoing characterization will be useful in planning of future therapeutic interventions.

# (#192) Gait, electromyography and synergies analysis of Freidreich's ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 192

Thursday, 3rd November - 11:51: Breakout: Clinical Outcome Assessments and Natural History Studies (Lalique Ballroom) - Flash talk - Abstract ID: 192

***Ms. Sofia Campi [1], Ms. Susanna Summa [2], Ms. Camilla Pierella [1], Ms. Martina Favetta [2], Mr. Maurizio Petrarca [2], Dr. Enrico Castelli [2], Prof. Maura Casadio [1], Dr. Enrico Bertini [3], Dr. Gessica Vasco [2]***

***1.*** *Department Informatics, Bioengineering, Robotics and Systems Engineering (DIBRIS), University of Genoa, Genoa,* ***2.*** *Movement Analysis and Robotics Laboratory (MARLAB) Intensive Neurorehabilitation and Robotics Department, Bambino Gesù Children's Hospital, IRCSS, Rome,* ***3.*** *Unit of Neuromuscular and Neurodegenerative Disorders Laboratory of Molecular Medicine Department Neurosciences, Bambino Gesù Children's Hospital, IRCSS, Rome*

**Background and Objective**

Friedreich's ataxia (FA) is the most common autosomal recessive form of neurodegenerative ataxia. We present a study on the gait pattern of children and adolescents affected by FA using gait analysis (GA) and electromyography (EMG). To the best of our knowledge, most of the studies have mainly focused on spatio-temporal parameters, few on kinematic and kinetic; even less on EMG analysis and no research group, to date, has dealt with the analysis of muscle synergies. Synergy analysis could be appropriate to investigate and to characterize specifically these aspects and enrich the standard GA and EMG analysis.

**Methods**

We assessed the spectrum of changes over 14±4 months enrolling 10 genetically confirmed patients affected by FA together with 10 normally developing age-matched subjects. Standardized gait analysis with a motion capture system (Vicon MX, UK) and EMG system (Cometa, Italy), performed gait and muscular evaluation.

**Results**

By comparing the parameters of FA with the control group, we found that FA adopt a different walking strategy, characterized by a shorter stride length and an increase in temporal parameters. In particular, the increased knee and ankle extension in stance revealed a peculiar biomechanical pattern. These results confirm what emerge in muscles envelopes. The synergy analysis confirms a difference in the weights of the first synergy, associated with the leg flexors; synergies 3 and 4 highlight a greater participation of the gastrocnemius and soleus in the initial stance phase.

**Discussion and Conclusion**

In conclusion, our findings shows a different gait pattern of FA, characterized by greater knee extension during stance. Confirming these results, synergies could be a new biomarker for the assessment of motor coordination in FA. Functional outcomes integrated by instrumental evaluation, increase sensitivity, reliability and suitability assessing the disease progression in the application of clinical trials and of rehabilitative programs.

# Breakout: Emerging Therapies (Clinical)

# (#97) Safety and Pharmacokinetics of Single and 13 Day Multiple-Dose Administration of CTI-1601, a Frataxin Replacement Therapy for Friedreich's Ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 97

Thursday, 3rd November - 16:00: Breakout: Emerging Therapies (Clinical) (Crystal Ballroom) - Oral - Abstract ID: 97

***Dr. David Lynch [1], Ms. Jennifer Farmer [2], Ms. Teresa Galas [3], Dr. Russell Clayton [4], Dr. David Bettoun [3], Ms. Angela Miller [3], Dr. Nancy Ruiz [3], Ms. Noreen Scherer [3]***

***1.*** *Children's Hospital of Philadelphia,* ***2.*** *Friedreich's Ataxia Research Alliance,* ***3.*** *Larimar Therapeutics, Inc.,* ***4.*** *Aeremedea LLC*

**Background and Objective:** The safety and pharmacokinetics (PK) of CTI-1601, a frataxin (FXN) replacement therapy designed to deliver human FXN into mitochondria in development for Friedreich's ataxia (FRDA), were evaluated.

**Methods:** Safety and plasma PK of CTI-1601 were assessed in 2 phase 1, double-blind, placebo-controlled trials in patients with FRDA (aged ≥18 years). In a single ascending-dose (SAD) study (NCT04176991), patients (n=28) were randomized to subcutaneous (SC) CTI-1601 (25, 50, 75, or 100 mg) or placebo. In a multiple ascending-doses (MAD) study (NCT04519567), patients (n=27) were randomized to placebo or 1 of the following CTI-1601-dose regimens: 25 mg once daily (QD) for the first 4 days, then every 72 hours until day 13 (7 doses; 50 mg QD for the first 7 days, then every other day until day 13 (10 doses); or 100 mg QD for 13 days (13 doses).

**Results:** All CTI-1601 doses were well tolerated in both studies. Most treatment-emergent adverse events (TEAEs) were grade 1 or 2 with injection-site reactions most commonly reported (MAD: CTI-1601, 100%; placebo, 43%); these were brief, self-limited, and mostly grade 1 in severity. In SAD and MAD, CTI-1601 plasma concentrations reached a maximum shortly after SC injection, demonstrating rapid uptake of CTI-1601 into the intravascular circulation. In MAD, CTI-1601 exposure increased in a linear-dose–proportional manner across the 25- to 100-mg range; SAD data were consistent. Dose-dependent exposure increases were observed with QD dosing for 4 and 7 days; exposure decreased with less frequent dosing.

**Discussion and Conclusion:** CTI-1601 was generally well tolerated. PK data suggest that daily dosing may be required to maintain exposure. These data from the first studies of CTI-1601, a therapy intended to increase FXN protein in patients with FRDA, support a positive benefit-risk profile and warrant continued study of CTI-1601.

# Home Aerobic Training versus Balance Training for Cerebellar Ataxia: A Randomized Controlled Trial

Thursday, 3rd November - 16:15: Breakout: Emerging Therapies (Clinical) (Crystal Ballroom) - Oral - Abstract ID: 376

***Dr. Scott Barbuto*** [1], ***Dr. Sheng-Han Kuo*** [2], ***Ms. Lauren Winterbottom*** [1], ***Dr. Yaakov Stern*** [1], ***Dr. Seonjoo Lee*** [1], ***Dr. Joel Stein*** [1]

***1.*** *Columbia University Irving Medical Center,* ***2.*** *Columbia Univeristy Medical Center*

**Background & Objective:** Balance training has shown some benefits in cerebellar ataxia whereas the effects of aerobic training are relatively unknown. In this study we wanted to determine whether a phase III trial comparing home aerobic to balance training in ambulatory patients with cerebellar ataxia is warranted.
**Methods:** We conducted a single center, assessor-blinded, randomized controlled trial. Participants randomized to the aerobic group were expected to train for thirty-minutes, five times per week at 65-80% of their maximum heart rate for 6-months. Individuals randomized to home balance training conducted the same frequency and duration of exercise. The primary outcome was improvement in ataxia as measured by the Scale for the Assessment and Rating of Ataxia (SARA). Secondary outcomes included safety, training adherence, and balance improvements.
**Results:** Nineteen subjects were randomized to aerobic training and 17 subjects to balance training. There were no differences between groups at baseline. Thirty-one participants completed the trial, and there were no training-related serious adverse events. Compliance to training was over 70%. There was a mean improvement in ataxia symptoms of 1.9 SARA points (SD 1.62) in the aerobic group compared to an improvement of 0.6 points (SD 1.34) in the balance group. Although two measures of balance were equivocal between groups, one measure of balance showed greater improvement with balance training compared to aerobic training.
**Discussion & Conclusions:** This 6-month trial comparing home aerobic versus balance training in cerebellar ataxia had excellent retention and adherence to training. There were no serious adverse events, and training was not interrupted by minor adverse events like falls or back pain. There was a significant improvement in ataxia symptoms with home aerobic training compared to balance training, and a phase III trial is warranted.

# SpeechATAX: A rater blinded randomized controlled trial of intensive home-based biofeedback therapy for dysarthria progressive ataxia

Thursday, 3rd November - 16:30: Breakout: Emerging Therapies (Clinical) (Crystal Ballroom) - Oral - Abstract ID: 168

***Prof. Adam Vogel*** [1], ***Ms. Hannah Reece*** [1], ***Ms. Lisa Graf*** [2], ***Dr. Sabine Braat*** [1], ***Dr. Stephanie Borel*** [3], ***Prof. Alexandra Durr*** [4], ***Dr. Michelle Magee*** [1], ***Prof. Richard Roxburgh*** [5], ***Prof. Matthis Synofzik*** [6]

***1.*** *The University of Melbourne,* ***2.*** *University Hospital Tübingen,* ***3.*** *ICM Institute for Brain and Spinal Cord,* ***4.*** *ICM,* ***5.*** *The University of Auckland,* ***6.*** *University of Tübingen*

Background: Speech quality and clarity is affected by hereditary ataxia. The loss of the ability to speak can lead to significant declines in quality of life through social isolation, underemployment and reduced ability to complete daily tasks. Very limited evidence is available for treatments designed to improve speech in ataxia. This study investigated the effectiveness of a digitized intensive home-based speech rehabilitation, SpeechATAX, in people with progressive hereditary ataxia.

Methods: Participants with ataxia completed a 4-week intensive home-based rehabilitation for dysarthria (SpeechATAX), using a handheld tablet. Participants were recruited from clinics in Australia, Germany, New Zealand and France. SpeechATAX was designed to improve intelligibility, vocal control, and prosody. Feedback and practice were based on principles of motor learning and neuroplasticity. During the 4-week rehabilitation period, patients trained for 45 min per day, 5 days per week (20 days of active treatment). Subjects were assessed at 4 timepoints: 4 weeks prior to therapy (baseline), immediately prior to the 4-week home-based speech rehabilitation (pre), immediately after the 4-week home-based speech rehabilitation (post), and 4 weeks after to speech rehabilitation (retention). Speech and voice samples were recorded and analysed by expert blinded raters. The primary outcome measure was intelligibility. Objective acoustic analysis was also performed.

Results: 163 patients with progressive ataxia commenced treatment. In the cohort, approximately half had Friedreich's Ataxia, with the remaining Spinocerebellar Ataxia across (SCAs 1, 2, 3, 6) and about 10% with other types of progressive ataxia. Intelligibility, naturalness and SARA speech item all improved post rehabilitation when compared to baseline. This aligned with acoustic measures of speech.

Conclusion: SpeechATAX can alleviate dysarthria in progressive ataxias, with improvements noted in intelligibility, vocal control, and voice quality.

# (#120) Autophagy as a treatment pathway in Spinocerebellar Ataxia: SLS-005 (Trehalose injection, 90.5 mg/mL for intravenous infusion)

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 120

Thursday, 3rd November - 16:45: Breakout: Emerging Therapies (Clinical) (Crystal Ballroom) - Oral - Abstract ID: 120

***Dr. David Biondi*** [1], ***Mr. Luke Pilipski*** [1], ***Dr. Timothy Whitaker, MD*** [1], ***Mr. Alec Quintalino*** [1], ***Ms. Claudia Moore*** [1], ***Dr. Raj Mehra, PhD*** [1]

***1.*** *Seelos Therapeutics Inc*

Seelos Therapeutics is developing SLS-005 (an intravenous formulation of trehalose) a naturally occurring disaccharide, for the chronic treatment of spinocerebellar ataxia (SCA). Evidence of the potential effect of trehalose in the treatment of SCA is derived from nonclinical and clinical studies conducted to date.

Trehalose is able to prevent pathological aggregation of proteins within cells and animal models in several diseases associated with abnormal cellular-protein aggregation such as the polyalanine (PolyA) or polyglutamic (PolyQ) repeats disorders, such as spinocerebellar ataxia (SCA).

Trehalose is well known for its protein-stabilizing properties, and its ability to enter cells (for example, hepatocytes) via the GLUT8 glucose transporter might stabilize aggregating mutated proteins within cells. In addition to its ability to stabilize proteins, trehalose has a number of other potentially therapeutic mechanisms of action. It was shown to inhibit glucose transporters (SLCA2 family), creating a temporary glucose starvation that leads to enhanced autophagy. Trehalose's ability to enhance autophagy was also shown in cellular and animal models of Huntington disease, oculopharyngeal muscular dystrophy, spinocerebellar ataxia and other disorders.
Phase 2, dose-controlled study in 15 SCA3 patients, evaluated the safety and tolerability of 2 different IV doses of SLS-005, 13.5 and 27 g administered weekly. Trehalose appeared generally safe and well tolerated in this patient population. The primary efficacy endpoint was the change from baseline on the Scale for the Assessment and Rating of Ataxia (SARA). On average, all patients' SARA scores remained stable over the 6-month period.

Trehalose has demonstrated ability to prevent pathological aggregation of proteins within cell and animal models in several diseases associated with abnormal cellular-protein aggregation and has shown relevant activity in models of SCA. The sponsor, Seelos, intends to continue the evaluation of SLS-005 in a phase 3 clinical study in patients with SCA.

# (#210) Engage-Ataxia: Preliminary results after 1 year from a physical activity coaching intervention in individuals diagnosed with ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 210

Thursday, 3rd November - 17:00: Breakout: Emerging Therapies (Clinical) (Crystal Ballroom) - Flash talk - Abstract ID: 210

***Dr. Chelsea Macpherson*** [1], ***Ms. Bria Bartsch*** [1], ***Ms. Miriam King*** [1], ***Dr. Sheng-Han Kuo*** [2], ***Dr. Lori Quinn*** [1]

***1.*** *Teachers College, Columbia University,* ***2.*** *Columbia Univeristy Medical Center*

**Background and Objective:**
Physical activity (PA) can be a powerful neuroprotective tool for people with neurodegenerative diseases,[1–4] however rehabilitation programs often fail to address strategies to increase PA engagement.[5–7] The *Engage* intervention is a well-tested framework that has been shown to increase exercise self-efficacy and PA uptake in Parkinson's[8] and Huntington's disease.[9,10] This study evaluates the feasibility and acceptability of the *Engage* intervention in people with ataxia.

**Methods:** *Engage-Ataxia* is an ongoing single-cohort study. The intervention consists of a 5-session PA coaching program delivered by a physical therapist on telehealth over 12-weeks. The intervention is grounded in self-determination theory and includes a disease-specific workbook to guide sessions, with consideration of balance and gait impairments, motor learning deficits, and fatigue. Sessions include individualized exercise recommendations, goal-setting, and methods to overcome exercise barriers. Participants use a Fitbit to monitor PA and heart rate.

**Results:** 34 people were screened and 25 were enrolled (73.5% recruitment rate); 19 completed the intervention. Mean (SD) age of completed participants was 55.8(13.7) yrs; 8M/11F. Retention was 95% with 1 participant lost to follow up; adherence rate was 100%. Diagnoses included multiple system atrophy cerebellar type, spinocerebellar ataxia type 1, 2, 3, 6, and 28, ataxia associated with SPG7 mutation, anti-TPO ataxia, and ataxia with oculomotor apraxia type 2. Assessments were conducted by a clinical researcher. Mean differences (95% CI) comparing pre-post intervention were: modified Scale for Assessment and Rating of Ataxia 0.79 (0.41, 1.17); Cerebellar Cognitive Affective Syndrome Scale 4.90 pts (1.34, 8.45), Exercise Self-Efficacy 4.42 (-2.57, 11.41), Exercise Identity Scale 3.00 (0.59, 5.41), and Activities Specific Balance Confidence Scale 7.54% (1.14, 13.95).

**Discussion and Conclusion**: *Engage-Ataxia* provides an implementable framework to increase PA in people with ataxia. Preliminary data supports this intervention, with improvements in behavior change and disease-specific measures of motor and cognitive function.

# (#403) A Phase 1/2 Study of the Safety and Efficacy of LX2006 Gene Therapy in Participants with Cardiomyopathy Associated with Friedreich's Ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 403

Thursday, 3rd November - 17:07: Breakout: Emerging Therapies (Clinical) (Crystal Ballroom) - Flash talk - Abstract ID: 403

***Dr. Clarice Lee [1], Mrs. Jaime May [1], Dr. Richie Khanna [1], Mr. Allen Reha [1], Dr. Keith Wonnacott [1], Dr. Jay Barth [1]***

***1.*** *LEXEO Therapeutics Inc.*

**Background and Objective:** Friedreich's ataxia (FA) is a rare, autosomal recessive disease caused by a mutation in the autosomal frataxin (*FXN*) gene. Progressive cardiomyopathy with cardiac hypertrophy and fibrosis is observed in most individuals with FA. The disease is more severe in those with earlier onset. Presently, there is no therapy that alters the progression of cardiomyopathy in FA, which is responsible for 60% of FA-related deaths.

The primary objective of this dose-ranging study is to assess the safety and tolerability of two ascending doses of LX2006 in patients with FA-associated cardiomyopathy. LX2006 is an adeno-associated virus (AAV) gene therapy designed to intravenously deliver the human FXN (AAVrh.10hFXN) gene to cardiac cells, restoring frataxin levels in order to improve mitochondrial function. Assessments of cardiac function, biomarkers and other preliminary efficacy endpoints are also included in this study.

**Methods:** This a multicenter, first in human, Phase 1/2, 52-week dose-ascending, open-label study followed by a 4-year long-term follow-up (LTFU) portion of the study for all participants who receive LX2006.

Two sequential cohorts (N=5, adult FA participants in each cohort) will be enrolled at escalating doses of LX2006. Key eligibility criteria for this study include age ≥18 to ≤ 40 years, onset of FA prior to 25 years of age, left ventricular ejection fraction by cardiac MRI ≥ 45%, left ventricular hypertrophy, no contraindications to cardiac biopsies, normal kidney, lung and liver function, and controlled diabetes (if present).

**Discussion & Conclusion:** This is the first clinical study of a gene therapy for the treatment of FA-associated cardiomyopathy. LX2006 is hypothesized to be safe and tolerable as well as stabilize or improve cardiomyopathy associated with FA.

# A randomised placebo-controlled crossover trial of micronised resveratrol as a treatment for Friedreich ataxia

Thursday, 3rd November - 17:14: Breakout: Emerging Therapies (Clinical) (Crystal Ballroom) - Flash talk - Abstract ID: 387

***Ms. Geneieve Tai [1], Dr. Ian Woodcock [2], Dr. Eppie Yiu [3], Dr. Louise A Corben [4], Prof. Katherine J Lee [5], Dr. Christina Liang [6], Dr. John O'Sullivan [7], Prof. Phillipa Lamont [8], Prof. Martin B Delatycki [9]***

***1.*** *Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia,,* ***2.*** *Department of Paediatrics, University of Melbourne, Parkville, Victoria, Australia; Department of Neurology, The Royal Children's Hospital, Parkville, Victoria, Australia; Murdoch Children's Research Institute, Parkville, Victoria, Australia,* ***3.*** *Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia; Department of Paediatrics, University of Melbourne, Parkville, Victoria, Australia;,* ***4.*** *Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia, School of Psychological Sciences, The Turner Institute for Brain and Mental Health, Monash University, Clayton, Victoria, Australia,,* ***5.*** *Department of Paediatrics, University of Melbourne, Parkville, Victoria, Australia; Clinical Epidemiology & Biostatistics (CEBU), Population Health, Murdoch Children's Research Institute, Parkville, Victoria, Australia,* ***6.*** *Department of Neurology, Royal North Shore Hospital, St Leonards, NSW, Australia,* ***7.*** *Department of Neurology, Royal Brisbane and Women's Hospital, Herston, Queensland, Australia; Centre for Clinical Research, University of Queensland, Herston, Queensland, Australia,* ***8.*** *Department of Neurology, Royal Perth Hospital, Perth, Western Australia, Australia,* ***9.*** *Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia; Victorian Clinical Genetics Services, Parkville, Victoria, Australia*

**Background and Objective**

Resveratrol is a naturally occurring compound (1) postulated to have antioxidant and neuroprotective properties (2). Resveratrol has been identified as a potential treatment for Friedreich ataxia (FRDA) (3). An open-label, proof of principle study previously demonstrated evidence of clinical benefit in people with FRDA treated with 5g/day of resveratrol for 12 weeks (4). The aim of this randomised, blinded, placebo-controlled study was to assess the safety and efficacy of micronized resveratrol as a treatment for FRDA.

**Methods**

Participants were randomised to receive 1g twice daily of micronized resveratrol in Period 1 and twice daily placebo in Period 2; or placebo in Period 1 and micronized resveratrol in Period 2. Both periods lasted 24 weeks with a 4-week washout in between.

The primary objective was to compare change in the modified Friedreich Ataxia Rating Scale (mFARS) from baseline to 24 weeks following treatment with 2g/day of micronized resveratrol, to placebo.

Secondary outcome measures included functional measures (full FARS, 9-hole peg test, Berg Balance Scale, Ataxia Instrumented Measure-Spoon), patient reported outcome measures (Friedreich Ataxia Impact Scale, Modified Fatigue Impact Scale), tests of speech and hearing, cardiac parameters and biomarkers including frataxin levels.

The study will be analysed according to the intention to treat (ITT) principle. Comparisons between the two treatments will be made using mixed effects models applied to the change in primary and secondary outcome measures from baseline to 24 weeks during the two periods.

**Results**

Twenty-five participants were enrolled. Seventeen have completed the study. Four participants have withdrawn, one participant was lost to follow up, and the remaining 3 participants will complete the study by the end of August. Results will be available for presentation at the meeting.

# (#432) Cerebellar transcranial direct current stimulation in spinocerebellar ataxia type 3: a randomized, double-blind, sham-controlled trial

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Flash talk - Abstract ID: 432

Thursday, 3rd November - 17:21: Breakout: Emerging Therapies (Clinical) (Crystal Ballroom) - Flash talk - Abstract ID: 432

***Dr. Roderick Maas*** [1]***, Dr. Steven Teerenstra*** [2]***, Prof. Ivan Toni*** [3]***, Dr. Thomas Klockgether*** [4]***, Prof. Dennis Schutter*** [5]***, Prof. Bart van de Warrenburg*** [1]

***1.*** *Department of Neurology, Donders Institute for Brain, Cognition, and Behaviour, Radboud University Medical Center, Nijmegen,* ***2.*** *Department of Health Evidence, Biostatistics section, Radboudumc, Nijmegen,* ***3.*** *Donders Institute for Brain, Cognition, and Behaviour, Radboud University, Nijmegen,* ***4.*** *German Center for Neurodegenerative Diseases (DZNE), Bonn,* ***5.*** *Experimental Psychology, Helmholtz Institute, Utrecht University, Utrecht*

**Background:** Repeated sessions of cerebellar anodal tDCS have been suggested to modulate cerebellar-M1 connectivity and decrease ataxia severity. However, therapeutic trials involving etiologically homogeneous groups of ataxia patients are lacking.

**Objective:** To investigate if a two-week regimen of daily cerebellar tDCS sessions diminishes ataxia and non-motor symptom severity and alters cerebellar-M1 connectivity in individuals with SCA3.

**Methods:** We conducted a randomized, double-blind, sham-controlled trial in which twenty SCA3 patients received ten sessions of real or sham cerebellar tDCS (i.e., five days/week for two consecutive weeks). Effects were evaluated after two weeks, three months, six months, and twelve months. Change in SARA score was defined as the primary endpoint. Static posturography, SCA Functional Index tests, various patient-reported outcome measures, the cerebellar cognitive affective syndrome scale, and paired-pulse transcranial magnetic stimulation to examine cerebellar brain inhibition (CBI) served as secondary endpoints.

**Results:** Absolute change in SARA score did not differ between both trial arms at any of the time points. We observed significant short-term improvements in several motor, cognitive, and patient-reported outcomes after the last stimulation session in both groups but no treatment effects in favor of real tDCS. Nonetheless, some of the patients in the intervention arm showed a sustained reduction in SARA score lasting six or even twelve months, indicating interindividual variability in treatment response. CBI, which reflects the functional integrity of the cerebellothalamocortical tract, remained unchanged after ten tDCS sessions. Albeit exploratory, there was some indication for a decrease in SARA speech score after six and twelve months.

**Conclusion:** This study does not provide evidence that a two-week treatment with daily cerebellar tDCS sessions reduces ataxia severity or restores cerebellar-M1 connectivity in SCA3 patients at the group level. In order to potentially increase therapeutic efficacy, further research is warranted to identify individual predictors of symptomatic improvement.

# Late-Breaking Research

# Variegated silencing in Friedreich ataxia

Friday, 4th November - 09:00: Plenary: Late-Breaking Research (Crystal Ballroom) - Oral - Abstract ID: 528

***Ms. Morgan Tackett*** [1]***, Ms. Christina Lam*** [1]***, Dr. David Lynch*** [2]***, Prof. Sanjay Bidichandani*** [1]

***1.*** *University of Oklahoma Health Sciences Center,* ***2.*** *University of Pennsylvania & Childrens Hospital of Philadelphia*

Background and Objective: Variegated silencing, classically seen as position effect variegation in *Drosophila*, occurs when gene expression is suppressed in a proportion of cells due to proximity to repressive chromatin. The expanded GAA repeat in Friedreich ataxia (FRDA), located close to *FXN* gene regulatory elements, causes epigenetic silencing of the *FXN* gene. Bereft of mitigating chromatin insulators, we reasoned that this genetic defect could result in variegated silencing. Most patients are homozygous for alleles with >500 triplets; however, ~20% of patients have one allele (GAA1) with <500 triplets and a distinctly milder phenotype. We hypothesized that this milder phenotype stems from a higher proportion of somatic cells with *FXN* genes that have been spared from epigenetic silencing, consistent with variegated silencing.

Methods: Single-cell ATACseq and single-cell RNAseq (10xGenomics platform) were performed on PBMCs.

Results: Single cell ATACseq revealed that chromatin accessibility at the *FXN* locus is localized to the *FXN* promoter, and it occurs in a significantly smaller proportion of cells in FRDA (p=6.7E-61). Chromatin accessibility in FRDA with GAA1 <500 triplets is seen in a significantly higher proportion of cells than in conventional FRDA (p=1.3E-13). *FXN* chromatin access, when seen in FRDA cells, is qualitatively similar to non-FRDA cells, and in FRDA with GAA1 <500 triplets as many as a third of the cells have one normally accessible *FXN* gene. Single cell RNAseq revealed a significantly higher proportion of *FXN*-expressing cells in FRDA patients with GAA1 <500 triplets (p=8.2E-16).

Conclusion: Shorter GAA expansions permit enhanced sparing from *FXN* gene silencing in somatic cells, and this epigenetic mosaicism fits with variegated silencing as the molecular mechanism for the milder phenotype in patients with such repeats. This suggests that significant clinical benefit may be derived by correcting only a proportion of relevant cells in people with FRDA.

# Identification of β-III-spectrin actin binding modulators for treatment of SCA5

Friday, 4th November - 09:20: Plenary: Late-Breaking Research (Crystal Ballroom) - Oral - Abstract ID: 539

***Dr. Piyali Guhathakurta [1], Ms. Sarah Denha [2], Ms. Amanda Keller [2], Mrs. Anna Carter [1], Mrs. Alexandra Atang [3], Dr. Robyn Rebbeck [1], Dr. Bengt Svensson [1], Dr. David Thomas [1], Dr. Thomas Hays [1], Dr. Adam Avery [2]***

***1.*** *University of Minnesota,* ***2.*** *Oakland University,* ***3.*** *oakland universtiy*

**Background**

Mutations in the cytoskeletal protein β-III-spectrin cause the neurodegenerative disorder spinocerebellar ataxia type 5 (SCA5). Currently, there is no cure or therapy for SCA5. Numerous SCA5 mutations localize to the β-III-spectrin actin-binding domain (ABD). We previously showed that an ABD-localized L253P mutation causes a 1000-fold increase in actin-binding affinity. Here we report the molecular characterization of ten additional ABD-localized mutations and the results of high throughput screening (HTS) using a novel fluorescence assay to identify β-III-spectrin actin binding modulators.

**Methods**

Purified SCA5 mutant ABD proteins were characterized structurally and functionally using circular dichroism spectroscopy and actin co-sedimentation assays. An in vitro fluorescence lifetime FRET assay was developed using fluorescently labeled, L253P mutant ABD and F-actin. HTS was performed using the FDA-approved compound library. Hit compounds were repurchased and activity confirmed by FRET and orthologous binding assays.

**Results**

All ten ABD-localized SCA5 mutations caused increased actin binding, similar to L253P. The novel FRET assay, monitoring binding of L253P ABD to F-actin, was optimized to achieve a high, ~50% FRET signal, and a Z' score of >0.8 using the tool compound, swinholide A. Duplicate screens of the 3,000-compound FDA-approved Selleck library resulted in the identified 35 Hits that reduced FRET. The majority of Hit compounds showed reproducible activity following compound repurchase. Hit compound activity was further confirmed in orthologous actin co-sedimentation assays.

**Discussion and Conclusion**

Our results indicate that increased actin binding is a shared molecular consequence of many ABD-localized SCA5 mutations. Thus, a small molecule that reduces actin binding of mutant β-III-spectrin may be broadly effective as a SCA5 therapeutic. Our novel FRET drug screening platform was validated by screening of the 3,000-compound FDA-approved library. Our results warrant larger library screening, and medicinal chemistry to optimize current Hits towards a SCA5 therapeutic.

# (#518) Direct utility of natural history data in analysis of clinical trials: Propensity match-based analysis of Omaveloxolone in Friedreich ataxia using the FA-COMS dataset

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Oral - Abstract ID: 518

Friday, 4th November - 09:40: Plenary: Late-Breaking Research (Crystal Ballroom) - Oral - Abstract ID: 518

***Dr. David Lynch*** [1], ***Dr. Angie Goldsberry*** [2], ***Dr. Christian Rummey*** [3], ***Ms. Jennifer Farmer*** [4], ***Prof. Sylvia Boesch*** [5], ***Prof. Martin B Delatycki*** [6], ***Prof. Paola Giunti*** [7], ***Dr. Chad Hoyle*** [8], ***Dr. Caterina Mariotti*** [9], ***Dr. Katherine Mathews*** [10], ***Dr. Wolfgang Nachbauer*** [5], ***Prof. Susan L. Perlman, MD*** [11], ***Dr. Sub H. Subramony*** [12], ***Dr. George R. Wilmot*** [13], ***Dr. Theresa Zesiewicz*** [14], ***Dr. Lisa Weissfeld*** [15], ***Dr. Colin Meyer*** [2]

***1.*** *University of Pennsylvania & Childrens Hospital of Philadelphia,* ***2.*** *Reata Pharmaceuticals, Dallas, TX,* ***3.*** *Clinical data science GmbH,* ***4.*** *Friedreich's Ataxia Research Alliance,* ***5.*** *Center for rare neurological Disorders Innsbruck,* ***6.*** *Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia,,* ***7.*** *Ataxia Centre, Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, London,* ***8.*** *Ohio State,* ***9.*** *Unit of Medical Genetics and Neurogenetics, Fondazione IRCCS Istituto Neurologico Carlo Besta, 20133, Milan, Italy,* ***10.*** *Departments of Pediatrics and Neurology, University of Iowa Carver College of Medicine, Iowa City, IA,* ***11.*** *David Geffen School of Medicine at UCLA, Los Angeles, CA,* ***12.*** *McKnight Brain Institute, Department of Neurology, University of Florida, Gainesville, Florida, United States of America,* ***13.*** *Department of Neurology, Emory University School of Medicine, Atlanta, GA,* ***14.*** *Department of Neurology, University of South Florida Ataxia Research Center, Tampa, FL,* ***15.*** *Stat Lab*

Background/ Objective: The natural history of Friedreich Ataxia (FRDA) is being investigated in a multi-center longitudinal study designated the Friedreich Ataxia Clinical Outcome Measures Study (FA-COMS). To understand the utility of this natural history dataset in analysis of clinical trials, we performed a propensity-matched comparison of the data from the open-label MOXIe Extension (omaveloxolone) with that from FA-COMS.

Methods: All MOXIe Extension patients who had at least one post-baseline assessment were matched to FA-COMS patients using logistic regression to estimate propensity scores based on multiple covariates: sex, baseline age, age of onset, baseline modified Friedreich Ataxia Rating scale (mFARS) score, and baseline gait score. Selection of covariates was based on clinical relevance (i.e., factors considered prognostic for disease progression) and availability. The change from baseline in mFARS at Year 3 for the MOXIe Extension patients compared to the matched FA-COMS patients was analyzed as the primary efficacy endpoint using mixed model repeated measures analysis.

Results: Data from the MOXIe Extension show that omaveloxolone provides persistent benefit over three years when compared to an untreated, rigorously matched cohort from FA-COMS. At each year, and in all analysis populations, patients in the MOXIe Extension experienced a smaller change from baseline in mFARS score than the matched FA-COMS patients. In the Primary Pooled Population (136 patients in each group) by Year 3, patients in the FA-COMS matched set progressed 6.6 points whereas patients treated with omaveloxolone in MOXIe Extension progressed 3 points (difference =-3.6; nominal p value =0.0001). Thus, progression in mFARS was slowed by 55% with omaveloxolone treatment.

Discussion/Conclusion: These results suggest a clinically meaningful slowing of FRDA progression with omaveloxolone, and consequently details how propensity-matched analysis can contribute to the understanding of effects of therapeutic agents. This demonstrates the direct value of natural history studies in the evaluation of clinical trials.

# (#547) An intronic GAA repeat expansion in FGF14 causes autosomal dominant adult-onset ataxia (SCA50, ATX-FGF14)

Friday, 4th November - 10:00: Plenary: Late-Breaking Research (Crystal Ballroom) - Oral - Abstract ID: 547

***Dr. Haloom Rafehi [1], Dr. Justin Read [2], Dr. David Szmulewicz [3], Dr. Kayli Davies [4], Dr. Penny Snell [4], Dr. Liam Fearnley [1], Dr. Liam Scott [1], Dr. Mirja Thomsen [5], Dr. Greta Gillies [4], Dr. Kate Pope [4], Dr. Mark Bennett [1], Dr. Jacob Munro [1], Dr. Kathie Ngo [6], Dr. Luke Chen [7], Dr. Mathew Wallis [8], Dr. Ernest Butler [9], Dr. Kishore Kumar [10], Dr. Kathy Wu [11], Dr. Susan Tomlinson [12], Dr. Stephen Tisch [11], Dr. Abhishek Malhotra [13], Dr. Matthew Lee-Archer [14], Dr. Egor Dolzhenko [15], Dr. Michael Eberle [15], Dr. Leslie Roberts [16], Dr. Brent Fogel [6], Dr. Norbert Brüggemann [5], Dr. Katja Lohmann [5], Dr. Martin Delatycki [4], Mr. Melanie Bahlo [1], Dr. Paul Lockhart [4]***

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Adult-onset cerebellar ataxias are a group of neurodegenerative conditions that challenge both genetic discovery and molecular diagnosis. In this study, we identified an intronic (GAA) repeat expansion in the gene encoding Fibroblast Growth Factor 14 (FGF14). Genetic analysis identified 4/95 Australian individuals (4.2%) with (GAA)>300 and a further nine individuals with (GAA)>250. PCR and long-read sequence analysis revealed these were pure (GAA) repeats. In comparison, no controls had (GAA)>300 and only 2/311 control individuals (0.6%) encoded a pure (GAA)>250. In a German validation cohort 9/104 (8.7%) of affected individuals had (GAA)>335 and a further six had (GAA)>250, whereas no controls had (GAA)>335 and 10/190 (5.3%) encoded (GAA)>250. The combined data suggests (GAA)>335 are disease-causing and fully penetrant [P-value 6.0x10-8, OR 72 (95% CI=4.3-1227)], while (GAA)>250 is likely pathogenic with reduced penetrance. The core phenotypes associated with FGF14 (GAA)n-mediated ataxia are pure CA and cerebellar ataxia with bilateral vestibulopathy (CABV), with the variable presence of other features including hyper-reflexia and autonomic dysfunction. Notably, to date CABV is conspicuously under-represented in individuals with ataxia in whom a genetic diagnosis is achieved. A negative correlation between age at onset and repeat length was observed (R2=0.44 p=0.00045, slope = -0.12) and identification of a shared haplotype in a minority of individuals suggests that the expansion can be inherited or generated de novo during meiotic division. This study demonstrates the power of genome sequencing and advanced bioinformatic tools to identify novel repeat expansions via model free, genome-wide analysis and identifies SCA50/ATX-FGF14 as a frequent cause of adult-onset ataxia.

# Expansion of a Deep Intronic FGF14 GAA Short Tandem Repeat in Late-Onset Cerebellar Ataxia

Friday, 4th November - 10:20: Plenary: Late-Breaking Research (Crystal Ballroom) - Oral - Abstract ID: 548

***Dr. David Pellerin [1], Dr. Matt Danzi [2], Dr. Carlo Wilke [3], Dr. Mathilde Renaud [4], Dr. Sarah Fazal [2], Mrs. Marie-Josée Dicaire [1], Mrs. Carolin Scriba [5], Dr. Catherine Ashton [6], Mr. Christopher Yanick [2], Dr. Danique Beijer [2], Dr. Adriana Rebelo [2], Dr. Clarissa Rocca [7], Dr. Zane Jaunmuktane [8], Dr. Joshua Sonnen [9], Dr. Roxanne Larivière [1], Dr. David Genis [10], Dr. Laura Molina [11], Dr. Karine Choquet [12], Ms. Rawan Sakalla [1], Mrs. Sylvie Provost [13], Dr. Rebecca Robertson [14], Mr. Xavier Allard-Chamard [14], Dr. Martine Tétreault [15], Dr. Sarah Reiling [16], Dr. Sara Nagy [17], Dr. Vikas Nishadham [18], Dr. Meera Purushottam [19], Dr. Seena Vengalil [18], Dr. Mainak Bardhan [18], Dr. Atchayaram Nalini [18], Dr. Zhongbo Chen [20], Dr. Jean Mathieu [21], Dr. Rami Massie [1], Dr. Colin Chalk [1], Dr. Anne-Louise Lafontaine [1], Dr. François Evoy [21], Dr. Marie-France Rioux [21], Dr. Jiannis Ragoussis [16], Dr. Kym Boycott [22], Dr. Marie-Pierre Dubé [23], Prof. Antoine Duquette [24], Prof. Henry Houlden [25], Dr. Gianina Ravenscroft [5], Dr. Nigel Laing [5], Prof. Phillipa Lamont [26], Dr. Mario Saporta [27], Dr. Rebecca Schuele [28], Prof. Ludger Schöls [28], Dr. Roberta La Piana [29], Prof. Matthis Synofzik [30], Prof. Stephan Zuchner [31], Dr. Bernard Brais [32]***

***1.*** *Department of Neurology and Neurosurgery, Montreal Neurological Hospital and Institute, McGill University, Montreal, QC, Canada,* ***2.*** *Dr. John T. Macdonald Foundation Department of Human Genetics and John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL, USA,* ***3.*** *Division of Translational Genomics of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research and Center of Neurology, University of Tübingen, Tübingen, Germany,* ***4.*** *Service de Génétique Clinique et de Neurologie, Hôpital Brabois Enfants, Nancy, France,* ***5.*** *Centre for Medical Research University of Western Australia and Harry Perkins Institute of Medical Research, Perth, Western Australia, Australia,* ***6.*** *Department of Neurology, Royal Perth Hospital, Perth, WA, Australia,* ***7.*** *UCL Queen Square Institute of Neurology,* ***8.*** *Department of Clinical and Movement Neurosciences and Queen Square Brain Bank for Neurological Disorders, UCL Queen Square Institute of Neurology, University College London, London, United Kingdom,* ***9.*** *Department of Pathology, Montreal Neurological Hospital and Institute, McGill University, Montreal, QC, Canada,* ***10.*** *Ataxia and Hereditary Spastic Paraplegia Unit, Service of Neurology, Hospital Universitari de Girona Dr. Josep Trueta (ICS) & Hospital Santa Caterina IAS, Girona, Spain,* ***11.*** *Neurological Tissue Brain Bank, Biobanc-Hospital Clínic-IDIBAPS, Barcelona, Spain,* ***12.*** *Department of Genetics, Harvard Medical School, Boston, MA, USA,* ***13.*** *Montreal Heart Institute, Université de Montréal, Montreal, QC, Canada,* ***14.*** *Department of Human Genetics, McGill University, Montreal, QC, Canada,* ***15.*** *Department of Neurosciences, Faculty of Medicine, Université de Montréal, Montreal, QC, Canada,* ***16.*** *McGill Genome Centre, Department of Human Genetics, McGill University, Montreal, QC, Canada,* ***17.*** *Department of Neurology, University Hospital Basel, University of Basel, Basel, Switzerland,* ***18.*** *Department of Neurology, National Institute of Mental Health and Neurosciences, Bengaluru, India,* ***19.*** *Molecular Genetic Laboratory, National Institute of Mental Health and Neurosciences, Bengaluru, India,* ***20.*** *Institute of Neurology, UCL, National Hospital for Neurology and Neurosurgery (UCLH), London UK.,* ***21.*** *Faculty of Medicine and Health Sciences, Sherbrooke University, Sherbrooke, QC, Canada,* ***22.*** *Children's Hospital of Eastern Ontario Research Institute, University of Ottawa, ON, Canada,* ***23.*** *Department of Medicine, Faculty of Medicine, Université de Montréal, Montreal, QC, Canada,* ***24.*** *Département de neurosciences, Université de Montréal,* ***25.*** *Department of Neuromuscular disorders, UCL Queen Square Institute of Neurology, University College London, London, United Kingdom,* ***26.*** *Department of Neurology, Royal Perth Hospital, Perth, Western Australia, Australia,* ***27.*** *Department of Neurology, University of Miami Miller School of Medicine, Miami, FL, USA,* ***28.*** *Hertie Institute for Clinical Brain Research, Tübingen,* ***29.*** *Department of Diagnostic Radiology, McGill University, Montreal, QC, Canada,* ***30.*** *University of Tübingen,* ***31.*** *Department of Neurology, University of Miami Miller School of Medicine, Miami, FL 33136, USA.,* ***32.*** *McGill University*

*Background:* Late-onset cerebellar ataxias (LOCA) have until recently largely resisted molecular diagnosis. Contributing to this diagnostic gap is that non-coding structural variations, such as tandem repeat expansions, are not fully accessible to standard short-read sequencing analysis.
*Methods:* We analyzed whole-genome sequencing performed on six cases from three large French-Canadian families with unsolved autosomal dominant LOCA and identified a candidate GAA repeat expansion in the first intron of the Fibroblast Growth Factor 14 gene (*FGF14*). We determined a pathogenic threshold of $\geq(GAA)_{250}$following segregation study within the three families and tested for an association between the repeat expansion and disease in (1) 66 French-Canadian cases and 209 controls, and (2) 228 German cases and 199 controls. We also tested for the presence of the repeat expansion in 20 Australian and 31 Indian cases.
*Results:* We identified 128 cases carrying a dominant GAA repeat expansion in the first intron of *FGF14*. The repeat expansion was present in 61%, 18%, 15% and 10% of index cases in the French-Canadian, German, Australian and Indian cohorts, respectively. We found a significant association between *FGF14* $\geq(GAA)_{250}$expansions and LOCA in the French-Canadian ($p$<0.001; OR=105.60, 95% CI=31.09-334.20) and the German ($p$<0.001; OR=8.76, 95% CI=3.45-20.84) case-control series. Our data suggest that $(GAA)_{250\text{-}300}$ expansions are incompletely penetrant while large expansions are fully penetrant. Cases developed a slowly progressive cerebellar syndrome at an average age of 59 years. The ataxia was episodic at onset in 46% of cases. Downbeat nystagmus was observed in 42% of patients. Cerebellar atrophy was found in 74% of cases on MRI.

*Discussion and Conclusion:* This novel dominantly inherited intronic GAA repeat expansion in *FGF14* is associated with LOCA. Our study demonstrates how advanced bioinformatics tools and long-read sequencing can lead to the identification of the genetic basis of hitherto undiagnosed late-onset neurodegenerative diseases.

# Poster Sessions: Disease Mechanisms

# (#13) Sleep changes in a mouse model of Spinocerebellar ataxia type 3

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 13

***Dr. Maria-Efstratia Tsimpanouli [1], Mr. Anjesh Ghimire [1], Ms. Anna Barget [1], Mr. Ridge Weston [1], Prof. Brendon Watson [1], Dr. Maria do Carmo Costa [1]***

***1.*** *University of Michigan*

Spinocerebellar ataxia type 3 (SCA3) or Machado-Joseph disease is a fatal, incurable, dominantly inherited ataxia, typically of adult-onset, and the most frequent SCA worldwide. SCA3 patients show a broad spectrum of motor and non-motor symptoms, including ataxia, parkinsonism, and sleep disorders. In other neurodegenerative diseases, sleep disturbances alter brain homeostatic mechanisms leading to deterioration of neurologic function. Sleep research has provided insights into their pathophysiology, disease prediction, and symptom management. Such studies have not been performed in SCA3. The aim of this study is to characterize sleep EEG in SCA3 transgenic mice.

Homozygous, hemizygous, and wild-type YACMJD84.2 mice, 22 to 31 weeks old, were tested for locomotor and exploratory functions in the morning and evening. We then implanted 6 electrodes in the frontal, parietal, and cerebellar areas. About two weeks after, we recorded their sleep activity for 15 hours per day for three consecutive days.

Compared with wild-type, homozygous SCA3 mice showed significantly decreased locomotor and exploratory activities, increased REM sleep duration, and increased β spectral power band activity during sleep, particularly in REM sleep.

Our data suggest that sleep architecture and EEG spectral power are dysregulated in homozygous SCA3 mice. Changes in β band have been observed in SCA3 patients during wake, and in patients with REM sleep behavior disorder. Therefore, future studies analyzing the sleep EEG of SCA3 patients are needed to confirm whether our findings are translatable. Further studies should also investigate the causal relationship between the observed differences in sleep and disease progression. Gaining greater insight into the role of sleep in SCA3 could provide translatable biomarkers and lead to improved assessment of the disease progression and therapeutic interventions.

# (#28) Hepcidin-Ferroportin axis in Friedreich´s Ataxia: preliminary findings

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 28

***Dr. Elisabetta Indelicato*** [1], ***Dr. Manuel Grander*** [2], ***Dr. Matthias Amprosi*** [1], ***Dr. Carina Gatt*** [1], ***Dr. Wolfgang Nachbauer*** [1], ***Dr. Andreas Eigentler*** [1], ***Dr. David Haschka*** [2], ***Dr. Christian Kremser*** [3], ***Dr. Benjamin Henninger*** [3], ***Prof. Günter Weiss*** [2], ***Prof. Sylvia Boesch*** [1]

***1.*** *Center for rare neurological Disorders Innsbruck,* ***2.*** *Department of Internal Medicine, Innsbruck,* ***3.*** *Department of Radiology, Innsbruck*

**Background**

Hepcidin (HAMP) is the main regulator of systemic iron metabolism. The liver synthetizes HAMP in condition of iron excess and it binds to ferroportin (FPN), the only cellular iron-exporter, inducing its internalization and degradation. Up to date, no study addressed the HAMP-FPN axis in Friedreich´s Ataxia (FA) patients. Namely, it is not known if this systemic regulatory feedback recognizes and properly reacts to the iron accumulation in FA.

**Methods**

We measured HAMP levels, iron and copper parameters in serum, as well as the expression of iron regulating factors in PBMCs of FA patients (n=40), carriers (n=30) and matched control subjects (n=40). Additionally, FA patients underwent a standardized MRI protocol to quantify iron content in the liver, pancreas and spleen.

**Results**

Our FA cohort (19 females; Age 39, 95% CI[34, 44]) showed lower iron and transferrin saturation level in serum (FA Iron 14.8 µmol/L ,[12.9, 16.7] versus 20.5,[18.3, 22.7] in controls, $p$<0.001; FA transferrin saturation 22.5,[19.4, 25.6] versus 29.8,[26.6, 33.0] in controls, $p$<0.006). Serum HAMP levels were comparable in FA, carriers and controls. In the preliminary analyses in PBMCs, we found robust differences in the mRNA expression of HIF1a, DMT1-IRE, Ferritin heavy chain, HAMP, lipocalin-2 and mitoferrin-2 mRNA level with reduced levels in FA and to a less extent in carriers. None of the 34 out of 40 FA patients who underwent the abdominal MRI displayed an absolute iron accumulation in the liver; a comparison with controls is pending.

**Conclusion**

These preliminary Findings from hint a dysregulation of the HAMP-FPN feedback mechanisms at the cellular level. At a systemic level, circulating HAMP level were comparable in FA and controls, although FA patients displayed a subtle iron-deficiency state compared to controls. The completion of study procedures is awaited to clarify if this finding represents an *inadequate* feedback response in FA.

# (#35) The enigmatic grumose reaction of the dentate nucleus in hereditary ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 35

***Dr. Arnulf H. Koeppen*** [1], ***Dr. Joseph Mazurkiewicz*** [2], ***Dr. Rahman Rafique*** [3]

***1.*** *VA Medical Center,* ***2.*** *Albany Medical College,* ***3.*** *Albany Research Institute*

Background and objectives: Grumose reaction, formerly called grumose degeneration, is an unusual neuropathological observation in the dentate nuclei in Friedreich ataxia (FA) and spinocerebellar ataxia type 3 (Machado-Joseph disease) (SCA-3/MJD). The objective is to illustrate the derivation of grumose reaction from gamma aminobutyric acid (GABA) containing axon terminals of Purkinje cells and correlate it with the clinical phenotype.
Methods: Dentate nuclei were harvested by autopsy from 63 FA cases and 8 cases of SCA-3/MJD.The principal methods were immunohistochemistry and laser scanning confocal immunofluorescence microscopy.
Results: Antibodies to synaptophysin identify the derivation of the grape-like clusters of grumose reaction from synaptic terminals. Glutamic acid decarboxylase immunohistochemistry confirms their GABA-ergic nature and origin from Purkinje cell axons. Gephyrin immunohistochemistry suggests a deficit of GABA-receptors on neuronal dendrites. The clusters of grumose reaction surround dendrites and cell bodies of large and small dentate neurons, but only the large glutamatergic neurons undergo atrophy. In contrast to the large neurons, small nerve cells are GABA-ergic and send axons to the contralateral inferior olivary nuclei where GABA has trophic properties and assures survival of neurons and their climbing fibers. Grumose reaction does not persist indefinitely. In FA and SCA-3/MJD cases of long duration, grumose reaction may be entirely absent, in parallel with loss of large neurons.
Discussion and conclusion: Loss of afferent GABAergic fibers in the dentate nucleus correlates with the undulating ataxia of patients with FA and SCA-3/MJD. In FA, “onset” of ataxia may be attributed to the first formation of grumose clusters.
Funding: Friedreich’s Ataxia Research Alliance; laboratory: Department of Veterans Affairs

# (#60) Multi-omic profiling reveals the ataxia protein sacsin is required for integrin trafficking and synaptic organization

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 60

***Dr. Lisa Romano [1], Dr. Wen Yih Aw [2], Dr. Kathryn Hixson [2], Dr. Tatiana Novoselova [3], Dr. Tammy Havener [2], Dr. Charlotte Hall [3], Ms. Laura Perna [3], Prof. Konstantinos Thalassinos [4], Prof. Paul Chapple [3], Dr. Justin Wolter [2]***

***1.*** *William Harvey Research Institute, Barts and the London School of Medicine, Queen Mary University of London,* ***2.*** *UNC Catalyst for Rare Diseases, Eshelman School of Pharmacy, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina,* ***3.*** *William Harvey Research Institute, Barts and the London School of Medicine, Queen Mary University of London, London,* ***4.*** *Institute of Structural and Molecular Biology, Division of Biosciences, University College London, London*

**Background and objectives:** Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a childhood-onset cerebellar ataxia caused by mutations in *SACS*, which encodes the protein sacsin. Cellular ARSACS phenotypes include mitochondrial dysfunction, intermediate filament disorganization, and loss of Purkinje neurons. It is unclear how the loss of SACS causes these deficits, or why they manifest as cerebellar ataxia.

**Methods:** We used multi-omic and cellular profiling of CRISPR/Cas9 generated sacsin knockout cell lines and the ARSACS mouse model. This included quantitative proteomic and phosphoproteomic profiling of *SACS* KO cells, kinome analysis, identification of a sacsin interactome and cell surface proteome. RNASeq analysis to reveal differential expressed genes in SACS KO cells was also performed.

**Results:** Multi-omic profiling in *SACS* knockout (KO) cells identified alterations in microtubule structure and dynamics, protein trafficking, and mislocalization of synaptic and focal adhesion proteins. We observed mislocalization of synaptic adhesion proteins in Purkinje neurons, and drastic synaptic disorganization in ARSACS mice. Targeting *PTEN*, a negative regulator of focal adhesions, rescued IF disorganization and focal adhesion deficits. Interactome analysis revealed that sacsin regulates vesicular transport and interactions between structural and synaptic adhesion proteins.

**Discussion and conclusions:** In all, this study suggests that disrupted trafficking and localization of synaptic adhesion proteins is a causal molecular deficit underlying ARSACS. It also suggests ARSACS should be classified with several other poorly understood ataxias based on its molecular pathology.

# (#64) Mitochondria in Friedreich cardiomyopathy

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 64

***Dr. Rahman Rafique*** [1], ***Dr. Arnulf Koeppen*** [2], ***Dr. Joseph Mazurkiewicz*** [3]

***1.*** *Albany Research Institute,* ***2.*** *Albany Research Institute at Veterans Affairs Medical Center, Albany, NY, USA,* ***3.*** *Albany Medical College*

Background and objectives

The mutation in Friedreich ataxia (FA) causes a deficiency of frataxin (*Fxn)* but the residual protein is normal. The objective in this research effort was to determine Fxn levels in FA cardiomyopathy and the localization of mitochondria in affected cardiomyocytes.

Methods

Lysates of left ventricular wall (LVW) were prepared for enzyme-linked immunosorbent assay (ELISA) of Fxn and sodium dodecyl sulfate polyacrylamide gel electrophoresis and Western blots. Matching samples of LVW were fixed and processed for the visualization of mitochondria by immunofluorescence with an antibody to adenosine triphosphate synthase F1 beta subunit (ATP5B) and electron microscopy. Double-label immuno-fluorescence of ATP5B and the major cytoskeletal protein, desmin, was used to show the localization of mitochondria in relationship to the cardiomyocyte cytoskeleton.

Results

In normal myocardium, mitochondria are aligned in parallel rows between heart fibrils. In FA, desmin-reactive intercalated discs undergo chaotic restructuring, and mitochondria are present in clusters surrounded by fragments of intercalated discs. Mitochondria appear enlarged but their cristae are regular. Western blots disclosed accumulation of a desmin fragment of 45 kDa, but the intensity of the ATP5B band did not suggest a change in this mitochondrial protein in FA.

Conclusion

FA is a mitochondrial disease, and the evidence supports a deficit in mitochondrial energy production. The mutation does not involve mitochondrial DNA. It is unknown whether mitochondrial clustering in FA cardiomyopathy contributes to energy deficiency or whether the chaotic modification of intercalated discs contributes to mitochondrial dysfunction. In addition to its role as a cytoskeletal protein, desmin is also viewed as a signaling protein. Therefore, the entrapment of mitochondria in a rim of desmin may be an important pathomechanism in FA cardiomyopathy.

# (#67) Metabolic rewiring in a cellular model of ARSACS

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 67

***Ms. Laura Perna*** [1], ***Dr. Oliver Haworth*** [1], ***Dr. Grace Salsbury*** [1], ***Prof. Paul Chapple*** [1]

***1.*** *William Harvey Research Institute, Barts and the London School of Medicine, Queen Mary University of London, London*

**Background and Objective:** Autosomal Recessive Spastic Ataxia of Charlevoix Saguenay (ARSACS) is an early onset neurodegenerative disease that also has a neurodevelopmental component. It is caused by loss of function of sacsin, a 520 kDa protein with multiple domains linked to protein quality control systems. Impaired mitochondrial health is a feature of cellular models of ARSACS. This includes altered mitochondrial network organisation, reduced oxidative phosphorylation and increased levels of superoxide in sacsin deficient cells and patient fibroblasts (Bradshaw et al., 2016). These phenotypes maybe a consequence of impaired recruitment of the mitochondrial fission factor dynamin-related protein 1 (Bradshaw et al., 2016). In this study we investigate if the mitochondrial dysfunction caused by loss of sacsin impacts on cellular metabolism.

**Methods:** Using CRISPR/Cas9 we generated a sacsin knockout SH-SY5Y (neuroblastoma-derived) cell line. Then, to compare the metabolite profiles of wild-type control and sacsin knockout cell lines, we performed mass spectrometry-based metabolomic flux analysis with both glucose and glutamine traced carbon

**Results:** This revealed that sacsin knockout cells have increased lactate production and alterations in the glutaminolysis pathway, suggesting an increased reliance on aerobic glycolysis in the absence of sacsin. Our analysis also revealed decreased levels of GABA in sacsin knockout cells, which given its neurotransmitter function may be directly relevant to neuronal dysfunction in ARSACS.

**Discussion and Conclusion:** Further analysis of our metabolomic data set will increase understanding of the molecular consequences of sacsin loss and may identify metabolic deficiencies that could potentially be targeted to treat ARSACS.

Bradshaw, T. Y., Romano, L. E., Duncan, E. J.,. . . Chapple, J. P. (2016). A reduction in Drp1-mediated fission compromises mitochondrial health in autosomal recessive spastic ataxia of Charlevoix Saguenay. Hum Mol Genet, 25(15), 3232-3244. doi:10.1093/hmg/ddw173

# (#68) Frataxin controls ketone body metabolism through regulation of OXCT1

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 68

***Dr. Yina Dong*** [1], ***Dr. Clementina Mesaros*** [2], ***Dr. Jimmy Xu*** [2], ***Ms. Elizabeth Mercado-Ayon*** [2], ***Ms. Sarah Halawani*** [1], ***Ms. Lucie Ngaba*** [1], ***Mr. Nathan Warren*** [3], ***Dr. Patrick Sleiman*** [4], ***Dr. Layne Rodden*** [4], ***Ms. Kimberly Schadt*** [4], ***Dr. Ian A. Blair*** [5], ***Dr. David Lynch*** [2]

***1.*** *CHOP,* ***2.*** *University of Pennsylvania,* ***3.*** *Childrens Hospital of Philadelphia,* ***4.*** *The Children's Hospital of Philadelphia,* ***5.*** *University of Penn*

Friedreich's ataxia (FRDA) is an autosome recessive neurodegenerative disease characterized by progressive ataxia, scoliosis, cardiomyopathy and increased incidence of diabetes. It is caused by the deficiency of frataxin, a mitochondrial protein crucial for iron-sulphur cluster formation and ATP production. The precise cellular function of frataxin is not entirely known. The aim of this study was to explore the function of frataxin by targeting its binding partners. The binding partners of frataxin in mouse cortex were identified by Co-immunoprecipitation coupled with mass spectrometry analysis. Western blot and Immunohistochemistry were used to measure changes in frataxin binding partners upon frataxin deficiency. Metabolites of ketogenesis were measured by mass spectrometry.

3-Oxoacid CoA-Transferase 1 (OXCT1), an enzyme catalyzing the rate limiting step in conversion of extrahepatic ketone bodies to aceto-acetyl-CoA, was identified as an interacting protein of frataxin. Overexpression of frataxin increases OXCT1 protein levels in human skin fibroblasts while frataxin deficiency decreases OXCT1 in multiple cell types both acutely and sub-acutely. OXCT1 reduction also occurs in cerebellum and skeletal muscle of frataxin knock-in/knockout (KIKO) mice as well as in skeletal muscle from FRDA patients, suggesting that frataxin directly regulates OXCT1 protein levels. This regulation is mediated by frataxin-dependent suppression of ubiquitin-proteasome system-dependent OXCT1 degradation. Concomitantly, plasma ketone bodies are significantly elevated in frataxin deficient KIKO mice with no change in the levels of other enzymes involved in ketone body production or intermediate metabolites of ketogenesis, suggesting that ketone body elevation is caused by OXCT1 reduction leading to tissue utilization deficits.

Our results suggest a new role for frataxin in energy metabolism through regulation of OXCT1. These findings not only provide mechanistic insights into the pathophysiology of FRDA such as exercise intolerance but also identifies OXCT1 as one of downstream mediators of frataxin deficiency and a novel therapeutic target for FRDA.

# (#85) Evidence of Oxidative Repair Abnormalities and Poly(ADP-ribose) Dysregulation within Spinocerebellar Ataxia Type 1

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 85

***Ms. Celeste Suart*** [1], ***Dr. Tamara Maiuri*** [1], ***Dr. Ray Truant*** [1]

***1.*** *McMaster University*

Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant neurodegenerative disorder caused by a CAG triplet repeat expansion in the ATXN1 gene, leading to a polyglutamine expansion in ataxin-1. Single-nucleotide polymorphism analysis has demonstrated that DNA repair genes significantly modify the age at onset of SCA symptoms. The oxidative DNA damage repair and poly(ADP-ribose) (PAR) pathways are altered in Huntington's Disease (HD), another polyglutamine expansion disease. Due to the similarities between HD and SCA1, we hypothesized that PAR and oxidative DNA damage repair may also be dysregulated within SCA1.

Immunofluorescent microscopy was used to examine DNA damage and PAR markers in SCA1 (TruSCA1-Q52Q29M), HD (TruHD-Q43Q17M) and wildtype fibroblasts (TruHD-Q21Q18F). In vitro PAR overlay assays with FLAG-ataxin-1 were used to assess ataxin-1 PAR binding capability

SCA1 fibroblasts had decreased oxidative DNA damage indicators, OGG1 and pATM, compared to wildtype cells. SCA1 and wildtype fibroblast had similar levels of double-stranded damage marker γH2AX. Unexpectedly, SCA1 fibroblasts had lower levels of PAR than wildtype and HD fibroblasts in control conditions. However, SCA1 and HD fibroblasts had a significant increase in PAR following combined oxidative stress treatment and PAR glycohydrolase inhibition. PAR overlay analysis demonstrated polyglutamine-expanded ataxin-1 had less PAR binding capacity than wildtype ataxin-1.

