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**Ataxias and Beyond:
Shared Pathways, Shared Progress**

ABSTRACT BOOK

Atlanta, GA | 10-13 November 2026

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FA: THE HEART OF THE MATTER

In Friedreich ataxia (FA), a heavy heart isn't just a feeling; it could be cardiomyopathy.

With FA cardiomyopathy (FA-CM), the heart becomes thicker and has to work harder than it should, which affects the heart's function. While there are currently no approved treatments for FA-CM, research is moving forward, bringing new focus and understanding.

Together we have the potential to change the heart of FA.



SUNRISE-FA 2 IS A LATE-STAGE CLINICAL TRIAL NOW ENROLLING

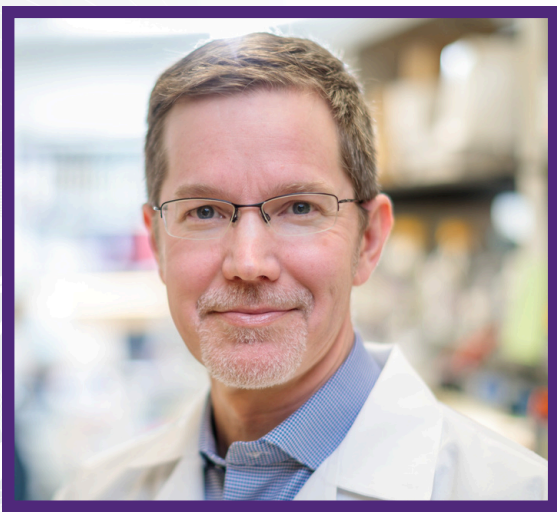
to evaluate how LX2006, an investigational gene therapy, may improve heart function and limit progression of FA-CM.



SUNRISE FA2



ICAR 2026 INVITED SPEAKERS



Timothy Miller, MD, PhD
Vice Chair of Research for the
Department of Neurology,
co-director of the ALS Center,
Washington University School of
Medicine



Sonia Vallabh, PhD
Director, Prion Therapeutic Science
Broad Institute of MIT and Harvard



Steven McCarroll, PhD
Dorothy and Milton Flier Professor of
Biomedical Science and Genetics, Director
of Genomic Neurobiology, Stanley Center
for Psychiatric Research, Broad Institute of
MIT and Harvard



Stephan Zuchner, MD, PhD
Professor of Human Genetics and Neurology;
Chief Genomics Officer, University of Miami Miller
School of Medicine



Alexandra Durr, MD PhD
Professor
Paris Brain Institute



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Keynote Speakers

Timothy Miller

Tuesday, November 10, 1:20 PM-2:00

RNA-Targeted Therapies for Neurodegenerative Diseases

Washington University in St. Louis, St. Louis, MO

Antisense oligonucleotide (ASO) therapeutics have been successfully applied to multiple neurological diseases. Tofersen, an ASO that lowers superoxide dismutase 1 (SOD1), was tested in genetic subset of amyotrophic lateral sclerosis (ALS). Tofersen treatment substantially lowered levels of the neurodegeneration-associated neurofilament proteins in biofluids. Treatment with tofersen more than doubled the survival of a rapidly progressing participant subgroup and, in about 20-25% of participants, completely stabilized disease progression at three years since starting the clinical trial. Diranersen, a tau-lowering ASO for Alzheimer's Disease and other dementias, is another successful example and will be discussed. Other ASO programs of interest include those that change RNA splicing, inhibit miRNAs, or target RNA regulation. Tofersen, diranersen, and other RNA-targeted therapeutic strategies serve as a blueprint for multiple ASO programs and, thus, this approach may be readily applied to ataxias.

Steven McCarroll

Wednesday, November 11, 9:10 AM-9:40 AM

The ticking DNA clock: Lifelong somatic expansion of repeated DNA as a cause of DNA-repeat disorders

Broad Institute, Cambridge, MA

Inherited DNA-repeat disorders such as Huntington's disease and the spinocerebellar ataxias have long presented fundamental challenges to our understanding: Why do patients enjoy decades of good health before symptoms commence? Why do such disorders profoundly affect certain types of neurons while sparing other types of neurons and other cells? I will describe experiments in Huntington's disease (HD) that taught us a surprising answer to such questions: the inherited DNA repeat that causes HD must undergo decades of somatic expansion – in specific types of neurons – and expands to several times its inherited length before it even begins to cause harm. This surprising model is turning out to explain many long-puzzling features of HD, such as its tendency to affect different brain areas (striatum, cortex, thalamus) in ways that had long appeared to have different mechanisms – but which, we have now found, have a shared mechanism in DNA-repeat expansion beyond a shared threshold of about 150 CAGs. This new model – which we call the "ticking DNA clock" – also helps us understand how common genetic modifiers of the disease shape its pathogenic trajectory. Since many DNA repeats are somatically unstable and tend to expand with age, an interesting possibility is that a similar dynamic may underlie many different DNA-repeat disorders, in particular the spinocerebellar ataxias.

Sonia Vallabh

Wednesday, November 11, 2:00 PM-2:30 PM

First steps into the era of targeted genetic therapies for prion disease

Broad Institute, Cambridge, MA

All cases of prion disease are caused by the templated misfolding of prion protein, or PrP, into so-called "prions" that spread across the brain and kill neurons, leading to a rapidly progressive and fatal dementia. Two therapies aimed at lowering the amount of native prion protein in the brain -- an antisense oligonucleotide and a divalent siRNA -- have now reached first in human clinical trials, and additional PrP-lowering modalities are under development. This talk will offer a patient-scientist perspective on recent therapeutic progress in prion disease, the pros and cons of current and envisioned treatment strategies, and the clinical and systemic challenges that must be surmounted for any of the above to deliver on its potential.

Stephan Zuchner

Thursday, November 12, 9:10 AM-9:40 AM

Accelerating repeat expansion disorder discovery: from linkage to single genomes

University of Miami, Miami, FL

Short tandem repeat (STR) expansions play an outsized role in the pathophysiology of inherited ataxias and neurodegeneration more broadly. Yet fewer than 100 STR loci have been reliably linked to disease phenotypes. Until recently, technical barriers have limited large-scale, genome-wide comparative studies of STR variation, despite the widespread use of such approaches to study single-nucleotide variants and small insertions and deletions in coding regions. Three key challenges remain: limited large-scale reference databases of STR alleles, a lack of robust molecular or statistical signatures for prioritizing potentially pathogenic STR loci, and a scarcity of high-quality long-read genome datasets from patients. This presentation will highlight advances addressing all three challenges: a catalogue of 100 billion STR alleles from the All of Us Research Program; the Pure Length Variability Index (PLVI), a novel metric for prioritizing STR loci in disease studies; and efforts to expand long-read genome sequencing in ataxia and enable effective data sharing. Together, these resources and approaches enable the prioritization of novel candidate disease-associated STR expansions from individual long-read genomes, accelerating discovery and laying the groundwork for new therapeutic targets.

Alexandra Durr

Friday, November 13, 9:20 AM-9:50 AM

The Presymptomatic Phase in Autosomal Dominant Ataxias: the optimal moment to improve disease trajectory?

Sorbonne University, Paris

Autosomal dominant cerebellar ataxias are relentlessly progressive neurodegenerative disorders for which no disease-modifying therapies currently exist. The presymptomatic phase—defined by genetic confirmation in at-risk individuals prior to clinical onset—represents a critical but underutilized therapeutic window. Emerging evidence from neuroimaging, biomarker studies, and preclinical models demonstrates that neurodegeneration begins years to decades before symptom onset, with detectable cerebellar and non-cerebellar dysfunction. This preclinical and neurodevelopmental pathology, coupled with the genetic predictability, provides a unique opportunity for intervention before irreversible neuronal loss occurs. However, the presymptomatic phase also presents challenges: the optimal timing of treatment initiation remains unclear, biomarkers for early disease activity are still being validated, and approaches to neuroprotection are missing. There is current evidence that support presymptomatic intervention and we will address the clinical, ethical, and logistical hurdles to implementing early treatment. We argue that the presymptomatic phase is not merely a therapeutic window but the optimal moment to improve the disease trajectory, offering the best chance to preserve neurological function and transform the natural history of these disorders.

Oral Presentations

Disease Mechanisms I - Recessive Ataxias

Tuesday, November 10, 3:30 PM-5:00 PM

DR40. RFC1, a gene involved in rare late-onset ataxia, regulates cerebellar neurogenesis in zebrafish

3:30 PM - 3:48 PM

Fanny Nobileau¹; Sébastien Audet²; Sanaa Tork³; Alexandra da Silva Babinet⁴; Charlotte Zaouter⁵; Meijiang Liao⁴; Nicolas Pilon³; Martine Tétreault²; Eric Samarut²; Kessen Patten⁵

1. Université de Montréal, Montréal, QC, 2. Université de Montréal, 3. UQAM, 4. Research Center of CHUM, 5. INRS

RFC1 is essential for DNA replication and repair. Beyond its traditional role in maintaining genomic stability, RFC1 has recently been implicated in rare cerebellar ataxia CANVAS, where pathogenic intronic repeat expansions lead to reduced RFC1 expression, particularly in the cerebellum. However, the fundamental role of RFC1 in cerebellar physiology remains unknown, and our work aims at investigating its role in the nervous system. Since knocking out Rfc1 in mammals leads to embryonic lethality, we generated a zebrafish loss-of-function model of rfc1 using CRISPR/Cas9. We found that cerebellar development in rfc1^{-/-} larvae is severely affected, with a marked reduction in Purkinje and granule cells. scRNAseq revealed that RFC1 is expressed in early neural progenitors where it maintains genomic integrity. Loss of RFC1 results in widespread apoptosis of these progenitors, disrupting neurogenesis and cerebellar formation. This work is the first to investigate RFC1's *in vivo* function.

RFC1 is not expressed ubiquitously but is a key regulator of cerebellar development, with enriched expression in the developing cerebellum of both mice and zebrafish. Combined with recent evidence supporting RFC1 loss-of-function as a pathogenic mechanism in CANVAS, these results suggest that cerebellar developmental defects may contribute to disease pathogenesis. This challenges the current view of CANVAS as a purely late-onset neurodegenerative disorder and supports a model in which developmental alterations predispose individuals to later neurodegeneration.

An important unresolved question is whether RFC1 also functions in mature cerebellar neurons and whether its deficiency contributes directly to the progressive nature of CANVAS. Thus, we have established conditional mouse and zebrafish models allowing temporal control of RFC1 depletion in adult cerebellum. These models will distinguish developmental from adult functions of RFC1 and assess its contribution to disease progression. With our developmental findings, these studies will provide a strong foundation for future biomarker discovery and therapeutic development in CANVAS.

Funding Sources: Since 2022, my research has been supported by competitive awards, including the Fonds de recherche du Québec Fellowship (\$25,000 CAD/year for 4 years), the NAF Graduate Research Fellowship (\$25,000 USD/year), and several University of Montreal merit-based scholarships (\$60,000 CAD total). These distinctions reflect the quality and relevance of my project.

DR25. The FRDA Transcriptomic Atlas: Identifying Cell Type-Specific Drivers of Selective Vulnerability and Candidate Biomarkers in Friedreich Ataxia

3:48 PM - 4:06 PM

Marnie Maddock¹; Sara Mielliet²; Anjila Dongol¹; Amy Hulme³; Chloe Kennedy⁴; Rocio K. Finol-Urdaneta⁴; Alberto Nettel-Aguirre⁴; Mirella Dottori¹

1. University of Wollongong, Wollongong, New South Wales, 2. University of Wollongong, Wollongong, 3. University of Oxford, 4. University of Wollongong

Background: Friedreich's ataxia (FRDA) is a neurodegenerative disease caused by a trinucleotide (GAA) repeat expansion within the first intron of the FXN gene, resulting in frataxin deficiency. Despite ubiquitous frataxin deficiency, dorsal root ganglia sensory neurons (SNs) are particularly vulnerable in FRDA, whereas lower motor neurons (LMNs) are relatively spared. The molecular basis underlying this selective vulnerability remains poorly understood.

Objectives: To identify molecular signatures associated with FRDA vulnerability across cell types and compare FRDA iPSC-derived SNs and LMNs to investigate sensory neuron-selective pathology.

Methods: We generated the FRDA Transcriptomic Atlas, a community-accessible resource integrating all available human bulk RNA-seq datasets in FRDA (23 datasets across 10 cell types). This framework spans disease-relevant (cardiomyocytes, SNs) and relatively FRDA-spared cell types (fibroblasts, LMNs), enabling systematic identification of recurrent gene and pathway-level dysregulation. Functional analyses were conducted in FRDA and isogenic corrected iPSC-derived SNs and LMNs using transcriptomic profiling, live-cell mitochondrial trafficking assays, and neurite morphology analysis.

Results & Discussion: Meta-analysis revealed recurrent transcriptional perturbations beyond FXN, involving long non-coding RNAs, translational control and cytoskeletal organisation. While some transcriptional themes were shared, the specific pathways engaged were strongly cell-type dependent. Candidate gene biomarkers MYH14, MEG9, and MEG8 showed preferential upregulation in disease-relevant cell types predominantly, including SNs and cardiomyocytes, supporting their potential relevance to selective vulnerability. Functional analyses demonstrated that FRDA SNs exhibited increased neurite beading, mitochondrial accumulation within neurite beads, altered mitochondrial spacing along neurites, and reduced mitochondrial motility along neurites (all $p < 0.05$ vs isogenic controls). These abnormalities were substantially less pronounced or absent in FRDA LMNs, supporting neuron subtype-specific responses to frataxin deficiency.

Conclusion: These findings suggest that FRDA selective vulnerability is associated with cell type-specific disruption and establish the FRDA Transcriptomic Atlas as a resource for investigating selective vulnerability, biomarker discovery, and therapeutic target prioritisation in FRDA.