The differences in DNA damage markers suggest SCA1 has a similar capacity for double-stranded break repair compared to wildtype fibroblasts, but an impaired response to oxidative damage. Similarly, our data indicates that SCA1 fibroblasts have decreased production of PAR. Combined with polyglutamine-expanded ataxin-1 having less PAR binding affinity, this supports the hypothesis that ataxin-1 polyglutamine expansions disrupts PARylation. Further research is needed to identify an underlying mechanism. This is an ongoing project, and we will have further results prior to the conference.

# (#95) Development of Fragile X-Associated Tremor Ataxia Syndrome Rating Scale (FXTAS-RS)

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 95

***Dr. Michelle Tosin*** [1], ***Dr. Glenn Stebbins*** [1], ***Dr. Christopher Goetz*** [1], ***Dr. Randi Hagerman*** [2], ***Dr. David Hessl*** [2], ***Ms. Melissa Zolecki*** [3], ***Prof. Peter Todd*** [4], ***Dr. Maureen Leehey*** [5], ***Dr. Deborah Hall*** [1]

***1.*** *Department of Neurological Sciences, Rush University Medical Center,* ***2.*** *MIND Institute, University of California Davis,* ***3.*** *National Fragile X Foundation,* ***4.*** *University of Michigan,* ***5.*** *University of Colorado School of Medicine*

**Background**: Clinimetric analyses of the first version of the Fragile X-associated Tremor Ataxia Syndrome Rating Scale (FXTAS-RS) showed weaknesses needing revision.

**Objective**: Develop a revised, improved version of the FXTAS-RS for motor signs assessment.

**Method**: We conduct a multimethod approach using Delphi panel and cognitive pretesting techniques. Nine specialists forming a Delphi panel conducted five-rounds of individual assignments and online group meetings to: 1) list the domains and subdomains, relevant to assessing FXTAS motor signs 2) establish consensus on salient domains and subdomains, 3) establish consensus on items addressing selected domains and considering the clinimetric properties of items from the first version, 4) develop and establish consensus on the type of item response options and anchors, and 5) write instructions for scale application after approving the revised version that would be ready for further field tested. Five FXTAS patients and five neurologists participated in two-rounds of cognitive pretesting to determine scale's readability, comprehensiveness, applicability, relevance, and missing themes.

**Results**: Delphi panel: In Round 1, six domains and 65 subdomains of FXTAS motor signs were listed. In Round 2, consensus was established for five domains and 13 subdomains. In Round 3, of the 61 items composing the first FXTAS version, 30 were excluded and 13 were revised according to the clinimetric property analysis. Panelists established consensus to keep 18 items, covering all five established domains. In Round 4, panelists agreed with nominal response options for most items. In Round 5, instructions for application of the resultant revised FXTAS-RS were created. Cognitive pretesting: In Round 1, 20 items were reviewed to meet participant's suggestions (Major Review=7, Minor Review=13). In Round 2, 11 items required minor edition to meet participant's suggestions.

**Conclusion**: This stepwise methodology provided a model strategy to develop a new version of the FXTAS-RS that is now ready for validation.

# (#102) Association of Genetic Polymorphisms with the Age at Onset in Patients with Spinocerebellar Ataxia Type 3 (SCA3) / Machado-Joseph disease (MJD)

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 102

***Mr. Torben Laidig [1], Ms. Jaqueline Jung [1], Ms. Leah Czisch [1], Ms. Charlotte Meyer [1], Ms. Rahel-Maria Burger [1], Ms. Priscila Pereira Sena [1], Dr. Jonasz J. Weber [1], Mr. Daniel Weishäupl [1], Dr. Thomas Ott [1], Prof. Laura Bannach Jardim [2], Prof. Maria Luiza Saraiva-Pereira [3], Prof. Marcondes C. França Jr [4], Prof. Carlos R. Gordon [5], Prof. Mario Cornejo-Olivas [6], Prof. Thorsten Schmidt [1]***

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**Background and Objective:** Spinocerebellar ataxia type 3 (SCA3) or Machado-Joseph disease (MJD) is an autosomal-dominantly inherited, neurodegenerative disorder caused by the expansion of a CAG repeat in the *ATXN3* gene resulting in an expanded polyglutamine repeat in the encoded ataxin-3 protein. SCA3/MJD, therefore, belongs to the group of polyglutamine diseases. Statistically, a correlation between the number of CAG repeats and the age at onset of SCA3 patients exists and patients with more CAG repeats have an earlier onset of symptoms. However, this statistical correlation is not perfect and the number of CAG repeats contributes only about 55% to the age at onset. Therefore, the remaining 45% are influenced by other factors, which we aim to identify in this study.

**Methods:** In order to identify modifiers of the disease progression, we genotyped in a combined European and South American approach more than 500 SCA3/MJD patients for promising polymorphisms in candidate genes.

**Results:** Candidate genes included ataxin-3 itself and known interaction partners of ataxin-3, functional modifiers identified in previous studies as well as genes with known relevance for the pathophysiology of SCA3/MJD. We selected polymorphisms with a high likelihood of having a functional relevance i.e. polymorphisms in the promoter regions as well as polymorphisms leading to amino acid changes. While controlling for ethnic origin we assessed the contribution of the respective polymorphism to the age at onset in addition to the already known modifying factor, the length of the expanded CAG repeat within *ATXN3*. We indeed identified interesting polymorphisms contributing to the age at onset including certain haplotypes within *ATXN3* itself.

**Discussion and Conclusion:** Subsequent functional characterizations will reveal the impact of these polymorphisms on pathogenic mechanisms in SCA3/MJD. We hope that our results will improve the prediction of clinical symptoms and contribute to the understanding of pathogenic processes in SCA3/MJD.

# (#103) Increasing power of clinical trials in SCA1, 2, 3 and 6 with efficient designs and SARA scales

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 103

***Dr. Emilien Petit*** [1], ***Dr. Thomas Klockgether*** [2], ***Prof. Alexandra Durr*** [1], ***Dr. Tetsuo Ashizawa*** [3], ***Dr. Gulin Oz*** [4], ***Dr. Henry Paulson*** [5], ***Dr. Sophie Tezenas du Montcel*** [6]

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**Backgrounds and Objective**. Choosing the right outcome and design is crucial for a powerful clinical trial. For spinocerebellar ataxias type 1, 2, 3 and 6, we describe here the power of several longitudinal trial designs with various follow-up time and number of visits, and the impact of selecting SARA, m-SARA, axial (ax-SARA) or appendicular SARA (ap-SARA) as the outcome.

**Methods**. The progressions of the four outcomes were estimated in 220 SCA1, 297 SCA2, 399 SCA3 and 191 SCA6 patients included in 3 cohorts (EUROSCA, CRC-SCA and SPATAX) with linear mixed models. First, we simulated datasets with two arms of 30 patients, with a treatment effect of 50% reduction of the patients' progression. Different designs were tested, with 1 to 3 years of follow-up and 3 to 9 visits during the trial. Second, we estimated the number of patients in each arm needed to reach 90% power for each outcome and each SCA, with a 2 years follow-up with visits every 6 months.

**Results.** All outcomes were progressing linearly since inclusion, and ax-SARA increased faster than ap-SARA except in SCA2. Increasing duration drastically increased power with visits every 6 months, from 53% for a 1-year trial to 97% for a 3-years one in SCA1, 90% being reached at 2 years. A higher number of visits with equal follow-up duration increased power: 71% for a 1-year trial with nine equally spaced visits. For all SCAs, SARA and ax-SARA were more powerful, followed by m-SARA and ap-SARA with respectively 34, 33, 51 and 102 patients needed in each arm for SCA1.

**Discussion and Conclusion.** Both the trial duration and the number of visits increased power of simulated clinical trials on a linearly progressing outcome. SARA and ax-SARA were more powerful than m-SARA and ap-SARA to detect a treatment effect.

Acknowledgements: NIH U01 NS104326

# (#113) The CAG repeats are somatically unstable in spinocerebellar ataxias

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 113

***Dr. Radhia Kacher*** [1], ***Dr. François-Xavier Lejeune*** [2], ***Mrs. Isabelle David*** [3], ***Mrs. Julie Six*** [1], ***Dr. Anne-Laure Fauret*** [3], ***Dr. Sandrine Humbert*** [4], ***Prof. Alexandra Durr*** [1]

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A pathological number of CAG repeats in coding regions is the most common cause of spinocerebellar ataxia (SCA). These repeats are unstable through the germline and larger repeats lead to earlier onset.

Here, we measured somatic expansion in blood samples collected from 30 SCA1, 40 SCA2, 75 SCA3, and 20 SCA7 mutation carriers over twenty years, along with post-mortem tissues and fetal tissues from SCA3 and SCA7 mutation carriers, to examine somatic expansion at different stages of life. To do so, we used specific PCRs for each locus on the four genes (*ATXN1, ATXN2, ATXN3*, and *ATXN7*), followed by fragment sizing to identify the number of CAG repeats in each sample.

We showed that somatic expansion in the blood increases over time. Expansion levels are significantly different among SCAs and correlate with CAG repeat length for SCA1, SCA2 and SCA7 but not for SCA3. Post-mortem brains had high expansions, whereas the fetal cortex was stable. Interestingly, the cerebellum has the lowest expansion scores among the studied brain regions. We analyzed clinical features and showed that somatic expansion correlates with age at onset for SCA1 and SCA7 and with the SARA scores for SCA2 and SCA7.

Overall, this study shows that CAG repeats are increasingly unstable during the lifetime in the blood and the brain, with different characteristics depending on the affected gene and potential association to disease progression.

# (#116) Toxicity of polyglutamine expanded ataxin-3 is regulated by its ubiquitin-binding site 1

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 116

***Mr. Matthew Prifti*** [1], ***Dr. Wei-Ling Tsou*** [2], ***Dr. Kozeta Libohova*** [1], ***Prof. Sokol Todi*** [3]

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Polyglutamine (polyQ) diseases are a family of nine neurodegenerative disorders caused by abnormal CAG triplet repeat expansions in different, widely-expressed, disease-causing genes. Abnormal glutamine expansions that are translated from these CAG repeats lead to clinically-distinct neurological disorders that include, but are not limited to, Huntington's Disease and several spinocerebellar ataxias (SCAs). SCA3, the most common, dominantly inherited ataxia worldwide, is caused by a polyQ expansion in the deubiquitinating enzyme (DUB), ataxin-3 (ATX3), which is involved in protein quality control. As of yet, there is no cure for SCA3. To understand the mechanisms of disease of SCA3, our lab has taken a systematic approach to studying the domains of ATX3 and their respective and collective roles in its toxicity. Ubiquitin-binding site 1 (UbS1), located in the catalytic domain of ataxin-3, plays a crucial role in its ability to function as a DUB by binding to ubiquitin and coordinating its spatial interaction with the catalytic site. We recently generated transgenic Drosophila lines that express pathogenic ATX3 with intact or mutated UbS1 and found that mutating UbS1 markedly exacerbates the toxicity of pathogenic ATX3. Additionally, we found that increased toxicity correlated with enhanced aggregation of the disease protein. Additional experiments indicate that UbS1 mutations enhance the ability of ataxin-3 to sequester ubiquitin species in neurons. Future studies are further probing into the mechanism of action.

# (#165) Mitochondrial deficits in a mouse model of spinocerebellar ataxia type 6 (SCA6)

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 165

***Ms. Tsz Chui Sophia Leung*** [1], ***Ms. Namrata Rana*** [1], ***Ms. RuYi Louisa Shen*** [1], ***Dr. Alanna Watt*** [1]

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**Background and Objective** Spinocerebellar ataxia type 6 (SCA6) is a rare, late-onset disease characterized by progressive ataxia and cerebellar degeneration. It is caused by an expansion of the CAG triplet repeats in the gene *CACNA1A*, which is highly expressed in cerebellar Purkinje cells. Disease-causing mechanisms remain incompletely understood for SCA6, and treatment options are limited. We used RNA sequencing (RNA-seq), transmission electron microscopy (TEM) and immunohistochemistry to characterize novel molecular dysregulations in hopes that understanding the molecular pathology in SCA6 will shed light on future therapeutics.

**Methods** We use a mouse model with an expanded triplet repeat ($SCA6^{84Q/84Q}$) that recapitulates the late-onset and progressive nature of the human disease. We performed RNA-seq on $SCA6^{84Q/84Q}$ and wildtype mouse cerebella at the onset of motor dysfunction (7 months) to identify dysregulated genes. Next, we used immunohistochemistry to study level of DNA damage in the cerebellum at disease progression stage (9 and 12 months). Finally, we used TEM to analyze morphological changes in mitochondria of Purkinje cells at an advanced disease stage (18 months).

**Results** We identified over 500 significant DEG in $SCA6^{84Q/84Q}$ cerebellum, with roughly half up- and half downregulated. Among the dysregulated gene sets, multiple mitochondrial related gene sets were downregulated. As a result of oxidative stress, the level of DNA damage is elevated in $SCA6^{84Q/84Q}$ cerebellum. We then characterized mitochondrial morphology, and found that mitochondria showed signs of damage at advanced disease stage in $SCA6^{84Q/84Q}$ cerebellar Purkinje cells, which is an indicator of mitochondrial functional deficits.

**Discussion and Conclusion** Mitochondria have important roles in many cellular processes, and abnormal mitochondria can render neurons vulnerable to dysfunction and death. Work is ongoing to explore the role of mitochondria in SCA6 disease-causing mechanism, and the therapeutic potential of mitochondria-targeting treatments.

# (#172) The displacement of frataxin from the mitochondrial cristae of Friedreich ataxia patients' cells is associated with defects in respiratory function

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 172

***Dr. Davide Doni*** [1], ***Dr. Federica Cavion*** [1], ***Dr. Silvia Muccioli*** [1], ***Dr. Elisa Palumbo*** [2], ***Dr. Elisa Baschiera*** [3], ***Dr. Roberta Peruzzo*** [1], ***Dr. Federica D'Ettorre*** [1], ***Dr. Natasa Dalinac*** [1], ***Dr. Daniele Ottaviani*** [1], ***Dr. Giovanni Rigoni*** [4], ***Prof. Antonella Russo*** [2], ***Prof. Ildikò Szabo*** [1], ***Prof. Maria Eugenia Soriano*** [1], ***Prof. Luigi Leanza*** [1], ***Prof. Milena Bellin*** [5], ***Prof. Leonardo Salviati*** [3], ***Prof. Paola Costantini*** [1]

***1.*** *Department of Biology, University of Padova,* ***2.*** *Department of Molecular Medicine, University of Padova,* ***3.*** *Department of Women's and Children's Health, University of Padova; IRP Città della Speranza,* ***4.*** *Department of Biology,* ***5.*** *Department of Biology,*

*Background and Objective.* Friedreich ataxia (FRDA) is a neurodegenerative disease resulting from a severe decrease of frataxin (FXN). Most patients carry a GAA repeat expansion in both alleles of the *FXN* gene, whereas a small fraction of them are compound heterozygous for the expansion and a point mutation in the other allele. FXN is involved in the mitochondrial biogenesis of the FeS-clusters. Distinctive feature of FRDA cells is an impaired cellular respiration, due to a deficit of key redox cofactors working as electrons shuttles through the respiratory chain. However, a definite relationship between FXN levels, FeS-clusters assembly dysregulation and bioenergetics failure has not been established. In this work, we performed a comparative analysis of the mitochondrial phenotype of lymphoblasts/fibroblasts/hiPSCs-derived cardiomyocytes from FRDA patients.

*Methods.* BN-PAGE, to analyze respiratory supercomplexes; spectrophotometry, to address the activity of single respiratory complexes ; oxygen consumption studies by Seahorse flux analyzer; morphometric analyses by TEM, to assess mitochondrial ultrastructure ; immunogold labelling, to determine the localization of FXN in mitochondrial subcompartments ; immunofluorescence/PLA, to explore the interaction between FXN, FeS-cluster assembly machinery and mitochondrial respiratory chain.

*Results*. We found that, in healthy cells, FXN and proteins of the FeS-cluster assembly machinery are enriched in mitochondrial cristae, the dynamic subcompartment housing the respiratory chain. On the contrary, FXN redistributes to the matrix in FRDA cells with defects in respiratory supercomplexes assembly and altered respiratory function. This impairs the interaction of frataxin with FeS cluster assembly machinery and respiratory chain.

*Discussion and Conclusion*. To date, we do not know exactly which metabolic consequences primarily occur after frataxin depletion and would be most relevant for early FRDA therapy strategies. Our finding opens up a new working perspective aimed at addressing if and how the perturbation of mitochondrial morphodynamics is involved in the bioenergetic defects afflicting FRDA cells.

# (#184) Spatiotemporal oligodendrocyte impairments in SCA3 mice are rescued by ATXN3 gene silencing

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 184

***Ms. Kristen Schuster [1], Ms. Annie Zalon [1], Ms. Danielle DiFranco [1], Ms. Alexandra Putka [1], Ms. Sabrina Jarrah [1], Mr. Nicholas Stec [1], Mr. Arsal Naeem [1], Mr. Zaid Haque [1], Dr. Hongjiu Zhang [1], Dr. Yuanfang Guan [1], Dr. Hayley McLoughlin [1]***

***1.*** *University of Michigan*

**Background and Objective:** Spinocerebellar ataxia type 3 (SCA3) is the most common dominantly inherited ataxia. It is a polyglutamine neurodegenerative disease for which there is no disease-modifying therapy. The polyglutamine-encoding CAG repeat expansion in the *ATXN3* gene results in the expression of a mutant form of the ATXN3 protein, which causes selective neurodegeneration despite being widely expressed. Our lab has recently shown that oligodendrocytes in SCA3 display some of the earliest and most progressive dysfunction in disease (Schuster et al. 2022, *JNeurosci*). Here, we extend that study to show that oligodendrocyte dysfunction can be rescued with the treatment of antisense oligonucleotides (ASO) against the ATXN3 gene in a symptomatic mouse model of SCA3.

**Methods:** We treated symptomatic SCA3 mice with our previously published anti-ATXN3 ASO (McLoughlin et al. 2018 *Annals of Neurol*) by intracerebroventricular injection and assessed transcriptional, biochemical, histological, and ultrastructural rescue of the oligodendrocyte maturation impairments relative to vehicle-treated mice.

**Results:** In transgenic SCA3 mice, we identified early and robust changes that implicate oligodendrocytes in disease pathogenesis and show that anti-ATXN3 ASO treatment rescues the genes important for oligodendrocyte maturation. We confirmed temporal rescue of oligodendrocyte impairments across vulnerable SCA3 brain regions by analysis of myelin proteins. We further validated ASO rescue of ultrastructural myelination defects that we previously reported with transmission electron microscopy of the SCA3 mouse corticospinal tract.

**Conclusions:** In summary, our data suggest a severe, but modifiable, deficit in oligodendrocyte maturation caused by the gain-of-toxic function of mutant ATXN3 early in SCA3 disease progression. We are actively exploring the role oligodendrocyte dysfunctions play in SCA3 pathogenesis and the possible implications of our studies in other neurodegenerative diseases.

# (#185) Importin-8 silencing reduces ataxin-3 aggregation, alleviates neuropathology and delays progression of Spinocerebellar Ataxia Type 3 (SCA3)

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 185

***Mrs. Inês Morgado Martins*** [1], ***Dr. Anna Sergeevna Sowa*** [2], ***Mr. David Rufino-Ramos*** [1], ***Ms. Diana Duarte Lobo*** [1], ***Mr. Kevin Leandro*** [1], ***Dr. Ana Vasconcelos-Ferreira*** [1], ***Dr. Dina Pereira*** [1], ***Mrs. Rosário Faro*** [1], ***Prof. Olaf Riess*** [2], ***Prof. Thorsten Schmidt*** [2], ***Prof. Luís Pereira de Almeida*** [1]

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**Background and Objective:** Spinocerebellar ataxia type 3 (SCA3) is a genetic neurodegenerative disorder caused by an elongation of the polyQ tract within the Ataxin-3 (Atxn3) protein. The pathophysiology of SCA3 remains under investigation but the aberrant localization and the formation of nuclear Atxn3 aggregates in neurons of specific brain regions is an important hallmark for disease progression. The nucleocytoplasmic transport (NT) mechanisms are gaining increasing attention in the field of the neurodegenerative disorders since they can explain the mislocalization and dysfunction of disease-associated aberrant proteins but also of macromolecules intervening in multiple other cellular pathways. Our main goal was to disclose which NT proteins are modulating Atxn3 aggregation and playing a role in SCA3 neuropathology.

**Methods:** We screened a total of 37 NT proteins for their ability to modulate Atxn3 aggregation and selected Importin-8 (Ipo8) as an effective candidate. *In vitro* and *in vivo* validation were performed to evaluate whether Ipo8 silencing would alleviate SCA3 neuropathology. Additionally, behavioral analysis was performed to investigate Ipo8 silencing effects in disease progression of transgenic SCA3 mice.

**Results:** Our studies demonstrate that Ipo8 downregulation decreases Atxn3 aggregation, preventing neuronal dysfunction and death. Importantly, *in vivo* silencing of Ipo8 led to the alleviation of balance and motor coordination impairments. Our data also suggests that Ipo8 may exert its protective effect by modulating the activity of other proteins, such as NF-kB/p65 and Argonaute-2, that have been also linked to SCA3.

**Discussion and Conclusion:** Our results provide first-time evidence that Ipo8 is an important modulator of SCA3 by intervening in multiple neuropathological pathways, standing as a promissing therapeutic strategy for the disease. This work was Funded by: COMPETE 2020, National Funds through FCT(SFRH/BD/132618/2017), ViraVector(CENTRO-01-0145-FEDER-022095), SpreadSilencing (POCI-01-0145-FEDER-029716), by National Ataxia Foundation (USA), the American Portuguese Biomedical Research Fund (APBRF) and Richard Chin and Lily Lock MJD Research Fund.

# (#187) Using induced pluripotent stem cell derived cardiomyocytes as a model to study Friedreich's ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 187

***Ms. Soteroulla Ellina*** [1], ***Dr. Jackson Chan*** [1], ***Prof. Richard Festenstein*** [2]

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- Background and Objectives

Friedreich's ataxia (FRDA) is an autosomal recessive multisystem disorder. It is caused by a GAA triplet repeat expansion within the first intron of the *frataxin* locus which leads to its heterochromatinization and the subsequent downregulation of the protein. Although frataxin is a ubiquitously expressed protein, the most affected cell types are the proprioceptive neurons and the cardiomyocytes. The initial symptoms of FRDA are neurological including the loss of coordination, while the majority of patients die of hypertrophic cardiomyopathy. Objectives: a) to provide proof of principle that epigenetic silencing can be studied in cardiomyocytes and b) to understand why cardiomyocytes are more susceptible to frataxin downregulation.

- Methods

Induced pluripotent stem cells (iPSCs) derived from patients and healthy controls are differentiated to cardiomyocytes (CMs). Immunofluorescence for cardiac specific markers, ChIP for known silencing histone modifications and RNA sequencing of iPSC-CMs at different time points post-differentiation are performed.

- Results

Results indicate that FRDA-iPSCs-CMs recapitulate aspects of the disease such as the increase of silencing epigenetic markers (H3K9me3, H3K27me3) at the frataxin gene and defective calcium homeostasis. Also, a novel finding suggests that FRDA-iPSCs-CMs are structurally less mature on Day 30 compared to the control cells according to immunofluorescence data and confirmed by the downregulation of structural genes based on RNA-seq data of Day 22 and Day 30 cardiomyocytes. RNA-seq data also suggest an upregulation of known pathological hypertrophy markers in FRDA-iPSCs-CMs of Day 105.

- Discussion and Conclusion

This system provides a platform to study the effect of restoring frataxin levels in the affected cell-type. It is possible that the increased susceptibility of the FRDA-CMs is that while they attempt to compensate for their initial structural immaturity, they become pathologically hypertrophic.

# (#195) Non-expansion spinocerebellar ataxias are presenting with neurodevelopmental and neurodegenerative phenotypes in the same gene.

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 195

***Dr. Paulina Cunha*** [1], ***Dr. Emilien Petit*** [2], ***Dr. Marie Coutelier*** [3], ***Dr. Giulia Coarelli*** [4], ***Dr. Caterina Mariotti*** [5], ***Dr. Judith van Gaalen*** [6], ***Dr. Joana Damasio*** [7], ***Dr. Zofia Fleszar*** [8], ***Dr. Ginevra Zanni*** [9], ***Dr. Clarissa Rocca*** [10], ***Dr. Martina Minnerop*** [11], ***Dr. Elisabetta Indelicato*** [12], ***Dr. Penina Ponger*** [13], ***Dr. Elsa Besse*** [14], ***Dr. Mathieu Barbier*** [3], ***Dr. Berry Kremer*** [15], ***Dr. Alessandro Filla*** [16], ***Dr. Lubov Blumkin*** [17], ***Dr. Enrico Bertini*** [9], ***Dr. Mercedes Serrano*** [18], ***Dr. Michele Tosi*** [9], ***Dr. Mathieu Anheim*** [19], ***Dr. Stefania Magri*** [20], ***Dr. Daniela Di Bella*** [21], ***Dr. Lorenzo Nanetti*** [22], ***Dr. Tanja Schmitz-Hübsch*** [23], ***Dr. Jorge Oliveira*** [24], ***Prof. Matthis Synofzik*** [25], ***Prof. Ludger Schöls*** [8], ***Prof. Bart van de Warrenburg*** [6], ***Dr. Florence Riant*** [26], ***Dr. Franco Taroni*** [20], ***Prof. Alexis Brice*** [4], ***Prof. Alexandra Durr*** [27]

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Background and Objective: To better understand the spectra of genetic variants and associated phenotypes in non expansion spinocerebellar ataxias (SCAs), we launched an international collaboration to gather data on the most frequent SCAs not caused by repeat expansions.

Methods: We recruited patients who had undergone genetic testing, garnered their consent, then applied American College of Medical Genetics and Genomics (ACMG) criteria for defining pathogenic variants. With 963 patients and 22 genes, we analyzed SCAs for which we had detailed phenotyping for more than 30 patients. There were 756 patients carrying pathogenic variants in 7 genes. We compared age at onset, disease duration, progression, and disease features by gene and variant, then developed an AI decision tree.

Results: We characterized phenotypes associated with *CACNA1A* (239 patients); *PRKCG* (SCA14, 175 patients);

*AFG3L2* (SCA28, 101); *ITPR1* (SCA15/29, 91); *STUB1* (SCA48, 77); *SPTBN2* 3(SCA5, 39); and *KCNC3* (SCA13, 34). Although ataxias are notorious for phenotypic variability, the heterogeneity we found was astonishing: in some cases, the same variant produced phenotypes ranging from elderly-onset ataxia to congenital developmental delay with intellectual disability. Specific locations of missense variants in *CACNA1A* were associated with different phenotypes: congenital presentations were preferentially associated with variants located within the transmembrane domain 5 of repeat IV. Only *STUB1* and *PRKCG* did not have a congenital or infantile-onset versions of the disease; *STUB1* produced a specific phenotype with adult-onset, rapid progression of cognitive decline, extrapyramidal and psychiatric symptoms. *AFG3L2* variants were most likely to produce ophthalmoplegia. Although AI classifier performed better than randomly ascertain a causal gene (20% specificity *versus* 48%), extensive phenotypic variability was seen.
Conclusion: The phenotypic heterogeneity of variants in SCA-related genes is far greater than suspected with intellectual disability or developmental delay. This international effort allowed us to better understand phenotype-genotype correlations in non expansion SCA genes.

# (#196) Frataxin bypass by ISCU M141I substitution in dividing and non-dividing mammalian cells

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 196

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Iron sulfur (Fe-S) clusters are essential co-factors required for the functioning of a variety of proteins involved in key cellular processes. The conserved multiprotein machinery ISC initiate, in the mitochondria, Fe-S clusters biogenesis by *de novo* assembly of sulfur, provide by a cysteine desulfurase, and iron on a scaffold protein. Frataxin protein (FXN) plays a major role in this machinery acting as an regulator increasing Fe-S clusters biogenesis rate. Dysfunction of this process is associated to diseases like Friedreich's ataxia (FA) which is due to a drastic decrease of FXN protein production mainly leading to a deficit in Fe-S proteins, especially in mitochondrial Fe-S enzymes, intracellular iron deposits and sensitivity to oxidative stress. In eukaryotes FXN is essential, its absence leading to growth deficit in yeast and embryonic lethality in mice. Recently a substitution Met to Ile in position 141 of the scaffold protein *isu1* in yeast, allowing frataxin deficient yeast to grow was identified, demonstrating that this suppressor can bypass frataxin. This discovery raises the question if the same substitution in ISCU protein could allow to bypass FXN in mammalian cells. We introduced this mutation using a CRISPR-Cas9 system either in NC6 L3/L-mice fibroblasts or in mESC-derived neurons carrying a conditional allele allowing FXN deletion. We showed that in dividing cells ISCU M141I substitution can bypass FXN lethality but survivor clones presented a slower growth despite a normal cell cycle progression, and deficit in Fe-S enzymes in particular mitochondrial ones. We are currently characterizing the effect of the mutation in mESC-derived neurons. Investigation of this differential FXN bypass by ISCU M141I between dividing and non-diving cells will permit to better understand the function of ISC machinery in production and distribution of Fe-S clusters among the cell.

# (#197) Understanding dosage sensitivity in Pumilio1-associated diseases

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 197

***Mr. Salvatore Botta [1], Dr. Nicola de Prisco [1], Mr. Alexei Chemiakine [1], Mr. Maximilian Cabaj [1], Dr. Purvi Patel [1], Prof. Raejesh Soni [1], Prof. Vincenzo Gennarino [1]***

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**Background and Objective:** SCA47 is notable for encompassing very distinct phenotypes, ranging from a neurodevelopmental disorder to a late-onset, mild ataxia (Gennarino et al., *Cell* 2018). These differences are not caused by anticipation, as SCA47 is not a triplet repeat disease, but rather by different loss-of-function mutations in the RNA-binding protein (RBP) Pumilio1 (PUM1). Typically we think about mutations in RBPs de-regulating their targets, but PUM1 targets are de-repressed to equal degrees in both phenotypes. We therefore hypothesized that mild mutations that reduce PUM1 by ~25% cause target deregulation, but that the more severe mutations that reduce PUM1 levels by 40-60% disrupt PUM1's interactions with its protein partners and de-repress the targets of these complexes.
**Methods:** We developed a PUM1 interactome for the whole brain and for the cerebellum, hippocampus, and cortex. We validated the interactions *in vitro*, *in vivo*, and in patient-derived cell lines. We also identified PUM1-specific targets as well as targets that are shared with key RBP interactors.
**Results:** PUM1 partners are involved in various aspects of RNA metabolism such as silencing, alternative splicing, and polyadenylation. Certain interactions vary by brain region while others remain constant across the brain. As we hypothesized, the mutation causing adult-onset ataxia dysregulates only PUM1-specific targets, but the mutation causing developmental delay dysregulates the shared targets as well. Normalizing PUM1 levels restored the normal concentrations of the interactors and the regulation of the shared targets.
**Discussion and Conclusion:** The effects of changing protein concentration are not necessarily linear but can involve distinct mechanisms past a certain threshold. In studying RBP function, it is necessary to account for protein partners and not just targets. It is also necessary to perform studies *in vivo* in specific tissues to understand RBP functions in health and disease.

# (#198) Mitochondrial proteotoxicity by AFG3L2 mutations elicits an integrated stress response in SPAX5

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 198

***Dr. Francesca Maltecca*** [1], ***Dr. Camilla Aurora Franchino*** [1], ***Dr. Martina Brughera*** [1], ***Dr. Emmanuel Scalais*** [2], ***Dr. Valentina Baderna*** [1]

***1.*** *San Raffaele Scientific Institute,* ***2.*** *Centre Hospitalier de Luxembourg*

**Background and Objective.** Heterozygous *AFG3L2* mutations cause SCA28, while biallelic *AFG3L2* mutations result in the severe childhood-onset SPAX5.

AFG3L2 is a mitochondrial protease exerting protein quality control (QC) in the inner mitochondrial membrane (IMM). We previously reported that AFG3L2 mutation/absence leads to accumulation of mitochondria-encoded proteins, causing impaired oxidative phosphorylation and over-activation of the stress-sensitive protease OMA1, which over-processes OPA1 leading to mitochondrial fragmentation. Here, we investigated how mitochondrial and cellular stress responses contribute to SPAX5 pathogenesis.

**Methods.** We studied skin fibroblasts derived from SPAX5 patients and the cerebellum of the *Afg3l2*$^{-/-}$ mouse model, which recapitulates SPAX5 features. We performed RT-qPCR and Western blot to assess the activation of ISR, and silencing experiments to modulate it.

**Results.** We found that mitochondrial proteotoxicity in the absence/mutation of AFG3L2, as shown by the upregulation of matrix chaperones, elicits an integrated stress response (ISR) at a cellular level. In general, ISR reduces global protein synthesis and drives the expression of cytoprotective genes. In both SPAX5 skin fibroblasts and in the cerebellum of *Afg3l2*$^{-/-}$ mice, we indeed detected increased phosphorylation of eIF2alpha, increased levels of ATF4 and strong upregulation of its downstream targets (*Chop*, *Chac1* and *Ffg21*). Recently, it has been discovered that mitochondrial stress is relayed to the cytosol via a pathway involving OMA1, the IMM protein DELE1 and the cytosolic kinase HRI. Silencing of DELE1 in SPAX5 fibroblasts (where OMA1 is overactivated) reduces eIF2alpha phosphorylation and severely worsens cell survival. In agreement, pharmacological potentiation of ISR ameliorated SPAX5-related phenotypes in cell models.

**Discussion and Conclusion.** Our results document for the first time the activation of the OMA1-DELE1-HRI pathway leading to ISR in a human disease context. Genetic and pharmacological tuning of the ISR may represent a future therapeutic strategy for SPAX5 and other cerebellar ataxias caused by impaired mitochondrial proteostasis.

# (#213) Calpains as new players in the molecular pathogenesis of spinocerebellar ataxia type 17

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 213

***Dr. Jonasz J. Weber*** [1], ***Ms. Stefanie Cari Anger*** [2], ***Ms. Priscila Pereira Sena*** [2], ***Ms. Rana Dilara Incebacak Eltemur*** [1], ***Ms. Chrisovalantou Huridou*** [2], ***Dr. Libo Yu-Taeger*** [1], ***Mr. Caspar Gross*** [2], ***Dr. Nicolas Casadei*** [2], ***Prof. Olaf Riess*** [2], ***Prof. Huu Phuc Nguyen*** [1]

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**Abstract**

**Background and Objective:** Spinocerebellar ataxia type 17 (SCA17) is a neurodegenerative disease caused by the expansion of a polyglutamine repeat in the transcription factor TATA box-binding protein (TBP). While its underlying pathomechanism is not fully understood, fragments of polyglutamine-expanded TBP of unknown origin were shown to mediate the mutant protein's toxicity. Disease protein cleavage is a hallmark of many neurodegenerative disorders. Calpains, calcium-dependent proteases, were found to be a protagonist in this process. Therefore, we examined the potential contribution of calpains to TBP fragmentation in the molecular pathogenesis of SCA17.

**Methods:** We employed *in silico*, *in vitro* and cell-based strategies to analyse whether TBP is a substrate of calpain-mediated proteolysis. Using SCA17 cell models and TBPQ64 rats, we investigated if the calpain system is perturbed upon polyglutamine-expanded TBP expression and which consequences a dysregulation has on vital neuronal proteins. Potential impairments of underlying molecular pathways were investigated by RNA sequencing of SCA17 cells. Moreover, we tested whether pharmacological calpain inhibition or calpastatin overexpression ameliorates molecular disease hallmarks in SCA17 cells.

**Results:** *In vitro* and cell-based calpain activation induced fragmentation of both wild-type and polyglutamine-expanded TBP. In contrast to TBP's nuclear localization, its C-terminal cleavage products were mislocalized to the cytoplasm. In SCA17 cells and
TBPQ64 rat cerebellum, polyglutamine-expanded TBP induced a calpain system
overactivation. Moreover, the elevated calpain activity in TBPQ64 rat cerebellum triggered excessive fragmentation and depletion of neuronal proteins. Transcriptome analysis of SCA17 cells revealed polyglutamine-expanded TBP-induced perturbations of synaptogenesis and calcium signalling pathways. Calpain inhibition by pharmacological means or overexpression of calpastatin in cells reduced TBP cleavage and aggregation, consequently improving cell viability.

**Discussion and Conclusions:** We identified calpains and their activating pathways as novel players in the molecular pathogenesis of SCA17, emphasizing their general significance in neurodegenerative disorders and their consequent potential as therapeutic targets.

# (#236) The Massachusetts General Hospital (MGH) Ataxia Center: A three-decade retrospective

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 236

***Dr. Jeremy D. Schmahmann*** [1], ***Ms. Jin yun Helen Chen*** [1], ***Dr. Grace Crotty*** [1], ***Ms. Miranda Mize*** [1], ***Dr. Anoopum Gupta*** [1], ***Dr. Christopher Stephen*** [1], ***Mr. Jason MacMore*** [1]

***1.*** *Massachusetts General Hospital*

**Background and Objective:** Exponential increase in the range of diagnoses in patients with cerebellar ataxia represents a challenge to the clinician. Genetic testing facilitated this advance and heightened the need for meticulous phenotyping to determine who needs gene testing, and which results are meaningful. We reviewed the ~3-decade experience of the MGH Ataxia Center to identify the diagnoses in patients presenting with ataxia, and to determine success rate of diagnosis.

**Methods:** Our Patient List is an IRB-approved ongoing catalog of patients in the MGH Ataxia Center since its establishment in 1994, including patients followed by the PI since 1989. We reviewed medical records of each patient: clinical evaluations, imaging, and laboratory data. When indicated we contacted gene testing labs to update information, and in some we sought re-evaluation of gene data or autopsy findings because of new genetic developments.

**Results:** The Patient List includes 2,878 patients. Of these, 1,203 non-cerebellar cases were referred to the PI in other clinics and were excluded. In the remaining 1,675 Ataxia Center patients, diagnoses were “non-genetic” in 961, and “genetic” in 696. Non-genetic included acquired and degenerative cerebellar disorders such as multiple system atrophy, and non-cerebellar disorders with gait impairment, such as normal pressure hydrocephalus and functional neurological disorder. Genetic cases were categorized as molecularly confirmed, molecularly probable, candidate diagnosis, negative work-up with genetic tests available at the time, and inconclusive. Idiopathic late onset cerebellar ataxia was designated after exhaustive negative investigation including exome analysis. Using the clinically driven method the diagnostic success was 89.0% for all patients, and 73.0% in those with clinically suspected genetic diagnoses.

**Conclusion:** Diagnostic success in the MGH Ataxia Center provides support for the precision medicine approach of deep clinical phenotyping as the precursor to targeted testing and underscores the need for specialized expertise in ataxia centers of excellence.

# (#238) Modeling Friedreich Ataxia in human iPSC-derived sensory neuron subtypes.

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 238

***Dr. ETI MALKA GIBOR*** [1], ***Ms. Shirley Chen*** [1], ***Mrs. Kate Oliver*** [1], ***Dr. Jordi Magrané*** [2], ***Dr. Joriene De Nooij*** [1]

***1.*** *Columbia Univeristy Medical Center,* ***2.*** *Weill Cornell Medicine*

Friedreich's ataxia (FRDA) is a severe autosomal recessive genetic disorder, which is caused by the reduced expression of the mitochondrial protein Frataxin (FXN) due to an intronic GAA trinucleotide repeat expansion in the *FXN* gene. Frataxin dysregulation is associated with several mitochondrial dysfunctions, including impaired biosynthesis of iron-sulfur-clusters and mitochondrial energy production.

The reduction of FXN first manifest as degenerative changes in the dorsal root ganglia (DRG) and peripheral nerves; Many large myelinated fibers that correspond to proprioceptive and low threshold mechanoreceptive (LTMR) touch sensory neurons are lost. Interestingly, small caliber unmyelinated nociceptive (pain-sensing) sensory neurons, are largely unaffected in FRDA, thus indicating that loss of FXN causes a selective neuronal loss in DRG. In our studies we aim to explore, why some SNs are more vulnerable to the loss of FXN by modeling FRDA disease in induced pluripotent stem cell (iPSC) derived sensory neurons.

In our lab we successfully established an in vitro model for FRDA sensory neurons (SNs) subtypes. We developed protocols that enable us to differentiate iPSCs into the main DRG sensory populations (nociceptors, LTMRs, and proprioceptors). In addition, using Crispr/Cas9 gene-editing strategies, we generated sensory subtype reporters (trkA:tdTomato, MafA;tdTomato, and Runx3:tdTomato, respectively) for FRDA patient and isogenic control iPSC lines .

Our preliminary findings demonstrate that FRDA-associated phenotypes are already apparent in differentiating LTMR mechanoreceptive neurons but not nociceptive neurons This suggests that the differential sensory neuron FRDA vulnerability can be recapitulated in vitro using iPSC-derived sensory neurons. We will discuss these and other ongoing studies aimed at understanding the mechanistic basis of the neural subtype selective requirements of FXN.

# (244) Differential effects of enhanced Wnt-β-catenin signaling in Purkinje cells and glial cells in SCA1

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 244

***Ms. Kimberly Luttik*** [1], ***Dr. Leon Tejwani*** [1], ***Dr. Hyoungseok Ju*** [2], ***Dr. Terri Driessen*** [1], ***Dr. Cleo Smeets*** [1], ***Dr. Janghoo Lim*** [1]

***1.*** *Yale University,* ***2.*** *Yale*

Spinocerebellar ataxia type 1 (SCA1) is a dominantly inherited neurodegenerative disorder characterized by progressive ataxia and degeneration of specific neuronal populations, including Purkinje cells (PCs) in the cerebellum. SCA1 is caused by a nucleotide expansion of a glutamine-encoding (CAG) repeat tract in *ATXN1*, and can be characterized by progressive ataxia, mild cognitive impairments, and ultimately, respiratory failure, etc. Previous studies have demonstrated a critical role for various evolutionarily conserved signaling pathways in cerebellar patterning, such as the Wnt-β-catenin pathway; however, the roles of these pathways in adult cerebellar function and cerebellar neurodegeneration are largely unknown. In this study, we found that Wnt-β-catenin activity was progressively enhanced in several cell types in the adult SCA1 mouse cerebellum, and that activation of this signaling occurs in an Ataxin-1 polyglutamine (polyQ) expansion-dependent manner. Genetic manipulation of the Wnt-β-catenin signaling pathway in specific cerebellar cell populations revealed that activation of Wnt-β-catenin signaling in PCs alone was not sufficient to induce SCA1-like phenotypes, while its activation in astrocytes including Bergmann glia (BG) resulted in gliosis and disrupted BG localization, which was replicated in SCA1 mouse models. The role of BG in degeneration of PCs in SCA1 is currently under further investigation, as well as the necessity of Wnt-β-catenin signaling activation in BG towards SCA1 pathology. Our studies identify a novel mechanism in which polyQ-expanded Ataxin-1 positively regulates Wnt-β-catenin signaling, and demonstrate that different cell types have distinct responses to the enhanced Wnt-β-catenin signaling in the SCA1 cerebellum, underscoring an important role of astrocytes, specifically BG, in SCA1 pathogenesis.

# (#249) Sphingolipid Rheostat as a Potential Target for Friedreich's Ataxia (FRDA)

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 249

***Dr. Ester Kalef Ezra*** [1], ***Dr. Adamo Valle*** [2], ***Dr. Sara Anjomani-Virmouni*** [3]

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**Background:** Sphingolipids are the major lipid constituents of eukaryotic cell membranes that play crucial roles as bioactive molecules in regulating different cellular processes. Ceramide (Cer), ceramide-1-phosphate (C1P), sphingosine (Sph), and sphingosine-1-phosphate (S1P) are the best described bioactive sphingolipids which have opposite roles in cellular survival signalling, also known as "sphingolipid rheostat". Cer and Sph as cell death activators, while C1P and S1P promotes survival. Metabolic alterations observed in the course of neurodegeneration favour Cer-dependent pro-apoptotic signalling, while the levels of the neuroprotective S1P are reduced early in the diseases' development. The aim of this project is to explore the hypothesis that modulating sphingolipid metabolism and their related signalling pathway may present a potential therapeutic approach for FRDA.
**Methods:** Mass spectrometric-based methods, Rapid Evaporative Ionisation Mass Spectrometry (REIMS) and Liquid Chromatography Mass Spectrometry (LC-MS/MS) were employed to identify differential levels of metabolites and lipids in FRDA samples. Gene expression of sphingolipid-metabolising enzymes was assessed by qPCR and protein expression was evaluated by western blotting and In-Cell ELISA Colorimetric assay.
**Results:** Metabolic analysis using REIMS revealed potentially interesting sphingolipid species in particular Cer and C1P that were significantly different in FRDA human cells and mouse tissues. We also found alteration in the gene and protein expression of sphingosine kinase (SphK) and lipid phosphate phosphatases (LPP) enzymes, that are regulating the balance of the aforementioned sphingolipids and may contribute to the neurodegenerative processes in FRDA. To obtain a comprehensive map of sphingosine metabolism, we performed a targeted sphingolipidomic analysis in FRDA cell lines and found increased levels of Cer and reduced levels of S1P in FRDA human cell lines.
**Conclusion:** The findings of these studies suggest that modulating sphingolipid rheostat could serve as therapeutic targets. Moreover, the disturbances in the sphingolipid metabolism may deliver a potential biomarker for FRDA.

# (#273) Interruptions in the FXN GAA repeat tract delay age at onset of Friedreich's Ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 273

***Dr. Suran Nethisinghe*** [1], ***Mr. Maheswaran Kesavan*** [1], ***Dr. Heather Ging*** [1], ***Dr. Robyn Labrum*** [2], ***Dr. James Polke*** [2], ***Dr. Hector Garcia-Moreno*** [1], ***Prof. Martina Callaghan*** [3], ***Dr. Francesca Cavalcanti*** [4], ***Dr. Mark Pook*** [5], ***Prof. Paola Giunti*** [1]

***1.*** *Ataxia Centre, Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, London,* ***2.*** *Neurogenetics Service, Rare and Inherited Disease Laboratory, London Noth Genomic Laboratory Hub, Great Ormond Street Hospital for Children NHS Foundation Trust, London,* ***3.*** *Wellcome Centre for Human Neuroimaging, UCL Queen Square Institute of Neurology, University College London, London,* ***4.*** *Institute for Biomedical Research and Innovation (IRIB), Italian National Research Council (CNR), Mangone,* ***5.*** *Division of Biosciences, College of Health, Medicine and Life Sciences, Institute of Environment, Health and Societies, Brunel University London, UK*

**Background and Objective:** Friedreich's ataxia (FRDA) is the most common inherited ataxia. It is primarily caused by the homozygous expansion of a GAA trinucleotide repeat in intron 1 of the *FXN* gene. GAA repeat expansion causes gene silencing and consequent deficiency of the frataxin protein leading to mitochondrial dysfunction, oxidative stress, and cell death. The GAA repeat tract may be impure and have sequence variations called interruptions. However, large interruptions, determined by abnormal *MboII* digestion, are very rare. We used triplet repeat primed PCR (TP PCR) assays to identify small interruptions at the 5' and 3' ends of the GAA repeat tract through alterations in the electropherogram trace signal. We subsequently examined how these interruptions modulate the disease phenotype.

**Methods:** 101 peripheral blood genomic DNA samples from patients with FRDA were analysed by Reverse TP PCR (RTP) and Forward TP PCR (FTP) to examine the 5' and 3' ends of the *FXN* GAA repeat tract, respectively. The cohort was subsequently stratified based on the presence and location of interruptions. The age at disease onset was then modelled by a group-specific exponential decay.

**Results:** 71% of our cohort had an interruption at either end of the repeat tract, with 3' interruptions being most frequent. Interruption at the 3' end of the repeat tract is associated with shorter GAA1 repeat sizes and later ages at disease onset. Our modelling revealed that a 3' interruption delays disease onset by approximately 9 years relative to those lacking 5' and 3' interruptions.

**Discussion and Conclusion:** TP PCR can quickly and easily screen for small interruptions towards the ends of the GAA repeat tract, which are more common than large interruptions. These interruptions play a key role in modulating the disease phenotype. Interruption-specific stratification and modelling can therefore facilitate more tailored and precise patient prognoses.

# (#279) FXN gene methylation determines carrier status in Friedreich ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 279

***Ms. Christina Lam*** [1], ***Dr. Layne Rodden*** [2], ***Ms. Kimberly Schadt*** [2], ***Dr. David Lynch*** [3], ***Prof. Sanjay Bidichandani*** [4]

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Background: Friedreich ataxia (FRDA) is typically caused by homozygosity for an expanded GAA triplet-repeat (GAA-TRE) in intron 1 of the *FXN* gene. Some patients are compound heterozygous for the GAA-TRE and another *FXN* pathogenic variant. Detection of the GAA-TRE in the heterozygous state is essential for diagnosing compound heterozygotes and asymptomatic carriers, but it is technically challenging, with a high false-negative rate.
Objective: We explored if the FRDA differentially methylated region (FRDA-DMR) in intron 1, which is hypermethylated in *cis* with the GAA-TRE, effectively detects heterozygous GAA-TRE.
Methods: *FXN* DNA methylation was assayed by targeted bisulfite deep sequencing using the Illumina platform.
Results: FRDA-DMR methylation effectively identified a cohort of known heterozygous carriers of the GAA-TRE. In an individual with clinical features of FRDA, commercial testing showed a paternally-inherited pathogenic *FXN* initiation codon variant but no GAA-TRE. Methylation in the FRDA-DMR effectively identified the proband, his mother, and various maternal relatives as heterozygous carriers of the GAA-TRE, thus confirming the diagnosis of FRDA.
Conclusion: *FXN* DNA methylation reliably detects the GAA-TRE in the heterozygous state, and offers a robust alternative strategy to diagnose FRDA due to compound heterozygosity, and to identify asymptomatic heterozygous carriers of the GAA-TRE.

# (#287) Spinocerebellar Ataxia Type 48 Associated Mutations Disrupt CHIP Function

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 287

***Ms. Anna Umano*** [1], ***Dr. Jamie Scaglione*** [1], ***Dr. Kuili Fang*** [1], ***Dr. Matt Scaglione*** [2]

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**Background and Objective**

Spinocerebellar ataxia type 48 (SCA48) is a debilitating, adult-onset neurodegenerative disease that is inherited in an autosomal dominant manner. SCA48 is characterized by loss of fine motor skills, cognitive decline, and cerebellar degeneration. SCA48 is caused by mutations in the *STUB1* gene which encodes C-terminus of Hsp70-interacting protein (CHIP). CHIP plays a fundamental role in cellular proteostasis by ubiquitinating misfolded proteins and targeting them for proteasomal degradation. While the identification of mutations in CHIP have been established, the molecular mechanisms underlying SCA48 are unknown.

**Methods**

To investigate the effect of mutations on CHIP stability, HEK293 cells were transfected with CHIP expressing plasmid constructs. Protein levels were then assessed through Western blotting. To determine chaperone-binding activity of CHIP mutants, we used a cell-based nanoBiT assay and an *in vitro* fluorescence polarization assay. Ubiquitination activity of CHIP mutants was assessed through an *in vitro* ubiquitination assay.

**Results**

Using our array of assays, we have found that unlike in SCAR16, a recessive form of ataxia caused by mutations in CHIP, most mutations that cause SCA48 do not destabilize CHIP. Instead, we have found that mutations that cause SCA48 have mutation specific effects on a variety of biophysical, biochemical, and cellular properties.

**Discussion and Conclusion**

Together our data identify molecular defects in CHIP that cause SCA48. Understanding how mutations in CHIP cause SCA48 is an important first step in developing therapies for this uncurable disease. In the future the development of animal models of SCA48 will be important for understanding how disease specific mutations in CHIP result in the range of symptoms that cause SCA48.

# (#308) Nucleocytoplasmic transport is altered in Spinocerebellar Ataxia type 7

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 308

***Dr. Joshua Jones Macopson*** [1], ***Dr. Albert La Spada*** [2]

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**Background and Objective:** The objective of this project is to elucidate the cellular and molecular basis of SCA7 neurodegeneration. An emerging theme in inherited retinal degenerations and cerebellar ataxias is vulnerability to DNA damage. Nuclear pore complexes are required for the transport of proteins that contribute to efficient DNA repair. Studies from a few labs, including our own research group, have now shown that nucleocytoplasmic (NC) transport is impaired in age-related neurodegenerative diseases, including ALS and HD. We chose to evaluate the status of NC transport in SCA7 model mice and SCA7 patient stem cells.

**Methods:** We used histological techniques to evaluate nuclear membrane morphology in mouse retina and cerebellar neurons. We also used Fluorescent Recovery After Photobleaching (FRAP) to assess NC transport within human neural progenitor cells (NPCs) and SCA7 patient neurons derived from induced pluripotent (iPSCs).

**Results:** We did not detect any significant differences in the number of neurons with nuclear membrane invaginations within the retina and in the cerebellum of symptomatic SCA7 266Q knock-in mice and age-matched littermate controls. To better clarify the status of NC transport in SCA7 disease, we measured nuclear import of two different shuttle constructs in SCA7 patient and control NPCs. Surprisingly, we found that SCA7 patient NPCs display significantly increased fluorescent recovery rates compared to NPCs from unaffected first-degree relatives, suggesting increased NC transport in cells expressing polyglutamine-expanded ataxin-7.

**Discussion & Conclusions:** Our findings indicate altered NC transport is a feature of SCA7 disease pathology. As ataxin-7 shuttles into and out of the nucleus it normally interacts extensively with the NC transport machinery. Whether increased NC transport is occurring in SCA7 neurons pathologically affected in the cerebellum and retina in human patients remains to be determined and raises the intriguing question of how such altered NC transport could be contributing to SCA7.

# (#321) Alteration of Intracellular Calcium Dynamics in Friedreich's Ataxia Skin Fibroblasts

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 321

***Dr. Anna Stepanova*** [1], ***Ms. Jenipher Tenesaca*** [1], ***Dr. Veronica Granatiero*** [1], ***Dr. Gabriella Casalena*** [1], ***Dr. Marek Napierala*** [2], ***Prof. Giovanni Manfredi*** [1], ***Dr. Hibiki Kawamata*** [1]

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Background and Objective:
Friedreich's ataxia (FA) is a multisystemic mitochondrial disease caused by transcriptional downregulation of Frataxin (FXN) due to intronic GAA expansion. Dorsal root ganglia and cerebellar degeneration, as well as cardiomyopathy are cardinal pathological features of FA. Research has focused on mitochondrial bioenergetics, oxidative stress, and iron dysregulation. Yet, despite being a potential therapeutic target, calcium homeostasis has not been extensively addressed in patient-derived cells. Calcium signaling is fundamental for many cellular functions, and mitochondrial calcium uptake is critical for the maintenance of calcium homeostasis. Mitochondria establish close contacts with the endoplasmic reticulum (ER), where calcium exiting the ER is taken up by mitochondria. We hypothesized that intracellular calcium is dysregulated in FA patients' cells.
Methods:
We used genetically-encoded calcium indicators for live-cell imaging of calcium dynamics in the cytosol (GCaMP6f), mitochondria (4mtGCaMP6f and CEPIA4mt) and ER (GCaMPer) in FA patient and control skin fibroblasts (n=12/group). Using these fluorescent calcium indicators delivered by lentiviral transduction, we measured basal calcium levels and calcium fluxes in different cell compartments, after stimulation of ER calcium release with histamine.
Results:
We found lower baseline free calcium levels in cytosol, mitochondria, and ER of FA fibroblasts, which also displayed altered intracellular calcium dynamics after histamine stimulation, with decreased mitochondrial uptake and overall increased levels of cytosolic calcium. While cytosolic peak calcium was unchanged, the area under the curve was increased.
Discussion and Conclusion:
We interpret these results as an indication that there are alterations in calcium handling in FA fibroblasts. Ongoing work is investigating molecular players of altered calcium dynamics in FA by assessing changes in expression and phosphorylation of calcium regulatory proteins. We are also extending calcium studies to iPSC-derived cardiomyocytes. The goal is to identify precise mechanistic targets and treat cells pharmacologically to ameliorate calcium dysregulation in FA.

# (#331) Alterations of lipid metabolism in CoQ deficient-cerebellar ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 331

***Dr. Alba Pesini*** [1], ***Dr. Giacomo Monzio-Compagnoni*** [2], ***Dr. Eliana Barriocanal-Casado*** [3], ***Mr. Giussepe Yanez*** [3], ***Dr. Giulio Kleiner*** [3], ***Dr. Agustin Hidalgo-Gutierrez*** [3], ***Dr. Mohammed Bakkali*** [4], ***Dr. Edoardo Monfrini*** [5], ***Dr. Delfina Larrea*** [3], ***Ms. Saba Tadesse*** [3], ***Dr. Caterina Mariotti*** [6], ***Dr. Barbara Castellotti*** [6], ***Dr. Luis C Lopez*** [4], ***Dr. Alessio Di Fonzo*** [7], ***Dr. Estela Area-Gomez*** [3], ***Dr. Catarina Marina Quinzii*** [1]

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**Background and Objectives**: CoQ deficiency is often a secondary feature of patients with cerebellar ataxias (CAs), such as ataxia-oculomotor-apraxia 1 (AOA1) due to *APTX (*aprataxin) mutations. However, the role of CoQ deficiency in the pathogenesis of neurodegeneration in CAs remains unknown.

The biosynthesis of CoQ and cholesterol share intermediate steps. Specifically, the first enzyme of CoQ biosynthesis, decaprenyl diphosphate synthase (PDSS1/PDSS2) uses as a substrate geranylgeranyl-diphosphate (GGdP), a metabolite from the mevalonate pathway, which is also responsible for the *de novo* synthesis of cholesterol. We have studied the interplay between CoQ synthesis and cholesterol metabolism to investigate whether CoQ deficiency causes alterations of cholesterol metabolism in CAs.

**Methods:** We used CoQ deficient-CA iPSC-derived neurons carrying mutations in APTX and PDSS2, to perform lipidomics, followed by RT-qPCR, western blots, and biochemical and molecular assays with radioactive substrates, to characterize lipid metabolism. Functional studies of pharmacological manipulation of the mevalonate pathway, and its CoQ and cholesterol branches were performed in SH-SY5Y neuronal lines to understand causal relationship between CoQ and cholesterol metabolism abnormalities.

**Results**: CA patient iPSC-derived neurons show CoQ biosynthesis defects associated with significant impairments in lipid metabolism, and in particular sphingolipids biosynthesis and cholesterol homeostasis.

**Discussion and Conclusions**: We propose that CoQ and cholesterol levels are concomitantly regulated, and dysfunction of the mevalonate pathway contributes to neurodegeneration in CA by altering the regulation of CoQ and cholesterol homeostasis.

# (#343) Bidirectional transcription at the PPP2R2B gene locus in spinocerebellar ataxia type 12

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 343

***Mr. Chengqian Zhou*** [1], ***Dr. Russell L. Margolis*** [1], ***Dr. Pan P. Li*** [1]

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Spinocerebellar ataxia type 12 (SCA12) is a neurodegenerative disease caused by a CAG repeat expansion in the gene *protein phosphatase 2 regulatory subunit Bbeta* (*PPP2R2B*). We tested the possibility that the repeat region of the *PPP2R2B* gene locus is bidirectionally transcribed. By strand-specific reverse transcription PCR (SS-RT-PCR) with linkered (LK) primers flanking the repeat in PPP2R2B exon 7, we detected expression of both a *PPP2R2B* transcript containing a CAG repeat and a *PPP2R2B* antisense (*PPP2R2B-AS*) transcript with a CUG repeat from the repeat locus. Using a similar protocol with RNAs extracted from human SCA12 iPSCs, and SCA12 iPSC derived NGN2 cortical neurons, we detected expression of normal and expanded alleles from both sense and antisense transcripts. We conclude that sense and antisense *PPP2R2B* transcripts containing expanded repeats may have a role in SCA12 pathogenesis. Which of these transcripts is the predominant faction in neurotoxicity, and whether the effect is at the RNA or the protein level, remains to be determined.

# (#357) Skeletal muscle RNAomics underpins a double hit in the pathogenesis of Friedreich´s Ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 357

***Dr. Elisabetta Indelicato*** [1], ***Dr. Alexander Kirchmair*** [2], ***Dr. Matthias Amprosi*** [1], ***Prof. Anne Krogsdam*** [2], ***Dr. Wolfgang Nachbauer*** [1], ***Dr. Andreas Eigentler*** [1], ***Prof. Zlatko Trajanoski*** [2], ***Prof. Rainer Schneider*** [3], ***Prof. Sylvia Boesch*** [1]

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**Background and Objective**

Gene expression profiling contributed to unravel key pathophysiological mechanisms and potential therapeutic targets in a variety of genetic diseases. In Friedreich´s Ataxia (FA), several studies investigated gene expression in PBMCs, cells which are not affected by the disease. One study in iPSC-derived sensory neurons failed to demonstrate a clear disease signature, probably due to the early maturation stage. Herein, we report on the first RNAomics study in a patient-derived tissue showing a disease phenotype: the skeletal muscle.

**Methods**

Seven adult FA patients underwent a gastrocnemius biopsy before and after a treatment with Erythropoietin within a previous clinical trial. Biopsies from 6 control subjects were also available. Total RNA extraction, 3'-mRNA library preparation and sequencing were performed according to standard procedures. Following quality control, we tested for differential gene expression with DESeq2 and used GSEA to perform pathway analyses of the results.

**Results**

1659 genes were differentially expressed between FA and controls. Pathway analysis highlighted two main alterations in FA: 1) a downregulation of gene sets related to oxidative phosphorylation and mitochondrial ATP synthesis and 2) an upregulation of gene sets related to RNA splicing, histone modification and translational initiation. FA samples exhibited a downregulation of *FXN* transcripts. Erythropoietin treatment upregulated several genes related to extracellular matrix as well lipid metabolism, including the key regulator leptin.

**Conclusion**

The present data bridge the cumulative evidence of two key pathophysiological signatures in FA: a first translational issue, and a consequential mitochondrial hit. Upregulation of the translational machine likely reflects a rescue mechanism for impaired *FXN* transcription, while mitochondrial failure is the expected consequence of *FXN* deficiency. Clear evidence of both hits in skeletal muscle of patients with manifest disease suggest the need of contemporary addressing both pathways in the design of future therapeutic approaches.

# (#366) Molecular manifestations of Frataxin Loss in human brain microvascular endothelial cells – implications for vascular barrier disease in Friedreich's Ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 366

***Mx. Frances Smith*** [1], ***Dr. Daniel Kosman*** [1]

***1.*** *The State University of New York at Buffalo*

Background and objective

Friedreich's Ataxia (FRDA) patients experience early atrophy of the cerebellar dentate nuclei followed by progressive brain iron accumulation (BIA) in the same region, leading to progressive loss of coordination and walking. That the BIA is progressive along with worsening disease symptoms warrants investigation of its role in central nervous system pathology. FRDA follows from a loss of the protein Frataxin (FXN) which incorporates iron into the iron-sulfur clusters essential to the enzyme complexes of the electron transport chain. FXN loss in cardiac endothelial cells is upstream of cell senescence, altered vascular branching, tube formation, and remodeling. These cell pathologies in FRDA endothelia likely compromise the integrity of the brain vasculature suggesting a potential role in progressive BIA. Metabolic and oxidative stress, two hallmarks of FRDA cell pathophysiology, promote cerebral vascular permeability. The blood-brain barrier (BBB) is responsible for aberrant solute trafficking to maintain brain homeostasis. Therefore, the BBB should be a target of FRDA investigation.

Methods

We have developed a FXN-knockdown brain microvascular endothelial cell model using lentiviral-mediated transfection, creating a stable cell line. We show that this model replicates important disease manifestations including free intracellular iron and mitochondrial dysfunction, the two key pathologies downstream of FXN loss.

Results

Our model displays altered cell size, increased paracellular permeability in an *in vitro* transwell model system, and an increase in iron flux without increase in markers of transcellular iron trafficking. These factors indicate that paracellular iron permeability is likely downstream of endothelial dysfunction and barrier defects.

Discussion and conclusion

Together, we show that FXN-knockdown brain endothelial cells support changes in cell morphology and paracellular solute extravasation. Investigation of the blood brain barrier will provide insight on the dyshomeostasis of the central nervous system in FRDA, contributing to disease progression and decreased patient quality of life.

# (#374) Hypoplasia and degeneration of the retina result in vision loss in Friedreich ataxia patients

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 374

***Dr. Layne Rodden*** [1], ***Ms. Kellie Mcintyre*** [2], ***Ms. Medina Keita*** [2], ***Ms. Courtney Park*** [2], ***Ms. Victoria Profeta*** [2], ***Ms. Mckenzie Wells*** [2], ***Dr. Christian Rummey*** [3], ***Dr. David Lynch*** [4]

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**Intro**: Friedreich ataxia (FRDA) is an inherited condition most commonly caused by a GAA triplet repeat (GAA-TR) expansion (pathogenic range: 40-1500 triplets) in the *FXN* gene. Clinical features of FRDA include ataxia, cardiomyopathy and scoliosis. Vision loss has also been reported, but the frequency, severity, and age of onset of vision loss in FRDA are all undefined. In this study, we define retinal and visual pathophysiology in adults and children with FRDA.

**Methods**: Using optical coherence tomography, we measured Retinal Nerve Fiber Layer (RNFL) thickness in 150 people with FRDA and 98 healthy controls. Early Treatment Diabetic Retinopathy Study (ETDRS) vision charts (full-contrast, 2.5% contrast, and 1.25% contrast) were used to determine visual acuity. RNFL thickness and visual acuity were compared to measures of disease severity obtained from the Friedreich Ataxia Clinical Outcomes Measures Study (FACOMS).

**Results**: Majority of patients, including children, presented with pathologically thin retinas (73±12 μm in FRDA and 99±10 μm in healthy controls) and low-contrast vision deficits early in their disease course. Variability in RNFL thickness (range=36-107 μm) was predicted by disease burden, a combination of GAA repeat length and age. Deficits in full contrast visual acuity (less than 20/20 vision) were apparent in patients with an RNFL thickness of 61±16 μm. RNFLs decreased in thickness at a rate of 1.0±1.1 μm/year and reached 61 μm at a disease burden of 27000 GAA-years

**Conclusions**: Retinal pathology early in disease is an indication of hypoplasia. Subsequent degeneration of the RNFL leads to high-contrast vision loss at a thickness of 61μm, a threshold that most FRDA patients will reach in the $4^{th}$ decade of life. These results suggest a need for vision specific treatment for people with FRDA early in the disease to prevent RNFLs from reaching the critical threshold of 61μm.

# (#401) Pathogenic variants in ITPR1 cluster in functional domains

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 401

***Dr. Jussi Tolonen*** [1], ***Dr. Ricardo Schnekenberg*** [1], ***Dr. Simon McGowan*** [2], ***Dr. Alexander Blakes*** [1], ***Dr. Meriel McEntagart*** [3], ***Dr. Tabib Dabir*** [4], ***Dr. Diana Johnson*** [5], ***Dr. Rachel Harrison*** [5], ***Dr. Abhijit Dixit*** [6], ***Prof. Henry Houlden*** [7], ***Dr. Jonathan Williams*** [8], ***Dr. Morag Shanks*** [8], ***Ms. Katariina Granath*** [9], ***Dr. Salla Kangas*** [9], ***Prof. Reetta Hinttala*** [9], ***Prof. Johanna Uusimaa*** [9], ***Prof. Esther Becker*** [1], ***Prof. Andrea Nemeth*** [1]

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**Background and Objectives.** The *ITPR1* gene encodes the inositol 1,4,5-trisphosphate ($IP_3$) receptor type 1 ($IP_3R1$), a critical player in intracellular calcium signalling. Pathogenic variants in *ITPR1* cause neurodegenerative Spinocerebellar Ataxia Type 15 (SCA15) and congenital SCA29, Gillespie Syndrome, and Severe Pontine/Cerebellar Hypoplasia, but the pathophysiological basis of these different phenotypes is poorly understood. Our objective is to interrogate genotype/phenotype associations of missense mutations in *ITPR1* and establish a library of induced pluripotent stem cell (hiPSC) lines carrying known SCA29 variants.

**Methods.** Variants in *ITPR1* were identified using next-generation sequencing as part of the Deciphering Developmental Disorders study, the 100,000 Genomes project, and clinical collaborations. A time-course study of *ITPR1* expression and alternative splicing in the human cerebellum and hiPSC-derived cerebellar organoids was performed by quantitative reverse-transcriptase (qRT)-PCR. Human cerebellum tissue samples were obtained from the Oxford Brain Bank. Established hiPSC lines were genome-edited using CRISPR/Cas9-mediated homology directed repair.

**Results.** Here we report a detailed genotype/phenotype analysis of 30 patients with 21 unique variants (eleven novel) that were predicted to be disease-causing based on the concordance of phenotype, the type of mutation present, and correlation with functional analyses reported in the literature. Genotype/phenotype analysis show that variants cluster in functional domains in the protein, most evidently in the N-terminal $IP_3$ binding domain, and the C-terminal transmembrane channel domain. Alternative splicing of *ITPR1* transcripts in the human cerebellum is stable at two known splicing sites, while the S3 site undergoes a switch from longer to shorter isoforms with age.

**Discussion and Conclusions**. The dataset represents the largest published cohort of patients with disease-causing variants in the *ITPR1* gene. Our findings have implications for gene-specific variant reporting and can guide *ITPR1* variant interpretation in the clinic. In the future, our library of SCA29-associated hiPSC lines enable us to model disease mechanisms in SCA29.

# (#408) Aberrant mGluR1-TRPC3 signalling in Spinocerebellar Ataxias

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 408

***Ms. Jodie Collingridge*** [1], ***Prof. Philip Biggin*** [2], ***Prof. Esther Becker*** [1]

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**Introduction**

The dominantly inherited spinocerebellar ataxias (SCAs) are a group of rare degenerative ataxia disorders characterised by a progressive loss of motor coordination and degeneration of the cerebellum. Over 40 subtypes exist, each arising from mutations in different genes, and no curative treatment is yet available. TRPC3 is a non-selective calcium-permeable cation channel highly expressed at the Purkinje cell (PC) postsynaptic membrane which facilitates mGluR1-dependent excitation, necessary for healthy development and functioning of PCs. Aberrant activation of these proteins is associated with several SCAs, including SCA41 and SCA44 (caused by mutations in TRPC3 and mGluR1, respectively), as well as SCAs with unrelated genetic backgrounds such as SCA1 and SCA2.

As TRPC3 integrates multiple signalling pathways at the plasma membrane, it presents a potential target for pharmacological modulation of signalling at the PC synapse. Here, we have used molecular dynamic (MD) simulations, coupled with cell-based functional assays, to explore drug binding to TRPC3.

**Methods**

MD simulations were performed using GROMACS (v2018 and 2020), and analysis with MDAnalysis packages. Autodock Vina was used for compound docking. TRPC3 activity was assessed using NFAT translocation assays in the mouse Neuro-2a cell line (ATCC).

**Results**

MD simulations revealed a consistent binding site and pose for a novel TRPC3 inhibitor, including potential key interacting residues. Experimental exploration of these residues through site-directed mutagenesis revealed a functional impact of a selection of these residues on inhibitor activity.

**Discussion and Conclusion**

Due to its localisation and functional importance, TRPC3 may be a viable route to treat several SCAs through modulation of aberrant signalling at the PC synapse. We have proposed a potential binding site and mechanism of a novel TRPC3 inhibitor, which may help elucidate fundamental mechanisms of the channel and guide development of further drugs for clinical use.

# (#439) Early changes in glia phenotype my contribute to the ataxic pathogenesis in an ARSACS mouse model

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 439

***Dr. Brenda Toscano*** [1], ***Mrs. Alison Aube*** [2], ***Prof. Anne McKinney*** [3], ***Dr. Alanna Watt*** [1]

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Background: Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is an early-onset neurodegenerative disease caused by loss-of-function of the *SACS* gene that encodes the protein sacsin. It is characterized by progressive loss of motor coordination and cerebellar degeneration. In the cerebellum of SACS knockout mice, we have shown progressive Purkinje cell disfunction and degeneration, as well as altered synaptic innervation into cells of the cerebellar nuclei (CN). A key player in neuronal and synaptic homeostasis are glial cells. Dysfunction of glial cells has been shown to contribute to the pathophysiology of several neurodegenerative disorders including many spinocerebellar ataxias. Here we explore whether glial function is dysregulated in a mouse model of the ARSACS.

Results: We quantified the number and reactive state of astrocytes and microglia in the both the cerebellar cortex and in the CN of SACS knockout mice at multiple stages of disease progression. We observed enhanced astrocyte reactivity with a progressive increase of GFAP labeling in the CN of ARSACS mice. We also observed an increase in the number of microglia in the cerebellum of SACS mice as well as morphological changes in microglia. These changes occur prior to cell death in the CN or of Purkinje cells.

Discussion and Conclusion: These findings suggest that changes in astrocytes and microglia may contribute to early pathogenesis in ARSACS.

# (#440) Preservation of bioenergetics and inhibition of ferroptosis with the novel compound SBT-588 in Friedreich's ataxia cell models

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 440

***Ms. Alyssa Handler*** [1], ***Dr. Hatim Zariwala*** [1], ***Ms. Yumi Park*** [1], ***Dr. Martin Redmon*** [1], ***Dr. David Brown*** [1], ***Dr. Laura Kropp*** [1]

***1.*** *Stealth Biotherapeutics*

Friedreich's ataxia (FA) is a progressive, neurological disease with a paucity of effective treatments. Mitochondrial dysfunction drives FA disease pathology and offers promising targets for novel therapeutics. Herein we describe a novel compound, SBT-588, that simultaneously targets two major contributors to mitochondrial dysfunction in FA: 1) dysregulated iron homeostasis that can trigger ferroptosis, and 2) disrupted bioenergetics due to dysfunctional mitochondrial respiration. In this study, we tested the effects of SBT-588 using human fibroblast lines derived from FA-affected (GM03665) and unaffected (GM08402) individuals. SBT-588 ameliorated iron-mediated cell death in experiments using two distinct ferroptosis-inducing agents: RSL3 and erastin. RSL3 treatment resulted in significant decreases in cellular viability as measured by endogenous ATP levels. In both FA-affected and unaffected cells, co-treatment with 500 nM of SBT-588 sustained cell viability at 111.4% and 84.5% of untreated controls, respectively. In erastin-induced ferroptosis assays, co-treatment with SBT-588 increased cell viability in a dose-dependent manner as compared to controls ($p=0.023$). Furthermore, SBT-588 treatment decreased levels of the lipoxygenase metabolic by-product, 15-HETE, which increases during ferroptosis and can serve as a biomarker for FA disease progression. Co-treatment of RSL3-injured cells with SBT-588 significantly reduced the concentration of 15-HETE in the supernatant from 970.38 pg/ml to 165.1 pg/ml after 4 hours in unaffected cells (1-way ANOVA $p=0.06$) and from 1587.92 pg/ml to 636.46 pg/ml in a trend towards significance (1-way ANOVA, n.s.) in FA affected cells. Finally, we performed assays to directly assess the impact of SBT-588 on defects in Complex I (CI) of the electron transport chain, which contribute to FA disease pathogenesis. Using high resolution respirometry, we measured a titratable increase in cellular respiration of CI-inhibited cells treated with SBT-588 ($EC50=1.86\mu M$). Together, our data highlight the dual pharmacology of SBT-588 and indicate its potential utility in mitigating FA disease pathogenesis.

# (#446) Temporal dynamics of the Scale for the Assessment and Rating of Ataxia (SARA)

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 446

***Mr. PAUL MOULAIRE*** [1], ***Mr. Pierre Emmanuel Poulet*** [2], ***Dr. Emilien Petit*** [2], ***Dr. Thomas Klockgether*** [3], ***Prof. Alexandra Durr*** [2], ***Dr. Gulin Oz*** [4], ***Dr. Henry Paulson*** [5], ***Dr. Tetsuo Ashizawa*** [6], ***Dr. Sophie Tezenas du Montcel*** [2]

***1.*** *Sorbonne Université, Paris Brain Institute, INSERM, INRIA, CNRS, APHP, 75013 Paris,,* ***2.*** *Sorbonne Université, Paris Brain Institute, INSERM, INRIA, CNRS, APHP, 75013 Paris,* ***3.*** *German Center for Neurodegenerative Diseases (DZNE), 53127 Bonn,* ***4.*** *Center for Magnetic Resonance Research, Department of Radiology, University of Minnesota, Minneapolis, MN 55455,* ***5.*** *Department of Neurology, University of Michigan, Ann Arbor, MI 48109,* ***6.*** *The Houston Methodist Research Institute, Houston, TX 77030*

Background

SARA scale is nowadays the reference scale to assess the severity of ataxia. In a context of therapeutics trials, a reliable clinical outcome is needed to assess the efficiency of the treatments. As there is a debate about the performance of the scale, we compared the temporal dynamic and the variability of the scale, globally and at the item level.

Methods

We analyzed data from four cohorts (EUROSCA, RISCA, CRC-SCA, SPATAX) for a total of 1210 patients and 4092 visits. The SARA scale is composed of eight items, four measuring axial ataxia and four appendicular ataxia. The linearity of the progression and the variability of each item and the total SARA score was assessed with an ordinal Bayesian mixed effect model (Leaspy). We did sample size calculations for therapeutics trials with different scenarios to improve the responsiveness of the scale.

Results

None of the eight different items had a linear progression. The speed of progression was different between most of the items with an average time for a one-point increase from 3.52 years [3.43, 3.63] (median, 95% credible interval) for the fastest item to 11.41 [10.90, 11.97] years. The total SARA score had a linear progression with an average time for a one-point increase of 0.95 [0.92; 0.98] years. After removing the appendicular items and re-scaling all items from 0 to 4, variability increased and progression was slower, involving larger sample size requirements for therapeutic trials.

Conclusion

Despite a heterogeneous temporal dynamic at the item level, the global progression of the SARA scale was linear. Changing the initial scale deteriorates the responsiveness. This new information about the temporal dynamic of the scale will help to design future clinical trials.

Acknowledgments: NIH U01 NS104326

# (#454) Pathogenic mechanisms underlying SCA3 are altered in primary oligodendrocyte cell culture

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 454

***Ms. Alexandra Putka*** [1], ***Ms. Kristen Schuster*** [1], ***Dr. Hayley McLoughlin*** [1]

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**Background and Objective:** Emerging evidence implicates non-neuronal cells, particularly oligodendrocytes, in the pathophysiology of Huntington's disease and spinocerebellar ataxia (SCA) types 1 and 3. We recently demonstrated that cell-autonomous dysfunction of oligodendrocyte maturation is one of the of the earliest and most robust changes in vulnerable regions of the SCA3 mouse brain. In this study, we use primary oligodendrocyte cultures to determine how known pathogenic mechanisms underlying SCA3 affect this cell type.

**Methods:** We isolated oligodendrocyte progenitor cells (OPCs) from 5- to 7-day old mice that overexpress human mutant ATXN3 (YACQ84) or lack ATXN3 (ATXN3-KO) for cell culture and subjected them to differentiation for up to 5 days in vitro (DIV). Immunocytochemistry was performed for ATXN3, markers of the oligodendrocyte lineage, and key proteins involved in protein quality control.

**Results:** At both DIV3 and DIV5, we find a cell-autonomous oligodendrocyte maturation defect in cells from YACQ84 mice but not ATXN3-KO mice, emphasizing that mutant ATXN3 acts by a gain-of-toxic function mechanism. Importantly, these *in vitro* results recapitulate observed RNA and protein changes in our mouse models, indicating that primary oligodendrocyte cell culture is a powerful model for translational research. Using high-magnitude confocal images, we observe cytoarchitectural differences between wildtype and diseased oligodendrocytes. Finally, we report changes in the proteasome and autophagy in disease, providing insight on how damaged or misfolded proteins are handled in SCA3 oligodendrocytes.

**Discussion and Conclusion:** We demonstrate the utility of primary oligodendrocyte culture for elucidating cell-specific mechanisms of SCA3. This novel method has broad applicability given recent work demonstrating oligodendrocyte dysfunction in other polyQ diseases, including SCA1 and Huntington's disease. We are currently exploring other disease relevant pathways in oligodendrocytes and how therapeutic intervention affects cell-autonomous dysfunction in SCA3.

# (#457) Uncovering mechanisms of SNX13-SNX14 interaction in cerebellar function and disease

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 457

***Ms. Vanessa Sanchez*** [1], ***Dr. Naiara Akizu*** [2]

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Our lab studies Spinocerebellar Ataxia Recessive 20 (SCAR20), a fatal, early-onset neurodegenerative disorder characterized by cerebellar ataxia, Purkinje cell loss, coarse facial features, and severe intellectual disability. Biallelic loss-of-function mutations in the ubiquitously expressed Sorting Nexin 14 (SNX14) protein have been reported in more than 45 patients with SCAR20 to date. We and others have uncovered that SNX14 regulates cerebellar lipid homeostasis and interacts with its paralog, SNX13, in neurons. We recently found *SNX13* mutations in three children with a cerebellar disorder like the one caused by the loss of SNX14. Like SNX14, SNX13 is an also an endoplasmic reticulum (ER) resident protein that has been shown to regulate lysosomal cholesterol homeostasis.

Taken together, we hypothesize that the interaction between SNX13 and SNX14 functions to regulate neuronal lipid homeostasis, which is critical for cerebellar function, survival, and integrity. To date, we are currently dissecting the molecular topology of this interaction, as well as determining the importance of this interaction for neuronal homeostasis through biochemical and cellular studies. Lastly, using the CRISPR-based epigenome editing tool, CRISPRoff, we are generating a cerebellar knockdown mouse model to study the role of Snx13 in cerebellar function and integrity.

This work will provide critical insights into our understanding how two sorting nexins are important for cerebellar lipid homeostasis. Like many ataxias, there are no effective treatment options for SCAR20 and patients with *SNX13* mutations. With this study, we hope to further understand disease mechanisms and identify novel targets for treatment of associated neurodegenerative disorders of the cerebellum.

# (#464) Respiratory dysfunction in a novel knock-in SCA1 mouse model

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 464

***Ms. Alyssa Soles*** [1], ***Ms. Jessica Grittner*** [1], ***Ms. Rebecca Barok*** [1], ***Mr. Shawn Miller*** [1], ***Dr. Brendan Dougherty*** [1], ***Dr. Marija Cvetanovic*** [1], ***Dr. Harry Orr*** [1]

***1.*** *University of Minnesota*

Spinocerebellar ataxia type I (SCA1) is an autosomal dominant polyglutamine disease characterized by motor deficits, cognitive decline, and premature lethality from bulbar dysfunction that disrupts normal swallowing and breathing functions. While SCA1 is foremost thought to affect the cerebellum, SCA1 patients also exhibit degeneration of the brainstem and spinal cord. The medulla and pons contain neuron populations that activate downstream spinal cord and muscle components to generate normal breathing. While respiratory dysfunction has been established in SCA1 patients and mouse models of SCA1, there is still a need to understand the regional, cellular, and molecular basis of such dysfunction.

Here we use methods including immunofluorescence confocal microscopy and whole body barometric plethysmography to elucidate mechanisms of breathing dysfunction in a novel knock-in SCA1 mouse model. The flox-Atxn1$^{146Q/2Q}$ mouse model contains human ATAXIN1 (ATXN1) exons 8 and 9 with 146 polyglutamine repeats flanked by lox-p sites inserted at the endogenous mouse locus. Plethysmography on fully conscious unrestrained mice at 35 weeks shows that flox-Atxn1$^{146Q/2Q}$ mice exhibit changes in breathing function compared to littermate controls. These mutant mice exhibit a largely increased respiratory rate, tidal volume, and minute volume. Initial immunofluorescence data shows decreased neuronal numbers and gliosis in medullary inspiratory and expiratory nuclei. Respiration and swallowing are closely associated and share similar neural and muscular substrates; we have also begun to investigate if swallowing is affected in flox-Atxn1$^{146Q/2Q}$ mice by examining feeding behavior.

In summary, this work shows evidence of respiratory dysfunction in a novel SCA1 mouse model. Future work aims to examine the cellular and molecular mechanisms of these deficits in flox-Atxn1$^{146Q/2Q}$ mice utilizing Nestin- and Acta1- Cre mice to remove mutant ATXN1 from neural cell types and from skeletal muscle respectively to examine their contribution to breathing phenotypes.

# (#486) Mechanism of STAU1 mediated mTOR hyperactivity in SCA2

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 486

***Dr. Daniel Scoles*** [1], ***Dr. Sharan Paul*** [1], ***Dr. Warunee Dansithong*** [1], ***Dr. Mandi Gandelman*** [1], ***Dr. Karla P. Figueroa*** [1], ***Dr. Stefan M. Pulst*** [1]

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**Background and Objectives**

The ATXN2 interacting protein STAU1 is an RNA binding protein that functions in mRNA trafficking and decay. We demonstrated that STAU1 is overabundant in SCA2 patient-derived fibroblast cells and SCA2 mouse cerebellum and spinal cord. STAU1 overabundance is associated with increased levels of mTOR and p-mTOR and impaired autophagic flux. Surprisingly, *MTOR* mRNA levels were unchanged. The objective of this study was to delineate the mechanisms underlying abnormal autophagy in SCA2 associated with STAU1 overabundance.

**Methods**

Previous reports indicated that mRNA translation could be activated by STAU1 binding to 5'-UTRs. We hypothesized that this may explain mTOR overabundance in SCA2. We performed three experiments to test this hypothesis: 1) we performed northwestern blotting whereby immobilized STAU1 was probed with dig-labeled *MTOR* mRNA fragments; 2) we performed *MTOR* RNA/STAU1 co-immunoprecipitation assays; 3) we developed a novel luciferase assay, in which we placed the *MTOR* 5'-UTR downstream of the *CMV* promoter, to report STAU1 mediated *MTOR* translation.

**Results**

STAU1 directly interacted with *MTOR* mRNA by northwestern blotting by co-immunoprecipitation. No increased luciferase was observed with STAU1 overexpression in control assays lacking the *MTOR* 5'-UTR. We also expressed STAU1 deletions in the *CMV- MTOR* 5'-UTR luciferase assay identifying the domain critical for *MTOR* translation. Expression of STAU1 lacking the *MTOR* binding domain in HEK-293 cells failed to elevate mTOR abundance. We confirmed that HEK-293-ATXN2-Q22/58-knockin cells treated with rapamycin or *MTOR* siRNA had normalized autophagy marker proteins p62 and LC3-II, and normalized STAU1 levels. We confirmed that lowering STAU1 expression normalized mTOR and autophagy markers.

**Discussion and Conclusion**

STAU1 directly drives mTOR overabundance, suggesting STAU1 as a potentially potent therapeutic target for normalizing disease-related autophagy. We had also observed STAU1 overabundant in C9ORF72 and TDP-43 patient fibroblasts and transgenic mice. These observations support targeting STAU1 in SCA2 as well as ALS.

# (#489) Defining elements necessary for expansion of GAA repeats in the endogenous FXN gene.

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 489

***Dr. Jixue Li*** [1], ***Dr. Yanjie Li*** [2], ***Dr. Jill Napierala*** [3], ***Dr. Marek Napierala*** [4]

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Background and Objective

In most cases, Friedreich's ataxia (FRDA) is caused by expansion of GAA repeats resulting in a pathological deficiency of frataxin protein. GAA repeats are unstable (contract and expand) in germline and somatic cells. Repeat instability has been demonstrated in model systems including E. coli, yeast, and transgenic mice. Practically every process acting upon DNA harboring expanded GAA repeats (e.g. replication, transcription, and repair) affects stability of these sequences. GAA repeats demonstrate a propensity for continuous expansion in cultured FRDA induced pluripotent stem cells (iPSCs), making them an ideal model to define factors influencing instability in the endogenous FXN locus.

Methods

Using CRISPR/Cas9, we created eight FRDA iPSC lines targeting transcription, its direction as well as proximity of the replication origin. Each cell line harbored one unedited allele with expanded GAAs, serving as an internal control, and the second CRISPR/Cas9 modified allele. Subsequently, we performed GAA repeat expansion assays to determine effects of altered transcription and replication on GAA expansions. Finally, we integrated expanded GAA repeats into a different genomic location (AAVS1 safe harbor locus) to evaluate the role of the genomic context on expansion.

Results

As expected, removal of the FXN promoter and ablation of transcription resulted in stabilization of the GAA repeats. Surprisingly, both restoration of transcription by an exogenous promoter and changing direction of DNA replication through the GAA tract did not reactivate expansions. Moreover, expanded GAAs were stably maintained through passaging when integrated into the AAVS1 locus. Simultaneously, the unedited GAA tract at the FXN gene demonstrated a continuous expansion pattern, indicating that the capacity of iPSC to stimulate expansions were unchanged upon editing.

Discussion and Conclusion

Results of our studies demonstrate that exact sequence context is essential, in addition to other processes, for expansion of long GAA tracts in the endogenous FXN locus.

# (#491) Effects of SCA48 disease-associated CHIP mutations on HSF1 activation

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 491

***Ms. Selin Altinok*** [1], ***Prof. Jonathan Schisler*** [1]

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Background: The heat shock response is an evolutionarily conserved protective mechanism. Molecular chaperones and the transcription factor Heat Shock Factor 1 (HSF1) coordinate the cellular response to cell stressors, including heat and consequent proteotoxicity. The Carboxy-terminus of Hsc70 Interacting Protein (CHIP) is a co-chaperone and ubiquitin ligase encoded by the *STUB1* gene. CHIP bridges molecular chaperones to the ubiquitin-proteasome system. CHIP was initially identified to facilitate HSF1 activation during heat shock via CHIP's TPR (tetratricopeptide repeat) domain, potentiating the trimerization and nuclear translocation of HSF1. Mutations in *STUB1* are associated with a newly identified form of autosomal dominant spinocerebellar ataxia: SCA48. However, the effects of SCA48-associated mutations on HSF1 activation are unknown. **We hypothesized that SCA48 mutations alter HSF1 activation and lead to an impaired heat shock response.**

Methods: We performed immunoblot analysis on cytoplasmic and nuclear fractions of Cos7 cells transfected with SCA48-associated mutations. We utilized an HSF1 dual-luciferase reporter assay to quantify HSF1 transcriptional activity in Cos7 cells during heat shock response.

Results: At 37°C, over-expression of SCA48 TPR mutations reduced levels of nuclear HSF1 localization. However, SCA48 mutations in the Ubox (ubiquitin ligase) domain retained HSF1 nuclear translocation. After 42°C heat shock, HSF1 activity of TPR mutations was similar to wild-type CHIP, however, Ubox mutants increased HSF1 activity.

Conclusion: SCA48-associated mutations have differential effects on HSF1 translation and transcriptional activity based on the mutation locale. Interestingly, TPR mutations retained HSF1 activity similar to CHIP wild-type during heat shock, indicating a compensatory mechanism. In contrast, disrupting the ubiquitin ligase activity of CHIP might result in reduced clearance of misfolded proteins during heat shock, exacerbating the heat shock response and hence increasing HSF1 activity. Ongoing studies include delineating the effects of CHIP mutants on HSF1 activity in human IPSC-derived neuronal cultures to characterize disease-related changes related to neuronal function.

# (#499) An EMG cocontraction index in Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay: a promising biomarker

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 499

***Dr. Isabelle Lessard*** [1], ***Dr. Olivier Audet*** [2], ***Dr. Mathieu Bielmann*** [3], ***Prof. Cynthia Gagnon*** [4], ***Mr. Raphael St-Gelais*** [5], ***Mr. Hubert Racine*** [3], ***Prof. Rubens A. Da Silva*** [6], ***Prof. Luc J. Hébert*** [7]

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**Background and objectives:** Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a neurological disorder in which lower limb impairments is one of the most disabling features leading to a loss of mobility. Recently, preliminary findings showed that excessive cocontraction of knee muscles, measured by muscle surface electromyography (EMG), could influence mobility limitations. However, no previous studies have documented the metrological properties of this knee muscle EMG cocontraction index (CI) in ARSACS. The main objectives of this study were to document the construct validity (known-group validity) and reliability of this knee muscle EMG CI in ARSACS population.

**Methods:** The knee muscle EMG activity of 51 ARSACS (mean age: 36.0 ± 11.0 years; 49.0% of men) and nine non-affected (mean age: 36.4 ± 14.7 years; 44.4% of men) participants was measured during two open and one closed chain movements related to three tasks: 1) knee extension, 2) knee flexion and 3) sit-to-stand transfer. For each task, the knee muscle CI was calculated using a ratio of the area under the curve (antagonist/agonist) from the EMG amplitude of the biceps femoris and rectus femoris muscles during the concentric phase of movement.

**Results:** Construct validity was partly supported as CI was significantly different between non-affected and ARSACS participants ($p<0.05$). The knee muscle CI was significantly higher in ARSACS participants ($p<0.05$). For the three tasks, intrarater reliability is excellent (ICCs ≥ 0.96). For the knee extension and flexion tasks, interrater agreement is excellent.

**Discussion and conclusion:** The three-task CI is a reliable and valid index to assess the level of cocontraction between agonist and antagonist knee muscles. These results showed the potential to use this CI to better understand lower limb impairments in the ARSACS population, to develop targeted interventions and to serve as an outcome measure for rehabilitation interventions.

# (#503) Nuclear frataxin and the regulation of macrophage activation

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 503

***Dr. Marco Carpenter*** [1], ***Mrs. Teresa Maltese*** [1], ***Dr. William Peranteau*** [1]

***1.*** *Center for Fetal Research, Children's Hospital of Philadelphia*

**Background and Objective:** Little is known about the role of frataxin (FXN) in genomic integrity or about the mechanisms that control its appearance within the nuclear volume. Key evidence suggest FXN has novel DNA repair functions and that this function is important in the pathogenesis of FRDA: 1) Iron accumulation is an inconsistent and late event in FRDA cells and animal models 2) Impaired DNA repair appears to be an early event in the pathogenesis of FRDA 3) FXN isoform expression and proteolytic processing target FXN protein to the nucleus in some cell-types. These observations encourage a new perspective on FXN beyond mitochondrial mechanisms, such as a role in the regulation of genome integrity and transcriptional regulation in macrophages. Here in, we optimize methodologies to profile and quantify frataxin interactions in the nucleus.

**Methods:** Our approach is the first to investigate nuclear frataxin-mediated genome integrity using a combination of classic and novel methodologies. First, we isolated primary monocytes, transduced them with FXN shRNA or control vectors, followed by differentiation into three macrophages subtypes (M0, M1, M2). We performed RNA-Seq to identify perturbed pathways in each subtype followed by qPCR validation. In addition, we used immunohistochemistry to visualize FXN localization in each macrophage subtype. Next, we used flow cytometry and Cleavage Under Targets and Release Using Nuclease to measure FXN function in the nucleus indirectly and directly, respectively.

**Results:** We identified inflammatory gene signatures regulated by FXN deficiency in macrophages. FXN localization showed differential localization to the nuclear compartment depending on the activation regimen. In addition, we found reduced quantities of DNA repair markers in FRDA patient fibroblasts.

**Discussion and Conclusion:** Careful analysis of the temporal progression of macrophage-dependent inflammation will lay the groundwork for investigations into immune-related FRDA pathology and therapies to mitigate it.

# (#505) Antisense RAN proteins accumulate in Friedreich's ataxia brain and spinal cord

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 505

***Dr. Lisa E.L. Romano*** [1], ***Dr. Monica Banez Coronel*** [1], ***Dr. Tao Zu*** [1], ***Dr. Arnulf H. Koeppen*** [2], ***Prof. Laura P.W. Ranum*** [1]

***1.*** *University of Florida, Center for NeuroGenetics, College of Medicine,Department of Molecular Genetics and Microbiology, Gainesville, FL,* ***2.*** *V.A. Medical Center and Albany Medical College, Albany NY*

**Background and Objective:** Friedreich's Ataxia (FRDA) is a recessive and relentlessly progressive neurodegenerative disorder caused by a GAA·TTC triplet repeat expansion in the frataxin gene (FXN). The expansion mutation results in reduced levels of the mitochondrial frataxin protein. While most therapeutic strategies aim to reestablish frataxin protein levels, other unexplored molecular mechanisms could contribute to disease progression and severity. These mechanisms may include toxicity of sense and antisense RNAs or repeat-associated non-AUG (RAN) proteins. RAN proteins have been reported in 11 diseases with non-coding or coding expansion mutations. This raises the possibility that sense and/or antisense mutant proteins could be expressed from the FXN GAA·TTC expansion mutation and potentially contribute to FRDA pathogenesis.
**Methods:** Our lab recently developed two antibodies that specifically target poly-Serine or poly-Leucine repeat motifs. Using these antibodies, we performed immunohistochemistry in FRDA postmortem tissue. We also developed novel FXN minigene constructs to test the production and toxicity of sense and antisense RAN proteins.
**Results:** Immunohistochemistry (IHC) shows that antisense poly-Serine and poly-Leucine RAN proteins accumulate in cerebellum and spinal cord from FRDA (n>5) but not control (n>4) autopsy cases as nuclear staining or perinuclear aggregates. Poly-Serine and poly-Leucine RAN protein staining is particularly abundant in the cerebellar dentate nuclei, deep white matter, Bergman glia, granular layer, and cortical white matter. RAN-positive white matter regions showed demyelination. In the spinal cord, nuclear poly-Leucine staining was found in oligodendrocyte-like cells in both the grey and white-matter regions.
**Conclusion and Discussion:** Antisense RAN proteins accumulate in FRDA autopsy brain and spinal cord. This is the first example in which RAN proteins have been detected in a disease caused by a non-hairpin forming repeat expansion. Understanding the role of RAN proteins in FRDA will provide novel insight into the mechanisms of disease and novel therapeutic targets.

# (#509) Vglut2-mediated Neurotransmission from Cerebellar Nuclei Neurons is Required for the Acquisition of Motor Control but not Social Behaviors

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 509

***Mr. Alejandro Rey Hipolito*** [1], ***Dr. Roy Sillitoe*** [1], ***Dr. Meike Van Der Heijden*** [2], ***Mr. Dominic Kizek*** [1], ***Mr. Ross Perez*** [1], ***Mr. Tao Lin*** [1]

***1.*** *Baylor College of Medicine,* ***2.*** *BCM*

**Background and Objective**

The cerebellum controls motor and non-motor behaviors, but it remains unclear how its canonical circuitry gives rise to distinct functions. We previously showed that the acquisition of motor control and social vocalizations in postnatal developing mice relies on the neurogenesis of *Atoh1*-expressing neurons, including glutamatergic cerebellar nuclei neurons and granule cells. However, the individual contribution of these cerebellar neurons to the development of motor and non-motor behaviors remains unresolved.

**Methods**

We eliminated Vglut2-mediated neurotransmission from *Atoh1* neurons by deleting the glutamate transporter (*Vglut2*). This manipulation affects granule cells and nuclei neurons during development, but in adulthood only impacts nuclei neurons due to a shift from Vglut2- to Vglut1-mediated neurotransmission in granule cells. We also selectively deleted Vglut2 from *Ntsr1* nuclei neurons starting during development. We tested for Vglut2 deletion using *in situ* hybridization and immunohistochemistry, measured the effects of eliminating granule cell neurotransmission using *in vivo* electrophysiology, and tested motor control and social behaviors using age-appropriate behavioral assays.

**Results**

*In situ* hybridization and immunohistochemistry confirmed the deletion of Vglut2 from *Atoh1* granule cells in developing mice and nuclei neurons in adult mice. We showed that silencing Vglut2-mediated neurotransmission from *Atoh1* neurons resulted in impaired motor control and social vocalizations in early postnatal mice. Moreover, the firing rate of developing Purkinje cells, the main downstream target of granule cells, was decreased. Interestingly, we show that pups lacking neurotransmission from *Ntsr1* nuclei neurons exhibited impaired motor control but intact social vocalizations. In adult mice with silenced neurotransmission from glutamatergic cerebellar nuclei neurons, only motor control was impaired while social behavior and Purkinje cell firing remained unaffected.

**Discussion and Conclusions**

Based on the data from our two genetic manipulations, we propose that glutamatergic cerebellar nuclei neurons regulate the development of motor control while granule cells also contribute to social behaviors.

# (#532) Repeat expansions in NOP56 are a cause of spinocerebellar ataxia type 36 in the British population

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 532

***Ms. Clarissa Rocca*** [1], ***Dr. Tanya Lam*** [2], ***Dr. Kristina Ibanez*** [3], ***Dr. Anupriya Dalmia*** [3], ***Dr. Marios Hadjvassiliou*** [4], ***Dr. Anke Hensiek*** [5], ***Prof. Andrea Nemeth*** [6], ***Dr. Stefano Facchini*** [1], ***Prof. Nicholas Wood*** [7], ***Dr. Andrea Cortese*** [8], ***Prof. Henry Houlden*** [9], ***Dr. Arianna Tucci*** [10]

***1.*** *Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London, UK,* ***2.*** *Great Ormond Street Hospital and St George's Hospital NHS Trust, London, UK,* ***3.*** *Clinical Pharmacology, William Harvey Research Institute, School of Medicine and Dentistry, Queen Mary University of London, London, UK,* ***4.*** *Royal Hallamshire Hospital, Sheffield Teaching Hospitals Foundation Trust, Sheffield, United Kingdom,* ***5.*** *Department of Clinical Neurosciences, Addenbrookes Hospital, Cambridge, UK,* ***6.*** *Nuffield Department of Clinical Neurosciences, University of Oxford,* ***7.*** *Department of Molecular Neuroscience, Institute of Neurology, University College London, London, UK,* ***8.*** *UCL Institute of Neurology,* ***9.*** *Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London,* ***10.*** *William Harvey Research Institute, Queen Mary University of London*

**Background**

Spinocerebellar ataxias (SCAs) form a clinically and genetically heterogeneous group of neurodegenerative disorders characterised by progressive cerebellar ataxia. Their prevalence varies among populations and ethnicities. SCA36 is caused by a GGCCTG repeat expansion in intron 1 of the NOP56 gene, and is characterised by late-onset ataxia, sensorineural hearing loss, and upper and lower motor neuron signs, including tongue fasciculations. SCA36 has been identified mainly in East Asian and Western European patients, and was thought to be absent in the British population.

**Methods**

Leveraging novel bioinformatic tools to detect expansions from whole genome sequencing, we analyse the NOP56 repeat in 1,257 British patients with hereditary ataxia and 7,506 unrelated controls.

**Results**

We identify pathogenic repeat expansions in seven patients, representing the first cohort of white British descent patients with SCA36. Employing in-silico approaches using whole genome sequencing data, we found an 87kb shared haplotype in the 7 cases around the NOP56 repeat region, although this block was also shared between several controls.

**Discussion and Conclusion**

Clinically, the patients presented with slowly progressive cerebellar ataxia with low rate of hearing loss and variable rates of motor neuron impairment. Our findings show that the NOP56 expansion causes ataxia in the British population, and that SCA36 can be suspected in patients with a late onset, slowly progressive ataxia, even without the findings of hearing loss and tongue fasciculation.

# (#536) Iron deficiency may be a key feature in the heart of Friedreich's ataxia at early stage

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 536

***Dr. Yuho Kim*** [1], ***Mr. Varun Dhorajia*** [1], ***Dr. Jonghan Kim*** [1]

***1.*** *University of Massachusetts Lowell*

Friedreich's ataxia (FRDA) is a rare genetic disorder that progressively develops neurodegenerative problems. Although FRDA is also known to develop cardiac dysfunction, the underlying mechanism is unclear. Using FRDA mouse model ($Fxn^{null}$::YG8s(GAA)>800; Jackson Laboratory #030395), we have sought to characterize the phenotype and related markers in the heart with a focus on iron transport. First, we observed cardiac hypertrophy at 2-3 months old, as evidenced by increased levels of both ANP and BNP by ~2-fold in the heart of FRDA mice as compared to control mice (FXN carrier). Notably, systemic and cardiac iron levels (i.e., total and non-heme iron) were lower in the FRDA mice, although brain iron level was elevated. In response to low cardiac iron status, the iron regulatory system was altered, as iron uptake proteins were significantly up-regulated, while the expression of iron storage proteins was decreased in the heart. Along with this iron imbalance, we also found that mitochondrial oxidative function was largely impaired in the heart of FRDA mice. In conclusion, iron deficiency could exacerbate cardiac dysfunction in the heart of the FRDA model, which may be a target for slowing the development of FRDA-associated cardiac problems.

# (#542) Hypomorphic Variants of SEL1L-HRD1 ER-associated Degradation Cause Early-Onset Cerebellar Ataxia and Neurodevelopmental Disorders

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 542

***Dr. Liangguang Lin*** [1], ***Dr. Huilun Wang*** [1], ***Dr. Mauricio Torres*** [1], ***Dr. Ling Qi*** [1]

***1.*** *Department of Molecular and Integrative Physiology, University of Michigan*

Cerebellar ataxia is a progressive neurodegenerative disorder with neurological symptom of defective motor coordination that can affect gait, balance, speech and gaze. Most recent studies in both patients and model systems support the concept that cerebellar ataxia is related to misfolded protein accumulation and aggregate. The SEL1L-HRD1 mediated Endoplasmic reticulum (ER)-associated degradation (ERAD) is the principal protein quality-control mechanism targeting misfolded proteins in the ER. However, the underlying molecular mechanism and significance of SEL1L-HRD1 ERAD in cerebellar ataxia remain largely unclear as no disease variants in human has been identified.

Using genome/exome sequencing, here we report the identification of three bi-allelic missense variants of *SEL1L* (p.Gly585Asp, p.Met528Arg) and *HRD1* (p.Pro398Leu) in six children from three independent families presenting with cerebellar ataxia/hypotonia, developmental delay and intellectual disability. An earlier study has identified a *SEL1L* variant (p.Ser658Pro) in new-born Finish hounds also suffering early-onset cerebellar ataxia. However, the causality of these variants and their underlying mechanism remain unknown. To understand the pathogenesis and molecular mechanism between the SEL1L-HRD1 variants and cerebellar ataxia, various state-of-art techniques were performed and knock-in mouse model was generated.

We found that these SEL1L-HRD1 variants are indeed disease-causing in human by causing ERAD dysfunction. Mechanistically, these variants impair ERAD function towards misfolded protein substrates via distinct mechanisms, including substrate recruitment, SEL1L-HRD1 interaction, and HRD1 activation. As a proof-of-principle, *SEL1L* S658P knock-in mouse model was generated, and the homozygous knock-in mice also exhibit developmental delay and early-onset cerebellar ataxia with motor incoordination caused by a reduction of Purkinje cells (a critical type of neuron controlling motor movement) in cerebellum.

These data established, for the first time, the pathophysiological importance and disease relevance of mammalian SEL1L-HRD1 and provided new insights into the SEL1L-ERAD in human cerebellar ataxia.

# (#544) The NF-kB inducing kinase (NIK) selectively regulates the abundance of mutant ataxin-3 in SCA3 brain cells

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 544

***Ms. Anna J. Barget*** [1]***, Ms. Naila S. Ashraf*** [1]***, Ms. Emily D. Shaw*** [1]***, Dr. Henry Paulson*** [1]***,***
***Dr. Maria do Carmo Costa*** [1]

***1.*** *University of Michigan*

Reducing levels of the SCA3 causative *ATXN3* gene products has been shown as a hopeful therapeutic strategy for SCA3, and nonallele-specific antisense oligonucleotides targeting *ATXN3* transcripts are currently being tested in clinical trials. However, whether sustained brain depletion of native *ATXN3* products is safe in humans is unknown. Therefore, identifying cellular mechanisms that specifically target mutant *ATXN3* products is an unmet need for SCA3 and our long-term goal. Because we have previously identified the NF-kB inducing kinase gene (*NIK*) as an enhancer of mutant ATXN3 abundance, here our goal was to determine whether *NIK* selectively modulates physiological levels of mutant ATXN3 in SCA3 human embryonic stem cells (hESCs)-derived cells and in vivo in a SCA3 transgenic mouse model.

We assessed whether NIK abundance is dysregulated in post-mortem SCA3 patients' brains, evaluated the ATXN3 levels in SCA3 hESC-derived neuronal progenitor cells (NPCs) depleted for *NIK* or overexpressing *NIK* or *NIK* kinase-mutant, and compared levels of human mutant ATXN3 and mouse Atxn3 in brains of SCA3 mice and SCA3 mice constitutively lacking *Nik*.

Comparing with controls, we found that NIK protein levels are reduced in the pons but not in the cerebral cortex of SCA3 patients suggesting that the spared cells in the post-mortem SCA3 pons, a region severely affected with neuronal loss, display low levels of NIK supporting its role as an enhancer of mutant ATXN3 abundance/toxicity. Further evidence of this novel NIK role are our findings indicating that *NIK* regulates levels of mutant, but not native, ATXN3 in SCA3 NPCs in a kinase-dependent way, and that *Nik* abrogation in SCA3 mice specifically decreases brain levels of human mutant ATXN3 but not mouse Atxn3.

NIK is a selective enhancer of mutant ATXN3 abundance in brain cells suggesting that NIK or the NF-kB pathway could be a target for SCA3 therapeutic intervention.

# (#546) Muscle-specific Frataxin Knockout Mice Exhibit Reduced Endurance

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 546

***Dr. Chen Liang*** [1], ***Dr. Robert Dirksen*** [1]

***1.*** *Department of Pharmacology and Physiology, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester, NY 14642*

**Background and Objective**

Though individuals with Friedreich's ataxia exhibit loss of balance and motor coordination, reduced muscle mass and weakness, the specific role of frataxin in skeletal muscle function is unknown. To address this question, we generated and characterized a novel, constitutive muscle-specific frataxin knockout mouse model.

**Methods**

Model generation: Exon 2 floxed Fxn (*Fxn*$^{fl/fl}$) mice were crossed to *HSA-Cre+* mice to generate compound *HSA-Cre+ : Fxn*$^{fl/+}$ mice. These mice were then intercrossed to generate *HSA-Cre+* : *Fxn*$^{fl/fl}$. *HSA-Cre+* : *Fxn*$^{+/+}$ were used as controls.

Model validation: Frataxin protein (western blot) levels were quantified across different skeletal muscles (*tibialis anterior, extensor digitorum longus,* soleus) and other tissues (heart, lung, liver, kidney, brain, spinal cord) in *HSA-Cre+* : *Fxn*$^{fl/fl}$ and *HSA-Cre+* : *Fxn*$^{+/+}$ mice.

Behavioral assessment of 2-month-old mice: 1) Wire hang 2) Wire-hang escape 3) Grip strength 4) Rotarod fatigue test 5) Treadmill endurance test.

**Results**

Postnatal body weight growth curves (0-30 days) were not significantly different between *HSACre+* : *Fxn*$^{fl/fl}$ and *HSA-Cre+* : *Fxn*$^{+/+}$ mice. Frataxin protein expression was reduced >95% across all skeletal muscles in *HSA-Cre+* : *Fxn*$^{fl/fl}$ mice compared to controls, while being similar in all other tissues tested. Though grip strength was similar for both genotypes, wire-hang escape scores and wire hang time to fall were both significantly reduced in *HSA-Cre+* : *Fxn*$^{fl/fl}$ mice. Additionally, compared with controls, *HSA-Cre+* : *Fxn*$^{fl/fl}$ mice exhibited increased fatigue in both treadmill and Rotarod endurance tests.

**Discussion and Conclusion**

Our findings demonstrate that *HSA-Cre+* : *Fxn*$^{fl/fl}$ mice represent a powerful animal model to assess the specific role of frataxin in skeletal muscle development and function. Using these mice, we found that constitutive muscle-specific reduction of frataxin results in reduced muscle strength and endurance. Current studies are investigating the cellular and molecular mechanisms by which frataxin deficiency results in muscle dysfunction.

# Poster Sessions: Cerebellar Non-motor Circuits and Functions

# (#147) A systematic review of the spectrum and prevalence of non-motor symptoms (NMS) in adults with progressive cerebellar ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 147

***Dr. Rajith de Silva*** [1], ***Dr. Yaqub Al Sami*** [1], ***Dr. Aram Aslanyan*** [1], ***Dr. Naveed Malek*** [1]

***1.*** *BHR University Hospitals NHS Trust*

*Background and Objective:*
Cerebellar ataxias comprise a large group of heterogeneous disorders with both motor and NMS. We wished to know what types of NMS had been described in adults with different forms of progressive cerebellar ataxia in the published literature, and their reported prevalence.
*Methods:*
Systematic review of studies of adults (>16 years) with cerebellar ataxias (involving >5 patients) who were assessed for NMS, published in the English literature in PUBMED and EMBASE databases from 1947-2020.
*Results:*
32 research papers, with data from 1181 cases of autosomal dominant spino-cerebellar ataxia (SCA), 160 cases of autosomal recessive cerebellar ataxia (ARCA) and 488 cases of multiple system atrophy (MSA) were included. Mean age for SCA cases was 39.8 (17.7) years, mean disease duration was 10.5 (11.1) years and 44.3% were male. Mean age for ARCA cases was 47.8 (21.6) years, mean disease duration was 20.1 (11.2) years and 48.1% were male. Mean age for MSA cases was 58.3 (7.3) years, mean disease duration was 4.2 (3.3) years and 59.2% were male. Our analysis showed that the prevalence of cognitive problems in SCA varied between 25-100%. Cognitive deficits are less common and/or severe in SCA6. The prevalence of depression in SCA was between 23-69% and sleep disorders 20-80%. Pain was reported by 60% of patients (most prevalent in SCA3), and fatigue by 57%. The prevalence of cognitive problems in ARCA varied from 12.5 -100% and depression between 21-50%. The prevalence of autonomic failure in MSA was between 48-96.5% at baseline.
*Discussion and Conclusion:*
In routine clinical practice, NMS in cerebellar ataxias are under-recognised and almost certainly under-reported. Consequently, they are unlikely to be managed adequately.

# (#361) Cerebellar Impulsivity-Compulsivity Assessment Scale

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 361

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**Background and Objective:** The cerebellum has been identified as the key brain region that modulates reward processing in animal models.[1-4] Consistently in humans, we recently found that people with cerebellar ataxia have impulsive and compulsive behaviors (ICBs), the main symptoms related to abnormal reward processing.[5,6] Due to the lack of a validated scale to quantitatively measure and monitor ICBs in cerebellar disorders, we aim to develop and validate a new scale, Cerebellar Impulsivity-Compulsivity Assessment (CIA).

**Methods:** We recruited 62 cerebellar ataxia cases, categorized into those with ICBs and those without. We developed a preliminary version of CIA, containing 17 questions. We studied the internal consistency, test-retest reliability, and inter-rater reliability to formulate the final version of CIA, which constitutes the final 10 questions. The receiver operating characteristic curve (ROC) was generated to assess the sensitivity and specificity of CIA.

**Results:** Cerebellar ataxia cases with ICBs have 3-fold higher total preliminary CIA scores than those without ICBs (12.06 ± 5.96. *vs.* 4.68 ± 3.50, $p = 0.038$). Cronbach's alpha revealed good internal consistency across all items ($\alpha > 0.70$). By performing the test-retest reliability and inter-rater reliability on the preliminary version of CIA, we excluded 7 questions ($r < 0.70$) and generated the final version of CIA. Based on the ROC, a score of 8.0 in CIA was chosen as the cut-off for ICBs in individuals with cerebellar ataxia with 81% sensitivity and 81% specificity.

**Discussion and Conclusion:** CIA is a novel tool to reliably and validly assess ICBs in cerebellar ataxia. Via its measurement and monitoring, CIA can broaden our understanding of the cerebellum-related cognitive and behavioral syndrome.

**References**

1. Nature 2017; 544: 96-100
2. Science 2019; 363: 6424
3. Neuron 2020; 106: 1055
4. Nature Communications 2021; 12: 6475
5. Movement Disorders. 2020;35(8):1491-1493
6. Neurology. 2022 Apr 15;10.1212/WNL.0000000000200349

# (#390) Determinant of the Cerebellar cognitive affective syndrome in Friedreich Ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 390

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Background

Individuals with Friedreich Ataxia (FRDA) display significantly lower performances in many cognitive domains compared to control participants with a pattern of impairment that falls within the cerebellar cognitive affective syndrome (CCAS).

Objective

Assess in a large cohort of individuals with FRDA the main determinant of the CCAS using multiple variable regression models.

Methods

38 individuals with FRDA were assessed. Ataxic Motor symptoms were evaluated with the SARA and cognitive functions with the CCAS-Scale (CCAS-S). The CCAS-S is composed of 10 separate cognitive tasks. A raw score is obtained for each task, with a minimum passing score. The number of failed tests determines the likelihood that the subject has CCAS (>3: definite CCAS). Age, SARA, GAA1, Age of symptoms onset (ASO) and disease duration (DD) were chosen as covariates in a linear regression model to predict CCAS-S score and in a logistic regression model to predict definite CCAS.

Results

Patients mean age, SARA score, ASO, DD and GAA1 were respectively of 28±14, 23±10, 13±10, 16±9 and 701±236. Mean CCAS-S raw score was of 86±16, mean number of failed items was 2.9±1.6. Twenty-two individuals had definite CCAS. Multiple linear regression model with Age, SARA, ASO, DD & GAA1 as covariates was statistically significant to predict CCAS-S ($R^2$ = 0.57, $p$ < 0.001). SARA was the only significant coefficient in the model and explained 34% of the variability of the CCAS-S raw score. Multiple logistic regression model was statistically significant to predict definite CCAS ($R^2$ = 0.53, $p$ = 0.008) with SARA as only significant coefficient in the model.

Conclusions

CCAS is highly prevalent in adult individuals with FRDA. CCAS is predicted by ataxic motor symptoms severity which supports common core cerebellar pathophysiology for cognitive and motor symptoms in FRDA. Screening for CCAS, especially in patients with SARA > 20 is warranted.

# (#391) Understanding the relevance of the cerebellar cognitive affective syndrome in SCA3 mice

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 391

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**Background and Objective:** Spinocerebellar ataxia type 3 (SCA3) is a neurodegenerative disorder caused by an expansion in the *Ataxin-3* gene. SCA3 patients present a very heterogenous clinical presentation, comprising mainly motor manifestations, but also report depressive or anxiety traits. These mood-related symptoms have often been attributed to the negative prospects of disease or to the prior knowledge of carrying the SCA3 gene mutation, but can, alternatively, be inherent to the disease state. This is of interest given that antidepressants, such as citalopram, have been shown to modify disease progression in animal models of SCA3. Interestingly, over the past years, cerebellar dysfunction has been associated to cognitive and mood-related disturbances, the so-called – cerebellar cognitive affective syndrome (CCAS). Currently, no studies are available in rodent models of SCA3 regarding cognition and mood-related disorders. Therefore, we questioned whether the animal models of SCA3 would also presents such deficits.

**Methods:** A longitudinal phenotypic characterization of mood-related, anxiety, and cognitive behavioral domains was performed in CMVMJD135 transgenic mice, beginning at an early symptomatic stage and along disease progression (from 6 to 36 weeks of age). Motor assessments were also performed to control for SCA3 progression.

**Results:** Overall, transgenic mice do not exhibit anhedonia when compared to wild-type littermates, at any disease stage. No anxiety, learned helplessness or cognitive deficits, in any of the measured paradigms, were detected in these mice either.

**Discussion and Conclusion:** Our results indicate that SCA3 mice do not present CCAS. Molecular analyses are being conducted to further examine neurotransmitters, as well as expanded Ataxin-3 protein levels in cognition and mood-related brain regions, such as prefrontal cortex, dorsal and ventral hippocampus, amygdala, and nucleus accumbens, in addition to the cerebellum of SCA3 mice.

# (#406) Increased month-to-month intra-individual variability in motor-cognitive performance in people with spinocerebellar ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 406

***Dr. Louisa P. Selvadurai*** [1], ***Mr. James Morgan*** [1], ***Ms. Sheryl Gullia*** [2], ***Prof. Adam Vogel*** [3], ***Dr. Kishore Kumar*** [4], ***Dr. David Szmulewicz*** [5], ***Dr. Ian Harding*** [6]

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Background: Autosomal dominant spinocerebellar ataxias (SCAs) are characterised by progressive movement incoordination. In addition to overall worsening of motor function, affected individuals experience short-term fluctuations in performance, which may be important clinical markers. Cognitive deficits may also be present. We investigated month-to-month variability in motor-cognitive tasks in SCAs compared to controls to determine whether there are short-term fluctuations in performance, over-and-above overall motor deficits.

Methods: 14 individuals with SCA and 13 controls completed monthly online tasks over 7 months: speeded finger tapping (FT); paced FT; simple visual reaction time (RT); choice RT; and cognitive interference RT. Intra-individual variability across time (standard deviation [SD]) was compared between the SCA and Control groups, and correlated against ataxia severity.

Results: Individuals with SCAs had greater SD compared to controls on paced FT ($d=1.0$, $p<0.001$), simple RT ($d=0.8$, $p=0.02$), and interference RT ($d=0.99$, $p=0.03$). More advanced disease correlated with greater SD on simple RT ($r=0.57$, $p=0.03$).

Discussion: These novel findings indicate that there is considerable short-term variability in psychomotor function in SCAs, unrelated to long-term progressive neurodegeneration. This variability has important implications for the reliability of even simple psychomotor tasks as treatment outcome markers, but also may reflect an important clinical marker. Inter-individual fluctuations in performance over time also highlight potential limitations in the use of performance-based ataxia rating scales as primary outcome measures in natural history studies and clinical trials, as changes in performance between assessments may be temporary fluctuations rather than reflections of longer-term progression. Further work is necessary to investigate modifiable correlates of this variability (e.g., fatigue; depression).

# (#435) Cerebellar transcranial direct current stimulation modulates timing but not acquisition of conditioned eyeblink responses in SCA3 patients

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 435

***Dr. Roderick Maas*** [1], ***Prof. Dennis Schutter*** [2], ***Prof. Ivan Toni*** [3], ***Dr. Dagmar Timmann*** [4], ***Prof. Bart van de Warrenburg*** [1]

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**Background:** Delay eyeblink conditioning is an extensively studied associative learning paradigm that critically depends on the integrity of the cerebellum. In healthy individuals, modulation of cerebellar excitability using tDCS has been reported to alter the acquisition and/or timing of conditioned eyeblink responses (CRs). It remains unknown whether such effects can also be elicited in patients with cerebellar disorders.

**Objective:** To investigate if repeated sessions of cerebellar tDCS modify acquisition and/or timing of CRs in SCA3 patients and to evaluate possible associations between disease severity measures and eyeblink conditioning parameters.