Funding Sources: Friedreich's Ataxia Research Alliance (FARA, USA), Friedreich's Ataxia Research Association (FARA, Australia), Medical Research Future Fund Stem Cell Therapies Mission, 2007421

DR13. Cardiac Clinical Findings in Friedreich's Ataxia Correlate with Functional and Multi-Omics Alterations in iPSC-Derived Cardiomyocytes

4:06 PM - 4:24 PM

Marta Moya Jodar¹; Yiangos Psaras²; Violetta Steeples²; Josephine M. Watkins²; Max Hill²; Maria Rudnytska³; Brian Wang²; Thomas Nicol²; Marcos Garcia Lacarte²; Betty Raman²; Hugh Watkins²; Christopher Toepfer²

1. University of Oxford, Oxford, Oxfordshire, 2. University of Oxford, 3. University of Oxford

Background and Objectives: Cardiomyopathy affects approximately 60% of patients with Friedreich's ataxia (FRDA) and represents the leading cause of mortality. However, the mechanisms linking frataxin deficiency to cardiac dysfunction remain poorly understood. Although frequently classified as hypertrophic cardiomyopathy (HCM) due to the hypertrophic left ventricular phenotype, FRDA-associated cardiomyopathy may represent a distinct metabolic cardiac disease. Our objective was to define the functional and molecular mechanisms underlying FRDA cardiomyopathy through integrated clinical analyses and human induced pluripotent stem cell-derived cardiomyocyte (iPSC-CM) models generated from FRDA patients.

Methods: Echocardiographic analyses were performed in FRDA patients and compared with healthy controls and patients with sarcomeric HCM. Functional phenotyping of FRDA and wild-type (WT) iPSC-CMs included electrophysiology, calcium handling, contractility, and mitochondrial respiration assays. Transcriptomic and metabolomic analyses were integrated to identify disease-associated pathways. In addition, pharmacological modulation using Omaveloxolone and Mavacamten was performed to evaluate therapeutic responsiveness.

Results and Discussion: FRDA patients exhibited impaired myocardial relaxation and diastolic dysfunction. FRDA iPSC-CMs recapitulated these abnormalities, demonstrating prolonged action potentials, delayed calcium reuptake, impaired relaxation kinetics, and altered sarcomeric dynamics. Furthermore, FRDA iPSC-CMs showed significantly reduced basal and maximal mitochondrial respiration, consistent with impaired bioenergetics. Integrated multi-omics analyses identified coordinated dysregulation of mitochondrial metabolism at both the transcriptomic and metabolomic levels, defining a molecular signature distinct from classical sarcomeric HCM. Pharmacological modulation with Omaveloxolone and Mavacamten partially rescued disease-associated phenotypes and altered metabolic pathways, supporting the utility of this human translational platform for therapeutic interrogation.

Conclusion: FRDA-associated cardiomyopathy is characterised by impaired relaxation and mitochondrial dysfunction rather than the hypercontractility observed in primary sarcomeric HCM. These findings support the concept that FRDA cardiomyopathy represents a metabolically driven form of hypertrophic remodelling distinct from classical sarcomeric HCM and establish a human translational platform for the development of novel therapies.

Funding Sources: This work was supported by the Christina Foundation.

DR47. Uncovering the contribution of Purkinje cells to early cerebellar pathogenesis in Friedreich's ataxia

4:24 PM - 4:42 PM

Brazil Bartholomew¹; Taquan Wilson²; CLEMENTINA Mesaros³; Heather Snell⁴; Jordi Magrane⁵

1. Yale University, New Haven, CT, 2. Hunter College, Brooklyn, NY, 3. University of Pennsylvania, 4. Yale School of Medicine, New Haven, CT, 5. Weill Cornell Medicine, New York, NY

Historically considered degenerative in nature, cerebellar pathology in Friedreich's ataxia (FA) is frequently characterized by atrophy of large neurons within the deep cerebellar nuclei (DCN). However, recent studies reveal a more complex pathogenic process, which includes both microstructural and functional abnormalities that affect a variety of cerebellar regions and may start early during disease pathogenesis. Surprisingly, few studies using FA mouse models have attempted to investigate cerebellar pathology outside DCN. Given this gap in research, we aim to investigate the contribution of early events affecting the formation and maturation of the cerebellar circuitry, with a focus on the Purkinje cells (PC) – DCN axis.

We study the early postnatal neurodevelopmental events (from day 7 to juvenile stages) in the knock-in/knock-out (KIKO) mouse model of FA. We combine targeted metabolomics, histology and imaging approaches (spatial reconstruction of synaptic inputs, automated image analysis), with neuron activity recordings (single cell electrophysiology in acute cerebellar slices) to uncover the events leading up to DCN pathology that ultimately causes the emergence of ataxia symptoms.

Our results indicate that PCs display aberrant firing in KIKO mice on postnatal day 15 (P15) and P30. While the number of PCs remains unchanged, parvalbumin levels are reduced, suggesting reduced activity. Moreover, markers for glutamatergic synapses and glutamate levels decrease in the cerebellum of P25KIKO mice. Finally, loss of coordination is apparent in KIKO mice at P90, when degenerative features in DCN are evident.

While different cerebellar micro-circuitries and cell types may contribute to FA, our data suggest that PCs could have abnormal physiology or receive altered synaptic inputs early during disease pathogenesis, which in turn could contribute to DCN degeneration and ataxia. Determining the most vulnerable regions in the FA cerebellum and the mechanisms underlying neurodevelopment and neurodegeneration will be important for our understanding of the pathogenesis of FA.

Funding Sources: The National Ataxia Foundation (NAF) to B.B. and the Friedreich's Ataxia Research Alliance (FARA) to J.M

DR36. Dentate Nucleus Hyperexcitability Triggers Adaptive Cerebellar Circuit Remodeling in Friedreich's Ataxia.

4:42 PM - 5:00 PM

Helene Puccio¹; Federica Pilotto²; Julia Novion Ducassou³; Yohann Coute³

1. INMG, Lyon, 2. INMG-PGNN, Lyon, 3. Univ. Grenoble Alpes

Friedreich's ataxia (FA) is a progressive neurodegenerative disorder caused by frataxin (FXN) deficiency, leading to mitochondrial dysfunction, oxidative stress, and degeneration of multiple neuronal populations, particularly dentate nucleus (DN) neurons, one of the principal output nucleus of the cerebellum. While DN pathology is a hallmark of FA, the contribution of cerebellar circuit dysfunction to disease progression remains poorly understood. We investigated how chronic frataxin (FXN) deficiency reshapes cerebellar network activity and explored whether compensatory or maladaptive circuit remodeling drives neurodegeneration and functional decline. Using the FxnG127V/G127V knock-in mouse model, we combined in vivo two-photon calcium imaging, chemogenetic manipulation of cerebellar circuits, longitudinal behavioral analyses, proteomics, and studies in human iPSC-derived DN neurons to characterize functional and molecular alterations associated with FXN deficiency.

Early symptomatic mice exhibited DN neuron hyperexcitability, increased oxidative stress, and altered motor behavior, preceding overt neurodegeneration. Longitudinal imaging revealed progressive remodeling of Purkinje neuron activity, indicative of an adaptive response to disrupted cerebellar output. Chemogenetic manipulation experiments in WT animals demonstrated that DN hyperactivity alone is sufficient to induce the network abnormalities observed in FA, whereas restoring the balance of cerebellar inhibition and excitation normalized circuit function, reduced oxidative stress, preserved DN neurons, and improved motor behavior in FA mice. Mechanistically, proteomic analyses of laser dissected DN identified dysregulation of ER-mitochondrial calcium signaling pathways, including altered store-operated calcium entry and calcium transfer machinery. Furthermore, pharmacological activation of SERCA normalized calcium handling and reduced oxidative stress in human FA iPSC-derived DN neurons.

Together, our findings identify calcium-dependent cerebellar circuit dysfunction as a key driver of FA pathogenesis and reveal modulation of DN excitability and intracellular calcium homeostasis as promising therapeutic strategies.

Funding Sources: Christina Foundation - ENDFA; Friedreich ataxia Research Alliance; Fondation pour la Recherche Médicale

Disease Mechanisms II - Dominant Ataxias

Wednesday, November 11, 9:40 AM-11:10 AM

DD9. A Sensory Neuron-Centric Mechanism for Peripheral Neuropathy in Polyglutamine Expanded ATXN3 Disease

9:40 AM - 9:58 AM

Juan Mato¹

1. University of Michigan

Background Repeat expansion mutations are linked to diverse neurogenetic disorders, many involving the peripheral nervous system. Despite peripheral neuropathy being the most common neurological disorder worldwide, its role in rare repeat expansion diseases remains poorly understood. Progress is limited by cross-sectional clinical studies involving small and heterogeneous patient populations. Genetically precise and experimentally accessible mouse models could bridge these gaps, but robust models recapitulating repeat expansion-associated neuropathy remain insufficiently characterized. Here, we establish a translational framework for investigating peripheral neuropathy in spinocerebellar ataxia type 3 (SCA3), a fatal multisystem neurodegenerative disease caused by a pathogenic CAG repeat expansion in ATXN3.

Methods SCA3 Knock-In Atxn3Q300(KI) and Atxn3^{-/-} Knock-Out (KO) mice relative to wildtype (WT) littermate controls underwent sensorimotor nerve conduction studies and behavioral assessments of patient-relevant peripheral neuropathy features. Immunohistochemistry, electron microscopy, and immunofluorescent imaging examined peripheral histopathology in KI and KO mice. Transcriptional and splicing dysregulation were analyzed in late-stage KI dorsal root ganglia (DRG). We then generated a conditional SCA3“OFF” Knock-In (cKI) mouse and crossed it with Avil-Cre mice to selectively silence mutant ATXN3 in sensory neurons. Electrophysiological, behavioral, and DRG transcriptional analyses were repeated in Avil-Cre;cKI mice to assess therapeutic effects on sensory function.

Results Comprehensive phenotyping of KI mice revealed progressive sensorimotor abnormalities and peripheral histopathology closely paralleling patient features. Conversely, KO mice exhibited normal peripheral nerve function, implicating toxic gain-of-function from mutant ATXN3 as the pathogenic driver. Selective silencing of mutant ATXN3 in sensory neurons dramatically attenuated peripheral nerve deficits, directly implicating sensory neurons as key mediators of SCA3 peripheral neuropathy.

Conclusions These findings define a pathogenic role for repeat expansion-mediated peripheral neurodegeneration, establish sensory neurons as critical therapeutic targets in SCA3, and provide a robust preclinical platform for developing peripheral intervention strategies in SCA3 and related repeat expansion disorders.

Funding Sources: National Ataxia Foundation: Pre-Doctoral Fellowship to Promote Diversity in Ataxia Research, R01NS122751, NS140063

DD4. Understanding the Role of PUMILIO1 in Spinocerebellar Ataxia Type 47

9:58 AM - 10:16 AM

Maximilian Cabaj¹; Serena Raimo¹; Vincenzo A. Gennarino²

1. Columbia University Irving Medical Center, 2. Columbia University Irving Medical Center, New York, NY

We initially identified 15 individuals with PUM1 deletions or missense mutations, defining two phenotypes: a severe early-onset syndrome, PUM1-associated developmental delay and seizures (PADDAS), and a milder, late-onset disorder, PUM1-related cerebellar ataxia (PRCA). OMIM subsequently unified these conditions under spinocerebellar ataxia type 47 (SCA47).

We have now assembled a cohort of 136 patients carrying diverse PUM1 variants, including deletions, microdeletions, microduplications, and 46 de novo missense mutations. This expanded cohort revealed three additional phenotypes: pure early-onset ataxia, seizures without motor features, and a syndrome beginning at approximately 2 years of age characterized by motor delays and cardiac defects. Notably, severely affected patients frequently present with neuropsychiatric disorders, cardiomyopathy, and sensorineural hearing loss, along with symptoms that wax and wane throughout the day, such as muscle weakness and fatigue, features reminiscent of mitochondrial ataxias.

We generated mouse models carrying the mildest and most severe mutations. These models faithfully recapitulate the human phenotypes: PADDAS mice exhibit developmental delay, seizures, hyperactivity, cognitive impairment, and motor incoordination beginning at 5 weeks of age, whereas PRCA mice show no detectable differences until approximately 44 weeks, when motor incoordination emerges. Both models display cerebellar degeneration, although at different time points.

RNA-seq analysis showed that mRNAs involved in RNA regulation and transport are similarly dysregulated in both disease models, with PUM1 targets biased toward upregulation. In contrast, quantitative proteomics revealed only a modest trend at the bulk-protein level. We therefore hypothesized that PUM1 variants may affect interactions with protein partners.

Co-immunoprecipitation coupled with mass-spectrometry from brains at presymptomatic ages revealed strikingly opposing interactome remodeling: PADDAS mice lose 48% of PUM1 interactors (n = 238), whereas PRCA mice gain 70% (n = 345). In both cases, the affected partners are predominantly nuclear-encoded mitochondrial proteins, pointing to mitochondria as a convergent, yet inversely affected, hub across the two diseases.

Funding Sources: R01NS109858, NIH/NINDS, National Ataxia Foundation Research Seed Money Total Award

DD14. Profiling the channelome to understand neurodegenerative cerebellar ataxia

10:16 AM - 10:34 AM

Praveen Kumar¹; Deborah M. Jones²; Satoshi Ishishita³; Deepa Elangovan⁴; Lucas Matt⁵; Robert Lukowski⁵; Ashwinikumar Kulkarni⁴; Allan H. Pool¹; Dale Swift⁴; Brett Whittemore⁴; Vikram Shakkottai
1. UT Southwestern Medical Center, Dallas, TX, 2. The University of Texas Southwestern Medical Center, 3. University of Texas Southwestern Medical Center, Dallas, TX, 4. UT Southwestern Medical Center, 5. University of Tübingen

Background and objectives: Ion channel gene mutations are significantly enriched as a cause of human degenerative cerebellar ataxia. The basis for why ion channel gene mutations in humans results in cerebellar atrophy whereas analogous loss of ion channel function in mice rarely leads to neurodegeneration remains unexplained.

Methods: To examine the hypothesis that allometric scaling of ion channel expression contributes to the increased vulnerability of human Purkinje neurons, we performed spatial transcriptome profiling of all ion channels using the MERSCOPE platform on acutely resected human cerebellar tissue and compared ion channel transcript abundance with that of the developmentally matched corresponding cerebellar region in mice. This analysis enabled characterization of the cerebellar channelome and identification of species-specific transcript distribution at the cellular level.