**Methods:** Eyeblink conditioning was examined in 20 individuals with SCA3 and 31 healthy controls. After the baseline assessment, patients were randomly assigned to receive ten sessions of cerebellar anodal tDCS or sham tDCS (i.e., five days/week for two consecutive weeks). The same eyeblink conditioning protocol was administered after the last tDCS session. The SARA, CCAS scale, and disease duration were used as measures of disease severity.

**Results:** At baseline, SCA3 patients exhibited significantly fewer CRs than controls. Acquisition was inversely associated with the number of failed CCAS-S test items but not with SARA score. Onset and peak latencies of CRs were longer in SCA3 patients and correlated with disease duration. Repeated sessions of cerebellar anodal tDCS did not affect CR acquisition, but had a significant treatment effect on timing parameters. While a shift of CRs toward the conditioned stimulus was observed in the sham group (i.e., timing became more similar to that of healthy controls, presumably reflecting the effect of a second eyeblink conditioning session), anodal tDCS induced a shift in the opposite direction (i.e., toward the unconditioned stimulus).

**Conclusion:** Our findings provide evidence that cerebellar tDCS is capable of modifying cerebellar function in SCA3 patients. Future studies should assess whether this intervention similarly modulates temporal processing in other degenerative ataxias.

# (#517) Respiratory Function in Friedreich's ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 517

***Dr. Andrea Martinuzzi*** [1], ***Dr. Elena Vinante*** [1], ***Dr. Gabriella Paparella*** [1], ***Ms. Elena Colombo*** [1], ***Dr. Michela MArtinuzzi*** [2]

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**Background**: Friedreich's ataxia is an inherited, rare, progressive disorder of children and young adults. It is characterized by ataxia, loss of gait, scoliosis, cardiomyopathy, dysarthria, dysphagia, with reduced life expectancy. Alterations of respiratory dynamics and parameters are frequently observed. However, in the literature there are few, dated studies with small cohorts.

Our study aims to make an objective analysis of the respiratory condition of both early and late stage FRDA patients, looking for correlations with the motor, skeletal, speech and genetic aspects of this condition.

**Materials and methods**: This retrospective observational study is based on the collection of clinical and instrumental respiratory data of 44 subjects between 13 and 51 years attending a tertiary rehabilitation centre in northern Italy. The analysis was carried out using Pearson's correlation test, ANOVA test and Post Hoc tests.

**Results:** data show the presence of a recurrent pattern of respiratory dysfunction of restrictive type, with reduction of forced vital capacity and of flow and pressure parameters. The severity of the respiratory condition correlates with disease severity (measured with disease-specific scales), with pneumophonic alterations and with the severity of the thoracic scoliotic curve.

**Conclusion**: the complex condition of incoordination and hyposthenia in FRDA affects daytime and night-time respiratory efficiency. Considering the concomitant postural difficulty and the presence of dysphagia, Wwe believe that the respiratory deficit and the inefficiency of cough are indeed a clinical problem deserving consideration, especially in the context of the concomitant postural difficulty and the possible presence of dysphagia. The rehabilitation project for the subject with FRDA should also consider the ventilation.

# (#526) Synaptic and Structural Abnormalities in The Cerebella of Friedreich Ataxia Mouse Models

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 526

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Friedreich ataxia (FRDA) is a life-shortening neurodegenerative disorder caused by deficiency of the mitochondrial protein frataxin. This deficiency leads to a multisystemic phenotype; however, neurological deficits remain the ubiquitous feature of FRDA patients. FRDA patients' clinical neurological features include progressive ataxia and speech problems, all of which are controlled to a large degree by the cerebellum. The cerebellum's role in fine motor coordination is well established; however, the mechanism by which frataxin deficiency impacts the cerebellum remains to be fully elucidated. In the present work, we utilized the inducible mouse model (FRDAkd) and the knock in knock out (KIKO) to examine the biochemical and structural properties of the cerebellum.

For c structural analysis, we immunostained FRDAkd cerebellar slides with Purkinje cell(PC) marker, calbindin and with hematoxylin & eosin. The slides were imaged with fluorescent microscopy and analyzed with ImageJ. Biochemical analysis was completed using whole FRDAkd and KIKO cerebella homogenates where PC and glial postsynaptic glutamate receptors were analyzed via Western blot and IHC.

Systemic knockdown of frataxin in FRDAkd mice leads to disrupted Purkinje cell morphology. PCs show distorted dendritic arbors and smaller cell bodies compared to wildtype. Protein analysis in whole cerebellar homogenates shows that AMPA receptors are significantly decreased in KIKO and FRDAkd mice while glial glutamate transporters are upregulated in KIKO and FRDAkd cerebella.The PC postsynaptic receptor NMDAR1 is significantly decreased in the FRDAkd cerebellum while other NMDA receptors subunits found in non-purkinje cells do not change.

Overall, we observe structural deficiencies in Purkinje cells from the FRDAkd mice and disregulated expression of glutamate transporters in the KIKO and FRDAkd mice models of Friedreich ataxia; these results suggest the importance of frataxin in maintaining Purkinje cells/cerebellar integrity, supporting our previous data that identified cerebellar synaptic degeneration as an important component of ataxia in FRDA models.

# Poster Sessions: Gene Discovery and Mechanisms

# (#137) Unique Ataxia oculomotor apraxia 2 (AOA2, OMIM #606002) case in Israel: Expanding the phenotypic spectrum, highlighting novel variants and possible identification of a poison exon

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 137

***Dr. Penina Ponger*** [1], ***Dr. Alina Kurolap*** [2], ***Dr. Hofit Gadot*** [2], ***Dr. Adi Mory*** [2], ***Dr. Yael Wilnai*** [2], ***Prof. Nir Giladi*** [3], ***Prof. Tanya Gurevich*** [3], ***Dr. Daphna Marom*** [4], ***Prof. Yuval Yaron*** [4], ***Prof. Hagit Baris Feldman*** [4]

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Background:
Ataxia oculomotor apraxia 2 (AOA2, OMIM #606002) is a rare progressive adolescent-onset disease. Phenotype includes cerebellar vermis atrophy, peripheral neuropathy, elevated serum alpha-fetoprotein (AFP) and oculomotor apraxia. The disease is caused by pathogenic bi-allelic variants in *SETX*, encoding senataxin, involved in maintaining genomic stability.
We describe a unique simplex case of AOA2, presenting with an atypical phenotype and compound heterozygosity for a nonsense and a deep-intronic variant seen at our center's Ataxia Clinic.

Methods: Trio whole exome sequencing (WES) was performed followed by *SETX* RNA analysis. RNA was extracted from patient's and parents' peripheral blood mononuclear cells and converted to cDNA to enable splicing analysis. qRT-PCR was performed to assess overall and mutant *SETX* mRNA expression.
Results: The proband is of non-consanguineous Persian Jewish family with early childhood onset of disease. Positive findings upon presentation in early 20's include ocular apraxia, dysarthria, and ataxia. MRI demonstrates cerebellar atrophy. AFP levels are normal. Trio WES of the proband and parents revealed two novel *SETX* variants in trans; a maternal nonsense variant in exon 6 (c.568C>T; p.Gln190*), and a paternal deep intronic variant in intron 12 (c.5549-107A>G). Intronic variant analysis and *SETX* mRNA expression revealed cryptic exon activation which introduces premature stop codon (p.Met1850Lysfs*18). qRT-PCR analysis revealed that intronic c.5549-107A>G variant induces aberrant splicing, leading to 20-30 times higher levels of cryptic exon activation compared to WT samples (mother and healthy controls). In combination with a second deleterious allele, this variant leads to low levels of *SETX* mRNA and disease manifestations.
Conclusions: Our case expands the phenotypic spectrum of AOA2, emphasizing the need for relentless tailored molecular work-up to ensure timely diagnosis of patients. Deep-intronic variant analysis potentially reveals a newly described poison exon in the *SETX* gene, contributing to tailored therapy development.

# (#139) Genes associated with ataxia in Initiative on Rare and Undiagnosed diseases (IRUD)

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 139

***Dr. Yuji Takahashi*** [1], ***Mr. Hidetoshi Date*** [1], ***Dr. Yuka Hama*** [1], ***Mr. Hideki OI*** [2], ***Prof. Shinji Kosugi*** [3], ***Prof. Naomichi Matsumoto*** [4], ***Prof. Kenjiro Kosaki*** [5], ***Prof. Yoichi Matsubara*** [6], ***Prof. Hidehiro Mizusawa*** [1]

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**Background and Objective:**
Initiative on Rare and Undiagnosed Diseases (IRUD) was launched in 2015 as a national project in Japan to provide accurate diagnosis, discover causes and provide cures for rare and undiagnosed diseases. IRUD belongs to Undiagnosed Diseases Network International (UDNI), an international collaborative network on rare disease research projects. The objective of this study is to investigate the frequency of genes associated with ataxia in IRUD.
**Design/Methods:**
The IRUD entry criteria are as follows: The patient remains undiagnosed for ≥6 months and suffers from disabilities in daily life, AND objective signs that cannot be attributed to a single organ are observed, OR direct or indirect evidence indicates a genetic etiology. In principle, spinocerebellar degeneration was classified as an undetermined disease, clearly distinguished from an undiagnosed disease. The participants were subjected to whole exome sequence analysis (WES) and diagnosed according to clinical phenotypes and candidate pathogenic variants. Genes associated with ataxia, “ataxia genes”, were defined as those which clinical description in Online Mendelian Inheritance in Man (OMIM) contained ataxia, which amounted to 612 genes on May 2020.
**Results:**
By the end of March 2021, 6301 pedigrees consisting of 18136 individuals were registered in IRUD. WES was completed in 5136 pedigrees and a final diagnosis was established in 2247 pedigrees. The total number of causative genes and pathogenic variants were 654 and 1718, respectively, among which 113 genes (18.0%) with 320 pathogenic variants including 202 novel and 118 known ones belonged to “ataxia genes”. Major disease categories included spinocerebellar ataxia, spastic paraplegia, Joubert syndrome, epileptic encephalopathy and neurodevelopmental disorders, although many disorders were not classified in a single disease category.
**Discussion and Conclusion:**
Genes associated with ataxia were not rare in IRUD, highlighting the significance of ataxia in rare disease research projects.

# (#141) New spinocerebellar ataxia subtype caused by SAMD9L mutation triggering mitochondrial dysregulation (SCA49)

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 141

***Dr. Antoni Matilla-Dueñas*** [1], ***Dr. Marc Corral-Juan*** [1], ***Dr. Pilar Casquero*** [2], ***Dr. Natalia Giraldo*** [2], ***Dr. Steve Laurie*** [3], ***Dr. Alicia Martinez-Piñeiro*** [4], ***Dr. Raidili Cristina Mateo-Montero*** [2], ***Dr. Lourdes Ispierto*** [5], ***Dr. Dolores Vilas*** [5], ***Dr. Eduardo Tolosa*** [6], ***Dr. Victor Volpini*** [7], ***Dr. Ramiro Alvarez-Ramo*** [5], ***Dr. Ivelisse Sanchez*** [1]

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Spinocerebellar ataxias consist of a highly heterogeneous group of inherited movement disorders clinically characterized by progressive cerebellar ataxia variably associated with additional distinctive clinical signs. The genetic heterogeneity is evidenced by the myriad of associated genes and underlying genetic defects identified. In this study, we describe a new spinocerebellar ataxia subtype in nine members of a Spanish five-generation family from Menorca with affected individuals variably presenting with ataxia, nystagmus, dysarthria, polyneuropathy, pyramidal signs, cerebellar atrophy and distinctive cerebral demyelination. Affected individuals presented with horizontal and vertical gaze-evoked nystagmus and hyperreflexia as initial clinical signs, and a variable age of onset ranging from 12 to 60 years. Neurophysiological studies showed moderate axonal sensory polyneuropathy with altered sympathetic skin response predominantly in the lower limbs. We identified the c.1877C > T (p.Ser626Leu) pathogenic variant within the *SAMD9L* gene as the disease causative genetic defect with a significant log-odds score ($Z_{max}$ = 3.43; $\theta$ = 0.00; $P < 3.53 \times 10^{-5}$). We demonstrate the mitochondrial location of human SAMD9L protein, and its decreased levels in patients' fibroblasts in addition to mitochondrial perturbations. Furthermore, mutant SAMD9L in zebrafish impaired mobility and vestibular/sensory functions. This study describes a novel spinocerebellar ataxia subtype caused by *SAMD9L* mutation, SCA49, which triggers mitochondrial alterations pointing to a role of SAMD9L in neurological motor and sensory functions.

# (#194) Identification of molecular pathways in the pathophysiology of frataxin deficient proprioceptive neurons

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 194

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Friedreich ataxia (FA) is a rare neurodegenerative disorder characterized by a mixed spinocerebellar and sensory ataxia, associating cardiomyopathy and increased incidence of diabetes. Proprioceptive sensory neurons (pSNs) of the dorsal root ganglia (DRG) are primarily affected in the disease. Axonal degeneration of these neurons has been reported, but the exact nature of their selective degeneration remains poorly understood. To gain insight into the molecular mechanisms involved in the neuropathophysiology of FA, it is important to uncover the transcriptomic signature of frataxin-deficient proprioceptive neurons in the DRG. We used single cell RNA sequencing (scRNA-seq), a powerful tool to allow comparison of individual cell transcriptomic signature, as this technology resolves cellular heterogeneity, while highlighting co-regulated gene modules in a single cell level. This allows us to selectively concentrate our studies on the proprioceptive neuronal sub-population. As a disease model, we use the recently generated *Fxn*$^{L3/L-}$; Pvalb$^{tm1}$(Cre)$^{Arbr/J}$ conditional model that has a depletion of frataxin in the pSNs.

DRGs scRNA-seq was performed at 3.5 weeks and 7.5 weeks of age, two important stages of the disease progression. At 3.5 weeks of age, the mice are at an early stage, with some signs of sensory ataxia. This will allow us to uncover the early molecular changes associated with frataxin deficiency. At 7.5 weeks, the mice are symptomatic with a strong decrease in the sensorimotor reflex. This will allow us to identify late molecular changes. Sc-RNA-seq was performed with 10X genomics technology. cDNA libraries have been processed to standard sequencing and preliminary data highlights key biomarkers and allow us to identify the proprioceptive sub-population. We are currently analyzing the generated data to identify new pathways that will be validated biochemically.

# (#206) Molecular analysis, repeat structure determination, and clinical spectrum in Italian ataxia patients with RFC1 expansion

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 206

***Dr. Daniela Di Bella*** [1], ***Dr. Stefania Magri*** [1], ***Dr. Elisa Sarto*** [1], ***Dr. Marinella Corbetta*** [1], ***Dr. Maria Balzo*** [1], ***Dr. Cinzia Gellera*** [1], ***Dr. Chiara Pisciotta*** [2], ***Dr. Ettore Salsano*** [2], ***Dr. Davide Pareyson*** [2], ***Dr. Mario Fichera*** [1], ***Dr. Lorenzo Nanetti*** [1], ***Dr. Caterina Mariotti*** [3], ***Dr. Franco Taroni*** [1]

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*Background*: CANVAS is an adult-onset, slowly progressive neurodegenerative disorder characterised in its full form by a combination of cerebellar ataxia, neuropathy, and vestibular areflexia [syndrome], whose phenotypic spectrum is expanding, ranging from late-onset ataxia to sensory neuropathy. It is caused by a biallelic intronic AAGGG expansions in the *RFC1* gene. Recently, expansions with alternative sequence conformations have been identified whose pathogenic role remains to be established.

*Objectives*: 1) To screen Italian patients with full or partial CANVAS phenotype (n=279) or late-onset (n=216) spinocerebellar ataxia for *RFC1* repeat expansion; 2) to assess the frequency of *RFC1* expansion carrier in a control population; 3) to sequence the expanded alleles to determine their repeat structure and role in disease.

*Methods:* The *RFC1* locus was analysed by a PCR flanking the repeat to identify the normal alleles $(AAAAG)_{11}$ followed by different fluorescent repeat-primed PCR to identify the normal [$(AAAAG)_n$, $(AAAGG)_n$] and the mutated $(AAGGG)_n$ expanded alleles. A long-read NGS approach has been set up to sequence the expanded alleles.

*Results*: Biallelic *RFC1* expansion was identified in 29% (81/279) probands with a phenotype in the CANVAS spectrum, and in 12.5% (27/216) of patients with ataxia. Heterozygous carrier rate was 12% in patients (24/198) and 10% (36/360) in healthy controls. Interestingly, approx. 5% of alleles showed complex repeat conformations with uncertain pathogenicity.

*Conclusions*: Biallelic *RFC1* expansion accounts for a large proportion of ataxia phenotypes in Italian patients (12-29%), and should always be considered in the diagnostic algorithm of patients with sporadic ataxia, ranking first in late-onset cerebellar and sensory ataxia. The high carrier rate suggests that the *RFC1* expansion might be the most common cause of ataxia in Italian patients. Sequencing data confirm the complex architecture of the expanded repeat which may have crucial implications for diagnosis and counseling. (Grant: Care4NeuroRare to FT (FRRB-CP-20/2018))

# (#254) The genetic and phenotypic landscape of STUB1/TBP-associated ataxia: Two genes, three patterns of inheritance, and a continuous phenotypic spectrum

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 254

***Dr. Stefania Magri*** [1], ***Dr. Lorenzo Nanetti*** [1], ***Dr. Cinzia Gellera*** [1], ***Dr. Elisa Sarto*** [1], ***Dr. Maria Balzo*** [1], ***Ms. Elena Rizzo*** [1], ***Dr. Alessia Mongelli*** [1], ***Dr. Giuseppe Di Fede*** [2], ***Dr. Caterina Mariotti*** [1], ***Dr. Daniela Di Bella*** [1], ***Dr. Franco Taroni*** [1]

***1.*** *Unit of Medical Genetics and Neurogenetics, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan,* ***2.*** *Unit of Neurology 5 and Neuropathology, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan*

**Background:** SCA17 and SCA48 are both characterized by cerebellar-cognitive-behavioural features and incomplete penetrance. While SCA17 is caused by polyQ repeat expansions in the *TBP* gene, with a full penetrance for >49-repeat alleles and a reduced penetrance for intermediate 41-49 alleles, SCA48 is attributed to heterozygous pathogenic variants in the S*TUB1* gene. Notably, biallelic *STUB1* pathogenic variants cause a recessive ataxia (SCAR16) characterized by a SCA17/SCA48-like phenotype. which manifests only in homozygotes or compound heterozygotes. We recently demonstrated the digenic inheritance of *STUB1*/*TBP* genotype which explains the incomplete penetrance in SCA17 and SCA48, showing that SCA17 is a monogenic disorder for $TBP_{>47}$ and a "true digenic" *STUB1/TBP* disorder for intermediate $TBP_{41\text{-}46}$ alleles.

**Objectives:** to investigate 1) the pathogenic role of "high-normal" 39-40 *TBP* alleles; 2) the discrepancy between the expected genetic prevalence of the *STUB1*/*TBP* genotype and the reported disease prevalence of *STUB1*/*TBP*-associated diseases; 3) the phenotypic overlap in monogenic and digenic SCA17, and SCA48.

**Results:** Analysis of 170 index cases with ataxia carrying "high-normal" $TBP_{39\text{-}40}$ alleles identified 10 novel families carrying a *STUB1* pathogenic variant (5.9%), supporting a pathogenic role for 39-40 alleles. Screening of 94 probands primarily referred for dementia revealed 18 cases with $TBP_{39\text{-}40}$ alleles, indicating a slight enrichment of "high-normal" alleles in this group of patients. Notably, *STUB1* pathogenic heterozygous variants were identified in 3 of these cases. Comparison of clinical and neuroimaging findings reveal that an earlier and more severe cognitive deterioration and a more widespread cerebellar atrophy characterize the $SCA17^{Digenic}$ phenotype in comparison with SCA17 monogenic disease. By contrast, highly homogeneous neuroradiological findings were observed in SCA48/$SCA17^{Digenic}$ and SCAR16 patients.

**Conclusions:** The data support the pathogenic role of $TPB_{39\text{-}40}$ alleles and indicate that *STUB1*/*TBP*-related disorders represent a very demanding task for genetic counseling, and are characterized by a wider and continuous spectrum of phenotypes.

# (#277) The Geographic and Temporal Distribution of Friedreich Ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 277

***Ms. Morgan Tackett*** [1], ***Ms. Christina Lam*** [1], ***Dr. Layne Rodden*** [2], ***Dr. David Lynch*** [3], ***Prof. Sanjay Bidichandani*** [1]

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Background: Friedreich ataxia (FRDA) patients are typically homozygous for an expanded GAA triplet-repeat (E allele) in the *FXN* gene. Prevalence is determined by the frequency of heterozygous carriers of E alleles, which varies globally. FRDA occurs in Europe, North Africa, Middle-East, the Indian subcontinent, and derived populations. FRDA does not affect East-Asians, Native-Americans, and Sub-Saharan Africans. Linkage disequilibrium (LD) indicates a rare duplication from short normal (SN) to long normal (LN) alleles led to the generation of LN+ alleles, including E alleles.

Objective: High-density haplotypes of LN and E alleles were generated to evaluate the temporal and geographic distribution of FRDA.

Methods: The *FXN* locus was sequenced using HaloPlex. GAA triplet-repeats were genotyped in individuals from the 1000G project. Haplotypes were analyzed from ~8000 present and ~3000 ancient humans.

Results: An LD block of 117 SNPs spanning the *FXN* gene characterized LN and E alleles, and identified a 12-SNP core haplotype representing LN+ alleles in susceptible populations. This haplotype is absent in East Asia. A haplotype with 11 of 12 SNPs was seen in ~10% of Sub-Saharan Africans, which was associated with LN alleles, indicating that SN to LN transition occurred in Africa. Acquisition of the $12^{th}$ SNP, transition to LN+ alleles, and concomitant susceptibility to FRDA occurred in West Eurasians and South Asians. Ancient DNAs revealed that the widespread Eurasian distribution of LN+ alleles, with the exclusion of East Asia and America, was established in Neolithic times (>5kya), likely via the spread of farming, which was further modified by subsequent Bronze Age migrations. Current worldwide distribution of the core haplotype identified several potentially susceptible populations that are underrepresented in the medical literature and in registries.

Conclusion: The geographic distribution of FRDA is explained by the ancient spread of LN+ alleles in susceptible populations. Genetic epidemiology revealed underrepresented susceptible populations.

# (#302) Role of motor protein KIF1C in the maintenance of myelin- Involvement in ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 302

***Dr. Khalid El Hachimi*** [1], ***Mrs. Liriope Toupenet*** [2], ***Mrs. Anne Vaiman*** [3], ***Mr. Johan Castille*** [3], ***Mrs. Flora Lasserre*** [2], ***Dr. Bruno Passet*** [3], ***Mr. Jerome Lecardonnel*** [3], ***Mrs. Nathalie Daniel-Carlier*** [3], ***Dr. Florence Jaffrezic*** [3], ***Mrs. Marthe Vilotte*** [3], ***Dr. Jean-Luc Vilotte*** [3], ***Dr. Giovanni Stevanin*** [2], ***Dr. Amandine Duchesne*** [3]

***1.*** *Paris Brain Institute (ICM), Sorbonne Université,* ***2.*** *Paris Brain Institute, Sorbonne Université,* ***3.*** *INRAE*

Mutations in the *KIF1C* gene have been associated with a rare form of spastic ataxia in human: spastic ataxia 2 (SPAX2/SAX2) and spastic paraplegia type 58 (SPG58). We have also previously shown that progressive ataxia of Charolais cattle, a frequent autosomal recessive disease, is due to a *KIF1C* gene mutation and mimics the human pathology. In this species, *KIF1C* mutation leads to disseminated demyelinating plaques due to anarchic oligodendrocyte membrane protrusions.

To further understand the role of KIF1C in myelin maintenance and integrity, we generated a *Kif1c* mouse model mimicking the bovine mutation using CRISPR/Cas9 technology. Mutant mice exhibited decreased locomotor performances, with an ataxic gait worsening with age. Histopathological and ultrastructural analysis evidenced abnormal lipophilic accumulations in all white matter tracts, which appeared as out-folding myelin aggregations and as ectopic myelin surrounding cells. These abnormalities were observed as early as 15 days postnatal and increased with age. The peripheral nervous system was not affected, even at later stages. In aged mice, microglial phagocytosis was often associated with myelin debris. Transcriptomic analysis was performed on cerebellum from wildtype and presymtomatic 6-8 weeks old *Kif1c* mutant mice. Amongst the 244 differentially expressed genes, 10 genes involved in oligodendrogenesis were highly downregulated, such as MBP, MOBP and RNA-binding protein QKI. MBP mRNA expression showed a dramatic change with its expression restricted to the perinuclear region as early as 7 days postnatal.

In summary, the preliminary data we present here, together with data published by us and others, strongly implicate KIF1C in myelination and/or myelin maintenance. We further present new evidences that KIF1C may be involved in RNA transport. Binding of KIF1C to MBP mRNA may be the link connecting KIF1C and myelin maintenance. This novel oligodendroglial function of KIF1C may contribute to a better understanding of myelin plasticity.

# (#310) Whole-exome sequencing study of fibroblasts from subjects affected by CoQ10 deficiency and cerebellar ataxia: unexpected findings

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 310

***Dr. Edoardo Monfrini*** [1], ***Dr. Alba Pesini*** [2], ***Dr. Catarina Marina Quinzii*** [2], ***Dr. Alessio Di Fonzo*** [3]

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**Background and Objectives**: Primary Coenzyme Q10 (CoQ10) deficiency is a clinically and genetically heterogeneous mitochondrial disorder. Cerebellar ataxia is the most common clinical presentation of primary CoQ10 deficiency. The aim of this study is to identify through whole-exome sequencing (WES) the genetic cause of cerebellar ataxia and CoQ10 deficiency in a collection of patient-derived fibroblasts.

**Methods**: WES was performed on genomic DNA extracted from patient-derived fibroblasts (16 samples). Sequencing data were filtered using a virtual panel of genes associated with CoQ10 deficiency and/or cerebellar ataxia.

**Results**: A definite genetic etiology was identified in 9 samples out of 16 (diagnostic yield = 56.3%). Pathogenic and likely pathogenic variants were found in *COQ8A* (*ADCK3*) gene and in five ataxia genes never associated with CoQ10 deficiency so far. Notably, among the pathogenic variants identified, six were novel

**Discussion and Conclusion**: The results of this study open a new scenario on the etiology of ataxia with CoQ10 deficiency. In fact, among the samples in which we identified pathogenic variants, only a minority were genes already known to cause cerebellar ataxia and CoQ10 deficiency (i.e., *COQ8A*). The majority of samples carried pathogenic variants in ataxia genes never associated with CoQ10 deficiency so far. These findings reveal that a limited gene panel containing only known CoQ10 deficiency genes is not sufficient to make a genetic diagnosis in these patients and a broader genetic screening should be performed (e.g., comprehensive ataxia gene panel, WES). Furthermore, this work suggests five novel likely genetic determinants of ataxia with COQ10 deficiency.

# (#342) Rescuing physiological expression of the frataxin gene in Friedreich Ataxia (FRDA) by translation modulation through genome editing

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 342

***Ms. Francesca Garilli*** [1], ***Ms. Chiara Ambrosini*** [1], ***Ms. Giuliana Palazzo*** [1], ***Ms. Giorgia Zappacosta*** [1], ***Prof. Alessandro Quattrone*** [1]

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**Background and Objective**

FRDA is a multisystemic disease characterized by neurodegeneration and degeneration/loss of cardiomyocytes. FRDA is caused by GAA-repeat expansion within intron 1 of frataxin gene that leads to a transcriptional deficiency and protein reduction. Frataxin is localized in mitochondria where is involved in FeS and redox metabolism. Different frataxin transcripts exist that codify for cytosolic isoforms but their role in disease has not been deeply investigated. Frataxin silencing leads to iron/ROS accumulation. Our aim is to boost protein production by modifying specific gene regulatory regions, restoring frataxin expression and rescuing disabilities.

**Methods**

We performed a screening using reporter-fluorescent protein to quantify the effect of modifications on protein production, focusing on modification feasible by CRISPR-Cas9 technologies. We performed a bioinformatic analysis of frataxin isoform transcripts expression in cell-line and tissue databases. We are studying the profile of expression of these frataxin isoforms by high-throughput techniques in a HEK293T-FXN-KO model created in the lab and in patients derived specific affected tissues. Indeed, we are currently creating a hiPSC-biobank, derived from the biggest cohort of patients in Italy and we are developing efficient 3D-protocols for hiPSC differentiation in sensory neurons and cardiomyocytes.

**Results**

We found that some specific isoforms are highly transcribed but negatively regulated at translation level. On the contrary, the canonical isoform is efficiently translated but its transcript level is low. We also confirmed that the organoids correctly recapitulate the sensory neurons population found *in vivo* and that can be used as *in vitro* model.

**Discussion and conclusion**

The investigation of the role of frataxin isoforms can represent a breakthrough in understanding FRDA molecular mechanism, and the possibility to manipulate target sequence of the gene to specifically increase the expression of these isoform to compensate or restore frataxin protein function could represent a potential therapeutic approach in the future.

# (#372) High-throughput expansion screening in inherited cerebellar ataxias with ExpansionHunter

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 372

***Dr. Jean-Loup Méreaux*** [1], ***Mrs. Claire-Sophie Davoine*** [1], ***Dr. Marie Coutelier*** [2], ***Mrs. Lena Guillot Noel*** [1], ***Dr. Anna Castrioto*** [3], ***Dr. Perrine Charles*** [4], ***Dr. Giulia Coarelli*** [1], ***Dr. Claire Ewenczyk*** [4], ***Dr. Anna Heinzmann*** [1], ***Dr. Aurélie Meneret*** [1], ***Dr. Anne-Laure Fauret*** [5], ***Prof. Alexis Brice*** [1], ***Prof. Alexandra Durr*** [1]

***1.*** *Sorbonne Université, Paris Brain Institute - ICM, Inserm, CNRS, APHP, Hôpital de la Pitié Salpêtrière, Paris,* ***2.*** *Sorbonne Université, Paris Brain Institute (ICM Institut du Cerveau), AP-HP, INSERM, CNRS, University Hospital Pitié-Salpêtrière, France,* ***3.*** *Grenoble Alpes University Hospital, Grenoble, France,* ***4.*** *APHP, Paris, France,* ***5.*** *Neurogenetics Laboratory, AP-HP, University Hospital Pitié-Salpêtrière*

Background and Objective.
Many inherited cerebellar ataxias transmitted dominantly are caused by expansions of repeat sequences in SCA (Spinocerebellar ataxia) genes. Molecular diagnosis requires a targeted sizing of one locus after another, which is resource consuming and poorly adapted to the large number of loci. We report the effectiveness of a global screening test for all possible repeats by high-throughput sequencing.
Methods.
253 exomes (TWIST capture) and 14 genomes were sequenced each in one run (Illumina, Novaseq6000). Genotyping of the main repeat sequences in SCAs was analyzed with the *ExpansionHunter* tool. Fragment length analysis by capillary migration was performed in all individuals for *ATXN 1,2,3,7 CACNA1A*, *TBP* and *ATN1*. In addition, TP-PCR was necessary to confirm *NOP56 ExpansionHunter* positive exomes.
Results.
Eight exomes revealed 3 *ATXN2*, 3 *ATXN3* and 2 *NOP56* pathogenic expansions corresponding to 3% detection rate. Interestingly, these cases were isolated and repeat expansion had not been excluded on a routine basis. Two genomes revealed *ATXN3* expansions. Fragment length analysis revealed that *ExpansionHunter* slightly underestimated the repeat size, particularly for large expansions. This underlines the need to lower the pathogenic screening threshold when using *ExpansionHunter* for genotyping.
Discussion and Conclusion.
The performance of *ExpansionHunter* had been demonstrated on genomes but the results obtained with exomes in this study allows us to propose this method as a first-line screening test with 100% of sensitivity and specificity. *ExpansionHunter* seems to be promising and can be used with high-throughput short-read sequencing. Like this, a single exome first-line analysis will allow to diagnose repeat expansions as well as single nucleotide variants in SCAs at once.

# (#380) Novel expanded ATTCT-ATXN10 alleles in Amerindian and Mestizo healthy populations from Peru: preliminary results

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 380

***Mr. Ismael Araujo-Aliaga*** [1], ***Ms. Carla Manrique-Enciso*** [1], ***Prof. Mario Cornejo-Olivas*** [1], ***Prof. Elison Sarapura-Castro*** [1], ***Prof. Maryenela Illanes-Manrique*** [1], ***Mr. Olimpio Ortega-Davila*** [1], ***Ms. Diana Cubas-Montecino*** [1], ***Prof. Luis Urbina-Ramirez*** [1], ***Prof. Jorge La Serna-Infantes*** [1], ***Prof. Andrea Rivera-Valdivia*** [1], ***Prof. Angel Medina-Colque*** [2], ***Prof. Julia Rios-Pinto*** [3], ***Prof. Ivan Cornejo-Herrera*** [4], ***Prof. Pilar Mazzetti-Soler*** [5], ***Dr. Tetsuo Ashizawa*** [6], ***Ms. Karina Milla-Neyra*** [1]

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Background and Objective: SCA10 is a dominant spinocerebellar ataxia caused by an ATTCT expansion within the ATXN10 gene. Non-affected individuals without family history of SCA10 carry up to 32 ATTCT repeats, while affected individuals are associated with 280 or more ATTCT repeats. Recent findings in Peruvian non-affected individuals showed alleles greater than 32 repeats, not previously reported. Our aim is to determine the variability of the ATTCT-ATXN10 alleles in Amerindian and Mestizo populations from Peru.

Methods: DNA aliquots from 661 neurologically healthy Amerindian and Mestizo individuals from Peru were provided by the DNA Bank-Neurogenetics according to the following selection criteria: legal age, no presence or history of neurological disease and self-defined as Mestizo or Amerindian. ATTCT-ATXN10 was amplified by PCR and measured by capillary electrophoresis. Samples not displaying both alleles were further genotyped by repeat-primed PCR (RP-PCR) followed by non-denaturing polyacrylamide gel electrophoresis.

Results: DNA aliquots and linked demographic data from 595 Mestizos and 66 Amerindian (n=661) have been included. A total of 1322 alleles were identified by PCR and RP-PCR, with the 14-repeat allele being the most common (545/1322; 41.2%), and a 7-repeat allele being found for the first time. RP-PCR analysis found individuals carrying an expanded allele greater than 32 repeats (50/1322; 3.8%), with significantly higher frequency among Amerindians (13/132; 9.8%) compared to Mestizos (37/1190; 3.1%).

Discussion and Conclusion: Our preliminary results suggest the presence of expanded ATTCT-ATXN10 alleles in Peruvian individuals, mainly in Amerindian, that should be carefully considered when genetically diagnosing SCA10 in this population. Further analysis including Southern blot and sequencing are required to confirm these observations.

# (#404) Recessive variants in SLC9A1 cause a syndrome of cerebellar ataxia, amelogenesis imperfecta and variable sensorineural hearing loss.

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 404

***Dr. David Pellerin*** [1], ***Dr. Reza Maroofian*** [1], ***Prof. Larry Fliegel*** [2], ***Prof. Henry Houlden*** [1]

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*Background:* The *SLC9A1* gene encodes the mammalian Na+/H+ exchanger isoform 1 (NHE1), a ubiquitously expressed membrane-bound enzyme involved in intracellular pH regulation. Ultra-rare recessive variants in *SLC9A1* have previously been described to cause autosomal recessive spinocerebellar ataxia type 19 (SCAR19) in two families. SCAR19 is characterized by early-onset ataxia and variable hearing loss. Here, we report 12 patients with recessive *SLC9A1* variants and a complex syndrome of cerebellar ataxia, amelogenesis imperfecta, developmental delay and variable sensorineural hearing loss.

*Methods:* Patient phenotyping was performed through serial clinical assessments, dental examinations, audiograms and brain MRI. Candidate variants in *SLC9A1* were first identified by whole-exome sequencing and next characterized *in vitro*. The expression and enzymatic activity of mutant NHE1 proteins were respectively examined by immunoblotting and transient induction with ammonium chloride of transfected NHE1-deficient cells. Intracellular targeting of mutant proteins was assessed by a combination of immunocytochemistry and cell surface biotinylation studies.

*Results:* We identified 12 patients belonging to eight consanguineous families with homozygous *SLC9A1* variants. Eight novel variants were discovered, including two nonsense, four missense, one frameshift and one splicing variant. Patients presented with moderate to severe cerebellar ataxia from infancy associated with cerebellar atrophy (10/11; 91%) and occasional thinning of the corpus callosum (3/11; 27%) on MRI. In addition to developmental delay, all patients exhibited amelogenesis imperfecta, which had not been previously reported with *SLC9A1* mutations. Sensorineural hearing loss of variable severity was present in nine out of 12 subjects (75%). All identified variants caused lower protein expression, reduced NHE1 enzymatic activity and protein mislocalization.

*Discussion and Conclusion:* This study expands the mutational and phenotypic spectrum of SCAR19 and provides functional evidence for the pathogenicity of the newly identified variants. Mutations in *SLC9A1* should be specifically sought for in the presence of early-onset cerebellar ataxia and amelogenesis imperfecta.

# (#422) Dysregulation of splicing in spinocerebellar ataxia type 1 (SCA1)

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 422

***Mr. Victor Olmos*** [1], ***Mr. Evrett Thompson*** [1], ***Ms. Serena Sim*** [1], ***Dr. Diane Krause*** [1], ***Dr. Janghoo Lim*** [1]

***1.*** *Yale University*

Spinocerebellar ataxia type 1 (SCA1) is caused by an expansion of the polyglutamine (polyQ) tract in the ATAXIN-1 protein. Ataxin-1 is broadly expressed throughout the brain, but the Purkinje cells (PCs) of the cerebellum degenerate earliest in this disease. Although it is still unclear why PCs are the most susceptible to degeneration in SCA1, it is known that nuclear localization of ataxin-1 is crucial for the development of SCA1 pathogenesis. Ataxin-1 is known to regulate gene expression, and most studies primarily focus on the regulation of transcription via interaction with transcription factors. Interestingly, ataxin-1 also interacts with many RNA binding proteins including splicing factors. It has never been shown that polyQ-expanded ataxin-1 indeed regulates alternative splicing events or what these splicing events are. To begin to answer whether and how the polyQ-expanded ataxin-1 modulates gene expression, via regulating splicing events, we performed RNA-sequencing using SCA1 mouse cerebellum over multiple timepoints by applying both polyA selection and rRNA depletion methods to capture both mature and immature RNA transcripts. We analyzed our data to look at both differentially expressed genes (DEGs) and alternatively spliced genes. We found many abnormal alternative splicing events occurring throughout disease initiation and progression in SCA1 mouse cerebellum and that they did not fully overlap with DEGs. These alternative splicing events are potentially critical for PC health and survival in SCA1 and should be further analyzed for their roles in SCA1 pathogenesis.

# (#527) Genetic modifier analysis in Friedreich ataxia: SNP Analysis and Whole Exome Results from the FACOMS Cohort

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 527

***Dr. David Lynch*** [1], ***Ms. Jennifer Farmer*** [2], ***Prof. Martin B Delatycki*** [3], ***Prof. Susan L. Perlman, MD*** [4], ***Dr. Giovannia Coppola*** [4], ***Dr. Khalaf Bushara*** [5], ***Dr. Manuela Corti*** [6], ***Dr. Sub H. Subramony*** [7], ***Dr. Christopher M. Gomez*** [8], ***Dr. Chad Hoyle*** [9], ***Dr. Bernard Ravina*** [10], ***Ms. Alicia Brocht*** [10], ***Ms. Cindy Casaceli*** [10], ***Dr. George R. Wilmot*** [11], ***Dr. Theresa Zesiewicz*** [12], ***Dr. Paul de Bakker*** [13], ***Dr. Darin Takemoto*** [13], ***Dr. Sarah Luchansky*** [13]

***1.*** *University of Pennsylvania & Childrens Hospital of Philadelphia,* ***2.*** *Friedreich's Ataxia Research Alliance,* ***3.*** *Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia,,* ***4.*** *David Geffen School of Medicine at UCLA, Los Angeles, CA,* ***5.*** *U Minnesota,* ***6.*** *Powell Gene Therapy Centre, University of Florida, Gainesville, Florida, United States of America,* ***7.*** *McKnight Brain Institute, Department of Neurology, University of Florida, Gainesville, Florida, United States of America,* ***8.*** *University of Chicago,* ***9.*** *Ohio State,* ***10.*** *U Rochester,* ***11.*** *Department of Neurology, Emory University School of Medicine, Atlanta, GA,* ***12.*** *Department of Neurology, University of South Florida Ataxia Research Center, Tampa, FL,* ***13.*** *Vertex*

Background /Objective: The causative mutation in Friedreich ataxia (FRDA), biallelic GAA expansions in the *FXN* gene, explains 40-50% of phenotypic variability in most models, with the remainder thus reflecting other environmental or genetic variability. We evaluated genomic DNA from participants in the Friedreich Ataxia Clinical Outcome Measures Study (FA-COMS) \to identify other genetic influences on the phenotype in FRDA.

Methods: 632 FRDA patient DNA samples were obtained from participants in FACOMS and subjected to SNP array analysis and exome sequencing. 613 samples passed quality control analyses and were matched with clinical data. Phenotypes of interest (such as age of onset) were analyzed by identifying SNPs in which the minor allele affects the slope of the genetic relationship among samples and the phenotype of interest (accounting for covariates). Phenotypes and covariates of interest included frataxin protein levels (accounting for GAA length), neurologic severity (FARS score adjusted for age of assessment, GAA repeat length), and age of onset (adjusted for GAA repeat length).

Results: Overall, the sample set behaves as expected, with patient samples clustering mostly with European genomes. In SNP analysis no loci were identified of genome-wide significance, but some loci passed a "suggestive" cutoff. No genes in the iron sulfur cluster synthesis pathway passed such criteria, and no gene was linked to all 3 outcome measures (age of onset, frataxin level, neurologic severity). By exome sequencing no genes passed a gene burden analysis test for significance.

Discussion/ Conclusion: This study, the first SNP array and exome sequencing analysis using FRDA patient samples, identified no modifiers reaching genome-wide significance, unsurprising given the sample number. While some loci reach the "suggestive" cutoff thus encouraging further study, the dataset also should prove useful for targeted analysis and in association with other data sets that increase the total number of patient samples.

# (#541) E4 APOE allele is associated with an earlier age of onset in SCA3 patients

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 541

***Dr. Emilien Petit*** [1], ***Dr. Tatiana Faroud*** [2], ***Dr. Tetsuo Ashizawa*** [3], ***Dr. Sophie Tezenas du Montcel*** [4]

***1.*** *Paris Brain Institute (ICM), Sorbonne Université,* ***2.*** *BioSEND,* ***3.*** *The Houston Methodist Research Institute,* ***4.*** *Sorbonne University*

**Backgrounds and Objective**. APOE is a protein coded by with three major alleles in human population: E2 (8.4%), E3 (77.9%) and E4 (13.7%). The E4 allele has been shown to be a risk factor for Alzheimer disease while E2 being slightly protective. Few studies were done in SCAs, presenting a potential earlier onset for E2 carriers in SCA3. We describe here the effect of such alleles on age of onset in patients with SCA1 and SCA3 mutation carriers.
**Methods**. APOE was genotyped in 32 controls, 32 SCA1 and 60 SCA3, participating in the US centers of the READISCA study. SCAs patients were either pre-ataxic or ataxic. Individuals were separated in 3 groups: E3 homozygotes, E4 carriers (homo- and heterozygotes) and E2 carriers without E4 allele (E2/E2 + E2/E3). We assessed the impact of these APOE statuses on the onset of ataxia through survival analysis with Kaplan-Meier estimates and CAG-adjusted Cox regression.
**Results.** In SCA3, median age at onset was 45 [37-50] for E3 homozygotes, 42 [39-55] for E2 carriers and 38 [30-47] for E4 carriers (p=0.09). In the CAG-adjusted Cox model the overall effect of APOE status was significant (p=0.041). E4 carriers had higher probability of onset than E2 carriers (HR = 3.04 [1.01-9.11], p=0.047), E3 homozygotes were intermediate (HR = 1.79 [0.73-4.41], p=0.31). In SCA1, adjusted Cox models showed similar trend without reaching significance (HR E4 vs E2: 2.78 [0.36-33.3]). CCAS means were similar between the different APOE categories.
**Discussion and Conclusion.** In the US patients of the READISCA study, APOE status seems to have a similar effect than in Alzheimer, E4 being associated with an earlier age of onset and E2 a later one. These results are inconsistent with previous findings.
Acknowledgments: NIH-U01-NS104326

# Poster Sessions: Animal and Cellular Models

# (#43) An improved mouse model for Friedreich Ataxia (FRDA)

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 43

***Dr. Catherine Gérard*** [1], ***Ms. Annabelle Fortin Archambault*** [1], ***Ms. Camille Bouchard*** [1], ***Prof. Jacques P. Tremblay*** [1]

**1.** *Laval university*

**Background and Objective :**

FRDA is a severe genetic disease with no curative treatment. A GAA repeat in the intron 1 of the frataxin gene leads to the reduction of frataxin protein involved in iron-sulfur metabolism, which leads to a progressive neuromuscular degeneration as well as a premature death due to heart failure. Many mouse models have been created but none of them reflects all aspects of the disease in human. The aim of our study was to determine whether the new mouse model developed by Jackson laboratory Inc. with 800 GAA repeats, called $Fxn^{null}$::YG8s$(GAA)_{>800}$ (YG8-800), is a good model for Friedreich Ataxia research.

**Methods :**

We used different behaviors tests (parallel rod floor, hanging wire test, notched and inversed T beams) to characterize the evolution of the disease from 8 to 26 weeks in YG8-800 mice compared to Y47 (normal mouse). The weights of the mice and of the heart were measured at 26 weeks and the frataxin concentration was quantified in different tissues.

**Results :**

The behavior of all mice was similar for the parallel rod floor test, only the number of foot faults per travelled distance was increased for the YG8-800 mice after 23 weeks. However, progressive loss of balance and of coordination were characterized on the two beams over time. A heart hypertrophy was also observed at 26 weeks. A strong reduction of frataxin concentration was observed in all tissues compared to Y47 mice.

**Discussion and Conclusion :**

The YG8-800 mouse has a strong reduction of frataxin concentration in all organs. Moreover, this mouse model developed a progressive loss of coordination and balance and a cardiac hypertrophy at 6 months. This mouse model is very close to the human disease it will thus be a very good model to test treatments for FRDA.

# (#47) Accentuation of the severity of the YG8sR phenotype with a shRNA against human frataxin

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 47

***Dr. Catherine Gérard [1], Dr. Solange Gni-fiene Yanyabe [2], Ms. Camille Bouchard [1], Ms. Annabelle Fortin Archambault [1], Dr. Nathalie Majeau [3], Ms. Gabrielle Buisson [1], Ms. Malek Aloui [1], Mr. Pouiré Yameogo [1], Ms. Vanessa Couture [1], Prof. Jacques P. Tremblay [1]***

***1.*** *Laval university,* ***2.*** *Universite Laval,* ***3.*** *Centre de Recherche du CHU de Québec-Université Laval, Québec city, Québec province, Canada*

**Background and Objective :**

YG8sR mouse is a model for Friedreich ataxia with a human transgene of 250-300 GAA repeats (Jackson Laboratories Inc.). The decrease in frataxin protein is due to such extended repeat in intron 1 of the frataxin gene leading in human to a progressive neuronal and cardiac degeneration and a premature death due to heart failure. We have followed this mouse model was followed for 14 months but no clear ataxic phenotype was observed. The goal of our project was to find a method to increase the phenotype of the YG8sR mice by using a shRNA against frataxin.

**Methods :**

Different shRNAs targeting different sequences of frataxin mRNA were created and tested on cells to identify the most effective to reduce frataxin expression. Four AAV-PHP.b were created with the best shRNA and injected to 2 months old YG8sR mice. These mice were followed with behavior tests for 45 days after IV injection. The frataxin concentration was determined in different tissues.

**Results :**

*In vitro*, the shRNA-1 and 3 showed the strongest decrease of frataxin. A scramble shRNA control not interacting with the frataxin mRNA was used as a negative control. After injection in mice of the AAV-PHP.B coding for shRNA-3, the hanging time on a grid was significantly reduced. The numbers of foot faults made when crossing over two types of beams were increased.

**Discussion and Conclusion :**

Even if shRNA-1 and 3 seems to have the same effect on cells, *in vivo* shRNA3 produced a more pronounced modification of mouse behavior. An AAV coding for a shRNA against frataxin mRNA can thus be used to increase the severity of the YG8sR symptoms. We have recently used an AAV coding for human frataxin and this treatment reduced the symptoms of this mouse model.

# (#59) Examining anatomical contributions of expanded ATXN1 to disease: Characterization of a novel knock-in mouse model of SCA1

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 59

***Ms. Lisa Duvick*** [1], ***Ms. Kellie Benzow*** [1], ***Dr. William Southern*** [1], ***Dr. Yun You*** [1], ***Mr. Orion Rainwater*** [1], ***Ms. Tessa Nichols-Meade*** [1], ***Ms. Hannah Kuivinen*** [1], ***Ms. Shannah Serres*** [1], ***Ms. Rachel TerHaar*** [2], ***Dr. James Ervasti*** [1], ***Dr. Huda Zoghbi*** [3], ***Dr. Marija Cvetanovic*** [1], ***Dr. Michael Koob*** [1], ***Dr. Harry Orr*** [1]

***1.*** *University of Minnesota,* ***2.*** *University of Chicago,* ***3.*** *Baylor College of Medicine*

Spinocerebellar ataxia type 1 (SCA1) is a neurodegenerative disease caused by expansion of the polyglutamine (polyQ) tract in ATXN1 protein. Much has been learned regarding disease pathology in the brain from both transgenic and knock-in mouse models. One major remaining challenge to understanding SCA1 pathology is linking dysfunction in specific cell populations to key disease phenotypes. To address this, we developed a mouse model (*Atxn1*$^{Flox146Q/2Q}$) containing 146 glutamines in which the endogenous coding region of the mouse gene was replaced with the human coding region. The human coding region is flanked by lox sites, which allow *ATXN1* excision in specific cell types by crossing to specific *Cre* recombinase lines.

*Atxn1*$^{Flox146Q/2Q}$ mice have motor and cognitive deficits, reduced body weight, muscle wasting, kyphosis, and reduced life span compared to healthy littermates. To distinguish between peripheral and neural contributions to disease phenotypes, we bred *Atxn1*$^{Flox146Q/2Q}$ mice to transgenic mice expressing *Cre* recombinase driven by either the human *ACTA1* promoter, expressed in striated muscle or the Nestin promoter, expressed in neurons and glia. We found mice without mutant ATXN1 in striated muscle have improved grip strength and absence of muscle wasting, kyphosis, and weight loss observed in the *Atxn1*$^{Flox146Q/2Q}$ mice. In addition, *ex vivo* muscle force was measured directly and found to be lower in *Atxn1*$^{Flox146Q/2Q}$ mice compared to the *Atxn1*$^{Flox146Q/2Q}$*;ACTA1 Cre* mice indicating an intrinsic problem in muscle. In support of previous studies, mice without mutant ATXN1 in neural cells showed improved motor performance on the rotarod, open field and Barnes maze but a deficit in grip strength. Ongoing studies include *Pcp2 Cre* and *Rgs9 Cre* mice crossed to *Atxn1*$^{Flox146Q/2Q}$ to examine the role of cerebellar Purkinje cells and striatal MSNs deficits in motor performance.

These results elucidate that neural and peripheral effects of ATXN1 contribute to disease.

# (#63) Cellular mechanisms driving toxicity in Cerebellar Ataxia with Neuropathy and Vestibular Areflexia Syndrome (CANVAS)

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 63

***Dr. Connor Maltby*** [1], ***Ms. Yomira Palacios*** [1], ***Prof. Peter Todd*** [1]

**1.** *University of Michigan*

Cerebellar ataxia with neuropathy and vestibular areflexia syndrome (CANVAS) is a late-onset, slowly progressive and recessively inherited ataxia characterized clinically by vestibular, cerebellar, and somatosensory impairments. CANVAS typically presents at middle-age, with a mean age of symptom onset in the 50's. Patients exhibit progressive motor imbalance, oscillopsia, and sensory neuropathy. Limited post-mortems have revealed cerebellar atrophy with diffuse cerebellar Purkinje cell loss, and peripheral neuropathy of the vestibular, facial, and trigeminal nerves with selective preservation of the auditory nerves. Recent studies and our own clinical experience suggest that it is a common cause of adult-onset cerebellar ataxia in the US.

A combined recessive linkage and whole-genome sequencing approach revealed a biallelic, non-reference, pentameric AAGGG repeat expansion within the second intron of replication factor complex subunit 1 (RFC1) in familial and sporadic CANVAS, and other cases of late-onset ataxia in patients of differing ethnicities. Unaffected individuals possess an average of 11 AAAAG repeats at this locus, which is replaced with 400-2000 AAGGG repeats in CANVAS patients.

The mechanism(s) by which this repeat expansion causes pathogenesis and neuronal death are yet to be identified. While CANVAS exhibits a recessive pattern of inheritance, studies suggest that RFC1 expression is maintained with repeat expansions. Therefore, this repeat expansion is likely to trigger toxicity through a combination of loss- and gain-of-function mechanisms, or through unknown mechanisms affecting wider cellular pathways.

To address this, we are conducting transcriptomic and proteomic analyses on mature neurons differentiated from patient-derived iPSCs, CRISPR-Cas9 corrected isogenic, and WT control iPSC lines to identify whether aberrations in RFC1 transcription, expression, or splicing are present, and whether the repeat is affecting other pathways or novel noncoding transcripts in a wider context. These studies will provide needed information to define the proximal mechanisms and pathways that underlie neuronal toxicity and neurodegeneration within CANVAS.

# (#74) Mice expressing mutant frataxin G127V protein survive and demonstrate mild neurobehavioral deficits

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 74

***Dr. Jill Napierala*** [1], ***Dr. Daniel Fil*** [2], ***Ms. Robbie Conley*** [2], ***Dr. Aamir Zuberi*** [3], ***Dr. Cathleen Lutz*** [3], ***Dr. Marek Napierala*** [4]

***1.*** *Department of Neurology, University of Texas Southwestern Medical Center, Dallas, TX,* ***2.*** *University of Alabama at Birmingham,* ***3.*** *The Jackson Laboratory,* ***4.*** *Peter O'Donnell Jr. Brain Institute, Department of Neurology, University of Texas Southwestern Medical Center, Dallas, TX*

Background and objective:Friedreich's ataxia (FRDA) is a neurodegenerative disease caused by reduced expression of the mitochondrial protein frataxin (FXN). Most FRDA patients are homozygous for large expansions of GAA repeats in intron 1 of *FXN*, while some are compound heterozygotes with an expanded GAA tract in one allele and a missense or nonsense mutation in the other. A prevalent missense mutation changes a glycine to valine at position 130 (G130V). We and others have demonstrated that levels of mature FXN protein in FRDA G130V samples are reduced below those detected in samples harboring homozygous repeat expansions. Little is known regarding expression and function of endogenous FXN-G130V protein due to lack of reagents and models that can distinguish the mutant FXN protein from the wild-type FXN produced from the GAA-expanded allele. We aimed to determine the effect of the G130V (murine G127V) mutation on Fxn expression and to define its multi-system impact *in vivo*.
Methods:We used CRISPR/Cas9 to introduce the G127V point mutation in the Fxncoding sequence and generated homozygous mice ($Fxn^{G127V/G127V}$; MUT). We performed neurobehavioral tests on cohorts of WT and MUT animals at three-month intervals for one year, and collected tissue samples to analyze molecular changes over the same timecourse.
Results:The endogenous Fxn-G127V protein is detected at much lower levels in all tissues analyzed from MUT mice compared to age and sex-matched WT mice without differences in *Fxn* transcript levels. MUT mice are significantly smaller than WT counterparts, but perform similarly in most neurobehavioral tasks.
Discussion and Conclusion:We are continuing to investigate molecular consequences in our mouse model expressing extremely low levels of Fxn. Results of our studies will increase our knowledge of a unique FXN G130V pathogenic mechanism and provide insight into the tolerable limit of Fxn/FXN expression *in vivo*.

# (#76) Postnatal sensorimotor development delay in a Friedreich's ataxia mouse model

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 76

***Dr. Anna Stepanova [1], Dr. Cristina Lao-Peregrín [1], Mr. Giussepe Yanez [1], Ms. Niharika Desiraju [1], Dr. Jordi Magrane [1]***

***1.*** *Weill Cornell Medicine*

*Background and Objective*: Abnormalities in the normal development of the nervous system may explain some of the earliest pathology in Friedreich's ataxia (FRDA). However, no studies have examined the consequences of frataxin reduction in the maturation of the nervous system during perinatal and postnatal stages in animal models.
*Methods*: We chose twelve neurobehavioral tasks to explore early maturation of sensory and motor circuitries in an FRDA mouse model (the KIKO mouse). We evaluated both the achievement of neurodevelopmental milestones and sensorimotor performances longitudinally from postnatal day 2 (P2) through P25.
*Results*: Observations onto the overall health of the newborns (assessed by weight gain and daily observations), as well as general somatic development (eye opening, auditory startle), revealed no differences between KIKO and control groups. However, KIKO mice exhibited specific delays in acquiring sensorimotor skills during the first two weeks after birth, as shown by delayed crossed extensor reflex disappearance and forelimb placing appearance, abnormal negative geotaxis responses, and reduced mid-air righting response. Moreover, KIKO pups presented delayed maturation of body posture, as shown by their impaired tube test performances and impaired locomotion. Contrarily, KIKO mice had normal motor behaviors as demonstrated by normal limb grip and forelimb strength tests, and normal surface righting responses.
*Discussion and Conclusion*: Our data demonstrate that frataxin-deficiency in mouse pups causes neurodevelopmental delay, which affects mainly sensory circuitries, body righting mechanisms, and labyrinthine function, but not muscle strength. We postulate that these delays may be the consequence of abnormal neuron development and neuronal network maturation.

# (#88) “Modeling Spinocerebellar Ataxia Type 1 (SCA1) using human induced pluripotent stem cell derived motor neurons.”

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 88

***Ms. Carrie Sheeler*** [1], ***Dr. Harry Orr*** [1], ***Dr. Marija Cvetanovic*** [1]

***1.*** *University of Minnesota*

Degeneration of the motor neurons (MNs) in the brain stem and spinal cord is hypothesized to contribute directly to premature lethality in spinocerebellar ataxia type 1 (SCA1) by reducing the strength of swallowing and respiratory drive. While we can recreate some aspects of MN pathology in mouse models, loss of MNs has only been seen in SCA1 patient populations. Thus, we seek to create a model of SCA1 which allows us to assess the effect of elongated ATXN1 on human MNs with the goal to provide insight to human cellular pathology in this cell type and facilitate the development of therapies to limit the lethal aspects of SCA1.

To investigate potential mechanisms underlying SCA1 pathology in human MNs, I have developed a human induced pluripotent stem cell (iPSC)-derived MN model using cells donated from three patients with SCA1 and three sibling controls. I have characterized these cells across a 35 day experimental time course, at the end of which MNs exhibit characteristic neuronal morphology and express key MN gene markers. To assess transcriptional changes in SCA1 human MNs, I have performed RNAsequencing and have identified disruptions in key cellular processes including extracellular matrix regulation, development, and intracellular signaling. I have further performed analyses investigating cellular proliferation and neurite outgrowth over the course of motor neuron development and have found that motor neurons from SCA1 patients exhibit slower neurite outgrowth and increased proliferation at the pre-MN stage of development, which may indicate disruptions in how these cells grow and mature.

In conclusion, we find that iPSC-derived MNs from SCA1 patients exhibit disrupted gene expression which contributes to functional changes in MN development and extracellular maintenance regulation.

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

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 93

***Ms. Anna Maria Schreiber*** [1], ***Dr. Yanjie Li*** [1], ***Dr. Jill Napierala*** [2], ***Dr. Marek Napierala*** [3]

***1.*** *University of Alabama at Birmingham,* ***2.*** *Department of Neurology, University of Texas Southwestern Medical Center, Dallas, TX,* ***3.*** *Peter O'Donnell Jr. Brain Institute, Department of Neurology, University of Texas Southwestern Medical Center, Dallas, TX*

Background and objective: Friedreich's ataxia (FRDA) is a neurodegenerative disease caused by transcriptional repression induced by expanded GAA repeats in intron 1 of the *FXN* gene. Rescuing defective *FXN* transcription remains as primary goal of potential therapy. A limited number of compounds have demonstrated modest *FXN* stimulation, however, to date, none of them effectively increased *FXN* in clinical trials. Lack of appropriate reporter systems: derived from patient cells, representing pathology-relevant cells, and based on expression of the endogenous *FXN* gene containing expanded GAAs, contribute to the limited success of these strategies. To address it, we utilized FRDA induced pluripotent stem cells (iPSC) to create an endogenous *FXN* reporter system, suitable for high throughput screening (HTS) campaigns. Methods: To generate and validate our reporter system, we reprogrammed FRDA patient fibroblasts to iPSCs. Subsequently, using a CRISPR/Cas9 approach, we engineered an in-frame fusion between the FXN gene and a small luciferase gene (NLuc). To validate the platform for HTS, we differentiated the iPSCs into neuronal progenitor cells (NPCs). The FXN-NLuc NPCs were validated using HDAC inhibitor 109 shown previously in different model systems to increase *FXN* transcription. Results: Treatment with this compound increased luminescence of FXN-NLuc cells in a concentration-dependent manner. As proof-of-concept, we tested the reporter line by screening a library of epigenetic modulators. We identified 15 novel compounds capable of increasing FXN levels in NLuc-NPCs. Validation studies confirmed three of them as the most potent, stimulating *FXN* expression at the transcript and protein levels. The stimulating effect was confirmed in terminally differentiated iPSC-derived FRDA cortical neurons. Discussion and conclusion: This new and sensitive reporter cell system can be utilized in HTS campaigns to uncover novel, robust regulators of endogenous *FXN* expression.