Results: We observed that ion channel transcripts, mutations in which result in human cerebellar degeneration, are invariably expressed in human Purkinje neurons. The allometric scaling of ion channel transcripts between mouse and human Purkinje neurons was, in general, substantially lower but not consistent across all ion channels. Additionally, significant interspecies differences were identified in both the localization and cellular distribution of individual ion channel transcripts. Complementary immunostaining analyses of human and mouse cerebellar tissue further revealed distinct expression patterns of several ion channels, suggesting that these proteins may possess previously unrecognized non-canonical functions beyond their traditional roles in plasmalemmal ion flux regulation.

Discussion and Conclusion: These findings indicate that the heightened susceptibility of human cerebellar neurons to degeneration may arise from a combination of altered transcript scaling and specialized ion channel functions unique to human Purkinje neurons. Collectively, this study highlights important molecular and spatial differences between human and mouse cerebellum and suggests that the vulnerability to degeneration of human cerebellar neurons is related to both allometric scaling of transcripts and novel roles for ion channels in human Purkinje neurons.

Funding Sources: Raynor Cerebellum Project

DD3. Mutant ATXN1 cell-autonomously modulates reactive phenotypes of human and mouse microglia that contribute to SCA1 pathogenesis

10:34 AM - 10:52 AM

Marija Cvetanovic¹; Selimovic Adem²; Harry Orr²; Gourango Talukdar¹; Gavin Fuchs³

1. University of Minnesota, Minneapolis, MN, 2. University of Minnesota, 3. University of Minnesota, Minneapolis

Background: Microglial activation is a hallmark of many neurodegenerative diseases, yet the specific role of microglia-intrinsic factors in microglial activation remains unknown. Spinocerebellar ataxia type 1 (SCA1), an inherited neurodegenerative disease caused by expanded CAG repeats in the ATAXIN1(ATXN1) gene, is characterized by cerebellar pathology, motor, cognitive and mood alterations. Increased microglia density and pro-inflammatory cytokines are present in cerebellum from early stages of disease, yet how mutant ATXN1(mATXN1) impacts microglial reactivity and disease pathogenesis in SCA1 remains unknown.

Objective: Determine the role of mATXN1 in microglia activation and SCA1 disease pathogenesis in human and mouse experimental systems.

Methods: We used SCA1 patient-derived and control iPSCs, imaging, RNA seq, proteomics, ELISA arrays, and flow cytometry to quantify morphology, gene and protein expression, cytokine secretion and phagocytosis in human microglia.

We removed mutant ATXN1 from microglia and macrophages by crossing Lyve-Cre mice with a conditional SCA1 mouse model, f-ATXN1146Q/2Q mice (ATXN1mKO mice) and performed behavioral testing, RNA sequencing and pathological analysis.

Results: SCA1 human microglia exhibited increased soma, and expression of mitochondrial and inflammatory genes and proteins, increased phagocytosis and secretion of pro-inflammatory cytokines compared to control microglia. Reducing mutant ATXN1 in microglia ameliorated changes in microglia morphology and the neuroinflammatory interferon type 1 pathway, reduced pathology of Purkinje neurons and cerebellar astrogliosis, and improved performance on cognitive, mood, and movement tests in ATXN1mKO mice.

Discussion and Conclusion: We found that mutant ATXN1 impacts reactive phenotypes in both human and mouse SCA1 microglia in a cell-autonomous manner. Our results indicate that microglial mATXN1 likely mediates behavioral SCA1-like phenotypes through reduced immune response leading to improvements in neuronal health and astrocyte phenotypes.

Funding Sources: NIH, grant numbers R01NS109077(to MC), R35NS127248(to HTO) and 1F31NS143172-01(AS).

DD39. AAV-Mediated hnRNP K Overexpression Rescues Type C SCA10 Phenotypes in Transgenic Mice

10:52 AM - 11:10 AM

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Background & Objective: Spinocerebellar ataxia type 10 (SCA10) is an autosomal dominant cerebellar ataxia found in the Americas and East Asia. Its pathogenic mechanism involves heterogeneously configured intronic AT-rich pentanucleotide repeat expansion and sequestration of RNA-binding proteins (RBPs) into expanded RNA repeats in spliced intronic transcripts, causing RBP loss of function. In Type C SCA10 expansion, configured as 5'-(ATTCT)_n-(ATCCT)_n-(ATCCC)_n-(ATTCT)₁₋₃-3', hnRNP K appears to play a critical pathogenic role. We tested whether hnRNP K loss of function drives Type C SCA10 pathogenesis by determining whether AAV-mediated hnRNP K overexpression rescues SCA10-like phenotypes in Type C transgenic mice.

Methods: Newborn Type C transgenic mice, which show robust, delayed-onset ataxia-like gait abnormalities, and age/sex-matched wildtype mice received intracisternal AAV2 expressing mCherry-hnRNP K under the CBA promoter, or control AAV2 expressing mCherry alone. mCherry expression was confirmed by in vivo IVIS imaging. Gait was assessed with DigiGait®. Representative mice underwent histopathology and hnRNP K immunofluorescence.

Results: At 3 months, control AAV2-treated transgenic mice showed approximately 25% shorter stride length and 50% higher stride frequency with increased irregularity. Rescue AAV2 completely reversed these gait abnormalities without adverse effects in wildtype mice. Type C transgenic mice showed severe Purkinje cell loss at 6 months of age that was unaffected by control AAV2 but prevented by rescue AAV2, although dendritic arborization remained abnormal. hnRNP K immunostaining showed irregular intracellular staining defects in surviving Purkinje cells of control-treated transgenic mice, whereas rescue AAV2 restored homogeneous hnRNP K staining.

Discussion & Conclusions: These findings indicate that hnRNP K sequestration causes hnRNP K loss of function in Purkinje cells and drives cerebellar ataxia in Type C SCA10. hnRNP K overexpression may represent a therapeutic strategy for Type C SCA10.

Funding Sources: NIH (NS083564, NS1150024)

Biomarkers I

Wednesday, November 11, 11:30 AM-1:00 PM

B3. Early and progressive spinal cord alterations in Spinocerebellar Ataxia Type 1

11:30 AM - 11:48 AM

Colette Reniers¹

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Background: Spinocerebellar ataxia type 1 (SCA1) is a rare autosomal dominant neurodegenerative disorder characterized by progressive degeneration of the cerebellum. Previous research has indicated that spinal cord atrophy is an important component of SCA1 pathology. As a result, MRI-based spinal cord measurements may function as potential biomarkers for disease staging and progression. However, most earlier studies were cross-sectional, leaving longitudinal changes and sensitivity to disease progression largely unexplored.

Objectives: This study aimed to examine both cross-sectional and longitudinal changes in the spinal cord in SCA1 mutation carriers compared with healthy controls, and to evaluate whether these measures can be used for disease staging and progression monitoring.

Methods: Baseline and one-year follow-up data were analyzed from 40 healthy controls, 16 preataxic carriers, and 58 symptomatic SCA1 mutation carriers. MPRAGE MRI scans were processed using FreeSurfer and the Spinal Cord Toolbox. Z-scores were associated with disease duration, while standardized response means were used to assess sensitivity to change. In addition, clinicogenetic relationships were analyzed using linear mixed-effects models.

Results: At baseline, significant differences were observed between the three groups in cross-sectional area (CSA) and eccentricity across all spinal cord levels. Over the one-year period, CSA and eccentricity at C1 and C2 showed a significant decline in both presymptomatic and symptomatic carriers compared to controls. Notably, presymptomatic individuals already exhibited marked spinal cord atrophy, whereas pontine changes appeared later in the disease course. The highest standardized response means were observed for C2 CSA in the presymptomatic stage, while pontine volume showed the greatest responsiveness in symptomatic individuals, suggesting stage-specific imaging biomarkers.

Conclusion: Cervical spinal cord atrophy represents an early and progressive feature of SCA1 and can be detected before clinical symptom onset, making it a promising biomarker for early disease stages. Overall, the findings suggest a caudal-to-rostral pattern of neurodegeneration in SCA1.

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Funding Sources: This study was supported by ZonMw (40-44600-98-606), NWO (NWA.1389.20.244; CureQ consortium, Dutch NWA-ORC), Italian Ministry of Health (RF-2011-02347420; GR-2013-02357821; RRC), and French Ministry of Health PHRC BIOSCA-ID (RCB:2010-A01324-35; AOM10094), BIOSCA study (NCT01470729).

B22. Neuroimaging Biomarkers for Friedreich Ataxia: A 24-Month Longitudinal Analysis of the TRACK-FA Study

11:48 AM - 12:06 PM

Nellie Georgiou-Karistianis¹; Louise Corben²; Eric F. Lock³; Isaac Adanyeguh⁴; Manuela Corti⁵; Daniel Chung⁶; Dinesh K. Deelchand⁴; Martin Delatycki⁷; Imis Dogan⁸; Jennifer Farmer⁹; Marcondes C. França¹⁰; Anthony Gabay⁶; William Gaetz¹¹; Ian H. Harding¹²; James M. Joers¹³; Michelle Lax⁶; Jiakun Li¹⁴; David Lynch¹⁵; Thomas H. Mareci¹⁶; Alberto Martinez¹⁷; Massimo Pandolfo¹⁸; Marina Papoutsis⁶; Myriam Rai¹⁹; Kathrin Reetz⁸; Thiago J. Rezende²⁰; Timothy P. Roberts¹¹; Sandro Romanzetti⁸; David Rudko²¹; Christian Rummey²²; Susmita Saha²³; Jörg B. Schulz⁸; Sub Subramony¹⁶; Veena Supramaniam⁶; Christophe Lenglet²⁴; Pierre-Gilles Henry

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Background and Objectives: Previous single-site longitudinal studies have identified promising neuroimaging biomarkers in Friedreich Ataxia (FRDA). However, cohort sizes have been insufficient for stratification by age and there is little data available in children. TRACK-FA aims to fill this gap.

Methods: Participants underwent repeated evaluation at baseline, 12, and 24 months, including multimodal imaging of brain and spinal cord and clinical assessments (mFARS, SARA). Linear mixed-effects models were used to compare rates of change between FRDA and control groups. Analyses were stratified by age (5-10, 11-17, and ≥18 years).

Results: Analyses included 248 participants (FRDA n=158; control n=90) with ≥2 timepoints. In young controls (5-10yrs), brainstem and cerebellum volumes and cervical spinal cord cross-sectional area (CSA) showed robust longitudinal increases, consistent with brain development. Conversely, young children with FRDA (5-10yrs) showed impaired growth, with stable brainstem and cerebellum volumes and longitudinal decreases in spinal cord CSA. In adults (≥18yrs), controls showed no significant longitudinal change in brainstem and cerebellum volume and spinal cord CSA, whereas adults with FRDA showed significant reductions across these outcome measures. The adolescent group (11-17yrs) exhibited intermediate structural changes, falling between the patterns observed in young children and adults. Quantitative susceptibility mapping showed dentate volume decreases only in FRDA adolescents and adults. Within-group effect sizes were highest for brainstem volume in young controls, and for cerebellum and brainstem volume in adolescents and adults with FRDA, outperforming clinical scales in these regions.

Discussion and Conclusion: TRACK-FA provides the first age-stratified longitudinal neuroimaging data in FRDA, including young children, adolescents and adults. Results demonstrate a lack of growth in brainstem, spinal cord and cerebellum in young children with FRDA from five years of age, and progressive atrophy in adolescents and adults. This provides evidence for neuroimaging endpoints in clinical trials, and for early intervention during brain development.

Funding Sources: TRACK-FA is funded by grants from the Friedreich's Ataxia Research Alliance (FARA, USA) to each of the academic sites and IXICO with financial support from Takeda Pharmaceuticals Company, Novartis Gene Therapies, IXICO, Larimar Therapeutics Inc., and PTC Therapeutics.

B56. MRI-derived Data-Driven Progression Patterns of Brain Atrophy in SCA1, SCA2, SCA3, and SCA6: An ENIGMA and ESMI Study

12:06 PM - 12:24 PM

Mónica Ferreira¹; Philipp Wegner²; Thiago J. Rezende³; Jennifer Faber⁴; Ian H. Harding⁵

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Background and Objectives: Brain degeneration in spinocerebellar ataxias (SCAs) is progressive in magnitude and spatial extent across cerebellar, brainstem, and subcortical regions. However, it remains unknown whether progression patterns are shared or distinct within and between SCA types. We applied data-driven disease modeling to characterize trajectories across SCA1, SCA2, SCA3, and SCA6.

Methods: Cross-sectional T1-weighted MRI data from 571 controls and 132 SCA1, 164 SCA2, 498 SCA3, and 119 SCA6 mutation carriers were analyzed. Regional volumes of the cerebrum, cerebellum, and brainstem were analyzed using genotype-specific and agnostic SuStaln models to identify progression patterns.

Results: Three progression subtypes were identified in the agnostic analysis. SCA6 exhibited a distinct trajectory characterized by early, largely selective cerebellar cortical degeneration. In contrast, SCA1, SCA2, and SCA3 were distributed across two subtypes with overlapping trajectories. The dominant subtype (SCA1: 76%, SCA2: 57%, SCA3: 92%) showed early brainstem involvement followed by cerebellar white-matter and subcortical abnormalities, with later cerebellar cortical degeneration. The less frequent subtype (SCA1: 22%, SCA2: 38%, SCA3: 6%) followed a more variable pattern with earlier cerebellar cortical and later subcortical involvement. Despite overlap among SCA1, SCA2, and SCA3 in the agnostic analysis, genotype-specific analyses identified a single distinct subtype for each disease.

Discussion: Data-driven progression modelling revealed shared and diverging patterns of neurodegeneration across SCA1, SCA2, SCA3, and SCA6. Consistent with previous literature, SCA6 showed relatively selective cerebellar degeneration, whereas SCA1, SCA2, and SCA3 exhibited broader olivopontocerebellar trajectories extending into subcortical structures. Accordingly, the disease-agnostic model did not separate all genotypes into distinct subtypes, with only SCA6 forming a clearly differentiated subtype. SCA1, SCA2, and SCA3 occupied partially overlapping trajectories despite distinct genotype-specific progression patterns and unique sequences of atrophy events. Our findings underscore the potential of data-driven modelling in large multicentre cohorts to identify shared and distinct progression patterns that do not fully recapitulate genotype-based classifications.

Funding Sources: JF was funded by ACCENT (BMFTR, FKZ 01EO2107), the iBehave Network (Ministry of Culture and Science of North Rhine-Westphalia), and the National Ataxia Foundation, and is a member of the European Reference Network for Rare Neurological Diseases. IH was funded by NHMRC Ideas Grant 1184403 and Investigator Grant 2026191.

B10. Mapping Brain and Spinal Cord Volumetric and Microstructural Changes in RFC1-Disease Across the Disease Course

12:24 PM - 12:42 PM

Lachlan T. Strike¹; Andreas Traschütz²; Thiago J. Rezende³; Matthias Synofzik⁴; Paula C. Alves⁵; Mathieu Anheim⁶; Orlando Barsottini⁷; Benjamin Bender⁸; Manoella G. Bueno⁹; Sirio Cocozza¹⁰; Jennifer Faber¹¹; Jemimah Harding¹²; Berkan Koyak¹³; Camila C. Lobo¹⁴; Paul J. Lockhart¹⁵; Nithin Manchery¹⁶; Alberto Martinez¹⁷; Wilson Marques-Jr¹⁸; José L. Pedroso⁷; Penny Snell¹⁹; Chloe Spiegel²⁰; Pedro J. Tomaselli¹⁸; Thomas Wirth²¹; David Szmulewicz²²; Marcondes C. França¹⁴; Ian H. Harding²³

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BACKGROUND: RFC1-disease is a common genetic cause of late-onset ataxia, typically presenting as Cerebellar Ataxia, Neuropathy, and Vestibular Areflexia Syndrome (CANVAS). Comprehensive characterisation of structural brain and spinal cord changes as potential biomarkers for its slowly progressive disease course remains limited.

METHODS: We conducted a multi-site volumetric and diffusion MRI study of the brain and cervical spinal cord including 87 individuals with RFC1-disease and 63 controls from Australia, Brazil, Germany, and France. We examined group differences relative to controls, correlations with ataxia severity, and cross-sectional patterns of atrophy across disease stages.

RESULTS: Atrophy was greatest in the spinal cord ($d=2.0-3.3$), medulla ($d=2.2$), and cerebellar anterior and flocculonodular lobes ($d=1.5-1.9$). Less pronounced volume loss was evident in the remainder of the cerebellum and brainstem, basal ganglia and thalamus. Limited cortical involvement included atrophy of the primary motor cortex. Spinal cord diffusion microstructural imaging revealed severe dorsal column (sensory) disruption ($d=1.9-3.2$), and more moderate lateral corticospinal tract (motor) involvement ($d=0.6-0.7$). Brain microstructural abnormalities were most prominent in the superior ($d=1.0$) and inferior cerebellar peduncles ($d=0.8$). Disease severity (SARA) correlated with decreasing cerebellar, cerebellar peduncle, medulla, and corticospinal tract integrity ($-0.30 > r > -0.46$). Cross-sectional staging revealed extensive dorsal column damage and moderate motor cerebellum atrophy already in individuals with mild ataxia ($SARA < 7$). Spinal cord atrophy and dorsal column (sensory) tract abnormalities appear stable across severity subgroups, suggesting a ceiling effect is reached at early disease stages. Widespread brain white matter integrity alterations, and primary motor cortex atrophy are predominantly features of late-stage disease.

DISCUSSION: RFC1-disease is characterised by severe sensory spinal tract degeneration, an evolving pattern of brainstem and cerebellar atrophy, and late-stage cerebral and descending motor tract involvement. These results suggest distinct timecourses of damage in sensory and motor systems and motivate longitudinal studies to identify markers of disease progression at different disease stages.

Funding Sources: Australian National Health and Medical Research Council (NHMRC), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Else Kröner-Fresenius-Stiftung, Connaître les Syndromes Cérébelleux, European Rare Disease Research Alliance (ERDERA), German Federal Ministry of Research, Technology and Space (BMFTR), German Ministry of Culture and Science of the State of North Rhine-Westphali

B28. Quantifying and predicting clinical meaningful change in Friedreich Ataxia.

12:42 PM - 1:00 PM

Louise Corben¹; Kanishka Ranaweera²; Malcolm Horne³; Martin Delatycki⁴; Christian Rummey⁵; Pubudu Pathirana⁶; Friedreich Ataxia Global Clinical Consortium Friedreich Ataxia Global Clinical Consortium⁷

1. Murdoch Children's Research Institute, Parkville, Victoria, 2. Deakin University, 3. Department of Medicine, St Vincent's Hospital, The University of Melbourne, 4. Murdoch Children's Research Institute, Hawthorn, 5. Clinical Data Science GmbH, Basel, Basel-Stadt, 6. School of Engineering, Deakin University, 7. Friedreich Ataxia Research Alliance

Background and objectives. Quantifying the clinical impact of Friedreich ataxia (FRDA) is crucial to clinical monitoring. Clinical rating scales fail to measure the impact of disease on quality of life (QoL) and function (Activities of Daily Living, ADLs). We used Item Response Theory to develop the Friedreich Ataxia Interpretable Rating (FAIR), an interpretable latent composite severity score that predicts ADL and QoL outcomes even when ADL and QoL scales are not directly measured. We tested the accuracy of the FAIR to predict ADL and QoL outcomes by integrating kinematic features extracted from the Ataxia Instrumented Measurement System (AIMs).

Methods. Participants with FRDA (n=105, 467 longitudinal observations) in the Friedreich Ataxia Global Clinical Consortium UNIFIED Natural History Study (UNIFAI) study provided the FAIR clinical data framework comprising: the Scale for the Assessment and Rating of Ataxia (SARA), subscales of the FARS -ADL and EuroQual-5D-5L (EQ-5D-5L). A further cohort (n=39, 204 longitudinal observations) confirmed convergent validity against the modified Friedreich Ataxia Rating Scale (mFARS) and FARS-ADL total scores. The FAIR score was mapped onto FARS-ADL sub-items providing an individual probabilistic functional profile. We then trained regression models on AIMs features to predict FAIR scores directly from motor task kinematics.

Results: The FAIR score outperformed mFARS in predicting all FARS-ADL domains i.e., cutting food (r = 0.86 vs 0.81), sitting (r = 0.86 vs 0.79) and personal hygiene (r = 0.83 vs 0.76). AIMs sensor derived data provided probability distributions predicting the individual functional impact (FARS-ADL and EQ-5D-5L scores) of FRDA at particular points in the disease trajectory.

Discussion: The FAIR score has a greater capacity to predict a meaningful aspect of health (i.e., capacity to feed self) than clinical scales. Moreover, the AIMs derived FAIRs score enables remote evaluation between clinic visits of the functional and QoL impact of FRDA.

Funding Sources: Friedreich Ataxia Research Alliance (FARA,USA).

Emerging and Existing Therapeutics

Wednesday, November 11, 2:30 PM-4:00 PM

EP32. Therapeutic development for Christianson syndrome ataxia

2:30 PM - 2:48 PM

Collin J. Anderson¹

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Background and Objectives: Christianson syndrome (CS) is an x-linked, recessive neurodevelopmental and neurodegenerative disorder caused by loss-of-function mutations in SLC9A6 encoding sodium hydrogen exchanger NHE6. NHE6 regulates acidification of early and recycling endosomes, with downstream consequences in late endosomes and lysosomes. Thus, NHE6 loss of function leads to dysfunction and cellular loss in several brain regions, particularly in the cerebellum, yielding severe symptoms, including intellectual disability, cerebellar degeneration, progressive ataxia, and treatment-refractory epilepsy. In 2023, we identified the Wistar Furth shaker rat as a naturally occurring rat model of CS, and we now utilize it with the objective of therapeutic development for CS.

Methods: We developed and tested several therapeutic strategies in the shaker rat, including recombinant adeno-associated viral (rAAV) vectors with Purkinje cell-specific, pan-neuronal, and ubiquitous promoters, as well as a small-molecule V-ATPase inhibitor, intended to regulate endosomal pH. We evaluated effects on cerebellar motor dysfunction using force plate actometry, applying methods and tools that we have recently published in a freely available, open-source capacity. During motor studies, we simultaneously collecting fecal and blood samples. Post-mortem, we conducted structural MRI and western blotting.

Results: Optimized rAAV vectors dosed early in life fully prevented the generation of ataxia and tremor, while chronic low-dose vATP-ase inhibition also generated motor benefit, albeit to a lesser extent. Structural protection of the cerebellum, key cerebellar protein expression levels, and microbiomic composition were all strongly associated with motor outcomes.

Discussion and Conclusion: Both gene replacement and vATP-ase inhibition represent potentially viable strategies to treat CS ataxia, without overt toxic effect, even when expressing NHE6 beyond wildtype levels. Importantly, even with post-natal treatment, it appears possible for optimized therapeutics to fully protect against motor symptoms of CS. Work is underway to determine whether these strategies may benefit intellectual and epileptic symptoms.

Funding Sources: 1. National Ataxia Foundation Junior Investigator Award 2. RTW Charitable Foundation Research Grant (3x) 3. Rare Village Foundation Research Grant

EP46. Purkinje cell-targeted silencing of the GABA(A) receptor rescues inhibitory circuit activity and motor function in Spinocerebellar Ataxia type 7 mice

2:48 PM - 3:06 PM

Fabiana Longo¹; Miranda Dunn²; Luke C. Bartelt³; Vikram Shakkottai; Albert R. La Spada⁴

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Background and Objectives Spinocerebellar Ataxia type 7 (SCA7) is a CAG polyglutamine repeat disease causing progressive cerebellar neurodegeneration and severe motor impairment. Increased GABAergic inhibition onto Purkinje cells (PCs) has emerged as a major contributor to PC dysfunction in SCA7; however, whether it is compensatory or deleterious is not yet established.

Methods Building on PC-enriched snRNA-seq data revealing early dysregulation of inhibitory synapse genes, we characterized GABA receptor remodeling and interneuron changes in SCA7266Q knock-in mice by immunofluorescence and western blot. To correct PC hyper-inhibition, we delivered an AAV-PHP.eB vector silencing Gabrb2 under a PC-specific promoter via single retro-orbital injection in presymptomatic mice at 4 weeks of age. Motor function was assessed longitudinally by rotarod and composite ataxia scoring; target engagement was validated by qRT-PCR, immunofluorescence, and whole-cell patch-clamp recordings.

Results We found that affected SCA7mice display bidirectional remodeling of PC GABA receptor expression with selective downregulation of GABA(B) subunits and upregulation of GABA(A) $\beta 2$ protein. After confirming that shGabrb2delivery achieves robust, sustained knockdown through 11 weeks, we documented that treated SCA7mice showed significant improvement in rotarod performance and composite ataxia scores at 8.5 weeks of age. Patch-clamp recordings revealed normalization of elevated IPSC frequency, and confocal quantification revealed rescue of PC inhibitory synapse density to wild-type levels. Furthermore, pharmacological enhancement of GABAergic transmission worsened ataxia and reduced survival, establishing that hyper-inhibition is indeed pathological. When we examined preterminal late-stage SCA7mice, we noted that PC rescue waned, reflecting the severity of this aggressive mouse model.

Discussion and Conclusion Our findings demonstrate that the hyper-inhibitory state in SCA7cerebellum is not compensatory but rather is pathological, establishing PC-targeted GABA(A) $\beta 2$ subunit silencing as a viable circuit-level therapeutic strategy. Given convergent excitatory-inhibitory imbalance across multiple polyglutamine ataxias, GABAergic circuit correction may have broader therapeutic relevance for such diseases.

Funding Sources: NIH R01EY014061and NIH R35NS122140

EC9. Safety and Preliminary Efficacy of AAVrh.10hFXN (LX2006) Gene Therapy in Friedreich Ataxia Cardiomyopathy

3:06 PM - 3:24 PM

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Background and Objectives: Friedreich ataxia (FA) is a rare, multisystem disease due to a FXN mutation, decreased frataxin (FXN), and mitochondrial dysfunction. Cardiomyopathy (CM) is the primary cause of death in FA, with no approved therapies targeting underlying cardiac pathology. In mice, intravenous (IV) AAVrh.10hFXN (LX2006) increased cardiac FXN and reversed CM. The objective is to evaluate the safety and efficacy of AAVrh.10hFXN in participants with FA-CM using pooled data from 2 ongoing, independent, open-label, dose-escalation Phase 1/2 trials by Lexeo Therapeutics (n=8) and Weill Cornell Medicine (n=9) (NHLBI HL151355).

Methods: 17 adult participants with FA-CM received an IV infusion of AAVrh.10hFXN in 3 dose cohorts (1.8x10¹¹, n=6; 5.6x10¹¹, n=7; 1.2x10¹²vg/kg, n=4). Efficacy was evaluated via cardiac biopsy (FXN expression) and cardiac questionnaire (KCCQ-12) in the Lexeo trial, and cardiac MRI and serum biomarkers in both trials (n=16, ≥6 months follow-up).

Results: AAVrh.10hFXN was generally well tolerated. One Grade 2 SAE of myocarditis at 12 months was possibly treatment-related; all other AEs were transient, non-serious, or unrelated. Cardiac FXN expression showed dose-dependent increases at 3 months (n=8; dose cohort 1, n=1, 29%; cohort 2, n=3, 69% [22-187%]; cohort 3, n=4, 115% [23-198%]). At latest follow-up, mean reductions of -10.5% (-50.9 to +63.4, n=16) in LVMI and -52.9% (-5.8 to -94, n=15, excluding participant with myocarditis) in hsTnI were observed; in those with increased baseline LVMI (n=6), mean reductions were -24.1% (-2.7 to -50.9%) and -61.1% (-26.8 to -88.7%), respectively. Among the 8 participants with KCCQ-12 at ≥6 months, the average improvement was +9.