# (#94) A new Drosophila model of SCA7

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 94

***Mrs. Alyson Sujkowski [1], Mr. Matthew Prifti [1], Mr. Bedri Ranxhi [1], Prof. Sokol Todi [1], Dr. Wei-Ling Tsou [1]***

***1.*** *Wayne State University*

**Background and Objective**

Spinocerebellar Ataxia Type 7 (SCA7) is an autosomal dominant neurodegenerative disease caused by a CAG repeat expansion in the disease gene. The polyglutamine (polyQ) tract in the pathogenic protein, ataxin-7, ranges from fewer than 34 repeats (wild type) to 35-460 repeats in SCA7 patients. A notable feature in all SCA7 patients is decreased visual acuity caused by progressive visual degeneration. More complications, such as problems with coordination, speech, and swallowing may also occur. How polyQ expansion in ataxin-7 leads to cytotoxicity in neurons - especially in the visual system - remains unclear.

**Methods**

To investigate the functional mechanism of the disease protein, we generated *Drosophila* SCA7 models that carry human, full length ataxin-7 with 10 or 92 polyQ repeats. A full characterization of the wild type and SCA7Q92 models was performed, including longevity, motility and eye morphology assays. We also performed mass spectrometry analysis to discover the abundance of protein species that interact with ataxin-7 Q10 or Q92.

**Results**

Expression of the SCA7Q92 protein in intact fly bodies significantly decreased fly longevity. Eye degeneration was observed in an age-dependent manner. In functional annotation of the mass spectrometry results, ataxin-7Q10 was found to associate with ubiquitin or microtubule associated complex cluster, while ataxin-7Q92 was associated with lipid particle- and nucleotide-binding clusters.

**Discussion and Conclusion**

These Drosophila models of SCA7 allowed us to probe important aspects of SCA7 pathogenesis and gain a better understanding of ataxin-7. This will help guide future development of therapeutic targets against SCA7.

# (#100) Modelling Friedreich's ataxia cellular phenotypes in patient induced pluripotent stem cell (iPSC)-derived cardiomyocytes

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 100

***Dr. Sandor Szunyogh*** [1], ***Dr. John Ho-Chun Lai*** [1], ***Dr. Gabriela Vilema-Enriquez*** [1], ***Prof. Richard Wade-Martins*** [1]

***1.*** *University of Oxford*

Cardiomyocytes differentiated from human induced pluripotent stem cells (hiPSC CM) represent an emerging model system in Friedreich's ataxia (FRDA) research, as the malfunction of these cells leads to hypertrophic cardiomyopathy, the predominant cause of death in FRDA patients. Our objective is to differentiate and characterize healthy control (HC) and FRDA hiPSC CMs to establish a baseline phenotypic difference between the two groups creating a platform to screen potential drug candidates to elevate FXN levels and, ultimately, to correct the disease phenotypes. The differentiation of hiPSC CMs is carried out by small molecule-based treatment of iPSCs regulating the WNT-signalling pathway in combination with a metabolic switch to fatty acid metabolism. We are using the differentiated CMs to measure gene expression changes by RT-qPCR, actinin localization by immunocytochemistry, ROS production, mitochondrial membrane potential measurements, contractibility and field potentials by microelectrode array (MEA). With high-resolution microscopy, we visualized the accurately aligned Z-disks, which marks mature CMs, in the case of HCs and FRDA patient hiPSC CMs.

We have recently shown that the compound A-196, a potent and selective inhibitor of the SUV4-20 H1 histone methyltransferase, elevates FXN mRNA and protein levels in patient-derived fibroblasts and PBMCs. We will test A-196 in our newly developed hiPSC CM model to investigate if FXN levels increase after treatment and if there is a phenotypic rescue in FRDA hiPSC CMs.

In addition, we have generated a SUV4-20 H1 CRISPR knockout in *FXN-GAA-Luciferase* HEK293 cells which serves as a reporter line to correlate modifications of GAA epigenetics with FXN levels. We observed an increase of FXN luciferase signal in SUV4-20 H1 CRISPR *FXN-GAA-Luciferase* knockouts, further validating SUV4-20 H1 as a target in FRDA.

# (#136) Transcriptional and metabolic profiles of cardiomyopathy and mitochondrial stress in mouse models of FXN deficiency

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 136

***Prof. Giovanni Manfredi*** [1], ***Ms. Nicole Sayles*** [1], ***Dr. Jill Napierala*** [2], ***Dr. Jordi Magrane*** [1], ***Dr. Marek Napierala*** [2], ***Dr. Hélène Puccio*** [3]

***1.*** *Weill Cornell Medicine,* ***2.*** *University of Alabama at Birmingham,* ***3.*** *Université Claude Bernard - Lyon I*

o **Background and Objective**

Hypertrophic cardiomyopathy is a prominent pathological feature of Friedreich Ataxia (FA), which is fatal in ~60% of patients. Surprisingly the FA heart often maintains adequate function until disease end stage. This suggests that the FA heart can initially adapt to loss of frataxin (FXN) through metabolic rewiring. Work in mouse models where FXN expression was completely ablated have evidenced transcriptional and metabolic profiles of cardiomyopathy and metabolic adaptation typical of the mitochondrial integrated stress response (mtISR). However, such parameters have not been systematically investigated in mouse models with disease-relevant partial decrease of FXN. Our objective was to characterize transcriptional and metabolic profiles of cardiomyopathy and mtISR in three mouse models of partial FXN loss.

**o Methods**

We compared heart RNASeq and metabolomics profiles in male and female 18 months old YG8-800, G127V-KI homozygote, KIKO700 mice and the relative littermate controls.

o **Results**

Only a few metabolites and genes were significantly different between YG8-800 mice and wild type, and they did not provide a signature of cardiomyopathy or mtISR. On the other hand, several metabolites were altered in both G127V-KI and KIKO-700 hearts, but they only partially overlapped. Branched chain amino acid metabolism was significantly altered in G127V-KI hearts, while nucleotide biosynthesis was among KIKO-700 most altered pathways. Transcriptional changes were found in both models, but profiles consistent with cardiomyopathy and mtISR were only identified in G127V-KI mice. Interestingly, a larger number of genes were affected in females than in males, in both lines.

**o Discussion and Conclusion**

Based on these initial findings, among the three models with reduced FXN, only G127V-KI mice show metabolic and transcriptional profiles indicative of both cardiomyopathy and mtISR. Going forward, it will be critical to delineate the time course, the pathophysiology, and the mechanisms of sex differences of the cardiac involvement.

# (#200) Characterization of a SCA47 mouse model bearing the disease-causing R1147W mutation in Pumilio1 (PUM1)

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 200

***Mr. Maximilian Cabaj*** [1], ***Mr. Salvatore Botta*** [1], ***Dr. Nicola de Prisco*** [1], ***Mr. Alexei Chemiakine*** [1], ***Mr. Winston Lee*** [1], ***Prof. Mu Yang*** [1], ***Dr. Chyuan-Sheng Lin*** [1], ***Prof. Vincenzo Gennarino*** [1]

***1.*** *Columbia University Irving Medical Center*

**Background and Objective.** Pumilio1 (PUM1) is a highly conserved RNA-binding protein that represses nascent mRNA targets via mRNA decay and translational inhibition. In the mouse brain, *Pum1* haploinsufficiency derepresses ATXN1, causing SCA1-like ataxia (Gennarino et al, *Cell* 2015). Patients bearing pathogenic loss-of-function *PUM1* mutations exhibit one of at least two distinct disease subtypes: the mild, late-onset Pumilio1-related cerebellar ataxia (PRCA) or the more severe, early-onset Pumilio1-associated developmental delay and seizures (PADDAS) (Gennarino et al, *Cell* 2018). So far, we have identified 61 SCA47 patients, with the most prevalent mutations being R1147W (PADDAS) and T1035S (PRCA). We generated mouse models therein and began characterizing the PADDAS mice behaviorally and pathologically, with the goal of understanding disorder-specific disease mechanisms.

**Methods.** The mice underwent several behavioral assays, including hind-limp clasping, open field maze, rotarod, and dowel-crossing. We performed immunohistochemistry and immunofluorescence assays to measure neuronal degeneration.

**Results.** Pum1-R1147W homozygous mice nearly always die *in utero*. Pum1-R1147W heterozygous mice are smaller in size than littermate controls and remain small throughout life. They show a clasping phenotype at 5 weeks of age, and hyperactivity from 7 weeks. They develop episodic seizures at 9 weeks. Initial pathological characterization has indicated potential neurodegeneration via progressive loss of IP3R1—a ligand-gated $Ca^{2+}$ channel regulating intracellular calcium release—in Purkinje cells at around 40 weeks of age.

**Discussion and Conclusion.** Our Pum1-R1147W heterozygous mice recapitulate the observed symptoms of PADDAS patients. We are now in the process of completing the behavioral and pathological characterization of these mice at different developmental stages, and performing molecular studies on the effects of this mutation on PUM1 interactors. Additionally, we have recently generated a T1035S mouse and will repeat the full experimental battery in this line as well to better understand disease-specific phenotypic recapitulation and disease mechanism.

# (#246) Analysis of the phenotype of new in vivo and in vitro models of Friedreich's Ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 246

***Dr. Saul Herranz-Martin*** [1], ***Mr. Miguel García López*** [1], ***Mr. Andrés Vicente-Acosta*** [1], ***Mr. Mario Amores*** [2], ***Dr. Ruth Pazos-Rodríguez*** [2], ***Dr. Frida Loria*** [2], ***Dr. Javier Diaz-Nido*** [1]

***1.*** *Universidad Autonoma de Madrid / Centro de Biología Molecular Severo Ochoa,* ***2.*** *Laboratorio de apoyo a la investigación / Hospital Universitario Fundación Alcorcón*

**Background and objective:**

Friedreich's ataxia (FRDA) is a rare autosomal recessive neurodegenerative disorder characterized by an early atrophy of the cerebellum and spinal cord. A molecular level, it is mainly caused by a GAA triplet repeat expansion within the first intron of the gene codifying for frataxin (FXN). The mutation leads to a decreased expression of this protein, whose role is the maintenance of FeS clusters. The absence of good experimental models that mimic the human disease is one of the challenges for the study of FRDA, likely due to the difficulty to recreate the pathological expansion. New models are currently emerging and in this work we aim to characterize a new mouse model which bear the human frataxin gene containing more than 800GAA repeats of the expansion (www.jax.org/strain/030395), herein referred as YG8JR, as well as some of their neuronal populations, such as the cerebellar granular neurons (CGNs)

**Methods**

To decipher the YG8JR phenotype, we carry out behavioural, histopatalogical and biochemical approaches at different time points. Moreover, we assess the mitochondrial functionality in the CGNs *in vitro* by seahorse, redox status and enzymatic assays, among others.

**Results**

In comparison to control animals, the YG8JR mouse displays an ataxic phenotype, with a decline in motor tests, such as rotarod or foot fault, severe loss of weight, atrophy of the cerebellum, loss of neuronal cells and synaptic alterations at the different time points analysed. Moreover, CGNs show low levels of some proteins in the mitochondrial respiration chain as well other proteins containing FeS clusters, triggering a dramatically decreased in the mitochondrial respiration and functionality.

**Discussion and conclusion**

Overall, this new model shows a stronger phenotype compared to previous models and will help to gain a better knowledge of the disease being also ideal for drug screening to treat the disease.

# (#263) Rapid Generation of Knock-out Stem Cell Models of ARCAs: ITPR1 as Proof of Concept

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 263

***Dr. Ricardo Schnekenberg*** [1], ***Dr. Hannah Sleven*** [1], ***Dr. Philip Hublitz*** [2], ***Prof. Andrea Nemeth*** [1]

***1.*** *Nuffield Department of Clinical Neurosciences, University of Oxford,* ***2.*** *Weatherall Institute of Molecular Medicine, University of Oxford*

**Background and Objectives**

Generating stem cell models of human genetic disease using genome editing relies on labour-intensive, time-consuming and expensive screening of hundreds of colonies. Our aim was to develop a system that significantly reduces the time needed to generate knockout human embryonic stem cells (hESCs) by avoiding large-scale screening of clones. We tested the system using *ITPR1*, encoding the Inositol 1,4,5-trisphosphate receptor type1 (IP3R1), a gene mutated in SCA15, SCA29 and Gillespie Syndrome.

**Methods**

Three *ITPR1* coding exons were separately targeted using CRISPR/CAS9. Plasmid carrying an antibiotic selection cassette was co-transfected with eSpCas9 and guide-RNAs into H9 hESCs. Antibiotic selection pressure was maintained for 3-5 weeks. Single-cell derived colonies for each target were genotyped using PCR and Sanger sequencing. Enrichment for membrane-bound proteins was achieved by digitonin fractionation. Knockouts were confirmed by Western Blot using antibodies targeting three different IP3R1 regions. Clones were differentiated into neurons using hNgn2 overexpression.

**Results**

Multiple H9 clones harbouring biallelic *ITPR1* knockout mutations were identified within 5 weeks post-transfection and minimal screening of colonies. Crude subcellular fractionation allowed IP3R1 detection directly from H9 hESCs, with 8/9 clones analysed by Western blot confirmed as IP3R1 knockouts. Early neuronal progenitors did not show morphological differences between knockouts and controls.

**Discussion and Conclusion**

We have developed a system to rapidly and inexpensively generate biallelic knockout human stem cells. The protocol provides a scalable method for cellular phenotyping, drug screening and other mechanistic research into recessive ataxias. Minor modifications to the protocol would generate models of Gillespie carriers and SCA15, and replacing *ITPR1* within the targeting vector with other genes would enable modelling of different ataxias. Preliminary data suggests that knockout of *ITPR1* does not impair early neuronal development; clinical phenotype is likely due to subtle effects on neuronal function requiring additional experiments to elucidate.

# (#265) Identification of Polyglutamine Aggregation Suppressing Factors in the social amoeba Dictyostelium discoideum

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 265

***Ms. Felicia Williams*** [1], ***Dr. Matt Scaglione*** [2], ***Ms. Yumei Wu*** [2]

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Background and Objective

Protein aggregation is a hallmark of neurodegenerative diseases, including the polyglutamine diseases. Among the polyglutamine diseases are six spinocerebellar ataxias (SCAs) including SCAs 1, 2, 3, 6, 7, and 17. These diseases are caused by expansion of a CAG repeat that encodes a homopolymeric polyglutamine tract. When the polyglutamine tract is expanded beyond a specific threshold, the protein aggregates and causes neurodegeneration. Notably, the social amoeba *Dictyostelium discoideum* normally encodes long polyglutamine tracts and previous work from our group and others identified it as being naturally resistant to polyglutamine aggregation.

Methods

To determine how *Dictyostelium* maintains these polyglutamine-rich proteins in a soluble state, we conducted a genetic screen that utilizes chemical mutagenesis to introduce mutations throughout the *Dictyostelium* genome in an unbiased manner with high coverage. Furthermore, we have used the Pyr56/5-FOA selection system to select for mutants which demonstrate polyglutamine aggregation, allowing for high-throughput screening and identification of genes of interest by whole genome sequencing and genomic analysis.

Results

Utilizing our genetic screen, we have successfully enriched mutagenized populations of *Dictyostelium* with polyglutamine aggregates. We have isolated these mutants, performed whole genome sequencing, and established a computational analysis pipeline to identify genetic variants. Genomic analysis revealed a variety of cellular pathways that may suppress polyglutamine aggregation. Currently we are validating our screen to verify that the genes identified are necessary to prevent polyglutamine aggregation in *Dictyostelium*.

Discussion and Conclusion

Here we have established a novel genetic screening platform to identify suppressors of polyglutamine aggregation. Moving forward, we plan to perform experiments to validate our screening results and investigate the therapeutic potential of these suppressors in mammalian cell line models. In the future, the discovery of novel suppressors of polyglutamine aggregation may aid in the development of novel therapies for the polyglutamine diseases.

# (#284) Development of a Novel Humanized Mouse Model and Silencing Reagents to Test Atn1-lowering as a Therapeutic Strategy for DRPLA

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 284

***Ms. Aliza Ben-Varon*** [1], ***Dr. Daniel O'Reilly*** [2], ***Dr. Joanna Korecka*** [3], ***Dr. Timothy Yu*** [4], ***Dr. Vikram Khurana*** [3], ***Dr. Anastasia Khvorova*** [2], ***Dr. Jeffrey Carroll*** [1]

***1.*** *Department of Psychology, Western Washington University, Bellingham, WA,* ***2.*** *RNA Therapeutics Institute, University of Massachusetts Medical School, Worcester, MA,* ***3.*** *Ann Romney Center for Neurologic Diseases, Department of Neurology, Brigham and Women's Hospital, Harvard Medical School,* ***4.*** *Division of Genetics and Genomics, Boston Children's Hospital, Harvard Medical School*

*Background and Objective:*
Dentatorubral-pallidoluysian atrophy (DRPLA) is a progressive neurodegenerative disease caused by a CAG repeat expansion in atrophin-1 (*ATN1*). We are developing a novel fully-humanized mouse, herein referred to as $Atn1^{Q112/+}$. These mice express a humanized *Atn1* allele with 112 pure CAG repeats. We will perform a longitudinal characterization of the behavioral and molecular phenotypes of the mice. In parallel, we have developed gene silencing techniques for *Atn1*, which potently lower *Atn1* levels across the brain. We will use these molecules in future interventional studies to determine their ability to modulate DRPLA symptoms in the $Atn1^{Q112/+}$ mice.
*Methods:*
Using CRISPR/Cas9-mediated genome editing, we have developed a fully-humanized *Atn1* ES cell line with 112 sequence-verified CAG repeats in exon5. This genome editing approach replaces approximately 14kb of the mouse genomic sequence, including all *Atn1* exons and introns, with human sequence. In parallel, we have developed potent siRNA sequences targeting *Atn1* and formulated them as divalent siRNAs, which we have delivered to mice via intracerebroventricular injection.
*Results:*
We have successfully completed genome editing in C57Bl/6NTac ES cells, and validated humanization using PCR and Southern blotting. Four ES clones were used to generate chimeric pups via blastocyst injection. We are conducting rapid expansion via IVF to generate large cohorts of $Atn1^{Q112/+}$ mice for longitudinal phenotypic characterization and *Atn1* knockdown rescue experiments. In addition, we are currently characterizing the functional impact of chronic *Atn1* lowering in the brain using divalent siRNA injections in wildtype mice.
*Discussion and Conclusion:*
Genome editing has increased the speed by which mouse models can be made to study DRPLA pathogenesis. In addition, the emergence of gene silencing technologies such as siRNA, and particularly divalent-siRNA, which produce sustained, brain-wide *Atn1* knockdown, enables us to test the hypothesis that adult-onset *Atn1* lowering will reduce DRPLA-relevant pathology.

# (#338) Characterisation of the first mouse model for Spinocerebellar Ataxia 44

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 338

***Dr. Sevda Boyanova*** [1], ***Dr. Mohamed Ibrahim*** [2], ***Ms. Alaa Baazaoui*** [2], ***Prof. Peter Oliver*** [3], ***Prof. Esther Becker*** [2]

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The spinocerebellar ataxias (SCAs) are a clinically and genetically heterogeneous group of neurodegenerative disorders characterized by the progressive dysfunction of the cerebellum. No effective treatments exist for these devastating diseases. A major challenge is to better understand the specific disease-causing mechanisms underlying this complex group of diseases and to identify common pathological pathways that could be targeted therapeutically.
Our work has uncovered a key role for the mGluR1-TRPC3 pathway in spinocerebellar ataxias. We identified the first patients and families harboring dominant mutations in the *TRPC3* and *GRM1* genes, causing SCA41 and SCA44, respectively. Using genome editing, we have recently created the first mouse model of SCA44 that harbors a gain-of-function patient mutation in the *Grm1* gene encoding the metabotropic glutamate receptor mGluR1. Here, we will present on the cellular and behavioral phenotypes of this novel ataxia model.
Importantly, disturbed mGluR1-TRPC3 signaling is linked to several other genetically distinct forms of SCAs, thus making it a strong candidate for a commonly affected disease pathway in cerebellar ataxia.

# (#425) Generation and characterization of a patient-derived neuronal model for spinocerebellar ataxia type 2

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 425

***Dr. Radhia Kacher*** [1], ***Dr. Nuno Palha*** [2], ***Mr. Morgan Devaux*** [3], ***Ms. Stephanie Bigou*** [3], ***Dr. Philippe Ravassard*** [4], ***Prof. Alexandra Durr*** [1]

***1.*** *Paris Brain Institute (ICM), Sorbonne Université,* ***2.*** *Neuroscience and Immunoinflammation Therapeutic Area, Institut de Recherches Servier,* ***3.*** *Paris Brain Institute (ICM), ICV-iPS core facility,* ***4.*** *Paris Brain Institute (ICM), Sorbonne Université, ICV-iPS core facility*

Polyglutamine related diseases are dominant genetic disorders characterized by progressive neurodegeneration, leading to behavioral and physical impairments. These conditions are caused by an abnormal expansion of polyglutamine-encoding CAG repeats in their respective causative genes. Such CAG repeat expansion in the *ATXN2* gene is the direct cause of spinocerebellar ataxia type 2 (SCA2), a progressive inherited ataxia often associated with Parkinsonism and lower motor neuron disease.

The characterization of the cellular and molecular mechanisms underlying SCA2 pathogenesis is still incomplete, and the development of new disease models is needed to better understand, diagnose and treat this disease. One of the current potential therapeutic strategies is the use of antisense oligonucleotides (ASO) to decrease the expression of the mutated gene. To test such method, we need robust and relevant models, especially patient-derived models. Therefore, we developed a novel *in vitro* SCA2 model by differentiating dopaminergic neurons from patient-derived iPSCs, carrying 40 repeats at a heterozygous state. We analyzed the characteristic markers of dopaminergic neurons and the expression of Ataxin2.

# (#448) Corticospinal disfunction in a novel Friedreich Ataxia mouse model

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 448

***Dr. Misa Nishiyama*** [1], ***Dr. John Kalambogias*** [1], ***Dr. Joriene De Nooij*** [2], ***Prof. Yutaka Yoshida*** [1]

***1.*** *Burke neurological institute,* ***2.*** *Department of Neurology, Columbia University Medical Center, New York, NY, U.S.A.*

Most patients with FRDA show signs of corticospinal neuron deficits e.g. diffuse volumetric reduction of motor cortex and corticospinal tract (CST). The main goal of this study is to examine neuronal dysfunction of corticospinal neurons (CSNs) in FRDA mice. We used a novel YG8sR mouse model that contains the human YG8sR FRATAXIN transgene with 800-900 GAA repeats ($YG8sR_{850}$) in the presence of the mouse Frataxin exon 4 deletion ($Fxn^{ex4-/-}$;$YG8sR_{850}$). The original YG8sR mouse model (200 repeats; Virmouni et al., 2015) shows mild symptoms compared to the human disease. Using this $YG8sR_{850}$ mouse model, we determined whether FRDA-associated CSN phenotypes will appear at an earlier developmental stage due to the higher number of repeats.

We labeled CSNs in $Fxn^{ex4-/-}$;$YG8sR_{850}$ mice using retrograde viral tracing to quantify CSN number and cell body size. 2-3 months old $Fxn^{ex4-/-}$;$YG8sR_{850}$ mice showed a significant loss of CSNs but no change in CSN cell body size when compared to littermate control animals. Consistent with these observations, we observed a reduction in the axonal CST marker PKC-γ in the dorsal column of the spinal cord.

In addition, to assess cortical driven muscle activity, we recorded motor-evoked potentials (MEPs) in forelimb muscles in response to primary motor cortex stimulation. We analyzed MEP amplitude to evaluate CST dysfunction and found a reduction in MEP amplitudes.

Behaviorally, we find that $Fxn^{ex4-/-}$;$YG8sR_{850}$ mice, are less capable to run at high treadmill speeds and on average, show a threefold increase in the time needed to cross a horizontal ladder. We also noticed a deterioration in skilled motor function, as $Fxn^{ex4-/-}$;$YG8sR_{850}$ mice show failures in grasping and increases in drops during pasta manipulation.

Thus, we present a novel FRDA mouse model harboring a human FXN genetic perturbation which exhibits predominant degenerative changes of the CST and sensorimotor behavioral deficits.

# (#460) Characterization and phenotype characteristics of cardiac and neuronal-specific Friedreich's Ataxia mouse models

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 460

***Dr. Elena Gonzalo Gil*** [1], ***Ms. Stephanie Williams*** [1], ***Ms. Hannah Knowlton*** [1], ***Mr. Dakota Jewett*** [1], ***Mr. Randy Walls*** [1], ***Mrs. Mia Bowden*** [1], ***Mrs. Crystal Davis*** [1], ***Dr. Cathleen Lutz*** [1]

***1.*** *The Jackson Laboratory*

**Background and Objective:** Friedreich's Ataxia (FRDA) is an autosomal recessive disorder caused by a GAA trinucleotide repeat expansion in intron 1 of the frataxin gene. Human patients develop cardiomyopathy, progressive neurodegeneration, glucose intolerance and skeletal deformities. Here we characterized the biochemical, molecular, and behavioral characteristics of the commonly used mouse models available from The Jackson Laboratory Repository used in drug discovery for FRDA.

**Methods:** Mice were monitored weekly for body weight and clinical observations. Echocardiogram was performed on Fxnflox/null::MCK-Cre (JAX#29720) mice to evaluate cardiac function. Succinate dehydrogenase (SDH) activity was measured in cardiac tissue to assess cell viability as a function of redox potential. Fxnflox/null::Pv-Cre (JAX#29721) mice were tested on rotarod and open field. Frataxin protein expression levels were analyzed by ELISA.

**Results:** $Fxn^{flox/null}$::MCK-Cre mutants exhibited a peak body weight between 7-8 weeks of age, with weight loss requiring euthanasia by 10.5 weeks of age (77 days survival). Mutants developed progressive cardiomyopathy, significantly decreased ejection fraction ($p<0.001$) and reduced stroke volume ($p<0.001$) compared to wildtype mice. SDH enzyme activity was significantly lower in Fxnflox/null::MCK-Cre mutants compared to wildtype controls ($p<0.001$). Fxnflox/null::PV-Cre mutants displayed progressive ataxia starting at 9-10 weeks of age, reflected in increased neuroscore compared to wildtype mice ($p<0.001$). Starting at 12 weeks of age, Fxnflox/null::PV-Cre mutants displayed a significant decrease in latency to fall on accelerating rotarod ($p<0.001$) and in total distance traveled in open field ($p<0.001$) compared to wildtype. Frataxin expression in brain (cortex and cerebellum) and spinal cord were reduced in Fxnflox/null::PV-Cre mutants compared to wildtype ($p<0.001$) controls.

**Discussion and Conclusion:** Conditional ablation of frataxin in cardiac and neuronal tissue generated mutant mice with robust phenotypes. These models play an integral role in understanding the biology of the FRDA as well as being one of the most efficient resource tools for pre-clinical testing of therapeutics.

# (#483) Deciphering the molecular effects of frataxin point mutations in neuronal models of Friedreich's ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 483

***Dr. Kinga Linowiecka*** [1], ***Dr. Jixue Li*** [1], ***Dr. Marek Napierala*** [1], ***Dr. Jill Napierala*** [1]

***1.*** *Department of Neurology, Peter O'Donnell Jr. Brain Institute, UT Southwestern Medical Center, Dallas, TX*

*Background and Objective*

Most Friedreich's ataxia (FRDA) patients are homozygotes for GAA trinucleotide expansions in intron 1 of the frataxin (FXN) gene (GAA/GAA). However, 2-4% patients are compound heterozygous with one allele affected by a point mutation in *FXN* and the second by GAA expansion. The most prevalent point mutation results in a change from glycine to valine in FXN position 130 (G130V). G130V patients have decreased levels of mature FXN in favor of the intermediate isoform and their clinical presentation differs from GAA/GAA FRDA patients. We hypothesize that the intermediate FXN isoform partially compensates for deficiency of the mature protein. Investigating the properties of FXN G130V in neuron development and function may reveal the molecular basis for distinct disease manifestation.

*Methods*

Skin fibroblasts of G130V FRDA patients, GAA/GAA FRDA patients and healthy individuals (CTRL) were reprogrammed to induced pluripotent stem cells (iPSCs). A doxycycline (Dox)-inducible human Neurogenin 2 (*hNGN2*) transgene was integrated at the *AAVS1* locus in G130V and CTRL iPSCs. Differentiation to cortical neurons (CNs) was initiated by addition of Dox to the culture medium, followed by supplementation with other neuronal growth factors for 21 days. Cells were collected at several timepoints during differentiation for molecular analyses.

*Results*

Expression of differentiated neuron markers and *FXN* were confirmed by RT-qPCR. Also, based on our initial observations, CNs differentiated from G130V FRDA iPSCs display distinct morphology from CTRL iPSCs.

*Discussion and Conclusion*

Our next step is assessing the development and morphology of CTRL, G130V and GAA/GAA CNs by analyzing growth cone area, axonal swellings and neurite outgrowth. We will also compare mitochondrial metabolic profiles between the different CN lines to determine how the G130V mutation or changes in FXN isoform equilibrium affect cellular respiration. These experiments could reveal unique molecular events underlying the atypical clinical manifestation of G130V FRDA patients.

# Poster Sessions: Biomarkers

# (#25) Objective assessment of motor impairments in spinocerebellar ataxia using wearable sensors

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 25

***Mr. Reza Mohammadi [1], Mr. Hung Nguyen [1], Mrs. Ana Enriquez [1], Dr. Christopher Stephen [2], Dr. Anoopum Gupta [2], Dr. Jeremy D. Schmahmann [2], Mr. Ashkan Vaziri [1]***

***1.*** *BioSensics,* ***2.*** *Massachusetts General Hospital*

**Background and Objective:** Robust tools for objective assessment of motor impairment in ataxia are needed. These tools enable progression of disease severity and can assist in evaluating the efficacy of new therapeutics. In this regard, the aims of this study are to: 1) develop a tool for objective assessment of upper and lower-limb motor functions in spinocerebellar ataxia (SCA) using wearable sensors; 2) investigate the possibility of reducing the number of assessment tests without adversely affecting the results.

**Methods:** 14 patients with genetically confirmed SCA (age 61.6 ± 8.6 years) and 3 healthy controls (age 49.0 ± 16.4 years) were recruited through the Massachusetts General Hospital Ataxia Center. Participants were instrumented with wearable sensors while performing motor assessments based on the Scale for the Assessment and Rating of Ataxia (SARA). Separate mathematical models were developed to extract sensor-derived features and predict the SARA ratings for lower and upper limbs. Furthermore, the redundancy of the upper-limb tests in the SARA was investigated. In doing so, a phase detection algorithm was developed to extract the kinematic measures of each phase of finger-to nose-test (FNT). Redundancies were characterized by predicting the SARA scores of the finger-chase and Dysdiadochokinesia tests using the extracted FNT feature set along with various machine-learning algorithms.

**Results:** A subset of most effective features in predicting the scores were obtained for each task. The predicted scores using the selected features show strong correlation with the subjective scores (high correlation with p-value<0.05). Also, obtaining relatively high accuracy of predicting upper-limb SARA scores using only the FNT features confirms the dependency of the tests.

**Discussion and Conclusions:** Sensor-derived metrics can potentially be used for automated objective assessment of motor impairment in SCA. Also, characterizing the redundancies of the assessment tests can potentially pave the way for shorter and more convenient assessments.

# (#154) Quantification of Whole Blood Isoform E as a Biomarker of Friedreich's Ataxia Using a Triple Quadrupole Mass Spectrometer

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 154

***Dr. Teerapat Rojsajjakul*** [1], ***Dr. Nicolas Eskenazi*** [1], ***Dr. Clementina Mesaros*** [1], ***Dr. Linfeng Wu*** [2], ***Dr. David Lynch*** [3], ***Dr. Ian A. Blair*** [1]

***1.*** *University of Pennsylvania,* ***2.*** *Agilent Technologies, Inc.,* ***3.*** *University of Pennsylvania & Childrens Hospital of Philadelphia*

Background and Objectives: Friedreich's ataxia (FA) patients have reduced levels of the mitochondrial frataxin protein, which results in progressive ataxia and hypertrophic cardiomyopathy. We have identified and characterized an N-terminally acetylated isoform of frataxin (Isoform E) erythrocytes. Because frataxin isoform E expression is also downregulated in FA patients; therefore, we have developed an assay for this isoform in erythrocytes.

Methods: A targeted, quantitative assay using stable isotope dilution coupled with immunoaffinity-liquid chromatography-tandem mass spectrometry using an Agilent 1290 Infinity HPLC system coupled to a 6495C triple quadrupole mass spectrometer. Recombinant stable isotope labeling by amino acids in cell culture (SILAC)-isoform E was used as the internal standard. Proteolytic digestion was conducted using Asp-N prior to analysis by liquid chromatography multiple reaction monitoring mass spectrometry (LC-MRM/MS).

Results. Dynamic MRM conditions for selected peptides were optimized using immunopurified SILAC-isoform E-spiked whole blood homogenate. Method validation was conducted with 5 replicates of quality control samples in a surrogate matrix on three separate days to determine, and both inter-and intra-day assay variability. This method enabled the quantification of endogenous Isoform E in FA patients, FA carriers and control whole blood.

Discussion and Conclusion: The new method is 8-10 times faster than the use of a high-resolution mass spectrometer. Furthermore, the accuracy and precision of the instrument for analysis of isoform E frataxin were comparable with those obtained with the high-resolution instrument. The correlation of whole blood isoform E levels with the age of onset of the disease and with the patients' numbers of GAA triplet repeats was superior to that for whole blood mature frataxin. Our new assay will be particularly useful for monitoring the natural history of the disease, for assessing the effects of novel therapies for FA, and for exploring the potential role of isoform E in disease progression.

# (#160) Validating ataxia instrumented measures for guiding therapy in Friedreich ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 160

***Dr. Louise A Corben*** [1], ***Prof. Pubudu Pathirana*** [2], ***Prof. Martin B Delatycki*** [3], ***Prof. Malcolm Horne*** [4], ***Dr. David Szmulewicz*** [5]

***1.*** *Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia, School of Psychological Sciences, The Turner Institute for Brain and Mental Health, Monash University, Clayton, Victoria, Australia,,* ***2.*** *Deakin University,* ***3.*** *Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia,,* ***4.*** *The Florey Institute of Neuroscience and Mental Health,* ***5.*** *The Florey Institute of Neuroscience & Mental Health; Eye and Ear Hospital, East Melbourne, Victoria, Australia.*

**Background and Objective**

Therapies that modify the progression of Friedreich Ataxia (FRDA) are under active research and will likely become available soon. Accurate and objective measurement of the effects of FRDA are needed in clinical trials. Expert clinicians, regulatory bodies, industry and people with FRDA all recognize the need to measure severity and disease progression using objective measurements that reflect activities of daily living. We have developed a system of objective measures of ataxia - the Ataxia Instrumented Measurement (AIM) system which consists of data loggers each equipped with an inertial measurement unit (IMU) capture device, and algorithms that analyze these signals, to measure ataxia during daily activities. Specifically we have developed the AIM-Spoon (AIM-S) and the AIM–Cup (AIM-C) which measures ataxia of the upper limb during simulated eating and drinking and the AIM –Pendant (AIM-P) which measures balance while standing. Each device delivers a single composite score which is of direct relevance to the clinician and researcher. Our aim is to convert the algorithms that we have previously developed to medical grade software, build data loggers to regulatory standards and validate these devices for use in FRDA which will enable their use in the clinic and clinical trials.

**Methods**

This is a prospective cross sectional study comprising 30 individuals with FRDA and 30 matched control participants, repeatedly measured with all devices to establish validity and reliability.

**Results**

We have manufactured the AIM-S, AIM-C and AIM-P as clinical grade devices with regulatory approved software and commenced a validation study. We will present the results from this study.

**Discussion and Conclusion**

Accurate measurement of ataxia is needed to progress therapies for FRDA. We have developed an instrumented system that addresses this need. The measurement system is being developed to regulatory standards for use in key trials and by treating clinicians.

# (#225) The perspective of people with recessive ataxia: Developing a new patient-reported outcomes.

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 225

***Ms. Marjolaine Tremblay*** [1], ***Dr. Bernard Brais*** [2], ***Ms. Véronique Asselin*** [3], ***Mr. Martin Buffet*** [3], ***Mr. André Girard*** [3], ***Mr. Denis Girard*** [3], ***Prof. Cynthia Gagnon*** [1]

***1.*** *Universite de Sherbrooke,* ***2.*** *McGill University,* ***3.*** *Groupe de recherche interdisciplinaire sur les maladies neuromusculaires (GRIMN), Centre intégré universitaire de santé et de services sociaux du Saguenay-Lac-St-Jean, Québec, Canada*

Background and objective. Clinical trial readiness is a major issue in rare diseases in part related to the lack of outcome measures with documented metrological properties. Among the requirement, regulatory agencies' guidelines including the Food and Drug Administration emphasized the need to assess the treatment's benefits according to patient's perspective as a key outcome in clinical trials. Manifestations and impacts of the disease are assessed with a type of instrument called Patient-Reported Outcomes (PRO). Up to now, there is just one existing PRO for ataxia in general. There is no PRO specific for recessive ataxias, including Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS). It is therefore essential to develop a PRO that can be used specifically for this clientele. Methods. Using the *Patient-Reported Outcomes Measurement Information System* (PROMIS), a review of literature, individual interviews and discussion groups has been performed to develop an items bank. Items has been selected by the research team, including persons with ARSACS. Effectively, four people with ARSACS where include in the research team as patients as partners. Content validity has been documented by online survey, experts' consultation, and cognitive interviews. The psychometric proprieties were documented by an online questionnaire and factor analysis. Results. The patient-reported outcome in recessive ataxia includes 38 items in three dimensions of health: physical, mental, and social, confirmed by the factor analysis. It shows good psychometric proprieties: normal distribution, no ceiling-floor effect, and a good known-groups validity. Discussion and conclusion. This is the first PRO designed specifically for recessive ataxias. This allows us to determine what is important for people affected and which aspects of the disease have a significant impact in their daily living. It can help clinician in the annual follow-up of their patients and, can be use in future clinical trial as outcomes measure.

# (#232) Long non-coding RNA TUG1 is a potential novel biomarker for Friedreich's ataxia.

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 232

***Mr. Mert Koka [1], Dr. Hui Li [1], Prof. Susan L. Perlman, MD [2], Dr. Darice Wong [2], Dr. Brent Fogel [2], Dr. David Lynch [3], Dr. VIJAYENDRAN CHANDRAN [1]***

***1.*** *university of florida,* ***2.*** *David Geffen School of Medicine at UCLA, Los Angeles, CA,* ***3.*** *University of Pennsylvania & Childrens Hospital of Philadelphia*

Currently, there is no effective approved therapy for Friedreich's ataxia (FRDA), and there is a high necessity for good prognostic and predictive molecular biomarkers associated with FRDA for clinical trials. Sensitive biomarkers in the clinical trials that show a change in shorter time frames are of immense interest. Current clinical endpoint tests using assessment scales to examine the efficacy of drugs need to be investigated over 1-2 years. As trials that take over a year are expensive and difficult to conduct, identifying sensitive molecular biomarkers may offer an opportunity to make quicker evidence-based decisions on potential therapeutics.

By extensive analyses of human and mouse model genomics data, here we report a long noncoding RNA (lncRNA) taurine up-regulated gene 1 (Tug1) as a potential biomarker for FRDA. We show that: (1) Tug1 is down-regulated in whole blood, heart, cerebellum, DRGs, spinal cord, muscle, and whole-brain after Fxn knockdown, (2) Tug1 expression levels are reversed after FXN restoration, (3) Tug1 is significantly correlating with Fxn levels during knockdown and restoration, (4) Tug1 downstream target genes are also dysregulated after Fxn knockdown in multiple tissues, and (5) Tug1 is also down-regulated in the whole blood and serum data obtained from FRDA patients.

This is the first study to directly examine and report lncRNA Tug1 as a potential biomarker for FRDA. lncRNAs are considered as suitable diagnostic and prognostic biomarkers because they can be easily and noninvasively obtained from patients, and they remain relatively stable in body fluids. lncRNAs can be quite easily detected in whole blood, plasma, serum, urine, saliva, and gastric juice samples, by using common laboratory techniques. In summary, Tug1 as a biomarker has the potentially to be used for early evidence-based decisions on potential therapeutics or together with other different clinical rating scales for long term assessment in FRDA.

# (#235) Unique balance impairments distinguish Fragile X-associated Tremor/Ataxia Syndrome from Parkinson disease and essential tremor

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 235

***Dr. Erin Robertson-Dick*** [1], ***Dr. Deborah Hall*** [1], ***Dr. Gian Pal*** [1], ***Dr. Bichun Ouyang*** [1], ***Ms. Yuanqing Liu*** [1], ***Mr. Andrew McAsey*** [2], ***Ms. Alexandra Bery*** [2], ***Ms. Colleen Huml*** [2], ***Dr. Bryan Bernard*** [1], ***Dr. Elizabeth Berry-Kravis, MD, PhD*** [1], ***Dr. Joanne O'Keefe, PhD, PT*** [2]

***1.*** *Department of Neurological Sciences, Rush University Medical Center,* ***2.*** *Rush University Medical Center*

**Background:** Fragile X-associated tremor/ataxia syndrome (FXTAS), a neurodegenerative disease affecting carriers of a 55-200 CGG repeat expansion in the *fragile X mental retardation 1* gene, may be initially diagnosed as Parkinson disease (PD) or essential tremor (ET) due to overlapping motor symptoms. In this study, FXTAS, PD, ET and control participants were compared in order to examine whether unique balance profiles existed between groups, and to explore the impact of cognition on balance performance.

**Methods:** Participants with FXTAS (n = 22; 69.1 ± 8.1 yrs), PD (n = 23; 71.2 ± 7.7 yrs), ET (n = 20; 69.8 ± 8.6 yrs) and controls (n= 20; 62.7 ± 8.3 yrs) underwent quantitative balance testing using instrumented postural sway tests (APDM$^{TM}$) under single and dual-task (DT) cognitive interference conditions. A neuropsychological test battery was administered including the Behavioral Dyscontrol Scale II, Controlled Oral Word Association Test, Animal Naming test, and Symbol Digit Modalities test to measure response inhibition, verbal fluency, and information processing speed, respectively. ANOVA, correlation and multinomial logistic regression analyses were performed.

**Results:** FXTAS participants had greater postural sway compared to PD with base of support (BoS) reduced and during DT conditions with BoS reduced and vision removed ($p < 0.05$ to 0.001). They also had greater postural sway compared to ET when proprioceptive information was reduced, BoS was reduced and vision removed during a DT ($p < 0.05$). There were significant correlations between cognitive and balance measures in all groups. Regression analysis revealed that greater sway variability distinguished FXTAS from ET during DT with BoS reduced and vision removed ($p < 0.05$).

**Conclusion:** This is the first quantitative study demonstrating distinct balance profiles in FXTAS, PD, and ET, which could be used to distinguish these movement disorders and select optimal outcome measures in future studies.

# (#264) Ankle submovements and at-home computer mouse use reflect patient-reported motor function in adult degenerative cerebellar ataxias

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 264

***Ms. Nicole M. Eklund*** [1], ***Ms. Jessey Ouillon*** [1], ***Dr. Vineet Pandey*** [2], ***Ms. Akansha Pandey*** [1], ***Dr. Christopher Stephen*** [3], ***Dr. Jeremy D. Schmahmann*** [3], ***Dr. Krzysztof Z. Gajos*** [2], ***Dr. Jeremy Edgerton*** [4], ***Dr. Anoopum Gupta*** [3]

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Current clinical measures of spinocerebellar ataxias (SCA) are subjective and typically not suitable for frequent at-home administration, resulting in imprecise measurements of disease severity. This study aimed to evaluate the construct validity and test-retest reliability of at-home assessments to monitor disease severity in SCA and possible/probable Multiple System Atrophy-cerebellar type (MSA-C) patients. Developing accessible tools to precisely measure disease severity will support clinical trials and care for individuals with ataxia.

Subjects participated in a remote study to collect speech biomarkers, Hevelius computer mouse task performance, and wrist and ankle accelerometer data. Participants were mailed a standardized laptop, computer mouse, and 2 GENEActiv devices to wear continuously for one week. A physician-administered examination was conducted for each participant via Zoom video conference. During this session, BARS, SARA, and UPDRS components that could be completed remotely were performed by two neurologists. Following the clinical assessment, participants completed speech surveys and Hevelius computer mouse tasks biweekly for 4 weeks and 5 quality of life and daily function questionnaires, including PROM-Ataxia and Neuro-QOL, at baseline and post-study.

Enrollment is projected to complete in August 2022. 16 subjects with SCA/MSA-C and 7 healthy controls have completed the study. Analysis of construct validity showed that out of 32 Hevelius features, 25 demonstrated a moderate-strong correlation with either BARS or SARA scores ($r$=0.60-0.75) and 4 demonstrated a moderate-strong correlation with PROM-Ataxia arm scores ($r$=0.65-0.69). Of those highly correlated features, 6 showed good-excellent test-retest reliability ($ICC$=0.797-0.904). The assessments demonstrated high feasibility, with participants completing 94% of Hevelius tasks.

These data demonstrate the potential use of Hevelius in tracking disease severity in SCA and MSA-C patients. We plan to extend this study longitudinally over a period of 12 months following the initial testing. Our final analysis will include additional modalities, such as data from GENEActiv devices and speech surveys.

# (#270) Free-living motor activity measurements in ataxia-telangiectasia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 270

***Ms. Anna C. Luddy*** [1], ***Ms. Nergis C. Khan*** [2], ***Ms. Jennifer Thornton*** [3], ***Ms. Sara Reiling*** [3], ***Dr. Anoopum Gupta*** [1]

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Background: As disease therapies for ataxias are being investigated, it is necessary to develop free-living motor biomarkers to evaluate disease severity and progression. Caregiver reported outcome measures (CROMs) also supply important information about disease severity that may not be captured by other measures. This study sought to test the hypothesis that metrics derived from a wrist worn accelerometer and CROMs can provide accurate, interpretable, and reliable information about disease severity and progression.

Methods: Data were collected remotely from 31 participants with ataxia-telangiectasia (A-T) and 27 control participants ranging from two to nineteen years of age. 14 participants with A-T and 13 controls completed the study at two time points separated by one year. Participants were asked to wear a GENEActiv accelerometer device on their dominant wrist continuously for one week. They were also asked to complete a speech survey three times over the course of a week. Following the study caregivers also filled out CROMs including the CPCHILD and Dysarthria Impact Scale.

Results: Features extracted from passive wrist sensor data were highly informative for distinguishing individuals with A-T from healthy controls, and correlated strongly with the Brief Ataxia Rating Scale, a motor subset of CPCHILD, and neurofilament light chain concentration. Several wrist sensor features captured disease progression over a 1-year interval. The CROMs were shown to have high feasibility as >85% of participants completed both the CPCHILD and the Dysarthria Impact Scale.

Discussion: These results demonstrate that data from wrist sensors produce reliable and informative measures of motor function and may be useful as an outcome measure in clinical studies and for monitoring disease. We plan to extend data collection over a period of two years to better characterize the longitudinal properties of wrist sensor features. The final analysis will additionally include data from the speech surveys.

# (#293) Using digital health metrics to capture upper limb impairment in the context of upcoming clinical trial in ARSACS

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 293

***Prof. Cynthia Gagnon*** [1]***, Dr. Christoph M. Kanzler*** [2]***, Dr. Isabelle Lessard*** [3]***, Dr. Roger Gassert*** [4]***, Dr. Bernard Brais*** [5]***, Dr. Olivier Lambercy*** [2]

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**Background and objectives**. Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is the second most frequent recessive ataxia and commonly features reduced upper limb coordination. This could be a potential this could be a potential key endpoint in upcoming clinical trials as it permits to include a larger patient population as opposed to walking capacity. However, current documented upper limb clinical assessments are insufficient to capture behavioral variability and detailed aspects of motor control. The Virtual Peg Insertion Test (VPIT) is a technology-aided assessment featuring a goal-directed pick-and-place task that requires arm and hand function and captures 10 digital health metrics such as movement smoothness. The objectives of this study were: 1) to document the metrological properties of the VPIT digital health metrics of the; 2) to document upper limb coordination in ARSACS population. **Methods.** Transversal design (n=57 participants) with a sub-study on reliability (n=23). Test-retest reliability and measurement error were documented using ICC. The digital health metrics were transformed in normalized VPIT scores and compared to the able-bodied control population using Mann-Withney-U tests. A cluster analysis was performed to identify subpopulations that exhibit similar kinematic and kinetic behavior with the *k-means* algorithm. **Results.** Eight metrics had excellent test-retest reliability, five low measurement errors. 98.3% of the participants were impaired in at least one of the metrics with grip force control during precise manipulations being the most commonly and strongly impaired. We identified high inter-participant variability in the kinematic and kinetic impairment profiles, which could be attributed to two behavioral subpopulations. These were especially discriminated by the ability to perform smooth and fast movements. **Discussion and conclusion**This establishes eight digital health metrics as valid and robust endpoints for cross-sectional studies and thereby promising novel insights into upper limb sensorimotor control in ARSACS.

# (#305) Factors influencing self-selected walking speed in adults with ARSACS

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 305

***Dr. Isabelle Lessard*** [1]***, Prof. Luc J. Hébert*** [2]***, Ms. Isabelle Côté*** [1]***, Mr. Raphael St-Gelais*** [1]***, Dr. Bernard Brais*** [3]***, Prof. Cynthia Gagnon*** [4]

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**Background:** Mobility limitations, including decrease of walking speed, are major issues for people with autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS). ARSACS is a hereditary neurological disorder presenting with pyramidal (i.e. lower limbs motor impairments), cerebellar (i.e. incoordination) and neuropathic (i.e. distal muscle weakness) impairments. The motor impairment progression lead to significant mobility and balance limitations, which induce participation restrictions and difficulty performing activities of daily living. ARSACS) is a hereditary neurological disorder presenting with pyramidal (i.e. lower limbs motor impairments), cerebellar (i.e. incoordination) and neuropathic (i.e. distal muscle weakness) impairments. Improving our understanding of the impairments and activities limitations that contribute to self-selected walking speed in ARSACS may inform the development of future interventions for gait rehabilitation and contribute to better clinical and rehabilitation practices.

**Objectives:** This study aimed to identify the factors influencing the self-selected walking speed in adults with ARSACS.

**Methods:** The dependent variable of this cross-sectional study was the self-selected speed and the factors assessed included: age, sex, balance, balance confidence, lower limbs coordination, lower limbs spasticity, and ankle dorsiflexion passive range of motion. Multiple regression model was used to assess the relationships between factors and self-selected walking speed.

**Results:** A total of 33 participants were included with a mean age of 31.9 (± 10.0) years with 45.5 % using a walking aid. All factors together explained 83.7% of the variability of the self-selected walking speed, and balance was the strongest one. All other factors significantly contributed to self-selected walking speed at a lesser extent.

**Discussion and conclusions:** Balance is the main contributor of self-selected walking speed. It is therefore important in clinical follow-ups to target interventions that stimulate balance, in order to maintain walking ability and functional independence as long as possible.

# (#352) Clinical Trial Design in FRDA: The Use of Bayesian borrowing methods to enrich the control group of future clinical trials with historical data.

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 352

***Mr. Pavel Mozgunov [1], Ms. Gaelle Saint-Hilary [1], Dr. David Lynch [2], Dr. Christian Rummey [3]***

***1.*** *Saryga,* ***2.*** *University of Pennsylvania & Childrens Hospital of Philadelphia,* ***3.*** *Clinical data science GmbH*

**Background and Objective** – Many clinical trials in FRDA are currently in the planning phase, and prior experience has shown that enrolment of reasonably sized studies (~80-100 patients) can easily take several years. Since large natural history datasets exist and pre-competitive efforts are in place to collect data from past clinical trials[1], advanced statistical methodology can facilitate accurate decision-making and powering of more studies than currently seems feasible.

**Methods** – Bayesian Dynamic Borrowing (BDB) allows incorporation of historical data into a predictive distribution, representative of what a new trial arms drawn from similar populations might look like. Into this distribution, information from any number of past trials might be synthesized, effectively enabling to reduce the size of The method is receiving growing interest from methodologists, clinicians, and regulators[2],[3] and was used in randomized controlled trials.[4]

**Results** – Recommendations on the selection of the historical data based on statistical considerations (feasibility to derive the endpoints, data heterogeneity) are provided, and several BDB designs for hypothetical future clinical trials are discussed. Their properties are assessed in a simulation study and are compared against classical frequentists designs.

**Discussion and Conclusion** – Using Bayesian designs and borrowing information from historical data permits to improve the efficiency and feasibility of clinical trials in FRDA and, ultimately, may lead to improved drug development and impact on patients.

[1] https://c-path.org/programs/rdca-dap/working-group/fa-icd

[2] FDA Guidance Document on Interacting with the FDA on Complex Innovative Trial Designs for Drugs and Biological Products, 2020. *www.fda.gov/drugs/development-resources/complex-innovative-trial-design-meeting-program?utm_medium=email&utm_source=govdelivery*

[3] EMA Regulatory science research needs, 2021. *www.ema.europa.eu/en/documents/other/regulatory-science-research-needs_en.pdf*

[4] Baeten, Dominique, et al. "Anti-interleukin-17A monoclonal antibody secukinumab in treatment of ankylosing spondylitis: a randomised, double-blind, placebo-controlled trial." *The Lancet* 382.9906 (2013): 1705-1713.

# (#377) Digital gait analysis in Spinocerebellar Ataxia type 44 (SCA44)

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 377

***Prof. Helen Dawes [1], Dr. Patrick Esser [2], Dr. Mario Inacio [2], Prof. Esther Becker [3], Prof. Andrea Nemeth [3]***

***1.*** *University of Exeter and Oxford Brookes University,* ***2.*** *Oxford Brookes University,* ***3.*** *Nuffield Department of Clinical Neurosciences, University of Oxford*

**Background and Objective:** Human SCA44 is caused by gain-of-function missense mutations in the metabotropic glutamate receptor type1 (mGluR1), a critical protein in coordinating movement and motor learning via intracellular signalling in cerebellum. A murine model of SCA44 also has been generated.

To facilitate rapid translation from animal models to human preclinical trials, we aim to develop an integrated platform for assessing biomarkers and clinical outcome measures in mGlur1-related ataxias in both humans and mice.

Here we report preliminary data on the first digital gait study to be performed in SCA44.

**Methods:** Spatial and temporal gait parameters were measured using an inertial measurement unit (IMU) comprising a triaxial accelerometer, gyroscope, and magnetometer.

Participants performed a 10m walking test. Time to completion as well as 15 spatial and temporal gait and variability parameters were measured, and compared to 60 controls. Additional data on gait, neurological examination, SARA, functional and ADL scores were obtained.

**Results:** Age of onset demonstrated intra- and inter-familial variability, ranging from 24 to 50 years. Gait and speech were notably affected. Subjects with the Y792C mutation had pathologically brisk reflexes suggesting cortico-spinal tract involvement. Total SARA scores reflected disease duration.

Average speed and temporospatial parameters were similar between subjects and controls. Temporal measures (i.e. step time left/right/average and cadence), demonstrated markedly increased intra-subject variability (>2 SD of controls; co-efficient of variation 0.13-0.45 compared to <0.1 Controls). Intra-subject spatial variability (eg. of step or stride length) was similar to controls. 8 year follow-up of 1 subject (Y262C) demonstrated further increases in temporal variability despite little change in average gait profiles or SARA score.

**Discussion and Conclusions:** Marked intra-subject variability, particularly in temporal measures was observed in SCA44 subjects. Our data is the first baseline digital gait data in SCA44 and provides a basis for on-going natural history studies.

# (#382) Harmonizing Results of Ataxia Rating Scales: mFARS, SARA & ICARS

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 382

***Dr. Christian Rummey*** [1], ***Dr. Ian Harding*** [2], ***Prof. Martin B Delatycki*** [3], ***Ms. Geneieve Tai*** [4], ***Dr. Thiago Rezende*** [5], ***Dr. Louise A Corben*** [6]

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**Background and Objective** – Ataxia research and rare disease drug development has expanded greatly during the last decade, and world-wide consortia are established to collect and compare data from various sources. Comparative assessment of disease severity requires the harmonization of the measures from a variety of sources.
**Methods** – The work was based on a dataset from a single center within the FACOMS study (Murdoch Children's Research Institutes, Melbourne). In total, 166 patients at 605 clinical visits had results from the three most commonly used rating scales in FRDA available. Conduct of all assessments aimed to minimize time and patient burden. Results from total scores, as well as axial and appendicular sub scores were transformed to a percentual scale and compared by functional disease stages boxplots. Correlation analyses made use of linear regression functions and analyzed by Pearson's $R^2$ statistics. Regression coefficients for transformation of the scales were derived using linear regression analysis.
**Results** – With correlation coefficients (Pearson's $R^2$) were generally high, i.e., at least 0.93 for total scores, 0.86 for axial scores, and 0.85 for the appendicular components, it could be confirmed that scales are reliably interconvertible. However, in particular the mFARS and it's axial component (FARS E) progressed numerically much faster than SARA and ICARS in early patients (stages 1-2). SARA on the other hand showed faster progression in late ambulatory patients (stages 3-4). This resulted in high intercepts in the conversion functions and leads to the conclusion that percentual scaling is not sufficient when combining results from these three rating scales.
**Discussion and Conclusion** – In conclusion, we provide conversion coefficients for mFARS and SARA, as well as their axial and appendicular components, useful for the harmonization and comparison of disease stage within cohorts from diverse origins. Comparing results on a percentual scale is not recommended.

# (#411) Saccade main sequence is affected in presymptomatic spinocerebellar ataxia type 2 and 7

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 411

***Dr. Pierre Pouget*** [1], ***Dr. Giulia Coarelli*** [2], ***Dr. Pierre Daye*** [3], ***Dr. Bertrabd Gaymard*** [4], ***Mrs. Rania Hilab*** [1], ***Dr. Candice Junge*** [5], ***Dr. Roger Lane*** [5], ***Dr. Moore Arnold*** [6], ***Prof. Alexandra Durr*** [7]

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**Background and Objective**

For spinocerebellar ataxias, reliable biomarkers with diagnostic and prognostic values are needed for upcoming trials. Our longitudinal study (CERMOI) aimed to identify oculomotor biomarkers in SCA2 and SCA7 at premanifest and manifest stages.

**Methods**

We included 15 carriers of the pathogenic expansion in *ATXN2*, 15 in *ATXN7*, and 10 controls. Three visits (baseline, at month-6, at month-12) are planned, including neurological examination (SARA and CCFS scores), neuropsychological evaluation, plasma neurofilament dosage, speech assessment (MBLF score), and brain MRI. Oculomotor recording used an EyeLink 1000 infrared eye tracking system (SR Research, Mississauga, Ontario) to record eye movements at 1 KHz. Each subject performed 4 blocks of 24 horizontal delayed and gap saccades. Multiple saccades during the oculomotor test were detected offline using P3lab software with a particle velocity detector (Daye and Optican, 2014) and the main sequence parameters were extracted for each subject.

**Results**

At baseline, SCA2 and SCA7 carriers had a median SARA score of 5.05 [0;9] and 12.25 [0;14.3] and a median CCFS score of 0.91 [0.88;0.97] and 1.07[1.06;1.1], respectevely. The saccade main sequence was altered in manifest (SARA score > 3) SCA2 (n=10) and SCA7 (n=5) expansion carriers versus controls (CI $p<0.05$). This was also significant in presymptomatic (SARA score <3) SCA2 expansion carriers (n=5) versus controls (CI $p<0.05$). With our current analysis, there was no significant correlation with the expanded CAG repeat size or SARA and CCFS scores. This preliminary analysis indicates that abnormal saccades are very early signs and detectable in the absence of cerebellar features evidenced by a neurological examination.

**Discussion and Conclusion**

Our preliminary results lead us to propose that a possible early marker of the evolution of the degenerative pathology by the variations of the saccadic eye movement execution parameters can be inferred in pre-symptomatic expansion carriers in ATXN2.

# (#414) Quantifying fine-motor impairment in ataxia: digital parameters of spiral drawing correlate with clinical severity, function, and ADLs

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 414

***Mr. Dominik Hermle*** [1], ***Mr. Robin Schubert*** [2], ***Mr. Pascal Barallon*** [2], ***Dr. Rebecca Schuele*** [1], ***Dr. Ralf Reilmann*** [2], ***Prof. Matthis Synofzik*** [1], ***Dr. Andreas Traschütz*** [1]

***1.*** *Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany,* ***2.*** *George-Huntington-Institute, Münster*

**Background and Objective:** Upper-limb function is insufficiently explored as digital-motor trial outcome in ataxia. The Archimedes spiral drawing task might serve as an excellent, patient-meaningful outcome measure as it requires fine-motor skills of everyday living like writing and is used in clinical scales and upcoming antisense oligonucleotide treatment trials. Here, we implemented Archimedes spiral drawing in the trial-ready quantitative motor (Q-Motor) system and explored digital parameters of its underlying movement kinematics as outcome measures.

**Methods:** Cross-sectional single-center validation study, including 46 patients with predominantly degenerative cross-genotype cerebellar ataxias (mean age: 50 years (18-80); mean SARA: 12 points (2-28)), and 48 age- and sex-matched controls. Subjects were instructed to draw – as accurately as possible at self-paced speed - two Archimedes spirals with their dominant hand on a paper template while traced by a Polhemus FASTRAK digitizer with a pencil lead. Thirty-five parameters in the temporal, spatial, spatiotemporal and frequency domain were extracted from positional data, and validated against the Scale for the assessment and rating of ataxia (SARA), activities of daily living (FARS ADL), and the 9-hole peg-test (9HPT).

**Results:** Parameters in the spatial domain, namely the summed-up $10^{th}$ decile, standard deviation, and maximum of the error distribution (distance from template), discriminated best between ataxia and controls (AUC: 0.83-0.89), and showed the strongest correlation with FARS ADL (Spearman rho: 0.38-0.47). In contrast, parameters in the frequency (Fourier power 1-4 Hz, rho: 0.75) and spatiotemporal domain ($90^{th}$ percentile of speed, rho: 0.72) correlated most with the total SARA and specifically with a composite of its upper-limb items, as well as the 9HPT (rho: 0.59-0.74).

**Discussion and Conclusion:** Quantitative analysis of Archimedes spiral drawing allows to capture fine-motor impairment, with differential correlations to clinical severity, function, and ADL across parameter domains. Longitudinal analyses are ongoing to investigate their respective sensitivity to change.

# (#417) Integrated Functional Evaluation of the Cerebellum (CERMOI) study: retinal baseline data on the spinocerebellar ataxia type 7 cohort

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 417

***Dr. Marco Nassisi*** [1]***, Dr. Giulia Coarelli*** [2]***, Mr. Benoir Blanchard*** [3]***, Mrs. Karima Drine*** [3]***, Dr. Nicolas Kitic*** [3]***, Mr. Serge Sancho*** [3]***, Mrs. Rania Hilab*** [4]***, Dr. Candice Junge*** [5]***, Dr. Roger Lane*** [5]***, Dr. Moore Arnold*** [6]***, Prof. Alexandra Durr*** [7]***, Prof. Isabelle Audo*** [3]

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**Background and Objective**

Reliable biomarkers with prognostic values are needed for upcoming therapy trials. One of the aims of our Integrated Functional Evaluation of the Cerebellum (CERMOI) longitudinal study was to identify ophthalmological biomarkers in SCA7.

**Methods**

Fifteen *ATXN7* pathogenic expansion carriers were included, all with a scale for the assessment and rating of ataxia (SARA) score <15/40 (9 premanifest and 6 manifest). Three visits (baseline, at month-6, at month-12) are planned, including the neurological examination (SARA and Composite cerebellar functional severity score [CCFS]) and ophthalmological examination (visual acuity [VA], microperimetry, full-field electroretinogram, optical coherence tomography [OCT] and fundus autofluorescence imaging). Here we report the baseline ophthalmic data from the cohort. A correlation between disease scores and ophthalmic results was attempted.

**Results**

At baseline, the mean age was 34.5 ± 13.1 years (median 38, range 18-60 years). Only three patients (20%) had no retinal abnormalities, all with a SARA ≤ 1 and a CCFS score <0.850. All other patients presented with cone dystrophy at variable stages. Both SARA and CCFS scores showed significant correlations (Spearman Rho coefficient [ρ]) with VA (ρ:-0,871 p<0.001 and ρ:-0.772 p=0.001, respectively) and the outer nuclear layer thickness on OCT (ρ:-0.861 p<0.001 and ρ:-0.560 p=0.037, respectively). The SARA score was also significantly correlated with the function of the cones tested by electrophysiology.

**Discussion and Conclusion**

From these baseline data of the CERMOI study, neurological scores and ophthalmological functional and structural tests are highly correlated in SCA7 patients, thus the latter have the potential to be outcome markers for treatment effect.

# (#418) Using Mathematical Modeling to Quantify the Progression of the modified Friedreich's Ataxia Rating Scale (mFARS) in Friedreich's Ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 418

***Dr. Megan Pane*** [1], ***Dr. Christian Rummey*** [2], ***Dr. Alexander Betourne*** [1], ***Dr. Jeff Barrett*** [1]

***1.*** *Critical Path Institute,* ***2.*** *Clinical data science GmbH*

**Background and Objectives:**

The objective of this work is to develop a disease progression model as the basis for a clinical trial simulation tool to optimize the design of FA efficacy studies. The dependent variable in the model is the modified Friedreich's Ataxia Rating Scale (mFARS), a primary outcome used in contemporary FA clinical trials. The data was extracted from the Friedreich's Ataxia Integrated Clinical Database (FA-ICD), a partnership between C-Path and FARA that contains de-identified patient-level data from clinical and observational studies. The FA-ICD is accessible to qualified researchers through the Rare Disease Cures Accelerator Data and Analytics Platform (RDCA-DAP) and is being leveraged in ongoing research presented at ICAR 22 (Abstract #352*).*

**Methods:**

Using a nonlinear mixed-effects modeling approach with NONMEM software, the longitudinal dynamics of mFARS throughout the disease continuum were modeled for a cohort of 1309 subjects and 5450 observations. Several measures commonly collected in FA trials were used as covariates and added in stepwise fashion to quantify the interindividual variability.

**Results:**

Baseline severity and progression for a typical patient of 23 years old is 45.2 points and 2 points/year on the mFARS scale, respectively. Significant covariates include age at time of observation on progression rate and functional disease staging score on baseline severity. A unit increase in functional disease staging score corresponds to a 0.676-point increase in baseline severity measured by mFARS.

**Discussion and Conclusion:**

The developed mathematical model describes the longitudinal dynamics of mFARS in a diverse cohort of patients with FA using a generalized Gompertz structural framework. This model provides the foundation for the development of a clinical trial simulation tool that can be used to optimize the selection of inclusion/exclusion criteria, selection of measures, and other trial parameters to improve trial design.

# (#421) Discovery of Frataxin-E in Monkey Blood Using Triple Quadrupole Mass Spectrometry

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 421

***Dr. Ian A. Blair*** [1], ***Dr. Teerapat Rojsajjakul*** [1], ***Dr. Clementina Mesaros*** [1], ***Dr. Rod Miller*** [1], ***Dr. Christian Hinderer*** [1], ***Dr. James Wilson*** [1]

***1.*** *University of Pennsylvania*

**Background and Objective:** Monkey frataxin (mFXN, 1-210), undergoes proteolytic processing into a 130-amino acid mature mitochondrial form (mFXN-M, 81-210). We discovered that the human *FXN* gene also undergoes alternative splicing to generate a 135 amino acid extra-mitochondrial protein that we named isoform E (hFXN-E) because it was identified in erythrocytes. Rhesus monkeys are widely used as model organisms in pre-clinical Friedreich's ataxia gene therapy studies, particularly in determining how much human mature frataxin (hFXN-M) is formed in tissues and whether its expression affects tissue levels of mFXN-M. The aim of this study was to develop a method to quantify and characterize mFXN-E in Rhesus macaques.

**Methods**: A stable isotope-labeled hFXN-E standard was added to blood samples (0.5 mL) obtained from Rhesus macaques. The blood was immunoprecipitated, protease digested with Glu-C, and then subjected to ultrahigh-performance liquid chromatography coupled with multiple-reaction monitoring (UHPLC-MRM/MS) using an Agilent 1290 Infinity II LC system coupled to a 6495C triple quadrupole mass spectrometer.

**Results:** hFXN-M differs from mFXN-M by only two amino acids; E92D and A187G. To characterize mFXN-E and distinguish it from hFXN-E, a Glu-C digest was performed and the resultant peptides analyzed. This process identified a unique N-terminal peptide that exhibited a triply charged protonated molecule ($MH_3^{3+}$) at *m/z* 778.7 with a retention time of 3.92-min. Major product ions at y5 *(m/z* 628.3), y6 (*m/z* 743.3), y7 (*m/z* 856.4), and y8 (m/z 943.4) characterized the N-terminal Glu-C peptide as acetyl-$M^{76}$NLRKSGTLGHPGSLDDTTYE$^{96}$. This differed from the N-terminal hFXN-E Glu-C peptide (acetyl-$M^{76}$NLRKSGTLGHPGSLDE$^{92}$) by a four amino acid extension.

**Discussion and Conclusion:** We established a procedure to quantify and characterize mFXN-E in Rhesus macaque blood. We determined that Rhesus macaques express mFXN-E, which is 98.5% identical with hFXN-E. This foundational work will enable investigations to be conducted to determine whether gene therapy effects mFXN-E blood levels.

# (#429) Characterization of Alternative Splicing in CAG-repeat expansion Spinocerebellar Ataxias

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 429

***Ms. Asmer Aliyeva*** [1], ***Ms. Emily Davey*** [1], ***Ms. Claudia Lennon*** [2], ***Ms. Cristina DeMeo*** [2], ***Dr. John Cleary*** [1], ***Dr. Hannah Shorrock*** [1], ***Prof. Andy Berglund*** [1]

***1.*** *RNA Institute, University at Albany-SUNY,* ***2.*** *The RNA Institute*

Background and Objective

The Spinocerebellar Ataxias (SCAs) are a genetically heterogeneous group of rare, dominantly inherited neurological disorders characterized by progressive ataxia. Numerous SCAs are caused by CAG-repeat expansions (SCA1, 2, 3, 6, 7 and 12) that involve expression of toxic expansion RNAs and, in most cases, toxic polyglutamine expansion proteins. Alternative splicing (AS), the process by which RNA transcripts are processed into distinct combinations, can be detected in various body fluids, and has great potential as a non-invasive biomarker. Very little is known about AS across SCAs. To address this knowledge gap, we seek to characterize the transcriptomic changes across CAG repeat expansion SCAs to identify splicing hallmarks.

Methods

We performed RNA-Seq analysis on SCA1, SCA3 and SCA7 patient derived fibroblasts and quantified changes in AS using rMATS. These analyses were compared to publicly available RNA-Seq data from multiple CAG expansion SCA mouse models. To assess the CAG dependence of AS in SCAs, we analyzed RNA-Seq data from a previously established CAG-repeat containing HEK293T screening cell line. Gene Ontology (GO) analysis was carried out using Metascape and DAVID, and Cytoscape was used to visualize overlapping enriched pathways.

Results

Across all datasets we found widespread splicing dysregulation with skipped exon (SE) events being the most frequent class. We identified overlap in disease relevant mis-spliced SE events between the datasets suggesting that CAG-expansion SCAs exhibit dysregulation of AS that may be shared across diseases. GO analysis of SE events demonstrated enrichment of pathways known to be impaired in CAG-expansion SCAs, such as ion channel function, neurotransmitter signaling, and protein transport along microtubules.

Discussion and Conclusion

Identifying AS events common among CAG-expansion SCAs will allow for further understanding of how splicing dysregulation contributes to the disease and whether these events could be used as potential biomarkers for monitoring disease onset and progression.

# (#433) Auto-Gait: Automatic Ataxia Risk Assessment with Computer Vision on Gait Task Videos

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 433

***Mr. Masum Hasan [1], Mr. Wasifur Rahman [1], Mr. Md Saiful Islam [1], Ms. Titilayo Olubajo [2], Mr. Jeet Thaker [1], Mr. Abdelrahman Abdelkader [1], Mr. Phillip Yang [3], Dr. Tetsuo Ashizawa [4], Dr. Ehsan Hoque [1]***

***1.*** *University of Rochester,* ***2.*** *Houston Methodist,* ***3.*** *Center for Health + Technology, URMC,* ***4.*** *The Houston Methodist Research Institute*

In this paper, we investigated whether we can 1) detect participants with ataxia-specific gait characteristics (risk-prediction), and 2) assess the severity of ataxia from gait (severity-assessment) using computer vision. We created a dataset of 155 videos from 89 participants, 24 controls and 65 diagnosed with (or are pre-manifest) spinocerebellar ataxias (SCAs), performing the gait task of the Scale for the Assessment and Rating of Ataxia (SARA) from 11 medical sites located in 8 different states across the United States. We develop a computer vision pipeline to detect, track and separate out the participants from their surroundings. We also construct several features from their body pose co-ordinates to capture gait characteristics like step width, step length, swing, stability, speed, etc. Our risk-prediction model achieves 83.06% accuracy and an 80.23% F1 score. Similarly, our severity-assessment model achieves a mean absolute error (MAE) score of 0.6225 and a Pearson's correlation coefficient score of 0.7268. Our models still performed competitively when evaluated on data from sites not used during training. Furthermore, through feature importance analysis, we found that our models associate wider steps, decreased walking speed, and increased instability with greater ataxia severity, which is consistent with previously established clinical knowledge. Our models create possibilities for remote ataxia assessment in non-clinical settings in the future, which could significantly improve the accessibility of ataxia care. Furthermore, our underlying dataset was assembled from a geographically diverse cohort, highlighting its potential to further increase equity. The code used in this study is open to the public, and the anonymized body pose landmark dataset is also available upon request.

# (#449) Mitochondrial dysfunction measured by cardiopulmonary exercise testing (CPET) in Friedreich's Ataxia (FA)

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 449

***Ms. Samantha Norman*** [1], ***Ms. Mackenzi Coker*** [1], ***Mrs. Tanja Taivassalo*** [2], ***Dr. Julie Berthy*** [1], ***Dr. Sub H. Subramony*** [3], ***Dr. Manuela Corti*** [4]

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Background: FA is an autosomal recessive disease caused by a mutation in frataxin, a gene that carries a mitochondrial targeting sequence. A relative absence of frataxin leads to dysfunction of multiple mitochondrial processes. FA is a neurodegenerative disorder characterized by impaired muscle coordination and cardiomyopathy. Lower endurance and physical energy levels are also prominent.

Objective: To quantify peak aerobic capacity in FA patients through CPET using two modalities (leg and arm ergometers) and evaluate the contribution of mitochondrial dysfunction to lower endurance capacity.

Methods

FA subjects (n=20) and health controls (HC) (n=9) completed both CPET modalities using a standardized ramp protocol (5-20 W/min). Peak exercise criteria werewas considered reaching 85% predicated peak heart-rateheart rate or a respiratory exchange ratio >1.05. Respiratory gas data was collected at rest and exercise to determine peak rate of oxygen consumption ($VO_2$) and anaerobic threshold (AT). Cardiac output (CO) was measured during arm CPET using a transthoracic impedance system, allowing calculation of arterio-venous oxygen difference (a-$vO_2$ diff), a non-invasive index of oxygen utilization by muscle mitochondria.

Results

90% of FA subjects reached peak exercise criteria during arm cycling compared to 60% during leg cycling. Peak-$VO_2$ was lower ($p<0.05$) in FA compared to HC using both modalities. However, while HC achieved lower values during arm (22.3+4.0) versus leg (32.5+ 5.1) due to less muscle engagement, FA patients reached similar peak-$VO_2$ for both (arm=15.4+4.3; leg=15.2+6.2 ml/kg/min). During arm cycling, peak CO was similar in FA and HC (14.1+2.2 vs. 15.9+1.6 L/min), whereas peak a-vO2 diff was lower in FA (10.9 +2.1 vs. 14.3 + 4.2 ml/dl, $p<0.05$).

Discussion and Conclusion

Peak aerobic capacity in FA is better assessed using arm CPET. The inability to increase a-$vO_2$ diff during peak exercise supports a low AT and suggests muscle mitochondrial dysfunction as the primary cause of low peak $VO_2$.

# (#465) The C□terminal crosslinked telopeptide of type I collagen (CTX-I) as a potential cardiomyopathy biomarker in Friedreich Ataxia patients

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 465

***Dr. chiara pane*** [1], ***Dr. Assunta Trinchillo*** [1], ***Dr. Andrea Salzano*** [2], ***Dr. Angela Marsili*** [1], ***Dr. Giorgia Puorro*** [1], ***Prof. Antonio Cittadini*** [3], ***Dr. Cinzia Valeria Russo*** [1], ***Prof. Francesco Saccà*** [3]

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**Introduction**: Friedreich's ataxia (FRDA) is the most common inherited recessive ataxia. Cardiomyopathy (CM) with myocardial hypertrophy is the predominant cause of death. The presence of CM is variable and the risk factors for cardiac involvement are not entirely clear. Markers of collagen degradation, such as C□terminal crosslinked telopeptide of type I collagen (CTX-I), seem to be associated with unfavorable cardiovascular outcomes. The aim of our study was to measure serum CTX-I as a marker of cardiac fibrosis in FRDA patients.

**Methods**: We measured serum CTX value in twenty-five FRDA patients (mean age, 31.3±14.7 yr) and nineteen healthy controls (mean age, 34.0±13.5 yr). Patients underwent Echocardiography and SARA scale evaluation.

**Results**: CTX values were significantly higher in the patients than in the control group (31.82±2.27 vs 16.44±1.6 µg/L; p=0.006). CTX-I was inversely correlated with age (R = - 0,535; n=44; p < 0.001). The regression model identified disease duration and TT3 levels to be independent predictors of CTX-I (model $R^2$=0.938; intercept -64.0, p=0.071; disease duration coefficient = -2.34, p=0.005; TT3 coefficient = 127.17, p=0.011).

**Conclusions**: CTX-I, a biomarkers of collagen turnover, is elevated in FRDA and should provide complementary information to identify patients with high cardiological risk even if longitudinal studies are needed to define the role of this serologic marker of collagen metabolism in the natural history of cardiomyopathy in FRDA patients.

# (#466) Progression over a 4-year period of lower limb impairments, balance and mobility limitations in ARSACS

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 466

***Dr. Isabelle Lessard*** [1], ***Ms. Isabelle Côté*** [2], ***Prof. Luc J. Hébert*** [3], ***Dr. Bernard Brais*** [4], ***Prof. Cynthia Gagnon*** [5]

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**Background and objective:** Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a hereditary neurological disorder presenting with pyramidal (lower limbs motor impairments), cerebellar (incoordination) and neuropathic (distal muscle weakness) impairments. The highest prevalence of ARSACS is found in Canada, but is now recognized as the second most prevalent recessive ataxia in Europe. The motor impairment progression lead to significant mobility and balance limitations, which induce participation restrictions and difficulty performing activities of daily living. Although impairments and mobility limitations in ARSACS are generally well documented, only one study assessed their progression rate through longitudinal design over a 2-year period. This study aims to document lower limb impairments and mobility limitations in ARSACS over a 4-year period.

**Methods:** A total of 40 ARSACS patients were recruited in this longitudinal study based on three data collections: T1 (2013-14), T2 (2015-16) and T3 (2017-18). Participants were assessed by trained physical therapists using standard operating procedures. Many outcomes measures were used, including the Lower Extremity Motor Coordination Test (LEMOCOT), the 10-Meter Walk Test at confortable speed (10mWT), the Six-Minute Walk Test (6MWT) and the Berg Balance Scale (BERG). Regressions were made with Mixed Models for repeated measures.

**Results:** A significant effect of time is observed for the BERG (F: 11.61, p<.001), the 10mWT (F: 6.05, p= 006) and the 6MWT (F: 11.34, p <.001), but not for the LEMOCOT (p= .107). Age is a significant covariate factor in all models (p<.001), excepted for the 6MWT (p=.144). A significant interaction Age*Time is observed for balance (BERG) and walking speed (10mWT), for which older participants do not have the same progression rate than younger participants.

**Discussion:** Despite the heterogeneity of impairments and activity limitations presentation and progression in this population, we found a significant progression over time for balance and walking activities (speed and long distance).

# (#470) Reliability of perceptual and acoustic assessments of speech in Spinocerebellar Ataxia type 3

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 470

***Prof. Laura Bannach Jardim*** [1], ***Ms. Elaine Cristina Miglorini*** [2], ***Ms. Vanessa Santos*** [3], ***Prof. Maria Luiza Saraiva-Pereira*** [3], ***Prof. Vanessa Bielefeldt Leotti*** [4], ***Prof. Maira Olchik*** [3]

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Background and aims: Data on reliability and validity of speech assessments for Spinocerebellar ataxia type 3 (SCA3) are lacking. We aimed (1) to establish test-retest reliability of auditory perceptual analysis (APA) and acoustic speech analysis (ASA) in ataxic subjects, obtained from face-to-face and from telephone calls; and (2) to get data on their external validity.

Methods: 17 ataxic SCA3 individuals performed two face-to-face APA and ASA evaluations, with a 10-minute interval between them; other 20 ataxic subjects collected their samples first in person and then remotely, by telephone, up to 15 days later. All subjects collected FARS-adl and demographic data. APA were performed in a blind way, and ASA were obtained by the open software Praat. Intraclass correlation coefficient (ICC) and weighted kappa were obtained in these two settings. Correlations with age, ataxia duration and FARS-ADL were used as external validation parameters.

Results: Test-retest reliability was good/excellent (ICC> 0.7) for all APA variables and for 30 of the 45 acoustic variables under study. In the remote vs in person comparisons, ICC >0.7 was achieved by phonation, breathing, resonance, prosody and the degree of dysarthria, in APA; and maximum phonation time, PATAKA-phonation-time, number of syllables in PATAKA and minimum fundamental frequency in interrogation, in ASA. Of note, all APA evaluations except prosody correlated with FARS-adl; for remote assessments, ASA parameters maximum phonation time and PATAKA-phonation-time correlated with ataxia duration and with FARS-ADL.

DIscussion and interpretation: Most of the variables of speech collected in person showed good/excellent reliability in SCA3 ataxic subjects. When the speech was collected by telephone, five variables from APA and four from ASA maintained good to excellent reliability. This data supports the application of these nine variables in future studies with simultaneous remote and face-to-face application, and of 36 variables in exclusively face-to-face studies.

# (#476) Impact of dual tasking on balance in FXTAS and potential prodromal postural sway deficits in asymptomatic FMR1 premutation carriers

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 476

***Dr. Joanne O'Keefe, PhD, PT*** [1], ***Ms. Nancy Cao*** [1], ***Dr. Erin Robertson-Dick*** [1], ***Ms. Nicollette Purcell*** [1], ***Ms. Emily Timm*** [1], ***Ms. Yuanqing Liu*** [1], ***Dr. Deborah Hall*** [1]

***1.*** *Rush University*

**BACKGROUND and OBJECTIVES:** Fragile X-associated tremor/ataxia syndrome (FXTAS) is a neurodegenerative disorder occurring in some *FMR1* premutation carriers (PMC) and is characterized by cerebellar ataxia, tremor and cognitive deficits which negatively impact balance and increase fall risk. Dual-task (DT) cognitive-motor paradigms may have the capacity to reveal impairments not present under single-task (ST) conditions. Markers of FXTAS onset are needed to provide preventative treatment interventions. Our aims were to determine: 1) the impact of DT interference on balance in FXTAS and 2) whether environmentally and cognitively challenging tasks uncover postural deficits in asymptomatic PMC.

**METHODS:** Participants with FXTAS (n = 34; 69.0 + 8.3 years), PMC without FXTAS (n=34; 54.9 + 9.5 years) and controls (n = 48; 64.0 + 10.5 years) underwent balance testing using an inertial sensor system (APDM$^{TM}$). Stance (feet apart (FA)/together (FT)/tandem), vision (eyes open (EO)/closed (EC)), surface stability (firm/foam), and cognitive demand (ST/DT) were manipulated in 30 second trials. A concurrent verbal fluency task was used in DT conditions.

**RESULTS:** FXTAS subjects had significantly greater total sway area, jerk, and RMS sway under all test conditions but less dual-task costs (DTC) for jerk than controls during the FTEC condition. PMC without FXTAS has significantly greater RMS sway compared to controls in the ST/FAEC and tandem/EC condition and less DTC for jerk in the FAEC condition. In several conditions PMC without FXTAS were approaching the FXTAS level of balance dysfunction.

**DISCUSSION and CONCLUSION:** Balance deficits under tandem/EC conditions in PMC without FXTAS might represent prodromal signs of the disease. Participants with FXTAS had reduced DTC for jerk which suggests they prioritize balance over cognition while dual tasking. This information may be useful in the design of treatment strategies to improve balance and prevent falls in individuals with FXTAS and to provide sensitive biomarkers of FXTAS onset.

# (#484) Clinical scales and quality of life in pre-ataxic and ataxic carriers of spinocerebellar ataxia type 10: preliminary results from CAHSCA10 study

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 484

***Dr. Ali Hasan [1], Ms. Rafaella Mergener [1], Mr. Gabriel Vasata Furtado [1], Dr. Tetsuo Ashizawa [2], Prof. Maria Luiza Saraiva-Pereira [3], Prof. Laura Bannach Jardim [1]***

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**Background and objectives:** Spinocerebellar ataxia type 10 (SCA10) is due to intronic (ATTCT)n repeat expansions at ATXN10. Variable expressivity, 85-96% of penetrance and slow progression are characteristic. We performed an exploratory study of ataxia scales and quality of life, to obtain knowledge about pre-ataxic and ataxic phases.
**Methods:** Data obtained from symptomatic carriers and relatives at 50% risk included: ages at onset of gait ataxia (AOga) and demographic information; EQ-5D and FARS-adl questionnaires; NESSCA, SARA, ICARS, and INAScount; and DNA. Three groups were analyzed after blind-genotypes: ataxic (SARA > 2.5), pre-ataxic, and controls. Non-parametric statistics were performed; mean±SD were presented for a p level of 0.05; p between 0.099 and 0.051 characterized “trends”.
**Results:** Twelve ataxics (15.5±10.4 years since AOga), 3 pre-ataxic carriers (20, 36 and 58 yo), and 7 controls with similar ages, were included. Epilepsy was present in 9/12 ataxics. AOga and of epilepsy were 32.1±9.7 and 34.6±5.2 years. Ataxics with and without epilepsy had similar ages and AOga, but EQ-VAS, FARS-adl, NESSCA and ICARS tended to be worse in epileptic subjects. When the three groups were compared, significant differences in all questionnaires and scales were obtained between ataxics and controls. There was a trend to a stepwise worsening from controls to pre-ataxics and then to ataxic carriers in FARS-adl (0.14±0.38, 1.33±1.52, and 13±7.6) and NESSCA scores (1.43±1.13, 2±1, and 11.8±5). Pre-ataxics presented sensory losses (2/3), limb ataxia, pyramidal findings, and cramps (⅓, each).
**Discussion and interpretation:** Quality of life, activities of daily living, and neurological scales were impacted in ataxic subjects. Seizures seemed to be associated with worse evolution. Fewer than expected pre-ataxic carriers were included. Their results suggest that some burden start earlier than ataxia.
Acknowledgements: CNPq and NIH

# (#495) Preliminary data on segregation analysis of SCA10 in families from South Brazil - CAHSCA10 study

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 495

***Dr. Ali Hasan [1], Ms. Rafaella Mergener [1], Mr. Gabriel Vasata Furtado [2], Dr. Tetsuo Ashizawa [3], Prof. Maria Luiza Saraiva-Pereira [4], Prof. Laura Bannach Jardim [1]***

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Background and objectives: Spinocerebellar ataxia type 10 (SCA10) is due to dominant intronic (ATTCT)n repeat expansions at ATXN10. Since data on allele segregation is lacking, we performed an exploratory study on this characteristic among SCA10 families.

Methods: Carriers and their relatives older than 18 years, from all SCA10 families from Rio Grande do Sul, Brazil, were invited to participate. Demographic data, symptomatic status and a DNA sample were obtained from all. Genotypes were performed in a double blind way. Two kinds of sibships were included in segregation analysis: those with >1 sib and completely genotyped; and sibships with >2 sibs and only one sib non genotyped. The group of non genotyped sibs was then arbitrated to have 50% of carriers and 50% of non-carriers (mimicking a mendelian segregation). Chi-square test was performed for a $p<0.05$

Results: seven SCA10 families were included and informed about 168 relatives (136 without symptoms), but only 22 subjects were genotyped. Six sibships from four families were informative. If the arbitrary genotype was not attributed, 11/16 (69%) sibs were carriers and 5/16 (31%) were non-carriers. If the arbitrary genotype was attributed, then 13/20 (65%) sibs were carriers and 7/20 (35%) were non-carriers (chi-square = 1.166, non significant).

Discussion and interpretation: there seems to be a trend pointing to a segregation distortion favoring the expanded alleles. However, the number of informative sibs is still quite small, preventing significance, and putting the analyzes still at risk of type II error. More sibships completely genotyped are needed to confirm or reject this hypothesis.

This work was supported by CNPq (Brazilian Government) and NIH (National Institute of Health)

# Poster Sessions: Imaging

# (#79) Localised Changes in Dentate Nucleus Shape and Iron Concentration in Friedreich Ataxia Assessed Using Quantitative Susceptibility Mapping

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 79

***Mr. Ikhsan Karim [1], Dr. Louisa P. Selvadurai [2], Dr. Sirio Cocozza [3], Dr. Scott Kolbe [4], Dr. Giuseppe Palma [5], Prof. Francesco Saccà [6], Prof. Nellie Georgiou-Karistianis [7], Dr. Ian Harding [4]***

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Background: The dentate nuclei of the cerebellum are key sites of pathology in people with Friedreich ataxia (FRDA). Previous studies have reported reduced dentate nucleus volume and increased mean iron concentration in people with FRDA using quantitative susceptibility mapping (QSM), a form of magnetic resonance imaging (MRI). However, it remains unknown whether these changes are spatially nonuniform, with some areas of the dentate nuclei affected more than others.

Methods: QSM data was acquired from 49 people with FRDA and 46 healthy controls in Melbourne, Australia and Naples, Italy using 3-Telsa MRI scanners. The dentate nuclei were manually traced. Regional changes in structure were assessed using 3D shape modelling to quantify areas of relative surface contraction or expansion. Localised differences in susceptibility – a proxy for iron concentration – were assessed using dentate-optimised voxel-based analyses. Between-group differences and correlations with ataxia severity, disease duration, and symptom onset age were assessed using regression models, with false-discovery rate correction for multiple comparisons ($p_{FDR}<0.05$).

Results: Individuals with FRDA, relative to healthy controls, showed bilateral surface contraction most strongly in rostral and caudal regions. The magnitude of surface contraction in these areas correlated with disease duration and ataxia severity. Conversely, increased susceptibility in the FRDA cohort was maximal in dorso-medial areas. These increases also correlated with disease duration and ataxia severity. No significant associations with symptom onset age were evident.

Discussion: Structural changes in the dentate nuclei in FRDA are not spatially uniform, and the spatial profile of atrophy and iron changes are unique. Evidence for atrophy is strongest in areas with high grey matter density, while progressive iron increases appear to predominate in the medial white matter. Regional changes within the dentate nuclei may therefore provide more specific biomarkers than whole-nucleus measures, a compelling hypothesis for future longitudinal studies.

# (#110) Spinal cord degeneration in Friedreich's Ataxia: Results from the Enigma-Ataxia working group

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 110

***Dr. Thiago Rezende*** [1], ***Dr. Isaac Adanyeguh*** [2], ***Dr. Filippo Arrigoni*** [3], ***Dr. Benjamin Bender*** [4], ***Dr. Fernando Cendes*** [1], ***Dr. Louise A Corben*** [5], ***Dr. Andreas Deistung*** [6], ***Prof. Martin B Delatycki*** [7], ***Dr. Imis Dogan*** [8], ***Prof. Gary Egan*** [9], ***Dr. Sophia Göricke*** [10], ***Prof. Nellie Georgiou-Karistianis*** [11], ***Dr. Pierre-Gilles Henry*** [12], ***Dr. Diane Hutter*** [12], ***Dr. Neda Jahanshad*** [13], ***Dr. james joers*** [12], ***Dr. Christophe Lenglet*** [12], ***Dr. Tobias Lindig*** [4], ***Dr. ALberto Martinez*** [1], ***Dr. Andrea Martinuzzi*** [14], ***Dr. Gabriella Paparella*** [15], ***Dr. Denis Peruzzo*** [16], ***Dr. Kathrin Reetz*** [8], ***Dr. Sandro Romanzetti*** [8], ***Prof. Jörg Schulz*** [8], ***Prof. Ludger Schöls*** [17], ***Prof. Matthis Synofzik*** [18], ***Dr. Sophia Thomopoulos*** [19], ***Dr. Paul Thompson*** [20], ***Dr. Dagmar Timmann*** [21], ***Dr. Ian Harding*** [9], ***Prof. Marcondes C. França Jr*** [1]

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**Background and Objective:** Spinal cord (SC) damage has been recognized as a hallmark of Friedreich Ataxia (FRDA) since its first description. However, the temporal course of such damage and its clinical correlates remain unclear. Therefore, we performed a characterization of SC structure and its relationship with measures of disease progression in a very large FRDA cohort.

**Methods:** We performed a retrospective cross-sectional analysis of cervical spinal cord structure (from C1 to C4) using MRI data from 8 sites within the ENIGMA-Ataxia working group, totaling 256 patients with FRDA and 223 age-gender matched healthy controls. Data processing was undertaken using harmonised protocols. Ataxia severity was quantified using normalized scores based on standard neurological rating scales (Actual score/Maximal possible score).

**Results:** Individuals with FRDA, relative to all controls, had significantly reduced cross-sectional area (CSA) at all examined vertebral levels, with very large effect sizes ($d$>2.1) and significant correlations with normalized disease severity ($r$<-0.4). Similarly, we found significantly increased eccentricity (ECC) in all vertebral levels, also with very

large ES ($d$>1.2) although substantially smaller in comparison to CSA and no significant correlations. Subgroup analyses based on disease duration and ataxia severity showed that CSA and ECC are already abnormal in early stages of the disease. However, while CSA decreased progressively with greater duration/severity, ECC remained stable.

**Discussion and Conclusion:** ECC and CSA are considered surrogate MRI markers of dorsal column (DC) and corticospinal tract (CST) damage in FRDA, respectively. Hence, our data support the hypothesis that damage to DC and CST follow distinct courses in the disease: non-progressive (ie, developmental) changes likely define the DC, whereas alterations in the CST may be both early and progressive (ie, developmental+degenerative) disease features. These results provide new insights about FRDA pathogenesis and indicate that SC MRI may be a useful biomarker to track the disease progression.

# (#111) Quantitative characterization of progressive neurodegeneration in Friedreich ataxia from a comprehensive time-efficient multi-parametric MRI protocol

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 111

***Dr. Koene Van Dijk*** [1], ***Dr. Courtney Bishop*** [2], ***Dr. James O'Callaghan*** [2], ***Dr. James Goodman*** [1], ***Dr. Laigao Chen*** [1], ***Dr. Lawrence Charnas*** [3], ***Dr. Eugenii A. Rabiner*** [4], ***Prof. Richard Festenstein*** [5]

***1.*** *Digital Medicine and Translational Imaging, Early Clinical Development, Pfizer,* ***2.*** *Invicro, London,* ***3.*** *Rare Disease Research Unit, Pfizer,* ***4.*** *Invicro, London; and Centre for Neuroimaging Sciences, IoPPN, King's College, London,* ***5.*** *Department of Brain Sciences, Imperial Clinical Research Facility and BRC (NIHR), Imperial College London*

Friedreich ataxia (FA) is a rare autosomal recessive disorder caused by genetic mutations that result in deficits in the mitochondrial protein frataxin. Patients experience sensory and cerebellar ataxia along with cardiac and other organ dysfunction. Here we quantitatively characterize progressive neurodegeneration in FA using a comprehensive, time-efficient multi-parametric MRI (mpMRI) protocol for potential use in natural history studies and clinical trials.

Twelve FA patients were scanned (4 males, 8 females, age = 34.1 ± 15.6 yrs, disease duration = 11.8 ± 7.5 yrs) on a 3T Siemens MRI scanner. Mean SARA score was 14.8 ± 4.7. The mpMRI protocol included: T1w and T2w imaging for anatomy; R2* mapping for iron content, specifically of the dentate nucleus; diffusion weighted imaging and a magnetization transfer imaging to characterize white matter structures; and magnetic resonance spectroscopy (MRS) for the estimation of metabolite concentrations in a single voxel covering the right dentate nucleus. Age-matched controls from the MIND-MAPS database (6 males, and 6 females, age = 35.7 ± 14.8) facilitated group comparisons for most MRI endpoints.

Evidence of cerebral and cerebellar volumetric changes were found in FA patients compared to controls, generally few regional R2* differences, but clear white matter microstructural changes (e.g. the inferior and superior cerebellar peduncle). Within the FA group, we found reduced myelin content in the superior cerebellar peduncle compared to a control/reference region, and generally good quality MRS.

The results suggest the potential for rich, longitudinal quantitative phenotyping of multiple features of the central nervous system in FA using a short but comprehensive mpMRI protocol with a total scan time of less than 1 hour. Larger cross-sectional and longitudinal studies are needed to confirm a role of non-invasive imaging biomarkers in patient stratification and assessment of treatment effects in clinical trials.

# (#203) Mapping Cerebello-Thalamo-Cortical Pathways in Friedreich Ataxia: The IMAGE-FRDA Study

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 203

***Dr. Louisa P. Selvadurai*** [1], ***Dr. Louise A Corben*** [2], ***Prof. Karen Caeyenberghs*** [3], ***Dr. Juan F Domínguez D*** [3], ***Prof. Gary Egan*** [4], ***Prof. Martin B Delatycki*** [5], ***Dr. Ian Harding*** [4], ***Prof. Nellie Georgiou-Karistianis*** [1]

***1.*** *Turner Institute for Brain and Mental Health, School of Psychological Sciences, Monash University, Clayton, VIC, Australia,* ***2.*** *Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia, School of Psychological Sciences, The Turner Institute for Brain and Mental Health, Monash University, Clayton, Victoria, Australia,,* ***3.*** *School of Psychology, Deakin University,* ***4.*** *Monash University,* ***5.*** *Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia,*

**Background:** Friedreich ataxia (FA) is associated with brain structural aberrations in the cerebellum, brainstem, and cerebrum. Dentato-thalamo-cortical white matter pathways, which connect the cerebellum and cerebrum, appear to be particularly implicated, but there has been limited work isolating these pathways in FA. Furthermore, it is unknown whether pathway disruptions are related to the magnitude of changes observed in the gray matter regions that they connect, namely the dentate nucleus, thalamus, and cerebral cortex.

**Methods:** Whole-brain T1- and diffusion-weighted magnetic resonance images (MRI) were acquired from 20 individuals with FA and 28 age- and gender-matched controls. Probabilistic tractography (MRtrix3) was used to reconstruct white matter pathways connecting the dentate nuclei to the contralateral thalami, and from thalami to ipsilateral primary motor, supplementary motor, and dorsal and ventral premotor areas. White matter connectivity within these pathways, based on white matter fiber volume, was compared between the FA and control groups and correlated against Friedreich Ataxia Rating Scale (FARS) scores and age of disease onset. Correlations between fiber connectivity and gray matter measures (dentate nucleus volume, thalamus volume, motor cortex thickness) were evaluated.

**Results:** Fiber connectivity between the dentate nucleus and thalamus, and the thalamus and primary motor area, was significantly lower in the FA group compared to the control group. Greater FARS scores were associated with lower connectivity between the thalami and dorsal premotor areas, and earlier onset age associated with lower connectivity between the left dentate and right thalamus. There was no strong evidence of relationships between white matter connectivity and adjacent grey matter structure.

**Conclusions:** White matter connectivity in FA is disrupted along all sections of the dentato-thalamo-cortical pathway, and is influenced by disease parameters. This work further confirms the loss of cerebello-cerebral connectivity in FA, with further investigation required to determine any relationship between this loss and cerebral structure.

# (#300) Advanced multimodal MRI detects preataxic and early-stage alterations in SCA1 and SCA3 with high sensitivity

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 300

***Ms. Jayashree Chandrasekaran [1], Dr. Young Woo Park [1], Dr. Emilien Petit [2], Dr. Sophie Tezenas du Montcel [3], Dr. Michal Povazan [4], Dr. Guita Banan [5], Dr. Romain Valabrègue [6], Dr. Philipp Ehses [7], Dr. Jennifer Faber [8], Dr. james joers [9], Dr. Pierrick Coupé [10], Dr. Chiadi U. Onyike [4], Dr. Peter Barker [11], Dr. Jeremy D. Schmahmann [12], Dr. Eva-Maria Ratai [13], Dr. Sub H. Subramony [14], Dr. Thomas Mareci [15], Dr. Khalaf O. Bushara [16], Dr. Henry Paulson [17], Prof. Alexandra Durr [18], Dr. Thomas Klockgether [19], Dr. Tetsuo Ashizawa [20], Dr. Christophe Lenglet [9], Dr. Gulin Oz [21]***

***1.*** *Center for Magnetic Resonance Research, University of Minnesota,* ***2.*** *Paris Brain Institute (ICM), Sorbonne Université,* ***3.*** *Sorbonne University,* ***4.*** *Johns Hopkins University School of Medicine, Baltimore, Maryland,* ***5.*** *University of Florida, Gainesville, Florida,* ***6.*** *Sorbonne University, Paris,* ***7.*** *German Center for Neurodegenerative Diseases (DZNE), Bonn,* ***8.*** *Neurology Univ. Hospital Bonn, DZNE,* ***9.*** *Center for Magnetic Resonance Research, Department of Radiology, University of Minnesota, Minneapolis, MN, USA,* ***10.*** *University of Bordeaux, Paris,* ***11.*** *Johns Hopkins University, Baltimore,* ***12.*** *Massachusetts General Hospital,* ***13.*** *Massachusetts General Hospital, Charlestown,* ***14.*** *McKnight Brain Institute, Department of Neurology, University of Florida, Gainesville, Florida, United States of America,* ***15.*** *Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL, United States of America,* ***16.*** *Department of Neurology, University of Minnesota, Minneapolis,* ***17.*** *University of Michigan,* ***18.*** *ICM,* ***19.*** *German Center for Neurodegenerative Diseases (DZNE),* ***20.*** *The Houston Methodist Research Institute,* ***21.*** *Center for Magnetic Resonance Research, Department of Radiology, University of Minnesota, Minneapolis, Minnesota, United States*

**Background:**

Morphometric, microstructural, and neurochemical alterations detected by MRI/MRS have been reported at early stage in SCAs. READISCA (https://readisca.org/) aims to validate MR biomarkers during preataxic (SARA<3) and early stages (SARA=3-9) of SCA1 and SCA3.

**Methods:**

Structural, diffusion and spectroscopic MR data were acquired from 107 participants at 3T at 6 sites with an adapted Lifespan Human Connectome Project protocol for MRI and a semi-LASER protocol for MRS.

Morphometric (whole-brain), microstructural (white-matter (WM) ROIs), and neurochemical (pons and cerebellar WM) metrics were analyzed blinded to diagnosis. MR measures were compared between preataxic (n=11 SCA1, 28 SCA3), ataxic (n=14 SCA1, 37 SCA3) SCA and control (n=17) groups using non-parametric testing accounting for multiple comparisons.

**Results and Discussion:**

Atrophy in brainstem and cerebellar structures, lower fractional anisotropy (FA) and higher diffusivity in the cerebellar peduncles, pons, corona radiata and striatum, lower total *N*-acetylaspartate (tNAA, neuronal marker), higher *myo*-inositol (Ins, glial marker) and total creatine (tCr) distinguished ataxic SCA1 and SCA3 from controls.

Notably, medulla, superior cerebellar peduncles were atrophied, and inferior cerebellar peduncles (ICP) and pontine crossing tracts showed microstructural abnormalities at preataxic stage in *both* SCA1 and SCA3. Consistently, pontine tNAA, Ins, and tCr in SCA1 and pontine Ins in SCA3 were significantly different from controls at preataxic stage SCAs.

MR metrics were significantly correlated with clinical measures including SARA, activities of daily living, INAS (Inventory of Non-Ataxia Signs) count and estimated time-to-onset for most of these regions.

Medulla, pons, and cerebellar peduncles are the earliest sites of involvement in both SCAs. Receiver operating

characteristics analyses showed that neurochemical (tCr) and microstructural (FA of ICP) markers have the highest sensitivity (AUC>0.9) to detect abnormalities at the preataxic stage of SCA1 and SCA3, respectively. Multimodal MR measures provide sensitive biomarkers of the earliest disease-related changes in the brain in SCAs.

**Acknowledgements**: NIH-U01-NS104326

# (#356) Accurate cerebellar cortex segmentation: a deep learning-based approach

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 356

***Mr. Diogo Hideki Shiraishi [1], Mr. Guilherme Wertheimer [1], Ms. Isabeli Miyoshi [1], Mr. Vinicius Yacoub [1], Mr. Thiago Haiter [1], Dr. Fabiano Reis [1], Dr. Fernando Cendes [1], Prof. Marcondes C. França Jr [1], Dr. Thiago Rezende [1]***

***1.*** *University of campinas*

Background/Objective: Cerebellum segmentation is a challenging task due to the intricately folded cortex and proximity to the cerebral cortex. Although several tools present reasonable results in healthy controls, the segmentation may be catastrophic in patients with cerebellar damage. Hence, we propose to develop an accurate deep learning-based MRI tool to segment abnormal and non-abnormal cerebellums, focusing on obtaining sensitive variables to measure the impact of modifier agents of new therapies for cerebellar neurodegenerative disorders.

Methods: We used T1-weighted MRI sequences acquired in a 3T Philips Achieva at University of Campinas. The dataset is composed of healthy controls, SCA1, SCA3, and FRDA patients, 67 images in total. Expert neuroradiologists segmented the images following a protocol, with attention to cerebellar fissures. To assess the relevance of our specialist-labeled data, we trained a baseline 3D U-Net model and compared it with ACAPULCO and CERES, state-of-the-art models.

Results: Our initial baseline model (BM) achieved 0.921 for Dice score on the validation set. A reproducibility test with the Kirby-21 dataset reported an intraclass correlation coefficient of 0.998 (ACAPULCO: 0.991, CERES: 0.998). Using a 14-image test set, we found a mean Dice of 0.928±0.030 for BM (ACAPULCO: 0.909±0.043, CERES: 0.918±0.039). In patients with the highest level of cerebellar damage, this difference is sustained (BM: 0.924±0.033, ACAPULCO: 0.904±0.047, CERES: 0.917±0.044). T-paired tests on out-of-sample data revealed a statistically significant difference between BM and ACAPULCO ($p<0.001$) and CERES ($p=0.001$) on control samples and again on SCA3 and FRDA patients ($p<0.001$ on both). Visual inspection of output masks produced by our model revealed precise segmentations, especially of cerebellar fissures.

Discussion/Conclusion: Indeed, by adopting a data-centric approach, our initial model showed promising results and was able to overcome established tools presenting precise segmentation of degenerated fissures.

# (#386) Spinocerebellar ataxia type 6 damage extends beyond cerebellum: data from spinal cord imaging.

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 386

***Dr. Lucas Branco*** [1], ***Mr. Breno Massuyama*** [2], ***Mr. Gustavo Lamas*** [1], ***Ms. Camila Lobo*** [1], ***Ms. Maria Paula Azevedo*** [1], ***Ms. Carelis Gonzalez-Salazar*** [1], ***Prof. Jose Luiz Pedrozo*** [2], ***Prof. Orlando Barsottini*** [2], ***Dr. Thiago Rezende*** [1], ***Prof. Marcondes C. França Jr*** [1]

***1.*** *Department of Neurology, School of Medical Sciences – University of Campinas (UNICAMP), Campinas, SP, Brazil,* ***2.*** *Department of Neurology, Federal University of Sao Paulo (UNIFESP), Sao Paulo, Sao Paulo, Brazil*

**Background and objective**: Spinocerebellar ataxia type 6 (SCA6) is a rare inherited disease and often considered a prototype of pure cerebellar ataxia. Although autopsy studies describe cerebellum and inferior olive atrophy as hallmarks of SCA6, spinal cord degeneration has also been related, but in vivo data are scarce. The present study aimed to assess spinal cord involvement through MRI and its clinical correlates in a Brazilian SCA6 cohort.

**Methods**: Seventeen SCA6 individuals with genetic confirmation underwent clinical evaluation and MRI examination on a 3T Phillips scanner at the same day. Four were excluded due to compressive myelopathy. Thirteen age-and-sex matched healthy controls were also assessed. The *Spinal Cord Toolbox* (SCT) was employed to assess spinal cord area and eccentricity in C1-T2 levels and white matter tracts integrity, by assessing fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD) and mean diffusivity (MD), in C2-C5 levels. Imaging data were compared between groups through a Mann-Whitney U test, employing age and sex as covariates, and Bonferroni correction for multiple comparisons was performed. Correlations between imaging and clinical data were assessed through Spearman's rank correlation coefficients.

**Results**: Mean age of patients, SARA score and disease duration were 68.1±7.0 years, 15.0±6.9 and 15.3±6.9 years, respectively. SCA6 group had increased AD in left tectospinal tract (p=0.023), left lateral vestibulospinal tract (p=0.027) and left ventral corticospinal tract (p=0.045), increased MD in right lateral vestibulospinal tract (p=0.032) and left tectospinal tract (p=0.019), and increased RD in right lateral vestibulospinal tract (p=0.032). These parameters did not correlate with clinical data but, in an exploratory approach, left ventral corticospinal tract RD was directly related to INAS scale (p=0.011, r=0.699).

**Discussion and Conclusion**: SCA6 damage extends beyond cerebellum and quantitative spinal cord MRI might be a useful biomarker in this setting.

# (#501) Nigroestriatal dysfunction: a frequent finding in RFC-1 related disorder

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 501

***Ms. Maria Azevedo [1], Ms. Camila Lobo [2], Dr. Lucas Branco [2], Mr. Gabriel da Silva Schmitt [1], Ms. Paula Matos [3], Dr. Alberto Martinez [1], Prof. Jose Pedroso [3], Prof. Orlando Barsottini [3], Prof. Barbara Juarez [1], Prof. Marcondes C. França Jr [4]***

***1.*** *Departament of Neurology, School of Medical Sciences – University Of Campinas (UNICAMP),Campinas, SP, Brazil,* ***2.*** *Department of Neurology, School of Medical Sciences – University of Campinas (UNICAMP), Campinas, SP, Brazil,* ***3.*** *Departament of Neurology, Federal University of São Paulo (UNIFESP), São Paulo, SP, Brazil,* ***4.*** *University of campinas*

**Background and Objective:** RFC1/CANVAS syndrome is a rare heredodegenerative disorder characterized primarily by cerebellar ataxia, sensory neuronopathy (ganglionopathy) and bilateral vestibular hypofunction, caused by biallelic AAAAG RFC1 expansions. Parkinsonism is now recognized as an additional feature in the disease, however no systematic evaluation of nigrostriatal dopaminergic function has been published so far. To describe striatal dopamine transporter (DAT) density in a sample of RFC1/CANVAS patients using dopaminergic transporter brain scintigraphy with 99mTc-TRODAT-1 (DAT imaging).

**Methods:** Observational, single-center study conducted in a tertiary referral hospital, which analyzed 10 patients with molecular confirmation of RFC1/CANVAS. Each subject was evaluated for the presence of parkinsonian features. Disease severity was assessed with the SARA scale. DAT imaging was acquired and reconstructed 4 hours after venous injection of 99mTc-TRODAT-1. An experienced nuclear physician performed the visual analysis of all images. Quantification was performed drawing region of interest (ROI) in the striatum, putamen and caudate bilaterally. The specific uptake (mean uptake ratio) of each region was calculated using occipital region uptake as the reference.

**Results:** Patients there were 7 women, had a mean age of 63 ± 9.2 years. Seven patients had abnormal DAT imaging results: reduced uptake in left putamen (100%), right putamen (85.7%), left caudate (71.4%) and right caudate (71.4%). Four patients (57.1%) had bilateral striatum abnormalities. The mean SARA score was 16.9 ± 5.8. Parkinsonism was noticed in 3/7 patients, all of which had abnormal DAT scans.

**Conclusions:** Nigrostriatal dysfunction is frequent in RFC1/CANVAS, suggesting that disease pathology is more widespread than previously thought. Additionally, the fact that patients without parkinsonism also had abnormal DAT imaging results might suggest that DAT imaging changes occur early along the disease course. These results have practical therapeutic relevance and suggest that dopaminergic agents might be useful at least for a subgroup of patients with RFC1-related disorder.

# Poster Sessions: Emerging Therapies (preclinical)

# (#12) Pharmacological inhibition of ATXN1 protein aggregation

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 12

***Mr. Ioannis Gkekas [1], Dr. Sotirios Katsamakas [2], Mr. Stelios Mylonas [3], Dr. Apostolos Axenopoulos [3], Dr. Theodora Kalogeropoulou [2], Dr. Petros Daras [3], Dr. Spyros Petrakis [1]***

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**Background and objective:** Spinocerebellar ataxia type 1 (SCA1) is a neurodegenerative disease caused by a trinucleotide (CAG) repeat expansions within the coding region of *ATXN1* gene. These expansions result in longer polyglutamine (polyQ chains) in the produced protein. A striking feature of polyQ-expanded ATXN1 is its ability to form toxic species and misfold into protein oligomers which slowly aggregate into larger insoluble intranuclear inclusions. Formation of polyQ inclusions is accompanied by selective neurodegeneration mainly in the Purkinje cells in the cerebellum.

It is widely accepted that the polyQ tract is the main determinant of ATXN1 protein aggregation. However, experimental evidence indicates that the AXH domain of ATXN1 is responsible for the dimerization/oligomerization of the polyQ-expanded protein. Here, we report the identification of compounds that are predicted to bind to the dimerization site (AXH domain) of ATXN1 and might suppress polyQ-expanded ATXN1 protein aggregation.

**Methods and Results:** We have simulated the dimerization of AXH domain and performed an *in silico*/virtual screening for molecules that would bind to it. Starting from large libraries of 500k+ molecules and by applying pharmacological criteria in pre- and post-filtering steps, we have identified 44 molecules that are predicted to bind to the AXH domain of ATXN1. In parallel, we have set up an *in vitro* cell-based assay (LuTHy) with high sensitivity and specificity for the quantification of AXH domain dimerization. Selected compounds will be tested whether they inhibit AXH domain dimerization in a high-throughput format and their effect will be quantified by luminescence measurements in LuTHy assays. Hit molecules will be further tested whether they affect ATXN1 protein aggregation in a previously established cell model.

**Discussion and Conclusions:** These experiments may lead to the identification of compounds that would block the aggregation of the pathological ATXN1 protein.

# (#24) AtaxiaV: A 3D-Environment Based System that Provides Quantitative Diagnosis and Treatment for Patients with Ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 24

***Mr. Donggun Kwak*** [1], ***Mr. Mincheol Song*** [2], ***Mr. Hemosoo Woo*** [2], ***Mrs. Chuyeon Lee*** [2]

***1.*** *Massachusetts Institute of Technology,* ***2.*** *Voice of Calling NPO*

Ataxia is a degenerative disease of the nervous system, similar to Parkinson's disease, caused by damage to the cerebellum. Patients can have trouble walking, speaking, swallowing, or moving their eyes. In some cases, Ataxia can even lead to death. Although it is a severe disease, it is rare, so data and research are lacking. To evaluate the ataxia of the upper limbs, neurological tests include the finger-to-finger test, the finger-to-nose test, and the peg test. The problem with these tests is that they are qualitative so the results may differ between physicians.

So, we made AtaxiaV, a program that works as both an evaluation system and a rehabilitation exercise system. It would initially allow patients to draw lines, rectangles, and other shapes while tracking the location of their fingers to obtain quantitative data on their movement paths and mobility. It has now been extended to have games in a 3D environment for rehabilitation exercises. Currently, AtaxiaV consists of several games like the Touching Rhythm Game, the Throwing Ball Game, a voice recognition maze game, and a drawing and coloring game. These games would help a patient practice speaking and treat a patient's lack of coordination, deterioration of fine motor skills, and eye movement abnormalities.

AtaxiaV will allow us to help doctors diagnose Ataxia objectively and accurately. The game-like environment of this program encourages patients to actively continue with their treatment on their own. AtaxiaV requires relatively simple equipment, just a laptop and a small motion sensor called the Leap Motion Controller. It also does not require a professional assistant and can be used anywhere. Furthermore, AtaxiaV is expected to assist in developing treatment methods and drugs for Ataxia patients and assist in testing these treatments' effectiveness. (ataxiav.com)

# (#40) Endurance exercise ameliorates phenotypes in Drosophila models of Spinocerebellar Ataxias

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 40

***Dr. Alyson Sujkowski*** [1]***, Ms. Kristin Richardson*** [2]***, Mr. Matthew Prifti*** [1]***, Prof. RJ Wessells*** [2]***, Prof. Sokol Todi*** [3]

***1.*** *Wayne State University School of Medicine Department of Pharmacology,* ***2.*** *Wayne State University School of Medicine Department of Physiology,* ***3.*** *Wayne State University*

Endurance exercise is a potent intervention with widespread benefits proven to reduce disease incidence and impact across species. While endurance exercise supports neural plasticity, enhanced memory, and reduced neurodegeneration, less is known about the effect of chronic exercise on the progression of movement disorders such as ataxias. Here, we focused on four different types of ataxias, Spinocerebellar Ataxias Type (SCAs) 1, 2, 3 and 6, belonging to the polyglutamine (polyQ) family of neurodegenerative disorders. In *Drosophila* models of these SCAs, flies progressively lose motor function and accumulate levels of toxic SCA proteins. Excitingly, we observe dramatic protection of speed and endurance in exercised SCA2 flies and modest protection in exercised SCA6 models, while no benefit is observed in either SCA1 or SCA3 flies. Importantly, causative protein levels are reduced in SCA2 flies after chronic exercise, but not in SCA3 models, linking protein levels to exercise-based benefits. Currently, we are focusing on the activation of exercise-mimicking genes in SCA-model flies in order to define the mechanisms by which exercise preserves function in polyQ ataxias. The exercise-inducible protein *dSestrin* suppresses longitudinal mobility decline and improves early mortality in SCA2 flies, even without exercise. Furthermore, overexpression of *dSestrin* mimics exercise-induced reductions in disease protein in SCA2 flies by increasing autophagic flux. These improvements critically depend on previously-established functions of *dSestrin* that reduce oxidative damage and modulate mTOR activity. Our study suggests differential responses of ataxia disorders to exercise, highlighting the potential for more extensive application of exercise-based therapies in the prevention of polyQ neurodegeneration. Defining the mechanisms by which exercise suppresses polyQ ataxias will inform disease targets driving individual polyQ disorders and will open the door for more effective treatment.

# (#48) Removal of the GAA repeat in the heart of a Friedreich's ataxia mouse model using CRISPR/Cas9 technology.

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 48

***Mr. Pouiré Yameogo*** [1], ***Dr. Catherine Gérard*** [1], ***Dr. Nathalie Majeau*** [2], ***Ms. Camille Bouchard*** [3], ***Prof. Jacques P. Tremblay*** [1]

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**Background and Objective**

Most Friedreich ataxia (FRDA) cases are caused by elongation of a GAA repeat (GAAr) sequence in the first intron of the *FXN* gene leading to a decrease of frataxin protein expression. Deletion of this GAAr with CRISPR/Cas9 technology leads to an increase in frataxin expression. We are therefore aiming to develop FRDA treatment using a single AAV to deliver a small Cas9, (CjCas9) and two single guide RNAs (sgRNAs).

**Methods**

We first identified two sgRNAs that efficiently target the FXN intron 1 near the GAAr in YG8sR cells and in FRDA cells. We constructed a single AAV9 encoding the CjCas9 protein and the two sgRNAs to optimize DNA editing. This AAV was administered by peritoneal injection to YG8sR mice (250-350 GAAr). Organs were recovered a month later and DNA was extracted from different tissues.

**Results**

Droplet digital PCR amplification of part of intron 1 of the *FXN* gene showed that the GAAr was removed in some cells in the heart and in the liver. This was confirmed by Sanger sequencing. The editing rate in the heart was 4.6% in heart and 17.1 % in liver. However, such deletions were not detected in various parts of the CNS despite de detection of abundant AAV vectors.

**Discussion and Conclusion**

We report for the first time a deletion of GAAr *in vivo* using CRISPR/Cas9 system. Targeting heart cells is the good thing because cardiac complications are responsible for 59% of deaths of FRDA patients. This interesting approach preserves the endogenous frataxin promoter, gene copy number, and the correction would be permanent. Our results constitute an advance for a treatment based on the elimination of the GAAr in intron 1 of the frataxin gene. However, we still have to understand why there was no deletion of the GAAr in the CNS.

# (#54) Engineered AAVs for efficient delivery to central nervous system and heart

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 54

***Dr. Nathalie Majeau*** [1], ***Dr. Dominique Ouellet*** [2], ***Dr. Catherine Gérard*** [3], ***Prof. Jacques P. Tremblay*** [3]

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**Background and Objective**

Advances in CRISPR/Cas9 technology that allow efficient gene correction give rise of a lot of expectation for gene therapy. However, *in vivo* delivery of gene editing tools remains challenging in current treatment efforts. Adeno associated virus (AAV) is the most promising vector as it is considered to be safe and versatile. However, there is an important need to improve AAV efficiency to transduce all targeted organs and more specially to bypass the blood-brain barrier (BBB).

**Methods**

In order to increase the accessibility of the Central Nervous System (CNS), we engineered AAV9 capsid by introducing 17 amino acid aleatory mutations in 3 different regions of the protein structure that were predicted to be exposed on the surface of the virus. According to the 3D structure, these 3 regions were closed to each other and could interact potentially with a same molecule receptor. The capsid gene including the mutated sequence was enclosed inside the AAV viruses in order to characterize the new viruses that display tissue tropism.

**Results**

Viruses were intravenously administrated in mice and after 10 days, the organs were recovered and DNA extracted. AAV variant sequences present in each tissue were analyzed by deep sequencing. Most AAV sequences recovered from CNS organs were highly divergent from the AAV9 initial sequence for the 3 modified capsid regions. We selected the most abundant clones found in the CNS and the heart and assessed their propensity to invade these tissues in mice.