Discussion & Conclusion: Interim data suggest a single IV dose of AAVrh.10hFXN was generally safe and biologically active, increased cardiac FXN, and improved key markers of cardiac structure and function in FA-CM.

Funding Sources: Lexeo Therapeutics

EP53. Autonomic Dysregulation Fuels Cardiac Dysfunction in Friedreich's ataxia: Therapeutic Benefit of Monoamine oxidase A Inhibition

3:24 PM - 3:42 PM

Francisco Figueroa¹; Sherif Bahriz²; Ryan Sellers¹; Lili Salinas³; Claire Montgomery-Bowie³; Bing Xu²; Gino Cortopassi¹; Yang K. Xiang²; Elena N. Dedkova⁴

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Background: Friedreich's ataxia (FA) is a neurodegenerative disease in which cardiomyopathy and arrhythmias are major causes of mortality. However, the contribution of autonomic nervous system (ANS) in FA cardiomyopathy remains poorly understood.

Objective: This study aimed to examine the ANS and cardiac outcomes in a mouse model of FA treated with monoamine oxidase A (MAO-A) inhibitor (clorgyline).

Methods: We used a muscle-specific frataxin knockout mouse model (FXN-cKO) that mimics severe cardiomyopathy. Clorgyline with and without the first FDA-approved treatment for FA: omaveloxolone (OMAV) were given to FXN-cKO mice. Proteins were quantified by western blotting, LC-MS/MS, and immunohistochemistry. Echocardiography, electrocardiography, qPCR, FRET, enzyme activity, and ELISA were performed.

Results: FXN-cKO hearts showed heightened catecholamine synthesis and metabolism (tyrosine hydroxylase, MAO-A expression and activity). β 1-adrenergic receptor signaling showed localized impairment at the sarcoplasmic reticulum (SR), while plasma membrane PKA signaling remained preserved. MAO-A inhibition restored norepinephrine-induced SR-localized PKA activity. FXN-cKO mice developed ECG abnormalities, including prolonged P-wave, QRS, and QT intervals, altered RR dynamics, and reduced heart rate variability. Clorgyline improved electrical propagation and rhythm features, while dual therapy improved echocardiographic measures of cardiac structure and function. In human FA differentiated myofibroblasts under oxidative stress, OMAV and dual treatment strongly protected cell survival.

Conclusions: Our findings identify MAO-A linked catecholamine dysregulation and SR-localized β 1-adrenergic signaling defects as novel contributors to arrhythmia and cardiac dysfunction in FA. Combined targeting of MAO-A and Nrf2 provides complementary benefit, with clorgyline improving adrenergic signaling and conduction, and OMAV enhancing antioxidant and functional cardiac protection.

Funding Sources: This project was supported by the National Institutes of Health 1R01HL155907-1 (to END), FARA Research Award (to END), American Heart Association Transformational Research Project 25TPA1478903 (to END), University of California Innovative Development Award (to END), FARA graduate student fellowship (to FF), and Freedland fellowship from UC Davis Dept. Mol. Biosciences (to FF).

EP11. Targeting Spt5-Pol II Interaction as a Broad Therapeutic Strategy for Repeat Expansion Diseases

3:42 PM - 4:00 PM

Anat Bahat¹; Rivka Dikstein¹

1. Weizmann Institute of Science

Repeat expansion diseases (REDs) comprise more than 40 inherited disorders, many of which are dominantly inherited neurodegenerative diseases caused by abnormal nucleotide repeat expansions. These include Huntington's disease (HD), spinal-bulbar muscular atrophy (SBMA), myotonic dystrophy type 1 (DM1), amyotrophic lateral sclerosis (ALS), and several spinocerebellar ataxias (SCAs). Despite their severe clinical burden, effective disease-modifying therapies remain unavailable.

The transcription elongation complex Spt4/Spt5 plays a critical role in the selective transcription of expanded-repeat genes, including HTT in HD, C9orf72 in ALS/FTD, and NOP56 in SCA36, making it an attractive therapeutic target across REDs. Using a high-throughput screen, we identified small molecules (SPIs) that inhibit the Spt5–RNA polymerase II interaction. Several SPIs selectively suppress mutant HTT expression while sparing wild-type HTT and other Spt4/Spt5 functions. Structure–activity relationship studies identified two potent analogs, SPI-24 and SPI-77, active in the low μM to nM range.

In the BACHD mouse model, direct striatal administration of these compounds reduced mutant huntingtin levels, improved mitochondrial function, restored BDNF expression, and ameliorated motor and anxiety-like phenotypes. Pharmacokinetic analyses demonstrated blood–brain barrier penetration, and prolonged subcutaneous or oral treatment significantly delayed disease progression in early-stage BACHD mice. Long-term SPI-24 treatment showed no apparent toxicity or major transcriptomic alterations.

Importantly, SPI-24 also reduced expression of mutant genes in patient-derived cells from multiple REDs, including DM1, DRPLA, SBMA, and SCA1, 2, 3, and 7, while sparing corresponding wild-type genes. These findings identify SPIs as a promising and broadly applicable therapeutic strategy for REDs, with advantages in mutant selectivity and systemic delivery potential.

Funding Sources: Weizmann - Azrieli Institute for Brain and Neural Sciences Research Centers:
Individual Research Grants

Disease Mechanisms III

Wednesday, November 11, 4:30 PM-6:00 PM

DR31. ZNF512-SUV39H pericentric heterochromatin initiation complex binds GAA·TTC repeats to drive H3K9me3 deposition in Friedreich's Ataxia

4:30 PM - 4:48 PM

Rémi NEPLAZ¹; Constance Kleijwegt²; Callum Burnard³; Antoine-Peio Uhart⁴; Jill Napierala⁵; Marek Napierala⁶; Jérôme Déjardin²; Helene Puccio⁷; Patrick Lomonte⁸

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Friedreich's Ataxia (FA) is the most common inherited autosomal recessive ataxia. FA results from strong downregulation of the FXN gene encoding the mitochondrial protein frataxin. The primary mutation is a GAA-TTC triplet repeat expansion in intron 1, causing heterochromatin formation. Main epigenetic modifications include DNA methylation and histone marks such as H3K9me3 and H3K27me3. However, the mechanism of GAA-TTC repeat recognition and heterochromatinization remains unknown. This work aims to identify protein complexes directly binding the GAA-TTC repeat and driving heterochromatin formation.

We performed Proteomics of Isolated Chromatin (PiCh) on GAA-TTC DNA fibers to identify potential repressor complexes binding this repeat. CUT&RUN was used on control, FA, and GAA-del isogenic FA primary fibroblasts and induced pluripotent stem cells (iPSCs) to validate binding at the endogenous FXN locus. Finally, KO cell lines were generated for the most promising proteins.

PiCh identified several proteins involved in chromatin repression. Among them ZNF512 and ZNF512B emerged as strong candidates as they contain three Zinc-finger DNA-binding domains predicted to recognize TTC triplets. They were also previously described to recruit SUV39H1/2 methyltransferases, catalyzing H3K9me3, to pericentric repeats to initiate heterochromatin formation. CUT&RUN confirmed ZNF512 and SUV39H1 binding on FXN in a GAA-TTC repeat-dependent manner in FA cells. Complete ZNF512/ZNF512B dKO decreased H3K9me3 deposition on FXN.

To our knowledge, this is the first demonstration of a repressor complex specifically binding the GAA-TTC repeat region in FXN. As FXN silencing involves multiple pathways, combining ZNF512 antagonism with other epigenetic treatments targeting histone deacetylases or DNA methyltransferases may be beneficial. Overall, we demonstrate that ZNF512's direct recognition of the GAA-TTC repeat expansion and SUV39H1 recruitment participate in de novo pericentric-like heterochromatin formation in FA.

Funding Sources: FARA : AIM 2025, AFM-Téléthon, ANR, CNRS, INSERM, UCBL1.

DR41. Longitudinal Assessment of Cerebellar Synaptic Morphology in the ARSACS Mouse Model Reveals Early-Onset Changes in Circuitry

4:48 PM - 5:06 PM

Samantha L. Boeshore¹; Huaxia Wang²; Edison King³; Anna Ohm⁴; Justin Wolter³

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Background/Objectives: In Sacs knockout mouse models of Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS), Purkinje neuron subtypes, marked by AldoC expression, are selectively vulnerable to synaptic changes and neurodegeneration. Our single nuclei RNA sequencing (snRNAseq) showed differences in genes related to synaptic dysfunction and glutamatergic dysregulation, in an AldoC dependent manner. Although Sacs knockout Purkinje presynaptic termini have been characterized, it is unknown whether other neuronal types are affected. This study aims to characterize the ARSACS mouse synaptic landscape across Purkinje inputs over time.

Methods: We collected mice from early postnatal development through progression of neurodegeneration (P30, P60, and P120) for histology. We performed immunohistochemistry for markers specific to excitatory/inhibitory synapses including VGLUT1, VGLUT2, and VGAT alongside ALDOC to separate subtypes. Quantification of synapse number, size, and intensity were performed using custom CellProfiler pipelines.

Results: We saw changes to both afferent, excitatory Purkinje inputs and intrinsic, inhibitory circuitry in the ARSACS cerebellum. We found an expansion of climbing fiber branching in the Purkinje dendrites at P60. We observed early alterations to inhibitory circuitry (P30) including Purkinje-Purkinje collateral coupling and interneuron inputs to the Purkinje axon initial segment. These changes were specific to vulnerable AldoC negative neurons. In parallel, we analyzed a proteomics dataset of cerebrospinal fluid (CSF) from ARSACS mice at P60 and P120, as CSF is enriched in synaptic proteins, and similarly found that synaptic deficits precede neurodegeneration.

Discussion/Conclusions: Longitudinal characterization of synapses indicates that multiple neuron types are affected in ARSACS. Changes in synaptic inhibition occur at the onset of changes to firing rate and motor deficits in the mouse (P20-P30) whereas excitatory input expansion occurs as deficits progress (P60), raising the hypothesis that neurons alter their synapses in a compensatory manner. These findings will be used to test therapeutic efficacy on putatively causal cellular deficits.

Funding Sources: ARSACS Foundation (Quebec) . Action for ARSACS USA Foundation. National Ataxia Foundation

DD17. From Anatomy to Function: Characterizing Serotonergic Circuits in a Spinocerebellar Ataxia Type 3 mouse model

5:06 PM - 5:24 PM

Cármén Vieira¹; Daniela Monteiro-Fernandes¹; Sara Duarte-Silva¹; Jonas Campos¹; Daniela Sofia Abreu¹; Nuno Dinis Alves¹; Rafael M Jungmann¹; João Filipe Oliveira¹; Lin Tian²; Kamran Khodakhah³; Ana João Rodrigues¹; Patricia Maciel⁴; Carina Soares-Cunha¹; Andreia Teixeira-Castro¹

1. Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal, 2. Max Planck Florida Institute for Neuroscience, Jupiter, FL 33458, USA, 3. Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY, USA. Department of Psychiatry and Behavioral Sciences, Albert Einstein College of Medicine, Bronx, NY, USA. Saul R. Korey Department of Neurology, Albert Einstein College of Medicine, Bronx, NY, USA, 4. ICVS, University of Minho, Braga

Background and Objectives: Spinocerebellar Ataxia Type 3 (SCA3) is characterized by progressive motor dysfunction and cerebellar degeneration, particularly affecting the deep cerebellar nuclei (DCN). Although serotonergic (5-HT) modulation improves disease phenotypes in preclinical models, the integrity and functionality of 5-HT circuits remain largely unexplored in SCA3. Given that the dorsal raphe nucleus (DRN) is one of the major sources of brain 5-HT, we sought to perform an anatomical and functional characterization of the DR-DCN circuit in a mouse model of SCA3.

Methods: To profile serotonergic circuitry in SCA3, we combined quantitative immunohistochemistry, viral circuit tracing, tissue clearing and whole-brain volume imaging, chemogenetic manipulation, fiber photometry coupled to genetically encoded calcium and serotonin sensors, and machine learning-based automatic behavior detection. To investigate circuit function during behavior, we monitored DRN 5-HT neuronal activity and DCN 5-HT release dynamics during motor tasks. Neural recordings were integrated with behavioral measures through an automated video-tracking analysis pipeline developed in-house.

Results: Initial analyses revealed preservation of the DRN, with no significant differences in serotonin transporter (Sert) expression, number of 5-HT-positive neurons, or accumulation of large ATXN3 aggregates in SCA3 mice. Similarly, Sert-positive innervation of the DCN was preserved. Circuit-tracing experiments established the anatomical connectivity of the DRN-DCN pathway and identified serotonergic inputs to the DCN arising from multiple raphe nuclei. In parallel, we are establishing tissue-clearing and whole-brain imaging pipelines enabling simultaneous visualization and quantification of serotonergic nuclei and projection targets across the brain. Ongoing studies are assessing DRN neuronal activity and DCN 5-HT dynamics during motor behavior.

Discussion and Conclusion: While the serotonergic system appears structurally preserved in SCA3, its functional contribution to disease progression remains unknown. The approaches established here will enable an unprecedented anatomical and functional characterization of serotonergic circuitry in SCA3, bringing modern systems neuroscience approaches to the study of ataxia.

Funding Sources: FCT: Fundação para a Ciência e a Tecnologia (Portugal). National Ataxia Foundation (NAF, USA)

DD37. STUB1 as a crossroad gene in hereditary ataxias: insights from human genetics and C. elegans models

5:24 PM - 5:42 PM

Stefania Magri¹; Giuliana Madonna¹; Elisa Sarto¹; Elena Mascazzini¹; Marinella Corbetta¹; Pamela Santonicola²; Elia Di Schiavi²; Caterina Mariotti³; Cinzia Gellera⁴; Daniela Di Bella⁴; Franco Taroni¹
1. *Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Milano*, 2. *National Research Council of Italy (CNR)*, 3. *Foundation IRCCS Carlo Besta Neurological Institute, Milan*, 4. *Fondazione IRCCS Istituto Neurologico Carlo Besta*

Background and objective: STUB1 is emerging as a master regulator associated with diverse diseases and distinct modes of inheritance: recessive in SCAR16, dominant in SCA48, digenic in SCA17-DI, and as a modifier in SCA8. Its pleiotropic, chameleon-like role raises key questions about its contribution to spinocerebellar ataxias and polyQ-diseases. Moreover, the E3-ubiquitin ligase CHIP, encoded by STUB1, also acts as a modifier in multiple in-vivo and in-vitro models of neurodegenerations.