**Discussion and Conclusion**

These experiments revealed new AAV candidates that could be interesting for Ataxia gene therapy.

# (#115) NRF2 Activating drugs, Potential Regulators of Mitochondrial Fragmentation in Friedreich's Ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 115

***Ms. Lucie Ngaba*** [1], ***Dr. Joseph Johnson*** [1], ***Mr. Jonathan Wong*** [2], ***Dr. Yina Dong*** [1], ***Ms. Elizabeth Mercado-Ayon*** [2], ***Ms. Sarah Halawani*** [1], ***Dr. David Lynch*** [3]

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

Friedrich's ataxia (FRDA) is an autosomal recessive neurodegenerative disorder that affects the nervous system; causing progressive ataxia, dysmetria and dysarthria. FRDA results from a deficiency of a mitochondrial protein called Frataxin; such deficiency is caused by an expansion of a GAA repeat in the first intron of the Frataxin(FXN) gene, leading to transcriptional inactivation. The inability of FRDA cells to handle oxidative stress (reflecting a loss of NRF2 activity) leads to an increase in mitochondrial fragmentation among other events. In the present study, we sought to understand whether clinically relevant Nrf2 activating drugs such as Omaveloxolone (RTA-408, which blocks proteolytic destruction of NRF2 by Keap1) and other NRF2 activators such LS102 (which blocks destruction of NRF2 through the HRD1 pathway) can block mitochondrial fragmentation in FRDA fibroblasts. Control fibroblasts (untreated and knock downed with shFXN) and patient fibroblasts were treated with either DMSO, RTA-408 (350nM) or LS102 (5uM). They were collected after 72 hours and further analyzed by Western Blots and IHC. We observed that NRF2 activating drugs like RTA-408 reduced mitochondrial fragmentation in FXN-knockdown and patient fibroblasts. Furthermore, RTA-408 significantly decreased the level of pDRP1 in FXN Knockdown fibroblasts as well as FRDA patient-derived fibroblasts. The reduction of mitochondrial fission after treatment with RTA-408 might indicate its effectiveness and use in a clinical therapy.

# (#131) INVESTIGATING THE THERAPEUTIC EFFECTS OF DIFFERENT ANTIOXIDANTS IN FRIEDREICH'S ATAXIA

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 131

***Mr. Fred Jonathan Edzeamey*** [1], ***Dr. Adamo Valle*** [2], ***Dr. Charareh Pourzand*** [3], ***Prof. Robert Hider*** [4], ***Dr. Ronan McCarthy*** [5], ***Dr. Sara Anjomani-Virmouni*** [5]

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**Background:** Aberrant frataxin expression in Friedreich's ataxia (FRDA) results in increased oxidative stress and mitochondrial dysfunction, driving disease progression. Different antioxidants have been tested in the past with quite satisfying and encouraging results *in vitro* and *in vivo*. So far there is no cure or effective treatment for FRDA. This project aims at investigating the effect of novel antioxidants on mitochondrial redox homeostasis and metabolism in FRDA.

**Methods:** Candidate compounds selected include Dimethylfumarate, N-acetylcysteine, Resveratrol, Vitamin C and E and Lipoic acid. *Galleria mellonella* model was used to determine the $LD_{50}$ of potential antioxidants. Toxicity of compounds in FRDA cells was assessed using PrestoBlue cell viability assay. Mitochondrial ROS (mROS)-decreasing capacity of compounds in FRDA fibroblast cells was assessed by MitoSOX-based flow cytometric assay, and *FXN* and NRF2 gene expression levels were assessed using qRT-PCR.

**Results:** Toxicity of the antioxidants was first assessed in the *Galleria* model and those compounds demonstrating minimal toxicity were further tested *in vitro* FRDA cell models. Cell viability studies in FRDA fibroblasts revealed 15μM Resveratrol, 2mM Vitamin E, 440μM Vitamin C, 0.1μM Lipoic acid, 10μM Dimethylfumarate and 1.5mM N-acetylcysteine as the tolerable concentrations. Significant reduction in mROS was observed in FRDA cells following treatment with N-acetylcysteine, Resveratrol, Dimethylfumarate Vitamin C and Vitamin E.These compounds were also found to significantly increase the levels of *FXN* and NRF2 gene expression. The effect of these compounds on frataxin, Nrf2, catalase and glutathione peroxidase protein expression and other mitochondrial enzymes are currently being assessed.

**Discussion and Conclusion:** The efficacy and safety of the antioxidants have been tested in FRDA fibroblasts. The ability of the compounds to upregulate the antioxidant response elements will be further assessed. Candidate compounds will be modified to improve their efficacy/bioavailability and facilitate their clinical translation. This study may provide a novel therapeutic avenue for FRDA.

# (#142) A single-intrathecal administration of a new gene therapy vector restores neurological and cardiac deficits in two mouse models of Friedreich ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 142

***Dr. Ivelisse Sanchez*** [1], ***Dr. Eudald Balagué*** [2], ***Mr. Daniel Cota-González*** [3], ***Ms. Kerrie Adrián-Campbell*** [3], ***Ms. Belen García-Lareu*** [4], ***Prof. Jaume Coll-Cantí*** [5], ***Dr. Miguel Chillón*** [6], ***Dr. Assumpció Bosch*** [4], ***Dr. Antoni Matilla-Dueñas*** [7]

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Friedreich's ataxia (FRDA) is a rare neurodegenerative genetic disease of autosomal recessive inheritance characterized mainly by progressive ataxia, sensory loss, and cardiomyopathy. The most prevalent genetic cause consists of a homozygous pathological GAA triplet expansion in the first intron of the FXN gene. This mutation results in a deficiency of frataxin and consequently mitochondria iron accumulation and dysfunction underlying the neurodegeneration of the neurons of the dorsal root ganglia, the peripheral sensory nerves, the spinal cord, and the cerebellar dentate nucleus. Although there is currently no effective treatment, several studies have clearly shown that gene therapy has great potential in the treatment of FRDA. However, multiple studies have highlighted the importance of paying special consideration to the regulation of frataxin levels since unregulated overexpression of frataxin appeared to be toxic in the target tissue. Therefore, we recently generated a new AAV9 gene therapy vector, rAAV9-hPGK1-hFXN, able to yield human frataxin levels within endogenous protein range under the metabolic regulation of the hPGK1 promoter following a single intrathecal administration in two mouse models of the disease, one showing chronic (YG8R) and the other acute (Pv-Fxn cKO) symptom presentation. Furthermore, a single intrathecal administration of the recombinant vector rAAV9-hPGK1-hFXN in FRDA mice provides a safe and durable expression of human frataxin in target tissues, including dorsal root ganglia (at least 24 months in mice). Evaluation of the *in vivo* therapeutic efficacy of the gene therapy vector demonstrated correction of ataxia and motor coordination, electrophysiological properties of sensory nerves, clasping reflex, mitochondrial function, cardiomyopathy, and mitochondrial iron deposition in treated mice. Therefore, we believe that this pre-clinical proof-of-concept study supports the therapeutic potential of our gene therapy strategy for Friedreich's ataxia.

# (#167) Assessment of the effect of Etravirine on frataxin levels in FRDA Fibroblasts

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 167

***Ms. Sarah Halawani*** [1], ***Dr. David Lynch*** [2]

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Friedreich's ataxia (FRDA) is a neurogenerative disease resulting from low expression levels of the mitochondrial protein Frataxin (FXN). It is caused by a biallelic GAA repeat expansion in the first intron of the *FXN* gene, with repeats varying in length from 70 to over 1000 repeats. Such repeats silence the gene leading to decreased frataxin expression with resultant mitochondrial dysfunction, oxidative stress, and cell death. There are no approved treatments for FRDA to date.

A previous study has reported an increase of Frataxin levels of approximately 50% in FRDA lymphoblastoid cells when treated with etravirine over a 24h period, rationalizing its use in clinical trials. Etravirine is a non-nucleoside reverse transcriptase inhibitor and is presently being used as an antiviral drug used to treat human immunodeficiency virus (HIV) infection. In this study, we used similar methodology to treat several FRDA patient fibroblast cultures over multiple time-points in order to attempt to verify etravirine as a possible therapeutic drug for FRDA. Several cell cultures of FRDA fibroblasts with dissimilar GAA repeat lengths on both alleles ranging from 700 to 1266 repeats and unaffected (Healthy subject) cell cultures were treated with 10uM of etravirine over various time-points of 24, 48, 72 and 96 hours.

Western blot analysis was performed to determine FXN expression levels, When compared to untreated and vehicle treated cell cultures, no significant evidence of any increase in FXN expression was detected. A dose response curve found etravirine to be toxic at higher concentrations at 30uM and 100uM, leading to complete cell death within 24 hours. The results from these experiments do not support the reported increase of FXN levels in any of the FRDA cell cultures, Thus, the present study did not provide rationale for therapeutic trials of etravirine.

# (#169) Bioactive fumarate improves cardiac function and expands lifespan in mice with Friedreich's Ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 169

***Ms. Lili Salinas [1], Mr. Francisco Figueroa [2], Ms. Claire B. Montgomery [3], Dr. Phung N. Thai [1], Prof. Nipavan Chiamvimonvat [1], Dr. Sundeep Dugar [4], Dr. Somdutta Sen [5], Prof. Gino Cortopassi [6], Dr. Elena N. Dedkova [7]***

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**Introduction:** Friedreich ataxia (FA) is a recessive ataxia caused by reduction of mitochondrial protein, frataxin (FXN). Cardiomyopathy is the leading cause of death in FA patients due to deficient FXN expression in the heart. Previously, we identified that bioactive fumarates are protective in FA cell models. We have developed a novel monomethyl fumarate prodrug, IMF, which has improved pharmacokinetic profile and compared its effect to fumarate prodrug dimethyl fumarate (DMF, Tecfidera).

**Aim:** To test the hypothesis that novel bioactive fumarate improves cardiac function and survival via Nrf-2 and $HCA_2$ signaling pathways activation.

**Methods and Results:** Cardiac-specific FXN knockout (Mck-Cre FXNKO) mice were used as a model of severe cardiomyopathy, a characteristic of late-stage FA. Animals were randomly split in vehicle and fumarate-treated groups. Animals were treated by IMF or DMF at equimolar doses of monomethyl fumarate. Treatment started at 3 weeks of age and continued for 5 weeks in the cross-sectional study and until death in the survival study. Cardiac function was examined *in-vivo* by echocardiography. We found that FXNKO mice developed severe heart failure with 45% reduction in ejection fraction, increased left ventricular (LV) mass (+80%) and diameter (+308%), decreased stroke volume (-26%) and cardiac output (-38%, n=10) as compared to wild-type mice (n=12). DMF and IMF partially recovered cardiac deficits in FXNKO mice but only IMF extended the lifespan in these mice by 13%. Expression of genes in Nrf2 (NQO1, SOD2, glutathione S-transferase) and $HCA_2$ ($HCA_2$ and Sirt1) signaling pathways were decreased in FXNKO and restored by DMF and IMF. Aconitase activity, used as a surrogate measure of frataxin's iron-sulfur biogenesis function, was decreased in FA by 49% and recovered 18% by IMF only.

**Conclusions.** Novel fumarate prodrug IMF improved cardiac function and expanded the lifespan in cardiac-specific FXNKO mouse model of FA more effectively than DMF.

# (#193) A drug combination rescues frataxin-dependent neural and cardiac pathophysiology in FA models

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 193

***Dr. Rosella Abeti*** [1], ***Dr. Mittal Jasoliya*** [2], ***Dr. Sahar Al-Mahdawi*** [3], ***Dr. Mark Pook*** [4], ***Dr. Cristina Gonzalez-Robles*** [1], ***Dr. Chun Kiu Hui*** [2], ***Prof. Gino Cortopassi*** [2], ***Prof. Paola Giunti*** [5]

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**Background and Objective**. Friedreich's ataxia (FA) is an inherited ultimately lethal multisystemic neuro- and cardio-degenerative disorder. Although clinical trials list 74 previous and ongoing in FA, there is no FDA/EMA approved therapy. Up to date, FA therapeutic strategies have focused along two main lines using a single drug approach: 1) increasing frataxin, 2) enhancing downstream pathways such as increasing antioxidants and mitochondrial function. Our novel strategy employed a combinatorial approach to screen approved drugs and supplements to determine if a combination of molecules provided an additive or synergistic benefit to FA cells or animal models.
**Methods**. Eight single drug molecules were administered to FA fibroblast patient cells: Nicotinamide Riboside, Hemin, Betamethasone, Resveratrol, Epicatechin, histone deacetylase inhibitor 109, Methylene Blue, and Dimethylfumarate. We measured their individual ability to induce *FXN* transcription and mitochondrial biogenesis. Pairwise combinations of 'most active' were evaluated for mitochondrial and antioxidant benefit in primary neural and cardiac explants from FA mice. Behavioural analysis was performed after drug administration.
**Results**. Single-drug testing highlighted Dimethylfumarate, Methylene Blue, and Resveratrol induce *FXN* mRNA expression and mitochondrial biogenesis. Paired-drug testing indicated that the drug pair- Dimethylfumarate and Resveratrol- was the most effective in terms of *FXN* mRNA and mitobiogenesis increase. This combination improved physiological functions and reduced mitochondrial reactive oxygen species generation in neurons and cardiomyocytes. Behavioural analysis of the Dimethylfumarate and Resveratrol combination in a FA mouse model demonstrated improved Rotarod performance.
**Conclusion.** Our data suggest that Dimethylfumarate is effective as a single agent, and the addition of Resveratrol further benefits some assays. Lastly, this combination is not toxic to cells. These results support the idea that Dimethylfumarate is a valuable compound to counteract FA pathophysiology, especially when administered with Resveratrol. Further studies will help to fully understand the potential of a combined therapeutic strategy in FA pathophysiology.

# (#258) Dual effects of BDNF in Atxn1154Q/2Q mouse model of Spinocerebellar Ataxia Type 1

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 258

***Mr. Stephen Gilliat*** [1], ***Mr. Juao-Guilherme Rosa*** [2], ***Ms. Katherine Hamel*** [1], ***Ms. Carrie Sheeler*** [1], ***Mrs. Ella Borgenheimer*** [3], ***Ms. Alyssa Soles*** [1], ***Mr. Fares Ghannoum*** [1], ***Mr. Orion Rainwater*** [1], ***Dr. Harry Orr*** [1], ***Dr. Marija Cvetanovic*** [1]

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Spinocerebellar Ataxia Type 1 (SCA1) is a genetic neurodegenerative disease that leads to motor impairment, cognitive decline, and premature lethality. Brain Derived Neurotrophic Factor (BDNF), a neuroprotective growth factor, has been shown to decrease with progression of SCA1. We previously showed in a cerebellum–specific mouse model of the disease that exogenous administration of BDNF ameliorated motor deficits and cerebellar pathogenesis. As SCA1 affects brain regions beyond the cerebellum, we wanted to determine the effects of increased BDNF throughout the brain.

To study this, we used *Atxn1*$^{154Q/2Q}$ mice where 154 CAG repeats were inserted into the endogenous *Ataxin1 (Atxn1)* gene. This allows the mutant form of the protein to be expressed throughout the central nervous system, more accurately representing the wider pathological effects of the ATXN1 mutation. Using Alzet pumps, we administered either exogenous BDNF or artificial cerebrospinal fluid into the right lateral ventricle of 7-week-old *Atxn1*$^{154Q/2Q}$ and wild-type (WT) mice. Motor and cognitive deficits were examined using rotarod, Barnes maze, and fear conditioning tests. Gene expression changes and pathology were examined using quantitative reverse transcription PCR and immunofluorescence.

Using immunohistochemistry, we verified that BDNF is decreased in cerebellum and medulla of SCA1 patients compared to age and sex matched healthy controls. Rotarod analysis showed that administration of BDNF improved motor performance in both *Atxn1*$^{154Q/2Q}$ and WT mice. We also observed that BDNF administration improved strategy development in both *Atxn1*$^{154Q/2Q}$ and WT mice, though treated mice also exhibited memory deficits. Finally, we found that BDNF increased expression levels of astrocytic homeostatic genes in the hippocampus and the cerebellum and rescued hippocampal neurogenesis deficits in *Atxn1*$^{154Q/2Q}$ mice.

Our results suggest that BDNF may improve motor and cognitive performance in *Atxn1*$^{154Q/2Q}$ mice and restore hippocampal neurogenesis. However, an excess of BDNF may cause possible side effects including memory deficits.

# (#261) Motor coordination and balance ameliorated after treatment of the CMVMJD135 mouse model of Spinocerebellar Ataxia Type 3 with befiradol

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 261

***Ms. Bruna Ferreira-Lomba [1], Ms. Sara Guerreiro [1], Dr. Sara Duarte-Silva [2], Ms. Daniela Cunha-Garcia [3], Dr. Joana Pereira-Sousa [4], Ms. Daniela Vilasboas-Campos [5], Ms. André Vidinha-Mira [3], Dr. Mark Kleven [6], Dr. Andreia Teixeira-Castro [3], Dr. Adrian Newman-Tancredi [7], Prof. Patrícia Maciel [8]***

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Spinocerebellar Ataxia Type 3 (SCA3) is a dominantly inherited neurodegenerative disease, characterized by motor dysfunction affecting gaze, speech, gait and balance, often accompanied by non-motor symptoms and a reduced life expectancy. This disease is caused by the expansion of a polyglutamine tract in the ataxin-3 protein (ATXN3). Other than some symptom-directed treatments of temporary efficacy, no effective disease-modifying therapy is currently available. However, recent studies of drugs targeting the serotonergic signaling pathway revealed promising results. Befiradol, a highly-selective and fully efficacious 5-$HT_{1A}$ receptor agonist ameliorated motor dysfunction and reduced mutant ATXN3 aggregation in a *Caenorhabditis elegans* model of SCA3. Therefore, this study aimed to assess the therapeutic impact of befiradol in the motor function of SCA3 mice, using another 5-$HT_{1A}$ receptor agonist, tandospirone (TD), as reference drug.

Two doses of each drug were selected based on plasma/brain exposure levels and animal welfare, and administered through the drinking water. Animals were treated for 35 weeks, starting immediately before symptom onset, at 6 weeks of age, with 0.625 or 5 mg/kg of befiradol and, in an independent experiment, 20 or 80 mg/kg of TD. Animals' welfare, body weight and temperature were regularly assessed, along with motor function, using the beam walking (BWT) and motor swimming tests.

These experiments revealed that doses up to 10 mg/kg for befiradol and 80 mg/kg for TD were safe and well-tolerated by the mice. Befiradol at 5 mg/kg significantly improved motor coordination and balance of SCA3 animals in the BWT, at advanced stages of the disease, while minor impact was observed in other motor parameters. Contrarily, TD exhibited no therapeutic effect on motor function, even after prolonged treatment.

Overall, befiradol showed a mild beneficial effect on the motor function of SCA3 mice, reinforcing the potential role of serotonergic signaling modulation as a promising therapeutic target for SCA3.

# (#267) Multi-modal analyses converge on the 5-HT1A serotonin receptor and CREB signaling as promising therapeutic targets for SCA3

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 267

***Dr. Andreia Teixeira-Castro*** [1], ***Dr. Joana Pereira-Sousa*** [2], ***Ms. Stéphanie Oliveira*** [3], ***Ms. Bruna Ferreira-Lomba*** [4], ***Ms. Sara Guerreiro*** [4], ***Ms. Daniela Cunha-Garcia*** [5], ***Dr. Mark Kleven*** [6], ***Dr. Adrian Newman-Tancredi*** [6], ***Prof. Olaf Riess*** [7], ***Dr. Jeannette Hübener-Schmid*** [8], ***Dr. Sara Duarte-Silva*** [3], ***Prof. Patrícia Maciel*** [3]

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The available treatments for Spinocerebellar Ataxias (SCAs), including SCA3, and the majority of other aging-associated neurodegenerative disorders only ameliorate symptomatology and do not stall disease progression. Using unbiased approaches, we and others found that modulation of the serotonergic signaling was beneficial to cellular and animal models of SCA3. We demonstrated that treatment with citalopram (CIT) and other selective serotonin reuptake inhibitors suppressed mutant ATXN3 aggregation and neuronal dysfunction in *C. elegans* and two independent transgenic mouse models. Importantly, we established 5-HT transporter SERT inhibition and the consequent increase in 5-HT extracellular levels as a novel therapeutic strategy in SCA3. Here, we aimed at further addressing the role of serotonergic signaling in SCA3 pathogenesis and therapeutics.

Investigation of the molecular determinants underlying these findings, by RNA sequencing, revealed a decrease in the mRNA expression of 5-HT1A receptors (5-HT1ARs) in transgenic mice, which was restored to WT levels upon CIT chronic administration. The mRNA profiling suggested that 5-HT1AR signal-transduction pathways, including the cAMP response element-binding protein (CREB) transcription factor, could be relevant in SCA3 pathogenesis and treatment. Importantly, the expression of 5-HT1ARs was also decreased in post-mortem brain samples of SCA3 patients. Moreover, direct targeting of 5-HT1AR using a novel, selective and potent 5-HT1A receptor agonist suppressed mutant ATXN3 aggregation and neuronal dysfunction *in C. elegans*. Using chemical genetics and pharmacological approaches, we found that potent and specific activation of 5-HT1A/SER-4 receptors is beneficial to SCA3 worms, highlighting the role of auto- and hetero-receptor function in the therapeutic outcome in this model. The validation of these findings in higher organisms leads us to propose the serotonin 1A receptor signaling as an important player in the pathogenesis and a novel therapeutic target in SCA3.

# (#274) Neuroprotective effect of a Hemerocallis citrina extract in a C. elegans model of Machado-Joseph disease

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 274

***Mr. Jorge Fernandes*** [1], ***Ms. Daniela Vilasboas-Campos*** [1], ***Dr. Marta Daniela Costa*** [2], ***Prof. Qiong Wang*** [3], ***Prof. Alberto Dias*** [4], ***Prof. Patrícia Maciel*** [2]

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Machado-Joseph disease (MJD) is an incurable neurodegenerative disease caused by mutations in the *ATXN3* gene. As a result, a form of ataxin-3 with an expanded polyglutamine tract is expressed, which can trigger different toxic events. Natural Products have been explored as a source for novel therapies for diseases with no effective treatments. The aim of this work was to explore the neuroprotective effects of extracts and purified compounds from Chinese medicinal plants, using *C. elegans* model of MJD.

The motor deficits displayed by the model were used as a readout for neuroprotection. We tested the impact of the treatments using the WMicroTracker and Motility Assay to assess movement in liquid and solid media, respectively. To unveil the mechanisms underlying the effect on these phenotypes, reporter strains for different antioxidant and proteostasis mechanisms were used. Given the described antidepressant properties of some of the plants, the involvement of serotonergic and dopaminergic signalling was assessed using *C. elegans* mutants lacking different receptors.

Our data indicates that an ethanolic extract of *Hemerocallis citrina* (HCE30%), improved the locomotion deficits displayed by the model. This effect was dependent on specific components of the serotonergic signalling, especially the serotonin receptors SER-1, SER-4, SER-5, SER-7 and the MOD-5 transporter. The referred extract also induced transcription of the *gcs-1* gene, involved in antioxidant response, and nuclear translocation of HLH-30, a transcriptional regulator of multiple autophagy-related genes.

The improvement of the motor phenotype supports the neuroprotective potential of HCE30%. Furthermore, the extract activated different adaptive responses, suggesting it could impact different aspects of the disease. It remains unclear which of the referred pathways is responsible for the effects on the motor function. Interestingly, we demonstrated that the effect on locomotion is dependent on the serotonergic receptors, indicating a role for the serotonergic signalling on the effect of the extract.

# (#303) Biodistribution and efficacy of a novel AAV-hFXN expression vector in the Rodent CNS

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 303

***Dr. Marshall Goodwin*** [1], ***Dr. Matthew Hamm*** [2], ***Ms. Tara McParland*** [2], ***Mrs. Elizabeth Butterworth*** [2], ***Dr. Nicola Rutherford*** [2], ***Dr. Fatima Shaerzadeh*** [2], ***Dr. Danielle Cucchiara*** [2], ***Dr. Ryan Spengler*** [2], ***Ms. Rebeca Everitte*** [2], ***Ms. Heather Stacy*** [2], ***Ms. Carli Brown*** [2], ***Mr. Sam Ewing*** [2], ***Ms. Tooray Fuller*** [2], ***Ms. Amber Calloway*** [2], ***Dr. Shyam Gajavelli*** [2], ***Dr. Edgar Rodriguez*** [2], ***Dr. Darin Falk*** [2]

***1.*** *Lacerta,* ***2.*** *Lacerta Therapeutics*

**Background and objective:** Friedreich's ataxia (FA) is an autosomal recessive disease in which the inheritance of a trinucleotide repeat expansion in the first intron of the human *FXN* (hFXN) gene results in deficient expression of the frataxin protein. We are developing an adeno-associated virus (AAV) gene therapy to deliver the hFXN gene to tissues critically affected in FA to restore functional levels of frataxin and prevent FA disease progression.
**Methods:** We engineered a gene expression cassette comprised of a human FXN (hFXN) sequence under the control of a synthetic Desmin promoter and a modified hFXN 5'UTR. This construct was packaged in AAV serotype 7 for injection into the CNS of parvalbumin-Cre conditional frataxin knockout (PV) mice which recapitulate features of FA neuropathophysiology. Cohorts of PV mice were injected at 5-6 weeks of age with either AAV7-hFXN or excipient via a combined intraventricular and intraparenchymal route. Motor performance was assessed using the accelerating rotarod every two weeks until 17 weeks of age. Tissues from all cohorts were harvested at endpoint for biochemical and histological examination.
**Results:** Delivery of AAV7-hFXN via this novel route of administration resulted in broad biodistribution across multiple CNS and PNS resident cells. Biochemical analyses revealed near-physiological human frataxin expression within tissues critically affected in FA including cerebellum and dorsal root ganglia. There were no gross histopathological findings and we failed to detect evidence of neurotoxicity, following long term CNS expression of AAV7-hFXN. In addition to its safety profile, AAV7-hFXN was effective at significantly improving and stabilizing the motor performance of the PV-Cre mouse model of FA.
**Discussion and Conclusion:** AAV7-hFXN is a lead clinical candidate for the treatment of myocardial and CNS-related pathologies in FA. Data from ongoing NHP studies will also be presented at the meeting.

# (#304) Biodistribution and activity of a novel AAV-hFXN expression vector in the MCK mouse model of Friedreich's ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 304

***Dr. Matthew Hamm*** [1], ***Dr. Marshall Goodwin*** [1], ***Ms. Tara McParland*** [1], ***Dr. Nicola Rutherford*** [1], ***Mrs. Elizabeth Butterworth*** [1], ***Dr. Fatima Shaerzadeh*** [1], ***Dr. Danielle Cucchiara*** [1], ***Dr. Ryan Spengler*** [1], ***Mr. Sam Ewing*** [1], ***Ms. Rebeca Everitte*** [1], ***Ms. Heather Stacy*** [1], ***Ms. Carli Brown*** [1], ***Ms. Christina Rodriguez*** [1], ***Ms. Tooray Fuller*** [1], ***Ms. Amber Calloway*** [1], ***Dr. Shyam Gajavelli*** [1], ***Dr. Edgar Rodriguez*** [1], ***Dr. Darin Falk*** [1]

***1.*** *Lacerta Therapeutics*

**Title:** Biodistribution and activity of a novel AAV-hFXN expression vector in the MCK mouse model of Friedreich's ataxia

**Background and objective:** Friedreich's ataxia (FA) is a mitochondrial disease with a heterogeneous presentation that commonly features spinocerebellar ataxia, sensory neuropathy, and hypertrophic cardiomyopathy. FA is an autosomal recessive disease in which inheritance of a trinucleotide repeat expansion in the first intron of the human *FXN* (h*FXN*) gene results in deficient expression of frataxin protein. We are developing an adeno-associated virus (AAV) gene therapy to deliver the *FXN* gene to tissues affected in FA to restore functional levels of frataxin and prevent FA progression.

**Methods:** We engineered a gene expression cassette comprised of a human FXN (hFXN) sequence under the control of a synthetic Desmin promoter and a modified hFXN 5'UTR. This construct was packaged in AAV serotype 7 and delivered intravenously to MCK mice at doses ranging from 3.75E12 to 1E14 vector genomes per kilogram (vg/Kg). The MCK mouse model used in this dose-escalation study lacks frataxin in striated muscle, resulting in a severe and aggressive cardiac phenotype. At 4- or 15-weeks post-administration, cardiac function was assessed via MRI. Subsequently, tissues from all cohorts were harvested for biochemical and histological examination.

**Results:** Intravenous delivery of AAV7-h*FXN* resulted in broad distribution to cardiac tissue, and MRI indicated a complete recovery of cardiac function at multiple doses. The 5E13 vg/kg dose resulted in near-physiological frataxin expression in the heart, and this correlated with normalization of cardiac ejection fraction and lifespan. Importantly, no treatment-related histopathological findings or toxicities were observed in heart or liver tissues at any of the tested doses.

**Discussion and Conclusion:** AAV7-hFXN is a lead clinical candidate for the treatment of myocardial and CNS-related pathologies in FA. Data from ongoing NHP studies will also be presented at the meeting.

# (#327) Gene Editing Strategies to Treat Spinocerebellar Ataxia Type 1

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 327

***Ms. Kelly Fagan*** [1], ***Dr. Megan Keiser*** [2], ***Dr. Beverly Davidson*** [3]

***1.*** *University of Pennsylvania, United States,* ***2.*** *Children's Hospital of Philadelphia,* ***3.*** *1. Raymond G. Perelman Center for Cellular and Molecular Therapeutics, The Children's Hospital of Philadelphia 3.Department of Pathology and Laboratory Medicine, University of Pennsylvania*

Spinocerebellar ataxia type 1 (SCA1) is a neurodegenerative disease that causes progressive loss of motor coordination and eventual death. SCA1 is caused by expansion of the polyglutamine repeat region in the *ATXN1* gene. The mechanism of SCA1 pathogenesis is unknown; however, some features of the disease include neuronal degeneration and formation of toxic mutant ATXN1 (mATXN1) nuclear inclusions. Although mATXN1 is expressed ubiquitously, it affects primarily Purkinje cells (PCs). There are currently no treatment options for SCA1. We hypothesize that CRISPR-Cas9 editing of ATXN*1* will reduce mutant ATXN1 and be therapeutically beneficial.

CRISPR Cas9 is a DNA editing tool used to induce knockout of the target gene. We designed two different strategies to reduce ATXN1; the first uses a single guide RNA (gRNA) to target near the exon-exon junction to induce nonsense-mediated decay, while the second approach employs a dual guide system to delete the CAG repeat region. gRNAs were optimized in vitro, with each approach significantly reducing *ATXN1* expression. The exon-exon approach reduced *ATXN1* mRNA levels by 40-45% ($p \leq 0.02$) and protein by approximately 20% ($p \leq 0.01$) and the dual guide approach reduced levels of mRNA and protein levels by 70-75% ($p < 0.001$) and 45-65% ($p \leq 0.03$), respectively.

For testing in vivo, SCA1 mice, expressing mutant human *ATXN1*, were crossed to *sp*Cas9 transgenic mice. Recombinant AAVs expressing the optimized gRNAs from each strategy were delivered directly to the deep cerebellar nuclei of 5-week-old SCA1/*sp*Cas9 mice for transduction of Purkinje cells. The exon-exon strategy reduced protein and mRNA levels by 55% ($p = 0.05$) and 50% ($p = 0.02$), respectively compared to saline injected controls. The dual guide strategy reduced *ATXN1* mRNA levels by 70-80% ($p < 0.001$). This shows that the dual guide strategy is more effective at reducing ATXN1 levels *in vivo* and studies are in progress to assess the impact of both strategies on SCA1 mice phenotypes.

# (#329) RNA-Seq in B05 SCA1 mice after Combined overexpression of ATXN1L and mutant ATXN1 knockdown by AAV

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 329

***Dr. Megan Keiser*** [1]***, Dr. Ellie Carrell*** [1]***, Ms. Ashley Robbins*** [2]***, Dr. Beverly Davidson*** [3]

***1.*** *Children's Hospital of Philadelphia,* ***2.*** *University of Pennsylvania Perelman School of Medicine,* ***3.*** *1. Raymond G. Perelman Center for Cellular and Molecular Therapeutics, The Children's Hospital of Philadelphia 3.Department of Pathology and Laboratory Medicine, University of Pennsylvania*

**Background and Objective**: Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant neurodegenerative disease caused by a (CAG) repeat expansion in the coding sequence of *ATXN1*. The primary mechanism of disease in SCA1 is toxic gain-of-function by polyglutamine-expanded mutant ATXN1 and is compounded by partial loss of wildtype function. Addressing both disease mechanisms, we have shown that virally expressed RNA interference targeting *ATXN1* can both prevent and reverse disease phenotypes in SCA1 mice, and that overexpression of the ATXN1 homolog, ataxin-1-like (ATXN1L) improves disease readouts when delivered pre-symptomatically.
**Methods:** Here, we combined these therapeutic approaches into a dual component recombinant adeno-associated virus (rAAV) vectors and tested their ability to reverse disease in symptomatic B05 SCA1 mice using behavior and next generation sequencing assays.
**Results:** Mice treated with vectors expressing human ATXN1L (hATXN1L) alone showed motor improvements and changes in gene expression that reflected increases in pro-development pathways and a decrease in angiogenic gene expression. When combined with miS1, a previously validated microRNA targeting hATXN1, motor improvements were complemented by robust normalization of disease allele-induced changes in gene expression.

**Discussion and Conclusion:** In summary, we show that overexpression of hATXN1L is sufficient to stabilize or reverse behavioral deficits following symptom onset in B05 mice, however broad disease-specific gene normalization requires concomitant knockdown of mutATXN1 by miS1. We demonstrate efficient processing of these therapeutic strategies from two different single rAAV vectors, simplifying production and delivery of a more effective SCA1 therapy.

# (#333) Long-read Nanopore sequencing reveals outcomes of AAV-CRISPR editing in the brain of transgenic SCA2 mouse models

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 333

***Mr. Bryan Simpson*** [1]***, Dr. Carolyn Yrigollen*** [2]***, Mr. Aleksandar Izda*** [2]***, Dr. Paul Ranum*** [2]***, Dr. Beverly Davidson*** [1]

***1.*** *1. Raymond G. Perelman Center for Cellular and Molecular Therapeutics, The Children's Hospital of Philadelphia 2. Department of Pathology and Laboratory Medicine, University of Pennsylvania,* ***2.*** *1. Raymond G. Perelman Center for Cellular and Molecular Therapeutics, The Children's Hospital of Philadelphia*

We are developing AAV-CRISPR strategies to treat spinocerebellar ataxia type 2 (SCA2), a neurodegenerative disease caused by a CAG trinucleotide repeat expansion in *ATXN2*, as well as broadly applicable methods to reliably assess editing. Standard methods analyze editing with PCR, a strategy that introduces biases. Furthermore, PCR of expanded repeat sequences is unreliable for assessing editing near repeats. We therefore developed a targeted CRISPR enrichment strategy followed by long-read Oxford Nanopore Technology sequencing of native genomic DNA from transgenic SCA2 mice previously co-injected with AAV-SpCas9 and AAV-gRNA in the brain. Nanopore MinION sequencing runs resulted in reads with a mean length of ~10 kb and 2500-6500x target coverage after alignment to the mouse genome and transgenic allele.

In two transgenic SCA2 mouse models treated with AAV-CRISPR expressing dual-gRNAs targeting sequences flanking the CAG repeat, CRISPR-enriched Nanopore sequencing detected deletions of the expected size range at frequencies of 2-4%. AAV vector integrations were found at on-target gRNA sites at frequencies of 10-20% and ranged from fragments to full-length genomes. While there was no evidence of CRISPR-mediated induction of longer repeat expansion, there were large deletions and rearrangements between tandem transgene copies—an unintended consequence of editing in a transgenic model. For comparison we performed PCR-based Nanopore sequencing, and detected deletions and only short AAV sequence integrations and no full-length genomes.

CRISPR-enriched Nanopore sequencing allowed unbiased assessment of native and edited genomic sequences. By combining long-read sequencing with polymerase-free enrichment, we captured large deletions between transgenes and full-length AAV genome integrations—outcomes missed by standard methods. To our knowledge this is the first study detecting full-length AAV genome integrations of clinically relevant sized vectors in the brains of animals after the delivery of editing machinery. Our results are important for development and safety considerations of CRISPR therapies for SCA2 and other neurodegenerative disorders.

# (#397) Autologous hematopoietic stem cell gene therapy for people with Friedreich's ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 397

***Ms. Nikoletta Jastrzebowska*** [1], ***Dr. Sian Baker*** [1], ***Ms. Chloe Moutin*** [1], ***Dr. Helen Scott*** [1], ***Prof. Neil Scolding*** [1], ***Dr. Oscar Cordero-Llana*** [1], ***Prof. James Uney*** [1], ***Dr. Kevin Kemp*** [1]

***1.*** *University of Bristol*

**Background and objective:**
Friedreich's ataxia (FA) is a neurodegenerative disease currently lacking any proven treatment. Experimental findings have provided evidence that allogeneic hematopoietic stem cell (HSC) transplantation may offer an effective treatment for the disease. When used in clinical practice, however, allogeneic transplantation is associated with significant morbidity and mortality. To mitigate this limitation, we propose to investigate whether viral vector-mediated genetic modification of autologous HSCs (insertion of a functional frataxin gene [*FXN*] ex vivo) and subsequent HSC transplantation, is of therapeutic benefit. The objective of this study was therefore to develop a lentiviral transduction protocol for efficient and stable *FXN* delivery to isolated murine FA HSCs.
**Methods:**
Lineage$^{-}$, Sca-1$^{+}$, c-Kit$^{+}$ hematopoietic stem cells (LSKs) were isolated from the bone marrow of FA transgenic mice (Fxn$^{null}$ ::YG8s(GAA)$_{>800}$) and transduced with a third-generation lentiviral vector carrying *FXN* and *EGFP* reporter (*lenti*-FXN.EGFP). Transduction efficiency and vector copy number (VCN) were assessed. Functional gene replacement was determined through analysis of frataxin protein levels, cell proliferation and differentiation, and mitochondrial function.
**Results:**
Using *lenti*-FXN.EGFP, a LSK transduction efficiency of 88% was achieved with an average VCN of 6 copies per cell. LSK cultures demonstrated a 12-fold increase in human frataxin protein expression following transduction, which was accompanied by a significant elevation in succinate dehydrogenase activity. *In vitro* quantification of LSK proliferation and differentiation showed no detrimental effects caused by lentiviral transduction on cell function.
**Discussion and conclusion:**
Our results demonstrate that lentiviral vector-mediated genome editing can provide an effective method for *FXN* gene correction and FA phenotype rescue in isolated HSCs. Further in vivo studies assessing transplantation of autologous *FXN*-corrected HSC are warranted to demonstrate the therapeutic potential of our approach. Future translation into clinical trials may offer the development of a novel disease-modifying treatment for people with FA.

# (#409) Preclinical development of TRPC3 inhibitors to treat spinocerebellar ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 409

***Dr. Bethan Cole*** [1], ***Dr. Tryfon Zarganis-Tzitzikas*** [2], ***Dr. Cassandra Adams*** [2], ***Prof. Paul Brennan*** [2], ***Prof. Esther Becker*** [1]

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Background

Spinocerebellar Ataxias (SCAs) are autosomal dominant neurodegenerative disorders primarily affecting the cerebellum, a key region of the brain responsible for movement and coordination. Progressive dysfunction of the cerebellum leads to severely impaired quality of life and for many patients is fatal. In addition to problems with balance, speech, and movement, patients often display cognitive disabilities, Parkinsonism, and seizures. There is an unmet need for therapeutic treatments.

Over 40 pathogenic variants are associated with SCAs, but common mechanisms likely underlie different genetic forms. One such mechanism is dysregulated $Ca^{2+}$ homeostasis in cerebellar Purkinje cells. The TRPC3 channel is essential for $Ca^{2+}$-signalling and excitability downstream of mGluR1 at the Purkinje cell postsynaptic membrane. Gain-of-function mutations in TRPC3 have been shown to cause SCA both in a patient with SCA41 and the *Mwk* mouse model. Notably, dysregulated TRPC3 signalling has been identified in several other SCAs, including SCA1 and SCA2. Inhibition of TRPC3 may therefore be a promising therapeutic strategy to treat multiple SCA subtypes.

Methods

In collaboration with medicinal chemists, we are generating and optimising drug leads to target TRPC3 channels.

Results

Compounds have been screened initially using an immunofluorescence-based assay in Neuro-2A cells, which has yielded a number of active inhibitors. Positive hits are being further validated using whole-cell electrophysiology to assess inhibition of TRPC3 channels overexpressed in HEK293-T cells.

Conclusions

We have identified small molecule inhibitors of TRPC3 using *in vitro* assays and have set up a pipeline to further optimise these compounds. Effects of lead compounds on Purkinje cell synaptic transmission in acute cerebellar slices will be assessed before testing in an *in vivo* SCA preclinical model. We are initially focusing on SCA2, since dysregulated mGluR1-TRPC3 signalling is well-described in mouse models of this subtype. Ultimately, we hope to translate these drugs into clinical testing in SCA patients.

# (#434) Exploring the SUV4-20 Methyltransferases as Potential Therapeutic Targets for Friedreich's Ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 434

***Mr. Saumya Maheshwari*** [1], ***Dr. Gabriela Vilema-Enriquez*** [2], ***Mr. Robert Quinlan*** [2], ***Dr. Loïc Cochard*** [2], ***Dr. Sandor Szunyogh*** [2], ***Prof. Paul Brennan*** [2], ***Prof. Richard Wade-Martins*** [2]

***1.*** *Universty of Oxford,* ***2.*** *University of Oxford*

**Background and Objective**

The molecular mechanisms associated with the reduced expression of the frataxin gene (*FXN*) in Friedreich's ataxia (FRDA) have been linked to its epigenetic modification. Expanded GAA repeats at the mutant locus induce a repressive heterochromatin environment, with accumulation of repressive methylated histone marks such as H4K20me3. We have identified SUV4-20, responsible for H4K20 me2/me3, as a potential novel therapeutic target for FRDA. Here, we aim to understand the effect of the inhibition of SUV4-20 in several cellular models of FRDA, including iPSC-derived sensory neurons.

**Methods**

To achieve this, we utilised the tool compound A-196, alongside structural analogues based on the known crystal structure of the tool compound/protein complex. We confirmed *FXN* expression before and after treatment with the compounds, in addition to measuring global levels of H4K20 methylation following treatment with A-196. Finally, we will assess the effect of inhibiting SUV4-20 in the phenotypes present in FRDA iPSC-derived sensory neurons.

**Results**

Chemical inhibition of SUV4-20 by the tool compound A-196 increased the expression of *FXN* by approximately 1.5 fold in a *FXN-GAA-Luc* reporter cell line. The magnitude of expression was further increased utilising A-196 structural derivatives. A-196 also increased *FXN* expression in various patient-derived cells, as A-196 derivatives did. Finally, A-196 and the structural derivatives also increased *FXN* expression in FRDA iPSC-derived sensory neurons, a relevant cell type where *FXN* expression is severely reduced.

**Discussion and Conclusion**

Our data in various cell types shows that methylation of H4K20, is important in the regulation of the expression of *FXN*. Thus, SUV4-20 could emerge as a potential therapeutic target for FRDA. Therefore, understanding the biology of SUV4-20 inhibition in sensory neurons is key to pursuing this as a promising target.

# (#453) Preclinical Evaluation of Intravenously Administered LX2006 Gene Therapy for the Treatment of FA-Associated Cardiomyopathy

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 453

***Dr. Richie Khanna*** [1], ***Dr. Dolan Sondhi*** [2], ***Mx. Carlos Munoz Zuluaga*** [2], ***Mx. Monica Gertz*** [2], ***Mx. Melissa Yost-Bido*** [2], ***Mx. Alessandria Greco*** [2], ***Mx. Alvin Chen*** [3], ***Mx. Vikrum Kooner*** [2], ***Dr. Jonathan B. Rosenberg*** [2], ***Dr. Bishnu P. De*** [2], ***Dr. Stephen M. Kaminsky*** [2], ***Dr. Nithya Selvan*** [4], ***Ms. Anju Nair*** [4], ***Dr. Anthony S. Fargnoli*** [4], ***Dr. Timothy D. Fenn*** [4], ***Dr. Ronald G. Crystal*** [2], ***Dr. Jay Barth*** [1]

***1.*** *LEXEO Therapeutics Inc.,* ***2.*** *Department of Genetic Medicine, Weill Cornell Medical College, New York, NY,* ***3.*** *2Department of Genetic Medicine, Weill Cornell Medical College, New York, NY,* ***4.*** *LEXEO Therapeutics, Inc, New York, NY*

**Background and Objective:** Friedreich's Ataxia (FA) is a rare autosomal recessive disease caused by mutations in the frataxin gene (*FXN*), which encodes FXN protein, required for mitochondrial energy production. Even though FA shows progressive neurologic disease, the cardiomyopathy is the main cause of death in majority of patients. LX2006 is an AAVrh.10 based gene therapy designed to promote cardiac FXN expression and is being developed for the treatment of FA-associated cardiomyopathy. The efficacy and safety of LX2006 was evaluated in several pre-clinical studies.

**Methods:** LX2006 was tested over a broad range of doses (E11 to E12 gc/kg) in animals following intravenous administration. Efficacy studies were conducted in the milder (aMyhc) and the severe (MCK) cardiac-disease mouse models. Studies were conducted in non-human primates (NHPs) to evaluate the biodistribution, expression, and safety of LX2006.

**Results**: LX2006 improved key cardiac efficacy measures to wild type levels in aMyhc mice. A dose-dependent improvement in efficacy was seen in MCK mice with minimally effective dose and significantly effective dose. These dose levels were associated with myocardial FXN expression that reached ~26% and above, relative to normal human levels. In the short- and long-term safety NHP studies, LX2006 was safe and well tolerated, with biodistribution and expression in various cardiac regions.

**Discussion and Conclusion:** It is anticipated that achieving about 30% of normal human FXN levels, the minimal FXN level in healthy FA heterozygote patients, will provide a clinically meaningful benefit to FA homozygote patients. The clinical dose range was determined using the doses in MCK mice that resulted in FXN levels about 30% or above normal human levels and were safe in NHPs. Collectively, these data demonstrated efficacy and safety of LX2006 and supported the initiation of First-In-Human study in FA patients associated with cardiomyopathy.

# (#456) Identification of Frataxin in mouse and monkey heart by UHPLC-MS following IV administration of LX2006 Gene Therapy

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 456

***Dr. Nithya Selvan*** [1], ***Dr. Teerapat Rojsajjakul*** [2], ***Dr. Nicolas Eskenazi*** [2], ***Dr. Bishnu P. De*** [3], ***Dr. Jonathan B. Rosenberg*** [3], ***Dr. Stephen M. Kaminsky*** [3], ***Dr. Dolan Sondhi*** [3], ***Dr. Ronald G. Crystal*** [3], ***Ms. Anju Nair*** [1], ***Dr. Jay Barth*** [4], ***Dr. Clementina Mesaros*** [2], ***Dr. Ian A. Blair*** [2], ***Dr. Richie Khanna*** [4]

***1.*** *LEXEO Therapeutics, Inc, New York, NY,* ***2.*** *University of Pennsylvania,* ***3.*** *Department of Genetic Medicine, Weill Cornell Medical College, New York, NY,* ***4.*** *LEXEO Therapeutics Inc.*

**Background and Objective:** Human frataxin (hFXN, 1-210), defects in the gene encoding which lead to Friedreich's Ataxia, undergoes proteolytic processing resulting in a 130-amino acid mature mitochondrial form (81-210). Mouse mature FXN (78-207, N) arises by the same mechanism, however, unlike primates, mice also have truncated extra-mitochondrial forms of unknown function where one or more N-terminal residues are lost (79-207, N-1, etc.). The objective of this study was to utilize a novel ultrahigh performance liquid chromatography-mass spectrometry (UHPLC-MS) method to (i) distinguish endogenous FXN levels in mice/monkeys from hFXN levels following LX2006 (AAVrh.10hFXN) administration; (ii) determine whether hFXN expressed in mice undergoes truncation and; (iii) determine heart hFXN levels post-LX2006 administration.

**Methods**: C57Bl/6 mice and green vervets were intravenously administered LX2006 at doses between E11-E13 gc/kg. Necropsied hearts were homogenized; endogenous FXN and hFXN levels quantified using stable isotope hFXN internal standard coupled with immunoprecipitation and 2D nano-UHPLC-MS with parallel/multiple reaction monitoring.

**Results:** Our method distinguished endogenous FXN in mice/monkeys from hFXN following LX2006 administration. Unlike mouse FXN, which was primarily processed to N-1, then N forms, hFXN was processed to N followed by N-2 forms in treated mouse hearts. Average total endogenous heart FXN levels were ~26 ng/mg tissue in mice and ~0.1 ng/mg tissue in monkeys. Average total heart hFXN levels were dose dependent and ranged from ~0.2-25 ng/mg in mice and ~0.2-0.5 ng/mg in monkeys.

**Discussion and Conclusion:** Unlike ELISA assays typically used for FXN detection, our UHPLC-MS method distinguishes mouse/monkey endogenous FXN from hFXN in a single assay. We show for the first time that hFXN expressed in mice undergoes truncation, albeit differently to mouse FXN; the effect of differential processing on treated mice is unclear. Importantly, at the doses administered, hFXN levels in treated monkeys were comparable to endogenous levels and not highly overexpressed.

# (#463) Limited Therapeutic Impact of Intermittent Fasting and Ketogenic diets in a Mouse Model of Spinocerebellar Ataxia Type 3

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 463

***Ms. Cármen Vieira*** [1], ***Ms. Sara Guerreiro*** [2], ***Dr. Sara Duarte-Silva*** [3], ***Dr. Carina Soares-Cunha*** [3], ***Ms. Bruna Ferreira-Lomba*** [2], ***Ms. Daniela Monteiro-Fernandes*** [3], ***Prof. Patrícia Maciel*** [3], ***Dr. Andreia Teixeira-Castro*** [1]

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Several studies reported that diet manipulations, both by prolonged reductions in caloric intake and periodic fasting increase longevity and delay disease onset, mainly due to the occurrence of a metabolic switch from the use of glucose to ketone bodies, as fuel source. These dietary regimens have, therefore, become very popular in the past years.

In Spinocerebellar Ataxia type 3 (SCA3), caloric restriction (CR) improved motor coordination and neuropathology in mice. However, studies in animals and humans are revealing beneficial effects of intermittent fasting (IF) on health beyond those resulting from CR without intermittent metabolic switching.

We are investigating the potential of time-restricted feeding (TRF) (restricts daily food consumption to an 8-hour window) and of ketogenic (KETO) diet (rich in fat and low in carbohydrates) regimens to delay SCA3 progression, by evaluating its impact on behavior, neuronal function, and neuropathology in a SCA3 transgenic mouse model.

At 7 weeks, animals were assigned to control, TRF or KETO diets. We validated that both dietary regimens were able to steadily induce ketosis, without causing body weight loss in SCA3 animals. We then evaluated the motor performance of the mice exposed to diet alterations across disease progression. Surprisingly, we found a limited impact of both diets on the animals' motor coordination and balance, suggesting that these approaches were unable to modify disease progression.

At 25 weeks, we performed *in vivo* single-cell electrophysiological recordings in affected brain regions, and we are currently characterizing how these dietary regimens modulate neuronal function. We are also determining the effects of the diets on the brain transcriptomic profile and neuropathology of SCA3 mice.

The importance of this work relies on describing the impact of diet-induced metabolic switching in SCA3 pathogenesis to inform clinicians about potential beneficial/detrimental effects to the patients that are willing to start these diets.

# (#478) Evaluation of RNA trans-splicing as a therapeutic strategy for spinocerebellar ataxia type 1

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 478

***Dr. Ronald Buijsen*** [1], ***Prof. Elena Avale*** [2], ***Dr. Jean-Marc Gallo*** [3], ***Prof. Karen Anthony*** [4]

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**Background and objective**

Spinocerebellar ataxia type 1 (SCA1) is caused by an expanded polyglutamine (polyQ) tract in the protein ataxin-1 encoded by the *ATXN1* gene. The exact pathogenic mechanism is not understood but phosphorylation of ataxin-1 at S776 is critical for the stabilisation and neurotoxicity of polyQ-expanded ataxin-1. Our objective is to evaluate the therapeutic potential of preventing pathogenic phosphorylation of ataxin-1 using an RNA reprogramming technology.

**Methods**

Spliceosome-mediated RNA *trans*-splicing (SMaRT) creates a hybrid mRNA through a *trans*-splicing reaction between an endogenous target pre-mRNA and an exogenously delivered pre-*trans*-splicing molecule (PTM). We constructed and tested, *in-vitro*, several PTMs designed to substitute S776 or S752 (the mouse homologue for S776) for alanine. PTMs were constructed in pcDNA3.1 and used to generate lentiviral constructs containing a GFP expression cassette. *Trans*-splicing in transfected, or transduced cells was analysed by RT-PCR and sequencing. Endogenous and ataxin-1 minigene transcripts were analysed, minigenes were constructed using the pSPL3 exon trapping vector.

**Results**

Human (SH-SY5Y) and mouse (N2a) cell lines were transfected with PTMs with and without minigenes. SMaRT successfully edited, *in-vitro*, mouse and human *ATXN1* transcripts to substitute S752 or S776 for alanine, with efficiencies of approximately 30% for endogenous human transcripts. We additionally observed *trans*-splicing of endogenous ataxin-1 in cultured primary cortical neurons from wild-type mice. The most efficient PTM design hybridises with the 3' end of intron 8, upstream of the branch point.

**Discussion and conclusion**

SCA1 is an excellent prototypic system to demonstrate that a SCA-causing protein can be converted into a non-toxic form by SMaRT. SMaRT can theoretically repair any mutation downstream of the PTM binding site and is particularly suited for dominant gain of function mutations characteristic of SCAs. Our work demonstrates the potential of SMaRT to prevent a pathogenic phosphorylation event and provides proof-of-concept for *in-vivo* preclinical development.

# (#498) Nrf2 agonist omaveloxolone improves cardiac function in mouse model of Friedreich's ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 498

***Mr. Francisco Figueroa [1], Ms. Lili Salinas [1], Ms. Claire B. Montgomery [2], Dr. Phung N. Thai [1], Prof. Nipavan Chiamvimonvat [1], Prof. Gino Cortopassi [1], Dr. Elena N. Dedkova [3]***

***1.*** *University of California, Davis,* ***2.*** *University of Califonia, Davis,* ***3.*** *University of California, Davis, California Northstate University College of Medicine*

**Introduction:** Friedreich's ataxia (FA) is an inherited, neurodegenerative disorder affecting ~1 in every 50,000 people in the USA and Europe. Initial symptoms include loss of balance and movement coordination, placing FA patients in wheelchairs. However, most FA patients die in their thirties from cardiac failure, caused by deficient frataxin (FXN) expression in the heart. Omaveloxolone (OMAV), a known Nrf-2 activator, has been proposed as the first treatment of neurological symptoms of FA; however less is known about OMAV effects on cardiac function.

**Aim:** To test the hypothesis that OMAV improves cardiac function in a mouse model of FA via Nrf-2 signaling pathway activation.

**Methods and Results:** Cardiac-specific FXN knockout (FXN Mck-Cre KO) mice were used as a model of severe cardiomyopathy. Animals of either sex were randomly split in vehicle and OMAV-treated groups. OMAV treatment (24 mg/kg) started at 3 weeks of age and continued for 5 weeks. Cardiac function was examined *in-vivo* by echocardiography. We found that KO mice developed severe heart failure with 45% reduction in ejection fraction, increased left ventricular (LV) mass (+80%) and diameter (+308%), decreased stroke volume (-26%) and cardiac output (-38%, n=10) as compared to wild-type mice (n=12). Cardiac deficits were more pronounced in FA males compared to females. OMAV (n=14) significantly decreased LV mass (-24%), LV diameter (-28%), and partially recovered a decrease in ejection fraction (+40%), stroke volume (+22%) and cardiac output (+35%). Gene expression of NADP(H) quinoline oxidoreductase-1 (NQO1), superoxide dismutase 2 (SOD2), and glutathione S-transferase were decreased by 60-90% in FA and recovered by 60-90% by OMAV. Aconitase activity, used as a surrogate measure of frataxin's iron-sulfur biogenesis function, was decreased in FA by 49% and recovered 33% by OMAV.

**Conclusions:** This is the first study to how that OMAV can improve cardiac function in FA mice with severe cardiomyopathy.

# (#535) Etravirine analogues promote frataxin upregulation

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 535

***Dr. Luca Panarello [1], Dr. Giulia Alfedi [2], Prof. Florence Malisan [1], Dr. Ivano Condò [1], Prof. Roberto Testi [3], Dr. Alessandra Rufini [3]***

***1.*** *Laboratory of Signal Transduction, Department of Biomedicine and Prevention; University of Rome "Tor Vergata", 00133 Rome, Italy,* ***2.*** *1. Laboratory of Signal Transduction, Department of Biomedicine and Prevention, University of Rome "Tor Vergata", 00133 Rome, Italy,* ***3.*** *1. Laboratory of Signal Transduction, Department of Biomedicine and Prevention, University of Rome "Tor Vergata", 00133 Rome, Italy; 2. Fratagene Therapeutics, 00144 Rome, Italy.*

**Background and Objective**

Etravirine, a non-nucleoside reverse transcriptase inhibitor (NNRTI) currently used as an anti-retroviral therapy to treat HIV infection, has recently raised interest in Friedreich ataxia (FRDA) for its ability to increase frataxin levels and rescue some phenotypic defects associated with frataxin deficiency. To further investigate the potential therapeutic of etravirine in FRDA, we studied the efficacy of different etravirine analogues and analysed their ability to upregulate frataxin in patients-derived cells, with the aim to identify molecules with increased activity and potency.

**Methods**

We evaluated the effects of two structurally related etravirine analogues, dapivirine and rilpivirine, and two structurally unrelated analogues, efavirenz and nevirapine, which share the same mechanism of action on their HIV target. We tested the effects of etravirine analogues on HEK293 cells stably overexpressing frataxin and in patients-derived lymphoblastoid cell lines and fibroblasts, and compared them with etravirine. We simultaneously evaluated their effect on cell viability.

**Results**

From our studies we can conclude that the structurally similar etravirine analogues, dapivirine and rilpivirine, show a slightly stronger potency in upregulating frataxin in FRDA cells, while the structurally different analogues, nevirapine and efavirenz, have no effect. None of the drugs tested affect cell viability. These data confirm the therapeutic potential of this class of NNRTI drugs in FRDA.

**Discussion and Conclusion**

The study of the effects of etravirine analogues in patients-derived cells could add to our understanding of etravirine function in FRDA. A comparative analysis between the structure and function of etravirine and its analogues is important for the identification of structural determinants that confer frataxin-upregulating properties to the drug. These data could provide a starting point toward the identification of the molecular targets involved in this pathway. Moreover, the identification of an analogue with increased potency and increased frataxin-upregulating properties could be of great therapeutic relevance.

# (#543) Novel fumarate prodrug and omaveloxolone improve cardiac and motor function in FXNKD mouse model of Friedreich Ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 543

***Ms. Lili Salinas [1], Mr. Francisco Figueroa [1], Ms. Claire B. Montgomery [2], Mr. Landon C. Sims [1], Dr. Phung N. Thai [1], Prof. Nipavan Chiamvimonvat [1], Prof. Gino Cortopassi [1], Dr. Elena N. Dedkova [3]***

***1.*** *University of California, Davis,* ***2.*** *University of Califonia, Davis,* ***3.*** *University of California, Davis, CA; California Northstate University College of Medicine, Elk Grove, CA*

**Background and Objective:** Friedreich's ataxia (FA) is a monogenic recessive ataxia for which there is no approved therapy. It is caused by reduction of a single mitochondrial protein frataxin (FXN) and eventually leads to the development of lethal cardiomyopathy. In this study, we tested the efficacy of 3 potential FA drugs, omaveloxolone, a known Nrf2 activator, and two monomethyl fumarate (MMF) prodrugs, dimethyl fumarate (DMF) and a novel MMF prodrug IMF, in their ability to improve cardiac function and neurobehavior in the inducible UCLA mouse model of FA (FXNKD).

**Methods:** FXNKD mice were fed doxycycline chow for 12 weeks, drugs or vehicle/placebo were dosed from week 4-12 at their most effective concentrations. Cardiac function was examined by *in-vivo* echocardiography. Neurobehavioral benefit was assessed by rotarod, 16 mm balance beam crossing and open field maze.

**Results:** Neurobehavioral deficits were progressive in FXNKD mice from week 4-12 and these deficits progressed significantly faster in FXNKD males vs females. Both IMF and DMF significantly improved the total time traveled in the open field maze in FXNKD males, while omaveloxolone did not. Rotarod performance was improved in FXNKD males by IMF and omaveloxolone only.

Echocardiography revealed a significant increase in left ventricular wall thickness, and a decrease in LV internal diameter, stroke volume, and cardiac output in FXNKD males as well as impairment in diastolic function. IMF and omaveloxolone significantly improved cardiac function in FXNKD males. Both drugs partially recovered expression of genes in Nrf-2 signaling pathway (NQO1, GPX4 but not HO-1) in FXNKD males.