Here, we aim to dissect genetic interactions involving STUB1 and clarify the functional interplay between CHIP and SCA17-associated TBP.

Methods: We performed whole-exome sequencing and repeat-expansion analysis in 81 index patients carrying pathogenic STUB1 variants. To investigate functional interactions, we generated *Caenorhabditis elegans* models of SCA17 and SCA17-DI by pan-neuronally expressing human TBP with wild-type (hTBP38Q), intermediate (hTBP43Q), or fully penetrant (hTBP54Q) polyQ tracts, in the presence or absence of *chn-1*, the worm ortholog of STUB1.

Results: Among 81 index cases, STUB1 variants were biallelic in 6 patients (SCAR16) and monoallelic in 75. In this latter group, 44 carried intermediate TBP alleles (>40 repeats), consistent with SCA17-DI, 15 carried high-normal TBP alleles (39 repeats), 4 showed expansions in ATXN8/ATXN8OS, and 12 had no additional pathogenic variants in ataxia-associated genes.

In C. elegans, hTBP54Q impaired mechanosensation, whereas hTBP43Q alone was insufficient to induce dysfunction. Notably, hTBP43Q expression caused mechanosensory defects in *chn-1* knockout animals, thus recapitulating SCA17-DI. By genetic and pharmacological approaches, we demonstrated that CHIP promotes proteasomal degradation of hTBP43Q, acting with the molecular chaperone HSP-1/HSP70 and the E4-ubiquitin ligase UFD-2/UBE4B. Moreover, using site-specific mutagenesis, we identified lysine 195 as the crucial hTBP residue that is specifically ubiquitinated by CHN-1.

Discussion and Conclusion: Our findings establish STUB1 as a master regulator of spinocerebellar ataxia pathogenesis and indicate CHIP-mediated degradation as a key therapeutic convergence point across diverse ataxias.

Funding Sources: This work was supported by the Italian Ministry of Health (Ricerca Corrente), Fondazione Cariplo 2021-1544 to SM and PNRR-2023-12377937 to SM.

DD40. Repeat RNA and RAN translation act synergistically to induce neurodegeneration in Fragile X-associated Tremor/Ataxia Syndrome

5:42 PM - 6:00 PM

Peter K. Todd¹; Samantha J. Grudzien²; Nurun Nahar Borna¹; Amy Krans²; M. Rebecca Glineburg³

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Background: Fragile X-Associated Tremor/Ataxia Syndrome (FXTAS) is caused by transcribed CGG repeat expansions in the 5' UTR of FMR1. CGG repeat RNA can sequester RNA-binding proteins and undergo repeat-associated non-AUG initiated translation to produce toxic proteins, especially FMRpolyG, which forms neuronal intranuclear inclusions. FXTAS is also marked by neuroinflammation and white matter abnormalities, but the mechanisms driving neurodegeneration remain unclear.

Methods: We used AAV-mediated expression of FMR1CGG repeats in distinct sequence contexts, along with transgenic CGG repeat expansion mice, CGG knock-in mice, and patient-derived neurons, to define the relative contributions of CGG repeat RNA and FMRpolyG to neuroinflammation, neuropathology, and neurodegeneration.

Results: Expression of RAN translation-competent CGG repeats, through AAV delivery or in transgenic mice, produced widespread neuronal intranuclear inclusions positive for FMRpolyG and P62. Inclusion formation was associated with gliosis, microglial activation, behavioral abnormalities, and Purkinje cell loss. In contrast, CGG repeats engineered to reduce FMRpolyG production, or FMRpolyG expressed from a non-repetitive sequence, did not induce behavioral deficits or gliosis. Transcriptomic analyses indicated that repeat RNA is required to activate innate immune pathway activation, whereas isolated FMRpolyG expression disrupts protein synthesis machinery. However, only RAN-competent repeat RNAs activated both pathways and caused neurodegeneration. In CGG knock-in mice, neuroinflammation preceded intranuclear inclusion formation, suggesting it is an early pathogenic event. Genetic deletion of PKR in cells and mice reduced CGG repeat-induced stress responses and RAN translation, rescuing behavioral deficits, neuroinflammation, and inclusion formation in AAV and transgenic CGG repeat models.

Conclusions: CGG repeat RNA and FMRpolyG act synergistically to drive neuroinflammation and neurodegeneration in FXTAS models. Genetic targeting of PKR mitigates these effects, supporting PKR as a promising therapeutic target for FXTAS.

Funding Sources: R01NS086810, P50HD104463

Advances in Genetics and Diagnostics

Thursday, November 12, 9:40 AM-11:10 AM

A16. Intergenerational instability of polyglutamine disease repeats and implications for genetic counselling: a whole-genome sequencing study of 10,929 families

9:40 AM - 9:58 AM

Arianna Tucci¹; Anupriya Dalmia²

1. Queen Mary University of London, 2. UCL

Background: Expansion of coding CAG trinucleotide repeats causes a group of inherited neurodegenerative disorders known as polyglutamine diseases, including Huntington's disease (HD) and several spinocerebellar ataxias. Repeat length is a major determinant of disease risk and repeat instability. While pathogenic alleles are highly unstable, the mutation risk of intermediate-length alleles remains poorly characterised, creating challenges for genetic counselling and risk prediction.

Methods: We analysed 166,551 parent–child transmissions across eight polyglutamine disease genes using whole-genome sequencing data from more than 10,000 families enrolled in the 100,000 Genomes Project. The study included 1,888 parents carrying intermediate-length alleles. We estimated mutation rates during intergenerational transmission and investigated the effects of repeat length, parental sex, repeat sequence structure, and rare variation in DNA repair genes on repeat instability.

Results: Parental repeat length was the strongest predictor of mutation risk, with instability increasing sharply once alleles exceeded approximately 30 CAG repeats. Although overall mutation rates did not differ significantly between maternal and paternal transmissions, paternal transmission of larger alleles was associated with a greater tendency towards large repeat expansions. Sequence interruptions within the CAG tract had a stabilising effect, reducing the likelihood of repeat-length changes. Rare predicted damaging variants in the DNA repair genes SLX1A, GTF2H2, and TOP3B were enriched among parents transmitting unstable alleles, implicating pathways involved in transcription-coupled repair and the processing of abnormal DNA structures in intergenerational repeat instability. Using current clinical allele classifications, the observed risk of expansion from an intermediate allele into the pathogenic range was 0.08% for HTT and 0.04% across all genes studied.

Conclusions: This study provides the largest population-based assessment of intergenerational instability in polyglutamine disease genes to date. Our findings identify repeat length as the primary determinant of mutation risk and provide quantitative estimates that will inform genetic counselling, particularly for carriers of intermediate alleles.

Funding Sources: This work was supported by the UK Medical Research Council with Clinician Scientist award (MR/S006753/1) and by Barts charity grant (MGU0569).

A7. FXN protomutations are the source of pathogenic expanded GAA alleles in Friedreich ataxia and explain its unequal population distribution

9:58 AM - 10:16 AM

Morgan C. Devore¹; Christina Lam²; Emily Xiao²; Jeremy Devore²; Cassandra R. McCain²; Courtney Park³; David Lynch⁴; Sanjay Bidichandani

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Background and objectives: Friedreich ataxia (FRDA) occurs in people of European, West/South Asian, and North African descent, and is not seen in people of sub-Saharan African and East Asian descent. FRDA is a recessive disease, and most patients inherit two copies of the FXN gene with an expanded GAA repeat (E allele) from heterozygous carrier parents. E alleles are evolutionarily derived from non-pathogenic long normal (LN) alleles (≥ 12 triplets) and occasionally arise de novo via intergenerational expansion of premutation alleles (34-60 triplets). However, why FRDA susceptibility is limited to Eurasians, and how the prevalence of E alleles is sustained are incompletely understood. We hypothesized that there must be an unrecognized step in the evolution of LN to premutation/E alleles that occurred exclusively in Eurasia, allowing FRDA to develop there.

Methods: Whole-genome longread sequencing was performed to determine high-density haplotypes of the FXN gene locus in FRDA patients and non-FRDA controls.

Results: Sequencing of the FXN locus revealed two major subclasses of E alleles, which have originated from a subset of Eurasian LN alleles, termed protomutations. Haplotype identity and evidence of intergenerational instability in protomutation alleles indicate that protomutations can transition to premutation/E alleles. Consistent with the exclusive prevalence of FRDA in Eurasia, protomutations are absent in sub-Saharan Africa, where E alleles did not develop despite a relatively high prevalence of LN alleles. However, genetic admixture has introduced a slight risk of FRDA in African Americans. Analysis of ancient DNAs revealed that protomutations have existed in Europe and Western Asia for thousands of years, with evidence of spread to Europe via early Neolithic farmers.

Discussion and Conclusions: These data indicate that FXN protomutations serve as a reservoir for the generation of premutation and E alleles, and for millennia have sustained the geographically-defined population distribution of FRDA.

Funding Sources: This work was supported by a FARA Graduate Research Fellowship (MCD), FARA Grant (SIB), US Department of Defense grant (USAMRAA) HT94252510541(SIB), US Department of Defense grant (USAMRAA) HT94252510542(DRL).

A14. Complex RFC1 genotypes and pseudodominant inheritance in an Australasian cerebellar ataxia cohort

10:16 AM - 10:34 AM

Kayli Davies¹; Haloom Rafehi²; Penny Snell³; Liam Fearnley²; Greta Gillies³; Genevieve Thompson³; David Bourke⁴; Kishore Kumar⁵; Colina McKeown⁶; Mark Paine⁷; Laura Perju-Dumbrava⁸; David Szmulewicz⁹; Martin Delatycki¹⁰; Melanie Bahlo²; Paul J. Lockhart¹¹

1. Murdoch Children's Research Institute, Parkville, Victoria, 2. Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, 3. Murdoch Children's Research Institute, 4. Wellington Hospital, 5. Department of Neurology and Molecular Medicine Laboratory, Concord Repatriation General Hospital, 6. Genetic Health Service New Zealand, 7. Department of Neurology, Royal Brisbane and Women's Hospital, 8. Royal Victorian Eye and Ear Hospital, 9. Royal Victorian Eye & Ear Hospital, Melbourne, Victoria, 10. Murdoch Children's Research Institute, Hawthorn, 11. Parkville, Victoria

Background and Objectives: RFC1-related disease, which includes cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS), is a late-onset neurodegenerative disorder predominantly caused by biallelic AAGGG(n) repeat expansions (RE) in RFC1. The locus is highly polymorphic, with multiple pathogenic and non-pathogenic repeat motifs identified. The carrier frequency has been reported in various control populations to be between 1.4% and 13%. We comprehensively characterised RFC1 repeat variation and inheritance patterns in an Australasian ataxia cohort.

Methods: Flanking-PCR, repeat-primed PCR, and genome sequencing were used to characterise the RFC1 locus in 269 Australian controls and 536 individuals with ataxia. Targeted long-read sequencing was used to resolve complex alleles.

Results: Biallelic pathogenic RFC1 variants were identified in 22.4% (n=120, 102 families) of affected individuals. While most individuals had biallelic AAGGG(n) RE, 10% encoded alternative pathogenic genotypes, including homozygous alternative pathogenic motifs (n=2) or compound heterozygous AAGGG(n) and alternative pathogenic alleles (n=10). Two of the compound heterozygous individuals carried a truncating RFC1 variant and had unusually early symptom onset (early 30s). The carrier frequency of the pathogenic AAGGG(n) RE was ~1 in 16 in controls. Apparent dominant inheritance was observed in nine pedigrees and genetically confirmed as pseudodominant RFC1-related disease in three families. Across controls and affected individuals, >16 distinct repeat motifs were identified, including at least six with allele frequencies >1%.

Discussion and Conclusion: RFC1-related disease genotypes are more heterogeneous than previously reported, with 10% of affected individuals encoding non-canonical pathogenic genotypes. Extensive repeat diversity at the RFC1 locus highlights the need for comprehensive molecular testing strategies. The high pathogenic allele carrier frequency (~1 in 16) and identification of pseudodominant pedigrees demonstrate that apparent dominant transmission can mask an underlying recessive disease. This has important implications for recurrence risk and cascade testing in affected families, supporting RFC1 testing in individuals with apparent dominant inheritance.

Funding Sources: Australian Government National Health and Medical Research Council grants (MRF2024989 and GNT2028681). KD was supported by an Australian Government Research Training Program Scholarship.

A19. CANVAS causing AAGGG repeat expansions cause tissue-specific reduction in RFC1 expression and increase sensitivity to DNA damage

10:34 AM - 10:52 AM

Riccardo Curro¹; Natalia Dominik¹; Ricardo Schnekenberg²; Stefano Facchini³; Cecilia Perini⁴; Riccardo Ronco⁵; Ilaria Quartesan⁶; Zhongbo Chen⁷; Mina Ryten⁸; Henry Houlden⁹; Puneet Opal¹⁰; Sheng-Han Kuo¹¹; James Jepson¹; Emmanuele Crespan¹²; Andrea Cortese¹³

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Background and Rationale: Biallelic AAGGG repeat expansions in RFC1 cause cerebellar ataxia, neuropathy, vestibular areflexia syndrome (CANVAS) and represent a common cause of adult-onset ataxia and sensory neuropathy. However, the pathogenic mechanisms underlying RFC1-related disease remain incompletely understood.

Methods: We combined in vitro functional assays, post-mortem brain tissue, patient-derived cell lines and iPSC-derived neurons, and a neuronal Drosophila model to investigate the molecular consequences of RFC1 AAGGG repeat expansions. We additionally assessed the effect of CRISPR/Cas9-mediated repeat excision.

Results: AAGGG repeat expansions were associated with tissue- and cell-specific reduction of RFC1 transcript expression, most prominently in cerebellar tissue and Purkinje cells. Patient-derived iPSC neurons demonstrated reduced RFC1 expression, impaired DNA damage response and increased sensitivity to platinum-based agents, with delayed repair of DNA adducts, increased γ H2AX accumulation, and enhanced apoptosis following cisplatin and oxaliplatin exposure. Biallelic CRISPR/Cas9 excision of the pathogenic repeat and flanking AluSx3 element restored RFC1 expression in iPSC-derived neurons and rescued DNA damage response abnormalities.

Conclusions: Our findings support reduced expression and partial loss-of-function mechanism in RFC1 disease. Rescue of molecular phenotypes following repeat excision provides proof-of-principle for targeted therapeutic approaches aiming at increasing RFC1 expression and function

Funding Sources: National Ataxia Foundation, Medical Research Council, European Research Council

A22. Somatic GAA repeat instability as a therapeutic target for Friedreich's ataxia

10:52 AM - 11:10 AM

Maheswaran Kesavan¹; Muzhou Wu²; Antonia Vitalo²; Anh Nhu²; Shota Shibata³; Paula Hornbostel³; Neil J. Doherty²; Marina Kovalenko²; Cameron J. Douglas²; Vanessa Wheeler²; Ricardo Mouro Pinto⁴*1. Massachusetts General Hospital, Somerville, MA, 2. Massachusetts General Hospital, 3. Massachusetts General Hospital Center for Genomic Medicine, 4. Massachusetts General Hospital Center, Harvard Medical School, Boston, Massachusetts, MA*

Friedreich's ataxia (FRDA) is a neurodegenerative disorder caused by an expanded GAA repeat in the FXN gene, which leads to transcriptional silencing and frataxin protein deficiency. While inherited repeat length correlates inversely with the age of onset, whether progressive somatic GAA expansion contributes to FRDA pathogenesis remains unclear. Based on the occurrence of large expansions in the disease-vulnerable brain tissues of FRDA patients and recent discoveries in Huntington's disease (HD), we hypothesized that somatic GAA expansions also contribute to FRDA onset and/or progression. To address this question, we first developed a densitometry-based method to quantify instability using the YG8s mouse model carrying 300 or 800 repeats. Through this method, we identified tissue-specific and age-dependent somatic GAA expansions in the striatum and cerebellum of these mice. To further characterize repeat-length-dependent phenotypes, we evaluated FXN mRNA levels using droplet digital PCR (ddPCR). Simultaneous detection of different FXN mRNA isoforms revealed a repeat-length-dependent decrease of full-length and FXN-E mRNA and an increase of FXN-ett levels in the striatum and cerebellum of both the YG8sR.300 and YG8sR.800 models. Furthermore, cerebellar bulk RNA-seq and BIS-seq identified clear repeat-length-dependent transcriptional dysregulation and altered methylation levels within the FRDA-DMR region, respectively. Utilizing an in vivo CRISPR/Cas9 screen previously established by our group, we screened a panel of modifiers and identified Msh3, Pms1, and Mlh3 as top candidates with low oncogenic liability. We crossed a constitutive Msh3 knockout mouse with the YG8sR model, which completely suppressed somatic expansion. We then evaluated FXN mRNA levels using ddPCR, performed bulk RNA-seq and BIS-seq, and assessed behavioral phenotypes. Ultimately, our results suggest that these phenotypes are repeat-length-dependent and that somatic expansions further exacerbate these phenotypes. Our findings position the MMR pathway as a potential therapeutic strategy for FRDA.

Funding Sources: NIH, Rappaport, NAF and Pfizer

Emerging and Existing Therapeutics - Pre-Clinical

Thursday, November 12, 11:30 AM-1:00 PM

EP52. Fluoxetine enhances cerebellar TrkB expression and function to rescue motor coordination deficits in a mouse model of spinocerebellar ataxia type 6

11:30 AM - 11:48 AM

Rana Abdelhalim¹; Élyse Zadigue-Dubé²; Juliana Gelber²; Alanna Watt²

1. McGill University, Montreal, QC, 2. McGill University

Background and Objectives: Spinocerebellar ataxia type 6 (SCA6) is an autosomal dominant inherited ataxia characterized by progressive motor incoordination and balance deficits beginning in midlife. We previously demonstrated that impaired brain-derived neurotrophic factor (BDNF) signaling through its receptor, tropomyosin-related kinase B (TrkB), contributes to disease pathogenesis in a mouse model of SCA6. Intriguingly, recent evidence indicates that fluoxetine (Prozac), an FDA-approved antidepressant, acts as an allosteric modulator of TrkB, enhancing BDNF-TrkB signaling. We therefore aimed to investigate whether fluoxetine could improve motor function and restore BDNF-TrkB signaling in SCA6mice.

Methods: Fluoxetine was administered in the drinking water of wild-type (WT) and SCA6litter-matched mice beginning before disease onset (6.5 months of age). At disease onset (7.5 months), motor coordination was assessed using the string-pull assay, which requires coordinated forelimb movements. Following behavioral testing, immunohistochemistry and confocal microscopy were used to quantify cerebellar levels of BDNF, TrkB, and phospho-Akt, a downstream effector of TrkB signaling.

Results: We found that fluoxetine-treated SCA6mice had a sustained and significant improvement in motor coordination compared to untreated SCA6mice, performing at levels similar to WT. This behavioural improvement was accompanied by significant increases in TrkB and phospho-Akt levels in fluoxetine-treated SCA6mice, whereas BDNF expression levels were unchanged.

Discussion and Conclusion: These findings demonstrate that fluoxetine enhances TrkB expression and function to ameliorate ataxia in SCA6. Overall, our findings suggest that repurposing fluoxetine could provide a safe, affordable, and translatable treatment option for SCA6 and other ataxias where BDNF or TrkB signaling are implicated.

Funding Sources: Canadian Institutes of Health Research (CIHR). Fonds de recherche du Québec (FRQ)

EP45. Small -molecule–regulated RNA switch achieves therapeutically safe levels of Frataxin in mouse models of Friedreich's Ataxia

11:48 AM - 12:06 PM

Jon Dempersmier¹; Mariam Elhawary²; Ian McLachlan²; Kai Li²; Diane Hamann²; Travis Wager²; Simon Xi²; Samuel Hasson¹

1. Rgenta Therapeutics, Woburn, MA, 2. Rgenta Therapeutics

Background and Objectives: Advances in engineered adeno-associated virus (AAV) capsids have improved delivery and tropism, yet precise control of transgene expression remains a critical unmet need for gene therapy safety. Friedreich's ataxia (FA) is an autosomal recessive disorder caused by repeat expansion–mediated silencing of the FXN gene, leading to impaired iron–sulfur cluster biogenesis, mitochondrial dysfunction, neurodegeneration, and cardiomyopathy. While constitutive AAV-mediated FXN replacement has shown preclinical efficacy, excessive Frataxin expression has been associated with cardiotoxicity, highlighting the need for controlled dosing. We describe a regulatory platform using DNA-encoded elements (RSwitches) modulated by orally bioavailable small molecules (RDrugs) to enable post-delivery transgene control.

Methods: An RSwitch-regulated FXN transgene was optimized through massively parallel sequence library screening coupled with deep learning–guided design. The construct was evaluated in vitro for dose-responsive Frataxin expression controlled by the brain-penetrant RDrug Cp7. Following AAV packaging, regulation was assessed in cultured cells and in vivo using MCK-Cre and Pvalb-Cre mouse models of FA, including dose range finding (DRF) and a 12-week efficacy study.

Results: Iterative engineering produced a construct with highly dynamic, dose-responsive FXN expression spanning nearly five orders of magnitude. Frataxin was restored to wild-type levels at single-digit nanomolar Cp7 concentrations in vitro. In MCK-Cre models, DRF studies demonstrated a clear relationship between daily Cp7 administration and steady-state cardiac Frataxin levels. The 12-week efficacy study showed that regulated FXN expression maintained non-toxic Frataxin levels and rescued disease-associated morbidity.

Discussion and Conclusion: These findings demonstrate that RSwitch-based regulation enables precise, dose-dependent control of Frataxin expression from AAV vectors in both cardiac and neurological tissues, supporting its potential as a single therapy to address both the cardiac and neurological manifestations of FA.

Funding Sources: Friedreich's Ataxia Research Alliance (FARA). Rgenta Therapeutics

EP10. A novel mRNA therapy for Friedreich ataxia cardiomyopathy developed using human iPSC-derived vascularised cardiac microtissues

12:06 PM - 12:24 PM

Li Li¹; Haoxiang Zhang²; Anne Kong²; Mirella Dottori³; Alice Pébay⁴; Stephen Wilcox⁵; Roger Peverill⁶; Martin Delatycki⁷; Jeffrey Pullin²; Jill Napierala⁸; Marek Napierala⁹; Louise Corben¹⁰; Shiang Y. Lim²; Jarmon Lees¹¹; Mark Chong¹²; Andrew Treller¹³

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Background: Friedreich ataxia (FRDA) is an inherited neurodegenerative disorder in which cardiomyopathy is a leading cause of premature mortality. Clinical evidence suggests that FRDA cardiomyopathy may involve abnormalities of the small coronary vessels, composed of endothelial cells (ECs) and smooth muscle cells (SMCs).

Objectives: To characterize cardiac phenotypes in FRDA patient-specific iPSC-derived ECs and SMCs and evaluate whether targeting dysregulated long non-coding RNAs rescues vascular dysfunction in a vascularized cardiac microtissue model.

Methods: Cardiac biopsies donated by Prof Arnulf Koeppen were stained for fibrosis and vascular markers to assess vascular pathology in FRDA myocardium. ECs and SMCs were differentiated from iPSCs derived from three FRDA individuals and CRISPR-corrected isogenic controls. Vascular phenotypes, mitochondrial function, transcriptomic changes, and cardiac injury were assessed using in vitro cellular assays and vascularized cardiac microtissues. All assays used three iPSC lines with ≥ 3 independent experiments; data were analyzed by one-way ANOVA.

Results: FRDA cardiac biopsies revealed focal interstitial fibrosis, endothelial thickening, and narrowed or partially occluded small vessels. FRDA-ECs exhibited impaired angiogenic potential and elevated mitochondrial reactive oxygen species, while FRDA-SMCs showed increased migration, mitochondrial dysfunction, and altered membrane potential. Replacement of control ECs and SMCs with FRDA vascular cells in cardiac microtissues induced cardiac injury, supporting a vascular contribution to FRDA cardiomyopathy. Transcriptomic analyses identified upregulation of the long non-coding RNAs MEG3 and MEG8 in FRDA vascular cells. In FRDA-SMCs MEG8 knockdown reversed mitochondrial ROS to control levels, while MEG3 knockdown improved mitochondrial membrane potential and reduced abnormal migration. In FRDA-ECs MEG3 knockdown restored angiogenic function and reduced mitochondrial ROS. Incorporation of MEG3-ASO-transfected SMCs, but not ECs, into FRDA vascularized cardiac microtissues prevented cardiac injury.

Conclusion: FRDA vascular cells contribute to cardiac injury. ASO-mediated targeting of MEG3 and MEG8 represent promising mRNA-based therapeutic strategy for FRDA cardiomyopathy.

Funding Sources: FARA

EP21. Antisense oligonucleotide-mediated removal of the CAG-repeat containing exon from ataxin-3 mRNA in SCA3 mice

12:24 PM - 12:42 PM

Willeke van Roon-Mom¹; Sean Visser²; Rick Minnard²; Mara Zuiderwijk²; David Mengel³; Matthias Synofzik⁴; Robyn Verpalen²; Lodewijk Toonen²; Mariana Guimarães Ramos²; Linda van der Graaf²; Barry Pepers²; Keith Sutton⁵; Richard Horgan⁵; Reinko Roelofs⁶; Laurens Bosman⁷; Ronald Buijsen⁸; Elsa Kuijper²

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Spinocerebellar ataxia 3 (SCA3) is an autosomal dominant brain disorder caused by a CAG repeat expansion in exon 10 of ATXN3. Previously, we showed that antisense oligonucleotide (AON)-mediated skipping of exon 10 leads to a truncated ataxin-3 protein without the toxic polyglutamine domain that still contains other important functional domains, preserving its function. In this study we optimized our ataxin-3 exon 10 targeting AON for improved safety. A novel lead AON was selected based on in vitro and in vivo efficiency studies, showing an effective exon 10 skip without increasing Gfap expression. The therapeutic efficacy of this AON was tested in homozygous MJD84.2 (SCA3) mice.

AON treatment resulted in an average exon skip percentage of 21% in cortex and 28% cerebellum on RNA level and on average 41% protein modification in cortex and 34% in cerebellum. The increased latency to fall on the beamwalk test in SCA3mice was improved by AON treatment and the reduced activity levels in the phenotypic cage were ameliorated. Overall the behavioral phenotype and treatment effect were more prominent in female mice than male mice. On molecular level, mild improvement was observed in nuclear accumulation of ataxin-3 and expression of 1 out of 4 genes related to oligodendrocytes and myelination was significantly rescued. However, the gait phenotype on the catwalk as well as levels of plasma NfL were not rescued.

Here, we provide promising data for the use of our novel lead AON to treat SCA3, showing stable and efficient modification sufficient to improve part of the behavioral and molecular phenotypes of SCA3mice. Further research into its safety, the extent of its effect on SCA3pathogenesis and potentially higher effect sizes at earlier treatment initiation should chart the way to clinical evaluation.