**Conclusions:** The progression of neurological and cardiac phenotype is faster in males than female UCLA FXNKD mice giving a broader dynamic range for detection of drug effect. Omaveloxolone and novel fumarate prodrug IMF delayed progression of some neurological and cardiac deficits, thus have potential for benefit in Friedreich's ataxia.

# Poster Sessions: Emerging Therapies (clinical)

# (#46) Open pilot trial testing safety and efficacy of Etravirine in Friedreich's ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 46

***Dr. Andrea Martinuzzi*** [1], ***Dr. Gabriella Paparella*** [1], ***Dr. Cristina Straga*** [1], ***Dr. Valentina Dal Molin*** [1], ***Dr. Gianantonio Martorel*** [1], ***Dr. Vasco Merotto*** [1], ***Dr. Arianna Piazza*** [1], ***Prof. Roberto Testi*** [2]

***1.*** *IRCCS Medea,* ***2.*** *University of Rome "Tor Vergata", Fratagene Therapeutics*

**Backgound and Objectives**: Etravirine, an anti HIV drug approved for use in children and adults with an excellent safety profile has been recently shown to promote Frataxin accumulation in cells and Fredreich's ataxia (FRDA) rodent models. In spite of continuous effort FRDA remains a disease without an effective approved treatment.
**Methods**. We designed a pilot open label phase 2 trial to test safety and efficacy of Etravirine in 30 FRDA molecularly confirmed FRDA patients aged 10-40 (NCT04273165). The trial was designed to allow the detection of change within the individual progression rate with a pre-treatment and a post-treatment observation. Recruited patients are stratified in 3 group according to severity. Half of them are treated with Etravirine for 4 months 200 mg/day and half with 400 mg/day. Safety indicators are: number and severity of adverse events reported by the patients or encountered at the clinical checks. Efficacy primary endpoint are changes in peak $VO_2$ as measured by incremental armo-ergometer exercise test. Secondary endpoints include: SARA score, heart wall thickness, Sokolov index, Frataxin levels and molecular analysis of Frataxin mRNA translation efficiency in peripheral blood mononuclear cells, perceived quality of life and disability. Complete sets of data are collected 4 months before the treatment start (T -4), at the start (T0), at the end of the treatment (T4) and 4 months after stop-treatment (T8).
**Results**. The recruitment of the expected number of patients (33) has been completed in January 2022. As of today 19 patients completed the trial, 6 finished the treatment and 8 are taking the drug. Thus far one serious adverse event was reported (not drug related). Final results will be available by January 2023.
**Conclusion**. The results of this pilot trial could open the way to a larger placebo controlled trial adding a potential new weapon to fight FRDA.

# (#82) A double-blind, randomized, placebo-controlled trial to test the efficacy, safety and tolerability of Dimethylfumarate in Friedreich Ataxia (DMF-FA-201). Study protocol.

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 82

***Dr. chiara pane*** [1], ***Dr. Cinzia Valeria Russo*** [1], ***Dr. Andrea Salzano*** [1], ***Prof. Antonio Cittadini*** [1], ***Prof. Francesco Saccà*** [1]

***1.*** *Federico II University*

**Background and Objective**

Several trials have been performed in Friedreich ataxia (FRDA) patients, but treatment is available. Compared to controls, *FXN*/mRNA is reduced to 20% in patients, and to 53% in carriers, and shows an inverse correlation with the size of the GAA repeat. Dimethyl fumarate (DMF) induces Nrf2 which binds to the *FXN* gene. DMF dose-dependently increases *FXN*/mRNA and frataxin in FRDA patient lymphoblasts, in the YG8 and KIKO mouse models (including the cerebellum). DMF can also increase *FXN*/mRNA by more than 70% in Multiple Sclerosis patients taking DMF as part of their standard of care treatment.

**Methods**

We will run a double-blind, placebo-controlled, randomized, phase II clinical trial to test the effect of DMF on *FXN*transcription and frataxin protein in FRDA patients. Secondary endpoints will be: the cardiopulmonary exercise test, cardiological measures, the nrf2 pathway, safety and tolerability, and clinical scales. The study is composed of two sequential phases of 12 weeks each: core and extension phase. During the core phase, patients will be randomly assigned to DMF 240 mg BID or placebo. During the extension phase, all patients will be treated with DMF. The study will enroll 40 patients with a molecular diagnosis of FRDA with a homozygous GAA expansion, age 12 years or higher, and with a body weight of at least 30 Kg.

**Results, Discussion and Conclusion**

The availability of an effective treatment able to halt or slow disease progression may be of immense value and could offer patients a therapeutic opportunity. The trial will take place at the Federico II University of Naples and is supported by a grant from the Agenzia Italiana del Frmaco (AIFA), the Tristan Allamby Research Fund (TARFfa) and the Friedreich's Ataxia Research Alliance (FARA).

# (#99) SARS-CoV-2 in patients with Friedreich Ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 99

***Ms. Megan Shen*** [1], ***Ms. Kimberly Schadt*** [1], ***Dr. David Lynch*** [1], ***Ms. Kellie Mcintyre*** [1], ***Dr. Layne Rodden*** [1]

***1.*** *Children's Hospital of Philadelphia*

*Background*

Friedreich ataxia (FRDA) is classically described as a neuromuscular disease that often presents with multisystemic findings including cardiomyopathy and diabetes that are concerning for COVID-19 risk. Best practices for treatment and vaccination in this population are unknown.

*Objective*

To describe COVID-19 outcomes in patients with FRDA and characteristics contributing to risk of illness and severity.

*Results*

Of 26 COVID-19 cases (8.8%) in a cohort of 297 patients, 2 (7.7%) were asymptomatic, 19 (73.1%) exhibited mild-moderate symptoms, and 5 patients (19.2%) were hospitalized, of which 2 died (7.7%). Comorbid diabetes mellitus (DM), including both early and late-onset types, was significantly associated with COVID-19 infection (RR 3.68, 95% CI 1.70-7.97) and hospitalization (RR 11.4, 95% CI 1.52-85.7). Other characteristics more common among those hospitalized were older age (p=0.038), more advanced neurological disease (p=0.038), and nonambulatory status (p=0.047). Presence of cardiomyopathy, age of FRDA-onset, and GAA repeat length were not associated with susceptibility to COVID-19 infection or severity. Five COVID-19 cases were observed post-vaccination, with an overall vaccination rate of 68.0%.

*Conclusions*

This study suggests that while FRDA-related diabetes may mediate increased COVID-19 risk distinctively from classical DM types, other features of FRDA including cardiovascular and neurological disease do not influence the risk of COVID-19.

# (#148) The impact of COVID-19 infection on individuals with progressive ataxia: results of an on-line survey undertaken by Ataxia UK

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 148

***Dr. Rajith de Silva [1], Ms. Adeela Ahmad [2], Ms. Emily Cutting [2], Ms. Suzanne Booth [3], Dr. Priya Shanmugarajah [4], Dr. Julie Greenfield [2], Prof. Paola Giunti [5]***

***1.*** *BHR Univ Hosp NHS Trust,* ***2.*** *Ataxia UK,* ***3.*** *Ataxia Centre, UCLH, National Hospital for Neurology & Neurosurgery, Queen Square, London,* ***4.*** *Academic Department of Neurosciences, Sheffield Teaching Hospitals, UK,* ***5.*** *Ataxia Centre, Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, London*

*Background and Objective:*
The impact of COVID-19 infection on individuals with progressive ataxia was evaluated- specifically to see how the infection influenced ataxia-related symptoms.
*Methods:*
An on-line survey conducted by the UK patient support organisation Ataxia UK.
*Results:*
There were 22 respondents from an estimated 4000 people with ataxia, and 17 provided data that could be analysed further. Twelve had hereditary ataxia (6 with Friedreich's Ataxia (FA)), and none received immunosuppressants (for immune-mediated ataxia). COVID-19 symptoms reported during the illness included fatigue (94%), cough (88%), dyspnoea (76%), weakness (76%) and pyrexia (59%). Loss of smell/taste was reported by 41%. Mean disease duration was 0.73 months. Ataxia-related symptoms that deteriorated acutely were fatigue (73%), balance (73%), gait/mobility (64%) and bulbar dysfunction (64%). After recovering, 75% reported no change in their level of mobility, but when asked if there were any complaints that were worse, 82% complained of fatigue.
*Discussion and Conclusions:*
The number of respondents was surprisingly low. At least 300 might have been expected (based on Ataxia UK's membership and the population risk of acquiring COVID-19). Not all cases might have been identified, but it is possible that the number of individuals with progressive ataxia who contracted COVID-19 was truly low. This vulnerable group may have heeded public health messages (including guidance publicised by Ataxia UK) to shield exceptionally well. The proportion of FA cases was slightly higher than expected. Co-morbidities in FA (cardiomyopathy, diabetes, etc.) may make it more likely for symptoms to develop. The impact of COVID-19 on balance appears to be transitory, with a marginal impact on mobility long-term. However, the majority report worse fatigue in the aftermath, a key symptom of "long COVID". This may have implications on the long-term management of progressive ataxia post-pandemic.

# (#161) A qualitative study exploring attitudes of individuals with Friedreich ataxia towards gene therapy

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 161

***Ms. Katherine Lieschke [1], Ms. Varlli Scott [1], Prof. Martin B Delatycki [2], Dr. Sharon Lewis [3], Prof. Megan Munsie [4], Dr. Claire Tanner [5], Dr. Louise A Corben [6]***

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Background and Objective

Scientists and pharmaceutical companies are working towards delivering gene therapy for Friedreich ataxia (FRDA). Understanding the views of individuals with FRDA and their parents toward gene therapy is essential to inform trial design and identify understanding and potential barriers to participation in clinical trials. The goals of this study are to (1) identify topics related to gene therapy important to individuals with FRDA and their families, and to identify perceived knowledge gaps about (2) gene therapy, and (3) what participating in a FRDA gene therapy trial will involve. Findings will inform the development of tailored educational resources and enhanced ethical consent processes to promote informed consent for potential trial participants.

Method

Twenty individuals with FRDA aged ≥14 years or parents/carers/guardians of individuals with FRDA were invited to participate, ensuring a proportional balance of both groups. Audio-recorded, semi-structured, qualitative interviews explored participants' experiences of FRDA, knowledge about clinical trials, views on gene therapy including risks and benefits, and potential barriers to participation in trials. Interviews were transcribed verbatim, de-identified and thematically analysed.

Results

Nineteen interviews have been conducted (11 adults, 2 adolescents with FRDA; 6 parents). Preliminary findings indicate low levels of knowledge about gene therapy, divergent opinions regarding participating in first-in-human trials with potential risks of gene therapy the greatest concern. Full results will be presented.

Discussion and Conclusion

Findings from this study indicate there is strong desire for information regarding gene therapy in FRDA. The level of uncertainty around gene therapy makes decision making challenging. It is critical that the FRDA community has access to trusted sources of high quality information around gene therapy to support their choices. The success of future gene therapy trials in FRDA is dependent on developing materials and strategies to engage with potential participants effectively.

# (#163) Best practice for rare diseases – developing Clinical Management Guidelines for Friedreich Ataxia.

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 163

***Dr. Louise A Corben*** [1], ***Dr. Sarah Milne*** [2], ***Dr. Veronica Collins*** [2], ***Ms. Ann Musheno*** [3], ***Ms. Jennifer Farmer*** [3], ***Dr. David Lynch*** [4], ***Dr. Sub H. Subramony*** [5], ***Prof. Massimo Pandolfo*** [6], ***Prof. Jörg Schulz*** [7], ***Dr. Kimberley Y Lin*** [8], ***Prof. Martin B Delatycki*** [9]

***1.*** *Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia, School of Psychological Sciences, The Turner Institute for Brain and Mental Health, Monash University, Clayton, Victoria, Australia,,* ***2.*** *Murdoch Children's Research Institute,* ***3.*** *Friedreich's Ataxia Research Alliance,* ***4.*** *University of Pennsylvania & Childrens Hospital of Philadelphia,* ***5.*** *Department of Neurology and Fixel Center for Neurologic Disorders, University of Florida,* ***6.*** *McGill University, 7. Department of Neurology, RWTH Aachen University, Aachen, Germany,* ***8.*** *Division of Cardiology, Children's Hospital of Philadelphia,* ***9.*** *Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia,*

Background and Objective

Individuals with Friedreich ataxia (FRDA) require access to specialised clinical management. In response, the Clinical Management Guidelines (CMGs) for FRDA were published in 2014. New evidence emerging since 2014 and a review of the guideline framework warranted an update of the CMGs. However, the lack of high certainty evidence and the inadequacy of accepted metrics to measure health status presents challenges in FRDA. We therefore adopted and expanded the RARE-Bestpractices Working Group Grading of Recommendations Assessment and Evaluation (GRADE) guideline framework for the second iteration of the CMGs for FRDA.

Methods

A panel representing international clinical experts and stakeholders provided oversight to guideline development. Over 140 Patient, Intervention, Comparison, Outcome (PICO) clinical questions guided the literature search. The GRADE framework was used to generate evidence profiles. Additional strategies to support the strength of recommendations for rare diseases including reviewing literature in like conditions, expert observation, utilising natural history registry data and seeking feedback from individuals with FRDA and their families were used.

Results

Seventy clinical experts from 13 countries contributed to 17 chapters. The 2014 CMGs were expanded to include new topics: emergency medicine, digital and assistive technologies and mental health. In total, 128 clinical practice recommendations and 92 best practice statements were generated. Eleven individuals with a lived experience of FRDA provided feedback on the development of topics, the lay summaries and process of dissemination of the CMGs.

Discussion and Conclusion

Evidence-based CMGs help facilitate the best clinical care for people with FRDA. Relative to the previous iteration of the CMGs, the RARE-Bestpractices Working Group GRADE framework enabled the development of higher quality CMGs for FRDA which should lead to better outcomes for people living with this condition. Our experience with using the guideline framework may be helpful to groups developing CMGs for other rare diseases.

# (#164) Facilitating best practice – Finding consensus in Clinical Management Guidelines for Friedreich ataxia.

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 164

***Dr. Louise A Corben*** [1], ***Dr. Sarah Milne*** [2], ***Dr. Veronica Collins*** [2], ***Ms. Ann Musheno*** [3], ***Ms. Jennifer Farmer*** [3], ***Dr. David Lynch*** [4], ***Dr. Sub H. Subramony*** [5], ***Prof. Massimo Pandolfo*** [6], ***Prof. Jörg Schulz*** [7], ***Dr. Kimberley Y Lin*** [8], ***Prof. Martin B Delatycki*** [9]

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**Background and Objective**

The first iteration of the Clinical Management Guidelines (guidelines) for Friedreich ataxia (FRDA), published in 2014, aimed to facilitate best practice healthcare for people with FRDA. Six years later, emerging evidence warranted an update of the guidelines. Adopting the RARE-Bestpractices Working Group Grading of Recommendations Assessment and Evaluation (GRADE) guideline framework provided an opportunity to ensure decision making around recommendations was transparent, the guidelines will be universally accepted, and the challenges associated with guideline development for rare diseases were addressed.

**Methods**

A guideline panel representing international clinical experts and consumers provided guidance as to topic selection and endorsement of the guideline recommendations. Individuals with FRDA and their families provided feedback and identified the priority of the topics. Seventy clinicians and researchers/scientists with expertise in FRDA developed clinical questions within topics that guided the resulting recommendations. A literature search was performed for each clinical question, with clinical observations and registry data used to support the recommendations.

**Results**

The second iteration of the FRDA guidelines comprises 17 chapters, 15 of which include recommendations and/or best practice statements. We will present the new chapters dedicated to fatigue, emergency medicine, mental health, digital and assistive technologies, endocrine and metabolism, orthopaedics and pulmonary function. The neurological chapter include upper limb dysfunction as a dedicated topic, and separate ambulant and non-ambulant management strategies for mobility disturbance and falls. A lack of high-quality published evidence was identified in almost all topic areas. In total, 128 clinical practice recommendations and 92 best practice statements were generated.

**Discussion and Conclusion**

Evidence-based guidelines help facilitate the best clinical care for people with FRDA. A review of the topics has enabled a more granular approach to the management of some previously overlooked issues related to FRDA. This should lead to better outcomes for people living with this condition.

# (#208) Telehealth High Intensity Interval Training prior to Balance in Cerebellar Ataxia: A Case Series

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 208

***Dr. Chelsea Macpherson*** [1], ***Ms. Bria Bartsch*** [1], ***Ms. Miriam King*** [1], ***Dr. Sheng-Han Kuo*** [2], ***Dr. Lori Quinn*** [1]

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**Background and objective**

Individuals with Hereditary Cerebellar Ataxias (HCAs) have symptoms that impact balance, gait, motor learning and control. Balance training (BT) and aerobic exercise (AE) improve motor function in HCA.[2,3] Studies in stroke[4,5] and Parkinson's disease[6,7] have evaluated AE prior to BT as a form of motor priming to enhance motor learning.[8,9] Motor priming has not been explored in HCA. This case series describes a telehealth (TH) delivered motor priming intervention in HCA.

**Methods**

Participants (n=4) were medically cleared for participation. The intervention consisted of 45-minute sessions, twice-a-week for 8 weeks delivered via TH. Using home equipment, participants completed AE via high intensity interval training prior to BT. Outcomes included: Cerebellar Cognitive Affective Scale (CCAS),[10] the first four items of the Scale for Assessment and Rating of Ataxia (mSARA), Static Postural Sway (SPS) and the Timed Up and Go test (TUG). SPS and TUG were evaluated via smartphone app (Mon4t LLC, Binyamina, Israel).[11–13] Participants were evaluated at baseline, mid- and post-intervention.

**Results**

Mean age (SD) of participants was 58.5 (4.4) yrs; 3M/1F. Diagnoses were early-mid stage Spinocerebellar Ataxia type 2, 3, 6, and ataxia without genetic confirmation with family history. Participants showed 100% adherence to the intervention, without adverse events. In 3 participants, heart rate (HR) response to exercise was blunted due to medication. A Beta Blocker Target HR Formula and the BORG rating of perceived exertion were used to monitor intensity. All participants improved on the CCAS (range 5-10 pt), mSARA (range 1-3 pt), SPS Neutral Stance (range 0.005-0.009 $m/s^2$), and TUG (range 0.6-8.0 sec).

**Discussion and Conclusion**

Preliminary data supports a TH-delivered motor priming intervention in HCA, with improvements in disease-specific motor and cognitive measures. Future research will explore exercise dosage for motor priming intervention, and outcomes of motor learning to capture functional cerebellar improvement.

# (#222) Efficacy of Omaveloxolone in Patients with Friedreich's Ataxia: Update of the Delayed-Start Study

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 222

***Dr. David Lynch*** [1]***, Dr. Melanie Chin*** [2]***, Prof. Sylvia Boesch*** [3]***, Prof. Martin B Delatycki*** [4]***, Dr. Angie Goldsberry*** [2]***, Dr. Caterina Mariotti*** [5]***, Dr. Katherine Mathews*** [6]***, Dr. Wolfgang Nachbauer*** [7]***, Dr. Megan O'Grady*** [2]***, Prof. Susan L. Perlman, MD*** [8]***, Dr. Sub H. Subramony*** [9]***, Dr. George R. Wilmot*** [10]***, Dr. Theresa Zesiewicz*** [11]***, Dr. Colin Meyer*** [2]

***1.*** *University of Pennsylvania & Childrens Hospital of Philadelphia,* ***2.*** *Reata Pharmaceuticals, Dallas, TX,* ***3.*** *Department of Neurology, Medical University Innsbruck,* ***4.*** *Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia,,* ***5.*** *Unit of Medical Genetics and Neurogenetics, Fondazione IRCCS Istituto Neurologico Carlo Besta, 20133, Milan, Italy,* ***6.*** *Departments of Pediatrics and Neurology, University of Iowa Carver College of Medicine, Iowa City, IA,* ***7.*** *Center for rare neurological Disorders Innsbruck,* ***8.*** *David Geffen School of Medicine at UCLA, Los Angeles, CA,* ***9.*** *McKnight Brain Institute, Department of Neurology, University of Florida, Gainesville, Florida, United States of America,* ***10.*** *Department of Neurology, Emory University School of Medicine, Atlanta, GA,* ***11.*** *Department of Neurology, University of South Florida Ataxia Research Center, Tampa, FL*

**Background and Objective**

Friedreich's ataxia (FA) is a rare, degenerative neuromuscular disease with no available therapies. Omaveloxolone (Omav), an investigational drug, is an activator of the transcription factor, Nrf2. MOXIe (NCT02255435) was a 2-part study of the safety and efficacy of Omav in patients with FA. MOXIe Part 2 showed that Omav significantly improved modified FA Rating Scale (mFARS) scores by 2.40 points relative to placebo after 48 weeks of treatment (p=0.014; n=82).

**Methods**

Patients in both MOXIe study parts were eligible to receive Omav in a double-blind open-label extension (OLE) study. The full analysis set (FAS) included patients without severe pes cavus. The current analysis compared the difference in mFARS scores between treatment groups (placebo-Omav versus Omav-Omav) using single mixed model repeated measures for all available data from the 48-week placebo-controlled period (MOXIe Part 2) and the 72-week delayed-start period (OLE).

**Results**

Patients previously randomized to placebo or Omav (n=42 and 40, respectively) in the FAS of MOXIe Part 2 enrolled in the OLE. The difference in mFARS scores between Omav and placebo groups observed at the end of MOXIe Part 2 (-2.25 ± 1.07 points, p=0.037) was preserved at the end of the delayed-start period (-3.51 ± 1.45 points, p=0.016). Patients in the placebo-Omav group had an annualized mFARS slope (0.45 ± 0.38 points) similar to the Omav-Omav group (0.27 ± 0.59 points); both slopes were less than the expected +1.9 points/ year observed in natural history data. Patients in the Omav-Omav group continued to show no worsening in mFARS scores relative to their original baseline through treatment week 120 (~2.5 years).

**Discussion and Conclusion**

The results of this study support the positive primary endpoint findings in the pivotal MOXIe Part 2 trial and are consistent with a persistent effect of Omav treatment on disease course in FA.

# (#230) Tissue Frataxin Increases After Administration of CTI-1601, a Frataxin Replacement Therapy in Development to Treat Friedreich's Ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 230

***Dr. David Bettoun [1], Ms. Jennifer Farmer [2], Mr. Devin Schecter [1], Ms. Teresa Galas [1], Dr. Nancy Ruiz [1], Dr. Russell Clayton [3]***

***1.*** *Larimar Therapeutics, Inc.,* ***2.*** *Friedreich's Ataxia Research Alliance,* ***3.*** *Aeremedea LLC*

**Background and Objective:** CTI-1601 is a frataxin (FXN) replacement therapy in development for Friedreich's ataxia (FRDA) designed to deliver human FXN into mitochondria. The pharmacodynamic (PD) and safety profiles of subcutaneous (SC) administration of multiple ascending doses (MAD) of CTI-1601 were evaluated.
**Methods:** In a phase 1, double-blind, placebo-controlled MAD trial (NCT04519567), patients with FRDA (n=27) received placebo or 1 of the following CTI-1601 doses SC: 25 mg once daily (QD) for the first 4 days, then every 72 hours until day 13; 50 mg QD for the first 7 days, then every other day until day 13; or 100 mg QD for 13 days. PD response was assessed by FXN concentrations measured by levels of the abbreviated n-terminal peptide segment for FXN in accessible tissue. Safety evaluations included incidence of treatment-emergent adverse events (TEAEs).
**Results:** Dose-dependent increases in FXN levels were observed in skin, buccal cells, and in platelets with QD CTI-1601 administration. The greatest increases in buccal FXN levels from baseline occurred with 100-mg CTI-1601, but concentration ranges with daily dosing of 50 and 100 mg at day 7 overlapped. Between days 7 and 13, buccal FXN levels were maintained with 100 mg QD. A switch to alternate-day dosing of 50 mg during days 7 and 13, decreased FXN levels. Most TEAEs were grade 1/2. Injection-site reactions were most common (100% of patients receiving CTl-1601; 43% for placebo).
**Discussion and Conclusion:** In this first clinical study of CTI-1601, increases in FXN levels were seen in multiple tissues after 7 days of QD dosing of 50- or 100-mg CTI-1601, reaching FXN levels at or above those observed in asymptomatic heterozygous carriers. CTI-1601 was generally well tolerated. These data support continued study of CTI-1601.

# (#241) The expanding clinical landscape of spinocerebellar ataxia type 47 (SCA47)

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 241

***Mr. Winston Lee*** [1], ***Mr. Salvatore Botta*** [1], ***Mr. Maximilian Cabaj*** [1], ***Prof. Vincenzo Gennarino*** [1]

***1.*** *Columbia University Irving Medical Center*

**Background and Objective**: SCA47 is caused by a mutation in *PUM1*, which is linked to two distinct clinical manifestations: *PUM1*-associated developmental delay, ataxia, and seizures (PADDAS) and the mild, late-onset *PUM*1-related cerebellar ataxia (PRCA). We have assembled a large cohort of patients to further understand the clinical and molecular characteristics of *PUM1*-associated disease since our initial description of these conditions.

**Methods**: We gathered comprehensive clinical histories, neuroimaging, and clinical photographs from 59 subjects with pathogenic (mostly *de novo*) variation in *PUM1*. Variant pathogenicity was assessed by computational modeling and functional cellular assays.

**Results**: The cohort's median age was 10.5 years, and the Female:Male ratio was 1.08. There were 14 subjects with deletions spanning the entire *PUM1* locus, 38 with exonic variants (31 missense, 1 nonsense, 1 synonymous, and 5 frameshifts), and 7 with intronic variants. Coding variants were found in four regions of the protein: the N-terminal low-complexity region, homology-domain 6, and the two regions flanking the RNA-binding domain. All canonical splice variants exhibited splice-altering defects. The most severe, early-onset neurological phenotypes are caused by de novo deletions, predicted null and missense variants flanking the RNA-binding domains. Milder disease phenotypes are caused by insufficiently penetrant alleles, such as the synonymous c.1941C>T (p.(Gly647Gly)), which reduces the amount of correctly spliced mRNA in HEK293T cells while creating an alternative splice donor within Exon 2, or familial variants that segregate from affected and/or unaffected relatives with slow-progressing, adult-onset ataxia and mild neurological features. Subjects with N-terminal variants have early-onset ataxia, suggesting the existence of a new clinical subgroup distinct from PRCA and PADDAS.

**Discussion and Conclusions**: The phenotypic spectrum of *PUM1* is far broader than previously thought. Continued clinical and molecular characterization of novel variants will help us understand the complex pathophysiology of *PUM1*-associated disease and bring us closer to developing personalized treatments.

# (#245) Assessing the value of specialist centres for the diagnosis and management of the ataxias in Europe.

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 245

***Dr. Julie Vallortigara [1], Dr. Julie Greenfield [2], Prof. Barry Hunt [2], Prof. Steve Morris [3], Ms. Emma Hudson [3], Dr. Carola Reinhard [4], Dr. Holm Graessner [4], Ms. Deborah Hoffman [5], Prof. Antonio Federico [6], Ms. Vinciane Quoidbach [7], Prof. Paola Giunti [1]***

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**Background and Objective**: The European Brain Council (EBC) initiated a European project called Value of Treatment for Brain Disorders. This project is looking at the value of early diagnosis and intervention, aiming to assess the benefits of coordinated care and multidisciplinary care patterns on patient outcomes and costs. In this context, a two-year study on ataxia, dystonia, and phenylketonuria was funded. We present here the ataxia case study.
**Methods**: This project explores the patient pathways of individuals with progressive ataxias and compares their experiences attending specialist ataxia centres (SAC) compared with care in non–specialist settings in terms of reaching a diagnosis, access to healthcare services and treatments, and quality of care. We collected data in the UK, Germany and Italy using a patient survey, to gather information about the diagnosis and the management of the ataxias in specialist and non-specialist settings, utilisation of other primary and secondary health care services, and satisfaction of treatment and care received.
**Results:** Patients gave positive feedback about the role of SAC in understanding their condition and ways to manage their ataxia, in coordinating referrals to other specialists, in offering opportunities to take part in research studies, among other aspects of the service. Overall, the symptoms management and care delivered to people living with ataxia in a specialist centre was better than in a non-specialist setting. The patients who attended a multidisciplinary clinic for their ataxia reported the care received there to be effective.
**Discussion and Conclusion:** This study successfully provided crucial information about the value of Specialist Centres in terms of diagnosis and management of patients living with ataxia. Ultimately the EBC Value of Treatment project aims to converge data evidence to policy recommendations on how to improve treatment and care for people with rare neurological diseases across Europe.

# (#257) Baseline Demographics in MOVE-FA: A Study to Assess the Efficacy and Safety of Vatiquinone for the Treatment of Subjects With Friedreich Ataxia (FA)

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 257

***Dr. Bert Yao*** [1], ***Dr. David Lynch*** [2], ***Prof. Antoine Duquette*** [3], ***Prof. Marcondes C. França Jr*** [4], ***Prof. Susan L. Perlman, MD*** [5], ***Prof. Alexandra Durr*** [6], ***Dr. Theresa Zesiewicz*** [7], ***Dr. Enrico Bertini*** [8], ***Dr. Alejandra Darling*** [9], ***Dr. Katherine Mathews*** [10], ***Prof. Ludger Schöls*** [11], ***Dr. Anne Fournier*** [12], ***Prof. Martin B Delatycki*** [13], ***Dr. Sub H. Subramony*** [14], ***Prof. Richard Roxburgh*** [15], ***Dr. Min Lin*** [16], ***Ms. Karen Hojnowski*** [16], ***Dr. Lee Golden*** [1]

***1.*** *PTC Therapeutics, Inc, South Plainfield, New Jersey, United States of America,* ***2.*** *University of Pennsylvania & Childrens Hospital of Philadelphia,* ***3.*** *Département de neurosciences, Université de Montréal,* ***4.*** *University of campinas,* ***5.*** *University of California, Los Angeles,* ***6.*** *Sorbonne Université, Paris Brain Institute - ICM, Inserm, CNRS, APHP, Hôpital de la Pitié Salpêtrière, Paris,* ***7.*** *Department of Neurology, University of South Florida Ataxia Research Center, Tampa, FL,* ***8.*** *Unit of Neuromuscular and Neurodegenerative Disorders Laboratory of Molecular Medicine Department Neurosciences, Bambino Gesù Children's Hospital, IRCSS, Rome,* ***9.*** *Hospital Sant Joan de Déu Barcelona, Spain,* ***10.*** *University of Iowa,* ***11.*** *University Hospital of Tübingen,* ***12.*** *CHU Sainte-Justine,* ***13.*** *Murdoch Children's Research Institute, Victoria,* ***14.*** *McKnight Brain Institute, Department of Neurology, University of Florida, Gainesville, Florida, United States of America,* ***15.*** *Auckland City Hospital,* ***16.*** *PTC Therapeutics Inc, South Plainfield, NJ*

**Background and Objective:** FA is the most common type of inherited ataxia, characterized by progressive neurological damage and loss of ambulation. FA usually begins in adolescence and is caused by mutations in the gene encoding frataxin. Decreased frataxin levels cause iron accumulation and oxidative stress, leading to cell death and neuronal degeneration through ferroptosis. Vatiquinone is an oral, first-in-class inhibitor of 15-lipoxygenase, a key enzyme in the ferroptosis pathway. In preclinical models, vatiquinone prevented cell death resulting from ferroptosis in mouse striatal cells and primary rat cortical neuronal cultures. In a phase 2 trial in patients with FA, vatiquinone was well tolerated and demonstrated significant improvement in neurological function relative to a natural history cohort.

MOVE-FA (NCT04577352) is a double-blinded, placebo-controlled, global phase 3 trial aiming to evaluate the safety and efficacy of vatiquinone in patients with FA. The primary endpoint is change from baseline in Modified FA Rating Scale (mFARS) at week 72. Secondary endpoints are change from baseline in activities of daily living (FARS-ADL), ambulation (1-minute walk test), and number of falls.

**Methods:** Patients with FA aged ≥7 years, mFARS 20–70 at baseline, and the ability to ambulate ≥10 feet in 1 minute +/- assistance were enrolled and randomized to oral vatiquinone (200 or 400 mg three-times-daily, based on age and weight) or matching placebo for 72 weeks. Subsequently, patients will enter a 24-week open-label extension phase and continue to receive vatiquinone at the same dose. Patients randomized to placebo will switch to vatiquinone at a dose determined by age and weight.

**Results:** 146 subjects (mean age 18.1 years, standard deviation [SD] 11.62 years) were enrolled. Most subjects are female (59.6%) and of European ethnicity (93.8%), with mean body mass index of 20.67 kg/m$^2$ (SD 5.36 kg/m$^2$) and a mean baseline mFARS score of 42.8 (SD 11.07).

# (#326) Identification of Differentially Expressed Genes in Friedreich's Ataxia Patients

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 326

***Dr. Matthew Baile*** [1], ***Mr. Devin Schecter*** [1], ***Mr. Mohamed Hamdani*** [2], ***Ms. Angela Miller*** [1], ***Mr. David Axilbund*** [1], ***Ms. Noreen Scherer*** [1], ***Dr. Ruihua Chen*** [1], ***Dr. Nancy Ruiz*** [1], ***Dr. David Bettoun*** [1]

***1.*** *Larimar Therapeutics, Inc.,* ***2.*** *Larimar Therapeutics*

Friedreich's ataxia (FRDA) is a genetic disease caused by decreased expression of the mitochondrial protein frataxin (FXN). FRDA is characterized by progressive cardiac and neuronal degeneration. Presently, there is no approved treatment for FRDA, and no molecular biomarkers have been identified to monitor disease progression and evaluate the activity of therapeutic approaches. FXN plays a major role in the activity of many enzymatic reactions involving electron transfer, and therefore is an important contributor to mitochondrial homeostasis. Bidirectional signaling between mitochondria and the nucleus has been documented in numerous organisms through which cellular homeostatic or proteostatic changes trigger changes of gene expression from both the nuclear and mitochondrial genomes. Accordingly, we and others have reported that variation of FXN levels impacts the expression of several genes.

Using several experimental models of FXN reduction, and our experimental drug CTI-1601 that repletes FXN levels, we defined a set of FXN-sensitive gene markers (FSGMs). Buccal and blood cells were collected FRDA patients treated with CTI-1601 or placebo in a double-blind, multiple ascending dose safety study. Samples were collected at similar timepoints from untreated, healthy individuals. RNA was purified and absolute gene expression levels were measured using Nanostring® technology. Data were normalized and relative gene expression for all FSGMs established.

We identified over 20 genes that are differentially expressed between patients and healthy individuals in a statistically significant manner ($p_{adj}<0.05$). Some of those genes are major regulators of the mitochondrial oxidative stress response, mitophagy, and neuron development and survival. Treatment with CTI-1601 significantly reversed the expression levels of 6 of those genes to levels observed in healthy individuals.

A small number of genes discriminate between healthy individuals and patients with FRDA. Treatment with CTI-1601 is effective in restoring the expression levels of 6 of those genes to levels similar to those observed in healthy individuals.

# (#330) The Attitude of Patients with Progressive Ataxias Towards Clinical Trials

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 330

***Dr. Gilbert Thomas-Black [1], Ms. Andrada Dumitrascu [1], Dr. Hector Garcia-Moreno [1], Dr. Julie Vallortigara [1], Dr. Julie Greenfield [2], Prof. Barry Hunt [3], Ms. Susan Walther [4], Ms. McKenzie Wells [5], Dr. David Lynch [6], Prof. Hugh Montgomery [7], Prof. Paola Giunti [1]***

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**Background and Objective**: The development of new therapies for ataxia requires human trials. Ethical considerations seek to protect the patient from risk, but few have sought to ascertain the attitude to such risk of patients with progressive conditions, for which no mitigating or curative therapies exist. We therefore sought to define the motivations for and barriers to trial participation amongst patients with progressive ataxias, as well as their condition-specific trial preferences.

**Methods**: Two ataxia charities, Ataxia UK and the Friedreich's Ataxia Research Alliance, sent a 29-question online survey to their members. This covered four key domains (demographics, personal motivation, drug therapy and study design) relating to the targets and design of, and participation in, clinical trials. Responses were analysed by disease and by ambulatory status.

**Results**: Of 342 respondents, 204 reported a diagnosis of Friedreich's ataxia (FRDA), 55 inherited cerebellar ataxia (CA) and 70 idiopathic CA. Respondents felt that the most important symptoms to be addressed were balance (47.3%) and ambulatory impairment (51.3%). When patients were wheelchair users, speech problems superseded (41.5%). Common motivations for participation were potential benefits to self and others. Reasons for non-participation included concerns about side effects, the burden and cost of travel. Financial reimbursement for expenses was likely to increase trial engagement. Phase two trials were the most popular to participate in, and the use of a placebo arm was seen as a disincentive. Across all subgroups, drug repurposing trials proved popular (75.2% for FRDA, 53.1% for inherited CA, 50.9% for idiopathic CA) and around 70% of participants would be prepared to undergo intrathecal drug administration.

**Discussion and Conclusion**: Knowledge of motivations for and barriers to trial participation (including acceptability of investigations, time commitments and routes of drug administration) should inform better, more patient focused trial design and thus participation.

# (#339) Epidemiology, etiology and clinical characteristics of pediatric cerebellar movement disorders - a follow-up study of 30 years

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 339

***Ms. Katariina Granath*** [1], ***Ms. Juulia Ellonen*** [1], ***Dr. Sanna Huhtaniska*** [1], ***Dr. Maria Suo-Palosaari*** [1], ***Dr. Salla Kangas*** [1], ***Prof. Reetta Hinttala*** [1], ***Prof. Johanna Uusimaa*** [1], ***Dr. Jussi Tolonen*** [2]

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**Background and Objectives.** Northern Finland has a unique genetic background due to recent bottleneck events and small founder populations. The objective of the present study is to characterise the epidemiology, etiology, and clinical characteristics of pediatric cerebellar disorders (PCDs) in a patient cohort established in Northern Finland.
**Methods.** All patients seen at the Oulu University Hospital Pediatric Neurology Clinic from the 1st of January 1990 onwards were screened for International Classification of Diseases (ICD)-10 codes pertaining to cerebellar movement disorders (CMD) and cerebellar malformations (CM). Medical records were reviewed for eligible patients to make note of disease course, diagnostic tests, and diagnoses.
**Results.** In total, 139 individuals were selected for patient chart review. After exclusion of 40 individuals, 80 and 20 cases met the inclusion criteria in the CMD and CM subgroups, respectively. In the CMD subgroup, 12 individuals had a non-genetic etiology. There were 24 and 3 cases with definitive genetic diagnosis, while 12 and 4 carried variants of unknown significance (VUS), in the CMD and CM subgroups, respectively. Genetic disorders such as Salla disease (*SLC17A5*) and Infantile-Onset Spinocerebellar Ataxia (*TWNK*), which belong to the Finnish Disease Heritage, were overrepresented. Cumulative incidences per 100,000 live births were 18.7 and 4.7 in the CMD and CM subgroups, respectively. Interestingly, the age at onset of symptoms, the age at which motor developmental delay was observed, and age at diagnosis were significantly lower for the CM subgroup. Median age at diagnosis for all cases was 4.0 years (range 0.2-27.1).
**Discussion and Conclusions.** While PCDs are rare, they are associated with a lengthy diagnostic odyssey and impose a burden on patients' quality of life. Altogether, 27 cases in the CMD subgroup lacked molecular diagnoses and are being recruited for whole exome sequencing studies. The pathogenicity of selected VUSs will also be evaluated.

# (#375) Pre-validation of a Virtual Reality Tool to Quantify the Progression of Friedreich's Ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 375

***Mr. Kevin Chénier*** [1], ***Dr. Lahoud Touma*** [2], ***Dr. Antoine Duquette*** [2], ***Dr. David Labbé*** [3], ***Mr. Minh Tri Le*** [4]

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**Background and Objective:** Patients who suffer from Friedreich's ataxia (FA) experience worsening of their impairments. Existing scales allow to evaluate the severity of ataxia, but these scales rapidly reach a plateau when patients lose the ability to walk, and they rely on the examiner to quantify the dysmetria. Therefore, more objective tools are necessary to complement the neurological examination and distinguish subtle changes in ataxia over time. Using virtual reality (VR), we aim to develop precise tools to measure the progression of FA.

**Methods:** A 3D virtual environment (VE) was developed where the Oculus Quest 2 is used to evaluate the patient's movement and coordination during different upper-limb motor tasks. A preliminary study is being conducted to qualitatively assess the experience of five (5) participants with FA, using a VR headset. Different parameters appearing in the FARS scale, such as tremor, intention tremor, dysmetria, dysrhythmia and dysdiadokokinesia (rapid alternating movements) are quantified through VR adaptations of tasks such as the nine-hole peg test, the finger-to-finger test and the fingerto-nose test.

In addition to these kinematic parameters, subjective data are collected through different questionnaires. These questionnaires aim to measure the feeling of presence in VR, cybersickness, appreciation of the experience.

**Results:** Eight different tasks have been implemented in the VE and are part of the preliminary study. The kinematic and qualitative results obtained will be used to improve the VE and the existing tasks as well as guide the development of additional tasks. The collected data and these identified improvements will be presented.

**Discussion and Conclusion:** Once the virtual environment has been adapted in response to the findings of the preliminary study, a larger study with 20 AF patients and 10 healthy participants will be conducted. The quantitative measures in VR will be compared to those of existing scales.

# (#385) An Overview of the CureDRPLA Global Patient Registry - Collecting Patient Reported Data to Advance Research

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 385

***Mrs. Andrea Compton [1], Mr. Paul Compton [1], Dr. Jeffrey Carroll [2], Dr. Julie Greenfield [3], Dr. Silvia Prades [3]***

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**Background and Objective**: DRPLA is a very rare neurodegenerative disorder with juvenile and adult onset. It is inherited in an autosomal dominant manner and is caused by expanded CAG repeats in the atrophin-1 gene. The patient advocacy groups CureDRPLA and Ataxia UK have established the CureDRPLA Global Patient Registry to collect patient-reported data on individuals affected with DRPLA.

**Methods**: This online registry is available in languages that reflect the prevalence of DRPLA in different countries (i.e., English, Japanese, Korean, Portuguese, French, and Italian). Upon enrolment, participants are asked to consent and answer questions about demographics, diagnosis, medical history, activities of daily living, mobility, research, and disease and economic burden. Participants are asked to complete the registry once a year. Ethics committee approval was obtained.

**Results**: To date, there are 27 participants in the patient registry from 7 different countries. The mean age is 30 ± 17.95 years old (mean ± SD) with a range of 64 ± 6.47 CAG repeats. For the juvenile-onset cohort, age at symptom onset was 7 ± 4.51 years old, 65 ± 6.42 CAG repeats, and 75% experience seizures (n = 12/16). The most bothersome symptoms reported were balance problems (n = 13/16), coordination problems (n = 12/16), and swallowing difficulties (n = 11/16). For the adult-onset cohort, age at symptom onset was 41 ± 10.80 years old, 60 ± 1.83 CAG repeats, and 25% experience seizures (n = 2/8). The most bothersome symptoms reported were balance problems (n = 6/8), coordination problems (n = 6/8), mood swings (n = 5/8), and personality changes (n = 5/8).

**Discussion and Conclusion**: This registry is creating a cohort of well-characterised DRPLA patients for participation in future research studies. In time, it will also enhance the understanding of DRPLA prevalence across the world.

# (#402) Disease Burden in Patients with Friedreich's Ataxia in the US: Real World Evidence (RWE) Retrospective Claims Analysis

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 402

***Ms. Christene Song*** [1], ***Dr. Daniel Brinza*** [1], ***Dr. Shobhana Natarajan*** [1], ***Ms. Nandini Hadker*** [2], ***Dr. Conrad Tenebaum*** [2], ***Ms. Adrienne Lovink*** [2], ***Dr. Seemi Khan*** [1], ***Mr. Oliver Jack*** [1]

***1.*** *Reata Pharmaceuticals,* ***2.*** *Trinity Life Sciences*

**Background and Objective**

Friedreich's ataxia (FA) is a rare, inherited, degenerative, neuromuscular disease without approved therapies. Prevalence estimates in the US indicate ~4000 patients are living with FA. Most patients have disease onset before 15 years of age, with a mean duration until wheelchair use of 11-14 years and a median survival of 34-37 years after disease onset. This analysis of RWE characterizes comorbidities and healthcare resource utilization (HCRU) associated with FA.

**Methods**

Retrospective, longitudinal, anonymous health insurance claims data from a commercially available database was analyzed. Patients diagnosed with FA with enrollment of ≥12 continuous months between 1/2016 and 2/2022 were selected. An age, gender and geographic region-matched non-FA control cohort was included with a ~5:1 (non-FA:FA) ratio.

**Results**

A total of 2,348 and 11,740 patients were included in FA and control cohorts, respectively. Patients with FA under age 65 had the most significant differences in disease burden as compared to control; data from this age-group are summarized. More patients with FA than control were non-ambulatory (32% vs. 4%). Patients with FA, as compared to control, had higher rates of cardiac disease (69% vs. 5%) and type II diabetes (29% vs. 12%). The proportion of patients in the FA cohort requiring 1 or more inpatient hospitalizations was 2X and mean length of stay was 4X that of the control. The proportion of patients using the emergency department (ED) was 2X and mean number of ED visits was 2.5X in the FA cohort as compared to control. The proportion of patients utilizing home health visits was 6X in the FA cohort as compared to control.

**Discussion and Conclusion**

This RWE analysis is the first large-scale, systematic assessment of healthcare claims of patients with FA, which demonstrates greater disease burden in comparison to a matched control cohort.

# (#423) Improving benefit-cost ratio of diagnosis amongst Cerebellar Ataxia patients – Lessons learnt from Israel Cerebellar Ataxia Clinic

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 423

***Dr. Penina Ponger*** [1], ***Dr. Alina Kurolap*** [2], ***Dr. Adi Mory*** [2], ***Prof. Nir Giladi*** [3], ***Prof. Tanya Gurevich*** [3], ***Prof. Roy Alcalay*** [1], ***Prof. Hagit Baris Feldman*** [4]

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Background:
Diagnostic yield of rare neurological disease, including Cerebellar Ataxia (CA), is increasing as next-generation sequencing (NGS) and advanced molecular testing are incorporated in routine clinical work-up. We present an overview of patients seen by the Ataxia Clinic at the Tel Aviv Medical Center Movement Disorders Unit (TAMDU) since 2019, emphasizing the importance of WES trio amongst both pediatric and adult population.
Methods:
We conducted a retrospective study of patients with CA seen at the TAMDU Ataxia Clinic between the years of 2019-2022, including analysis of demographic and clinical data, and genetic workup yield.
Results:
We report 105 patients (59 female) diagnosed with ataxia at ages 4-84 years, with childhood till late adulthood onset of disease. Of the newly referred 69 symptomatic ataxia patients, genetic diagnosis was reached in 18 patients (26%). Nine patients remain undiagnosed following completion of both repeat expansion and NGS analysis. Workup is pending amongst remaining 42 cases due to financial limitations.
Of these newly diagnosed patients, 4 (22%) were diagnosed using repeat expansion analysis and 14 (78%) were diagnosed using NGS testing. Diagnostic yield was increased by incorporating whole exome sequencing (WES) trio, as illustrated by three adult cases with autosomal recessive CA (AOA2 OMIM #606002 , SPG39 OMIM # 612020 and SCAR28 OMIM #618800 ) and two pediatric cases with autosomal dominant CA (SCA29 OMIM #117360 and CDCBM1 #OMIM 614039)
Conclusions:
Our center's Ataxia Clinic diagnostic yield is in accordance with current reported yield in CA cohorts (20-30%). WES trio increased diagnostic yield significantly, highlighting the need for such testing, irrespective of patient age and specifically amongst pedigrees of common ethnic origin. We propose that tailoring molecular workup according to local population characteristics, including completion of WES trio at initial workup stages under certain predefined conditions could improve benefit-cost ratio of diagnostic procedures.

# (#424) Estimated prevalence of Friedreich´s ataxia in the state of São Paulo, Brazil

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 424

***Dr. Marcondes Franca*** [1], ***Ms. Daiana Machado*** [2], ***Ms. Amalia Maranhão*** [3]

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Background and Objective

Friedreich´s ataxia (FRDA) is the leading cause of autosomal recessive ataxia worldwide, but prevalence estimates are not homogeneous across countries. In European countries, FRDA is found in one in every 50,000 people. No epidemiological data is available from Latin America, a region characterized by mixed ethnic background. Herein, we attempted to compute the prevalence of FRDA in São Paulo, the most populous Brazilian state.

Methods

This is an observational study using data from the main advocacy group for ataxic patients in Brazil (ABAHE - Brazilian Association of Acquired and Hereditary Ataxias). To calculate the prevalence of FRDA in São Paulo in 2021, the reported patients with confirmed genetic test and also who live in São Paulo state were considered, and this number was divided by the total population of the state. Demographic data from these patients is also described.

Results

We were able to find 137 patients with molecular confirmation of FRDA living in São Paulo in 2021. In the same period, the total population of the state was 46,649,132 inhabitants according the latest IBGE (The Brazilian Institute of Geography and Statistics). This results in a prevalence of 2.94 / 1,000,000. Mean age of patients is 36.9 years (SD = 12.2), mean age at first symptoms was 18 years (SD = 9.7), being 61.3% female and 38.7% male.

Conclusion

Prevalence of FRDA in São Paulo is much smaller than previously reported in European countries. Since São Paulo is the most populous state and the region that has the most migration in Brazil, we believe the computed prevalence is a good estimate for the whole country. The highly admixed background of the Brazilian population may explain at least in part the epidemiological differences relative to Caucasian populations.

# (#431) Friedreich ataxia 'Patient Journey'

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 431

***Dr. Mary Kearney*** [1]***, Dr. Carola Reinhard*** [2]

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**What is a patient journey?**

A "Patient Journey" is a visual record and shows a patient's path through their disease, starting with the first symptoms through to therapy and follow-up. Forks or crossroads along this path are depicted and provided with motifs and actions.

A "Patient Journey" connects professional expert guidelines—with foreseen medical interventions, screening, treatment—with patient needs –both medical and psychological.

To achieve this, patient representatives completed a mapping exercise of the needs of each rare inherited syndrome they represent, across the different stages of the Patient Journey.

The stages that are considered inherent to the specific disease in four main areas:

- The disease itself
- Clinical care for the disease,
- Challenges and needs identified by patients,
- Goals to improve care.

The final Patient Journey is reviewed by both patients and professional experts before publication. By visualizing this in a comprehensive manner, patients and their caregivers are able to discuss the individual needs of the patient, while keeping in mind the expertise of both professional and patient leads. Patient Journeys encourage experts to look for national guidelines on the rare disease and if they are not available, they may identify an evidence-based European and international guideline to help them care for the individual with the rare disease.

The aim is that medical doctors confronted with rare diseases, may use the 'Patient Journey' to explain the many facets for the disease to the person who had just been diagnosed. It can also be used by doctors and other healthcare professionals, who are not experts in the particular area to understand the disease. i.e. an orthopedic surgeon, general practitioner, accident and emergency team, physiotherapist, speech or occupational therapist

In summary, it provides knowledge of a broad community of expert medical professionals and expert patients.

# (#436) Therapeutic misestimation in ataxia patients: lessons from a randomized controlled trial

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 436

***Dr. Roderick Maas*** [1], ***Prof. Bart van de Warrenburg*** [1]

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**Background:** The absence of effective treatments may render patients with degenerative cerebellar ataxias susceptible to a placebo response, which could affect the outcome of clinical trials.
**Objective:** To examine expectations of benefit in participants of an ataxia trial and identify demographic and clinical determinants of possible therapeutic misestimation.
**Methods:** Individuals with spinocerebellar ataxia type 3 who participated in a randomized, double-blind, sham-controlled trial of cerebellar transcranial direct current stimulation (tDCS) received, before unblinding, a custom-designed questionnaire about short-term and long-term expectations of benefit, allocation preferences, and interpretation of treatment arm assignment based on the presence or absence of clinical improvement. To evaluate whether expectations were specifically related to the application of tDCS or more generally reflect an overly positive attitude of ataxia patients toward trial participation and results, the last questions involved a hypothetical scenario in which an oral drug was tested against placebo with an aim identical to that of our tDCS study.
**Results:** All twenty trial participants completed the questionnaire. If allocated to the active treatment arm, 75% of patients expected short-term health benefits and 55% thought they would still have less severe ataxia at one-year follow-up compared to baseline. After two weeks of real tDCS, an average reduction in ataxia severity of 31.5% (SD 22.2) was anticipated. Conversely, 65% associated a lack of improvement with probable or definite allocation to the placebo group. High expectations of benefit were neither related to the type of intervention – device-based or a (hypothetical) oral drug – nor to clinical or demographic characteristics, including age, disease duration, SARA score, CCAS scale score, and educational level.
**Conclusion:** In spite of an informed consent process with well-balanced information, therapeutic misestimation is common in patients with degenerative ataxia and requires special attention in future trials.

# (#467) The opinion of Friedreich's Ataxia patients towards gene therapy trials

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 467

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**Background and objective.** Gene therapy is a potential treatment for Friedreich's Ataxia (FA). In the coming years, multiple gene therapy programs may be available for the FA population. The purpose of this survey was to collect opinions about gene therapy from FA-affected individuals or caregivers, which might help develop and direct future clinical trials.

**Methods.** FA participants (³14 years old) or a parent/guardian (for FA children < 14 years old) were asked to complete a survey after reading brief educational materials regarding gene therapy. The survey included 42 questions and was distributed through the Friedreich's Ataxia Research Alliance (FARA) patient registry.

**Results.** 152 participants completed the survey; 37.5% were caregivers and 62.5% were individuals affected by FA. Ages ranged from 7-73 with a mean age of 31.61 (+/- 16.80). The mean age at diagnosis was 18.88 (+/- 11.76) and symptom onset was 15.09 (+/- 11.62), while the median ages were 15 and 11, respectively. Results indicated that the FA community is well-informed on clinical trials and gene therapy, and patient advocacy groups are instrumental in this. Most FA individuals felt that balance issues/inability to walk most interferes with their quality-of-life and 73% would choose to treat this symptom first. In contrast, only 2% report that cardiomyopathy most interferes with quality of life and 5% would choose to treat it first, despite the risk for cardiovascular mortality within this population. In further analysis of this question, 10.2% of caregivers would choose cardiomyopathy compared to 1.1% of patients. Finally, approximately half of respondents expect gene therapy to stop progression and/or improve symptoms.24.7% of all respondents believe gene therapy will cure them of FA.

**Discussion and Conclusion.** Respondents felt urgency in participating in gene therapy clinical trials, despite risks. There are high efficacy expectations, and treating neurological symptoms is more valued than cardiomyopathy.

# (#473) Diagnostic Delay of Hereditary Ataxias in Brazil: the Case of Machado-Joseph Disease

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 473

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**Background**: Spinocerebellar ataxia type 3/Machado-Joseph disease (SCA3/MJD) is a rare disease with diagnosis offered by the Unified Health System in Brazil. Our aim was to investigate the diagnostic delay in an interval of 23 years in a public university hospital, and some determining factors.

**Methods:** a retrospective review of the medical records of subjects identified at our institution between 1999 and 2017 was carried out, including residents of Rio Grande do Sul. The diagnostic delay was equivalent to the difference between age at onset of symptoms and age at molecular diagnosis. Calendar years, educational level, sex, distance from household, age and being the index case were studied as modifying factors. Nonparametric tests were used in the comparisons, for $p<0.05$.

**Results:** SCA3/MJD had a median diagnostic delay of 5 years. Index cases had delays of 6 versus 4 years ($p<0.001$) for subsequent family members. Delay correlated with age ($rho=0.346$, $p<0.001$), but not with age at disease onset ($rho=0.005$, $p=0.91$). No change was observed with the level of education of individuals or with the distance between household and hospital from 1999 to 2017.

**Discussion**: The diagnostic delay of SCA3/MJD is high in RS, where its occurrence has been reported for years. Failure to change the delay over the years suggests ineffective dissemination to the population; but a smaller lag among younger people can portray the effect of digital inclusion.

# (#474) Prospective evaluation of 2777 patients with progressive ataxia

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 474

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Background and Objective

Major advances in genetic testing and in our ability to diagnose immune ataxias means that the number of "idiopathic" sporadic cases is rapidly diminishing. We present our experience in the diagnosis of patients with progressive ataxia.

Methods

Patients were seen and assessed in a specialist national ataxia centre (Sheffield UK). Extensive investigations included gene testing using next generation sequencing and more recently whole genome sequencing. Immunological tests included antibody tests for immune ataxias including antigliadin and TG6 antibody testing for gluten ataxia.

Results

2777 patients were assessed over 25 years. 10% of patients developed ataxia under the age of 20. A family history of ataxia was present in 16% of 2777 patients. Amongst patients with a family history 69% had autosomal dominant (AD) ataxia. The commonest amongst AD ataxias were episodic ataxia type 2 (20%) and SCA6 (13%). The commonest autosomal recessive (AR) ataxia amongst AR ataxias were Friedreich's (26%) and SPG7 (13%). A genetic diagnosis was achieved in 14% of sporadic cases with Friedreich's ataxia being the commonest followed by CANVAS. Ataxia panel gene testing using NGS produced a positive result in 33%. Whilst subsequent testing as part of the 100K genome project also produced a positive result in 33% the introduction, under the NHS, of WGS produced a positive result in only 8% of cases. Amongst acquired ataxias the commonest was gluten ataxia (29% of all the ataxias), followed by other immune mediated ataxias (11%), alcohol-related ataxia (9%) and cerebellar variant of multiple system atrophy (7%).

Discussion and Conclusion

Advances in genetic testing and improved recognition of immune ataxias have been the most important steps in unravelling the aetiology of the ataxias. Amongst patients with sporadic ataxia only 9% remain idiopathic and amongst patients with familial ataxia no genetic cause has been found in 22%.

# (#504) A Feasibility Study of Dual-Task Treadmill Training to Improve Gait and Balance in Fragile X-Associated Tremor/Ataxia Syndrome (FXTAS)

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 504

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**Background:** Individuals with FXTAS have action tremor and cerebellar gait ataxia as major clinical manifestations, and optimising ambulatory capacity is vital to improving quality of life. Dual task (DT) treadmill training has shown to improve motor and cognitive deficits in patients with Parkinson's disease, Traumatic Brain Injury and stroke survivors, but its feasibility and efficacy have not been investigated in FXTAS. The primary objective of this study was to evaluate the feasibility of DT treadmill training in individuals with FXTAS.

**Methods:** Three individuals with FXTAS participated in the treadmill training combined with cognitive training 3x weekly for six weeks. One control participant with FXTAS did not perform any DT training. Feasibility was determined by a 70% or greater attendance rate and performing 45 min of moderate-intensity exercise per session (minimum of 60% maximum heart rate). Pre- and post-intervention assessments included: (1) gait and balance testing via inertial sensor based measures (APDM ™ ) and the Balance Evaluation Systems Test (mini-BEST); 2) disease severity using the FXTAS rating scale; (3) the Montreal Cognitive Assessment (MoCA), Controlled Oral Word Association Test (COWAT), Symbol Digit Modalities Test (SDMT) and Beck Anxiety Inventory (BAI); (4) step count with a pedometer; and (5) cardiopulmonary function via VO2 max testing.

**Results:** All participants attended 100% of the sessions and fully completed the required DT treadmill exercise for the 6-weeks, proving the intervention feasible. Several of the cognitive and balance outcome measures also showed improvements post-intervention, including the mini-BEST, COWAT, SDMT, BAI, and VO2 max. Due to the COVID-19 pandemic the study was halted, and data on three participants in the intervention arm who completed assessments through the 3-month follow-up visit is presented here.

**Conclusion:** Treadmill-training combined with a cognitive task is feasible and warrants future research with a randomized clinical trial in FXTAS.

# (#506) Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay: Clinical guidelines for physiotherapists and neurologists

Wednesday, 2nd November - 12:00: Lunch and Poster Session - Poster - Abstract ID: 506

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**Background and objectives:** Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a hereditary disease with cases reported worldwide but more prevalent in the regions of Charlevoix and Saguenay–Lac-Saint-Jean (Quebec, Canada), where 1/1932 person is affected. This disease is characterized by a triad of components, namely cerebellar, pyramidal and neuropathic impairments, which result in various signs and symptoms that lead to important limitations and restrictions. Consequently, persons with ARSACS need personalized multidisciplinary follow-up. Neurologists and physiotherapists play a key role in the follow-up of these persons to improve their quality of life. However, optimal care is limited by the lack of access to evidence-based clinical information.
This knowledge transfer project aimed to document signs and symptoms and provide recommendations to physiotherapists and neurologists for good clinical practice and improve their interventions with ARSACS patients.
**Methods:** The development of these clinical guidelines is based on the Rare Knowledge Mining Methodological Framework, specific for the development of knowledge transfer tools in rare diseases context.
**Results:** Many signs and symptoms present in ARSACS were identified through the Rare Knowledge Mining process:

- Musculoskeletal: muscle weakness and atrophy, tonus alteration, muscle retractions, deformities, spasticity, contractures and co-contractions;
- Nervous: hyporeflexia, hyperreflexia, balance and coordination troubles, tremor, epilepsy, locomotion and sensibility alteration, loss of voluntary movement and pain;
- Digestive: dysphagia, fecal incontinence and constipation;
- Genito-urinary: urinal incontinence;
- Cognitive, intellectual, and psychiatric: personality traits, cognitive impairments and fatigue;
- Auditory and speech: dysarthria.

In addition, recommendations for physiotherapists and neurologists for all these impairments have been documented to guide their clinical practice.
**Discussion and conclusion:** Physiotherapists and neurologists can now easily access evidence-based information, which will enhance the quality and efficiency of interventions offer to ARSACS patients worldwide.

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