Funding Sources: AFM: 20577. Cure Rare Disease. Donation of the family Laux to the Center of Rare Diseases, University Tübingen. Stiftung Hoffnung, Germany. Medical Clinician Scientist program of the Medical Faculty Tübingen (459-0-0). Elite Program for Postdoctoral researchers of the Baden-Württemberg-Foundation (1.16101.21). Health Holland TKI-LSH EMCLSH21017

EP4. BK Channel Activators Improve Motor Function and Rescue Disease Pathology in Spinocerebellar Ataxia 1

12:42 PM - 1:00 PM

Emma Palmer¹; Michael Bonomo¹; Sandhya Bala¹; Sharan R. Srinivasan²

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Background and Objectives: Spinocerebellar ataxias (SCAs) are fatal, incurable, inherited neurodegenerative disorders causing progressive motor incoordination and cerebellar degeneration. Although Purkinje cells (PCs) are a major site of pathology, astro- and micro-gliosis are prominent findings in murine and human studies, suggesting non-neuronal mechanisms of disease. How this neuroinflammation is related to PC pathology remains unclear. We previously demonstrated that pharmacological BK channel activation rescues PC firing in SCA1 *ex vivo*. Here, we investigated whether *in vivo* administration of a novel BK channel activator (BK-86) rescues motor ataxia and cerebellar pathology in SCA1 mice.

Methods: SCA1 knock-in (Atxn1154Q/2Q) mice and wild-type littermates were treated with BK-86 or vehicle using a subcutaneously implanted osmotic pump with cerebral ventricular cannulization for direct CNS delivery. Pumps were implanted at 10 weeks old and treatment continued for 6 weeks. Motor performance was assessed using rotarod and balance beam. Cerebellar degeneration and inflammatory responses were evaluated by immunohistochemistry and qPCR for PC, microglial, and astrocytic markers. Splicing alterations were analyzed by capillary electrophoresis.

Results: BK-86 significantly improved motor coordination and balance in SCA1 mice compared to untreated controls. Targeted splicing analysis revealed rescue of Kcnma1 splicing in SCA1 cerebella. Immunohistochemistry showed rescue of PC density and molecular layer thickness, suggesting neuroprotection. We also found marked reduction of astro- and micro-gliosis, with decreased GFAP and IBA1 immunoreactivity in the molecular layer of the posterior cerebellum.

Discussion and Conclusion: These findings suggest that BK channel activation is a promising therapeutic strategy for SCA1 improving multiple levels of disease pathology. The rescue of splicing and neuroinflammatory pathology suggests a broader disease-modifying effect of BK-86 beyond simple effects on neuronal firing and motor function. Future work will further define the scope of BK channel activation's impact on disease pathology as we advance these compounds for IND-enabling studies.

Funding Sources: NINDS K08NS13462402, UM MTRAC for Life Sciences Hub, UM Research Scouts, UM NGP Regents Fellowship

Biomarkers II

Thursday, November 12, 2:00 PM-3:30 PM

B62. Task-Dependent Saccade Variability as Early Oculomotor Markers of Pre-Ataxic Cerebellar Ataxia

2:00 PM - 2:18 PM

Hasnaa Ouadoudi Belabzioui¹; Annika R. Toivonen²; Kimberly Rosenthal³; Nathan S. Zhang²; Kaitlyn Arrambide²; Yi Zheng⁴; Matthew B. Walsh⁴; Franziska Hoche⁴; Chih-Chun Lin⁴; Christopher D. Stephen²; Jeremy D. Schmahmann⁵; Lawrence White⁴; Ryan Anthony J. de Belen⁶; Divya D. Kulkarni⁷; Siddharth Patel⁸; Anoopum Gupta⁴

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Clinical oculomotor assessments may have limited sensitivity to detect the earliest disease signs and change over time. We evaluated whether quantitative saccade and fixation features differentiate individuals with early stage ataxia from controls.

Neurobooth study participants included 197 ataxia participants (406 sessions; SCA1/2/3/6/8/27B, CANVAS, Friedreich's Ataxia, MSA-C, FXTAS, HSP7, ARSACS, and other ataxias) and 80 controls (222 sessions). 19 ataxia participants (28 sessions) had no observable oculomotor abnormalities (nOOA), 29 (52 sessions) were pre-ataxic (SARA < 3), and 163 (324 sessions) were ataxic (SARA ≥ 3). Eye movements were recorded at 1000 Hz (Eyelink Portable Duo). Saccade and fixation kinematics were extracted. Group comparisons were corrected for multiple comparisons and we highlight significant properties in pre-ataxic/nOOA groups, although many additional properties were significant in the ataxic group.

During passage reading, pre-ataxic and nOOA groups, but not ataxic participants, exhibited reduced variability in horizontal saccade durations compared with controls (pre-ataxic: Cohen's $d=-0.55$, $p=4.47e-03$; nOOA: $d=-0.66$, $p=2.42e-02$). Ataxic participants showed markedly reduced variability in horizontal saccade displacement ($d=-0.90$, $p=3.27e-17$), while pre-ataxic and nOOA groups showed trends in the same direction that did not reach significance (pre-ataxic: $d=-0.30$; nOOA: $d=-0.39$). In contrast, during horizontal and vertical saccade tasks, pre-ataxic and nOOA groups, but not ataxic participants, exhibited increased variability in horizontal saccade mean speeds on horizontal saccades task (pre-ataxic: $d=+0.52$, $p=1.13e-02$; nOOA: $d=+0.63$, $p=1.81e-02$) and increased variability in vertical saccade mean speed on vertical saccades task (pre-ataxic: $d=+0.50$, $p=1.37e-02$; nOOA: $d=+0.63$, $p=2.10e-02$).

These findings suggest that early cerebellar dysfunction affects saccade variability in a task-dependent manner — reducing it during naturalistic reading, reflecting a stereotyped oculomotor strategy, while increasing it during structured saccade tasks, reflecting reduced oculomotor precision. Next steps include training machine-learned composite scores to robustly quantify early oculomotor impairment, correlating features with clinical scales, and assessing longitudinal change.

Funding Sources: Massachusetts Life Sciences Center, U.S. Department of Defense, NIH R01NS117826, Biogen, Broad Institute, Duke Family Foundation

B36. Age and genotype shape longitudinal serum neurofilament light chain dynamics in Friedreich's ataxia

2:18 PM - 2:36 PM

Lucie Stovickova¹; Alena S. Zumrova²; Alexandra Mizikova³; Filip Kolman⁴; Jitka Hanzalova⁵; Valerij Semjonov⁶; Vit Matejicek⁴; Tomas Pozdena⁴; David Nemecek⁴; Jiri Bonaventura⁶; Libor Eichenmann⁶; Katerina Lebedova⁶; Adam Vogel⁷; Ludmila Novotna⁸; Simona Karamazovová⁹; Jaroslava Paulasová Schwabová¹⁰; Martin Vyhnalek¹¹

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Background and objectives: Blood neurofilament light chain (NfL) is an emerging biomarker of neuroaxonal injury in Friedreich's ataxia (FA), but published studies have reported inconsistent associations with age, GAA1repeat length, disease severity, and progression. We aimed to characterize serum NfL (sNfL) dynamics across pediatric and adult FA, evaluate the joint effects of age and genotype, and explore real-world sNfL changes after omaveloxolone initiation.

Methods: We analyzed 295 serum samples, including 230 longitudinal samples from 44 genetically confirmed patients with FA and 65 samples from 25 matched healthy controls. Serum NfL was measured using ELISA. Longitudinal mixed-effects models assessed relationships between sNfL, age, GAA1repeat length, and disease duration. Exploratory analyses evaluated associations with clinical progression, retinal measures, speech parameters, and sNfL changes after omaveloxolone initiation in 18 patients.

Results: sNfL levels were significantly elevated in FA compared with controls (16.9 ± 9.6 vs 7.2 ± 4.1 pg/mL; $P < 0.001$). Pediatric FA patients showed high values, whereas controls demonstrated the expected age-related increase. Cross-sectional GAA1subgroup comparisons did not support a simple genotype effect. However, longitudinal mixed-effects modelling identified a significant age $\times$ GAA1interaction ($P < 0.001$): longer GAA1expansions were associated with higher sNfL at younger ages followed by steeper age-related decline and partial convergence across genotypes in adulthood. Longitudinal trajectories showed heterogeneity and could be grouped into four patterns differing in age, genotype, and disease stage. Across exploratory analyses, sNfL related most consistently to annualized SARA and mFARS worsening, speech-motor abnormalities, and retinal nerve fiber layer thinning. In treatment-adjacent analyses, sNfL decreased at 6 months and remained lower at 12 months after omaveloxolone initiation.

Discussion and conclusion: Age and genotype (GAA1) jointly influence longitudinal sNfL trajectories in FA. These findings support stage-sensitive interpretation of sNfL in FA natural history studies and therapeutic trials. Early omaveloxolone data

Funding Sources: This work was supported by the Grant Agency of Charles University (2021-2022 No. 226423; 2023-2025 No. 309121), the European Friedreich's Ataxia Consortium for Translational Studies (EFACTS; NCT02069509, till 2024), the Friedreich's Ataxia Global Clinical Consortium UNIFIED Natural History Study (UNIFA; NCT06016946, from 2024), and the FARA through the MEABET-FA (2024-2026)

B37. Establishing and Validating a Patient-Meaningful Digital Gait Endpoint for 1-Year Progression in SCAs, exemplified for SCA27B and SCA3

2:36 PM - 2:54 PM

Kristina Bohn¹; Jens Seemann¹; Lisa H. Graf²; Sabine Zentner³; Lisa-Marie Hauser¹; Andreas Träschütz⁴; Matthias Synofzik⁵; Winfried Ilg⁶

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Background: Gait impairment is an early and disabling hallmark of cerebellar ataxias and a major target for clinical trials. Wearable sensor-derived gait metrics provide objective markers of motor dysfunction and progression, but their qualification as trial endpoints requires evidence that longitudinal changes are meaningful to patients, as emphasized by regulatory agencies. We aimed to identify and validate gait measures capturing patient-meaningful change in both natural-history and treatment settings. We studied two common and trial-relevant spinocerebellar ataxias, SCA27B and SCA3.

Methods: In a longitudinal study, gait was assessed in individuals with SCA27B (n=19) using three wearable inertial sensors during a 2-minute walk; a subgroup received 4-aminopyridine. Measures of gait variability and smoothness were evaluated for reliability, association with disease severity, and sensitivity to 1-year progression. Minimal Patient Important Change (MPIC) thresholds were derived using an anchor-based approach relating gait changes to Patient Global Impression of Change (PGI-C) ratings via linear mixed-effects models. Validation was performed in an independent SCA3 cohort (n=34).

Results: In SCA27B, gait measures correlated with disease severity (SARA) and patient-reported disease severity (PGI-S). Over 1 year, stride length variability (p=0.044) and vertical harmonic ratio (HR-V; p=0.006) changed significantly, whereas SARA did not. Anchoring HR-V to PGI-C yielded an MPIC_{SCA27B} threshold of ± 0.0138 (p=0.02). Previously reported 4-aminopyridine treatment effects exceeded this threshold, supporting its clinical relevance. In SCA3, HR-V was also successfully anchored to PGI-C, enabling determination of an MPIC_{SCA3} (threshold: ± 0.0181 ; p=0.02). HR-V declined significantly over time (p=0.018), exceeding both MPIC thresholds and indicating clinically meaningful progression. Sample-size simulations showed that HR-V reduced required trial sample sizes by >75% compared to SARA.

Conclusions: HR-V is a patient-meaningful digital gait biomarker that outperforms SARA in detecting progression in two independent SCA cohorts. These findings support HR-V as a clinically meaningful endpoint for future interventional trials in degenerative cerebellar ataxias.

B21. Structural Brain and Spinal cord Signature of Spinocerebellar Ataxia 27B: a Multisite MRI-based Study

2:54 PM - 3:12 PM

Nadson Bruno Serra Santos¹; Camila C. Lobo²; Thiago J. Rezende³; Alberto Rolim Muro Martinez⁴; Luíza Alves Corazza⁵; Luiz Eduardo Novis⁵; Orlando Barsottini⁶; José L. Pedroso⁶; Pedro J. Tomaselli⁷; Wilson Marques-Jr⁷; Antônio Carlos dos Santos⁸; David Pellerin; Bernard Brais⁹; Roberta La Piana¹⁰; Jemimah Harding¹¹; Penny Snell¹²; Paul J. Lockhart¹³; David Szmulewicz¹⁴; Ian H. Harding¹⁵; Marcondes C. França²

1. UNICAMP, Campinas, Sao Paulo, 2. University of Campinas (UNICAMP), Campinas, Sao Paulo, 3. Department of Neuroscience, University of São Paulo, Ribeirão Preto, Sao Paulo, 4. University of Campinas (UNICAMP), 5. Federal University of São Paulo (UNIFESP), 6. School of Medicine – Federal University of São Paulo (UNIFESP), 7. School of Medicine – University of São Paulo at Ribeirão Preto (USP-RP), 8. University of São Paulo at Ribeirão Preto (USP-RP), 9. McGill University, Montreal, QC, 10. Montreal Neurological Institute, Montreal, QC, 11. Bionics Institute & University of Melbourne, Melbourne, Victoria, 12. Murdoch Children's Research Institute, 13. Parkville, Victoria, 14. Royal Victorian Eye & Ear Hospital, Melbourne, Victoria, 15. Queensland Institute of Medical Research, Brisbane, Queensland

Objective: To characterize the structural brain and spinal cord MRI signature of spinocerebellar ataxia 27B (SCA27B) using multimodal quantitative imaging.

Methods: In this cross-sectional, multisite study, 28 genetically confirmed SCA27B patients underwent standardized 3T brain and cervical spinal cord MRI. A group of age- and sex-matched healthy controls was recruited at participating centers using harmonized acquisition protocols. Cerebral and cerebellar volumetry were performed using FastSurfer and CerebNet, respectively. Diffusion tensor imaging was used to assess microstructural integrity in supratentorial white matter. Spinal cord gray and white matter areas were quantified at the cervical level using the SCT toolbox. Between-group comparisons were conducted using general linear models adjusted for age, sex, and site.

Results: Mean age and SARA score of the SCA27B cohort were 68.7 ± 13.3 years and 10.5 ± 5.8 points. Compared with healthy controls, SCA27B patients exhibited significant volumetric reduction of the cerebellar vermis (lobules I to IV and Vermis VIII). Diffusion analyses revealed widespread fractional anisotropy reduction in subcortical cerebral white matter, consistent with microstructural disruption. At the cervical spinal cord level, patients demonstrated significant gray matter area reduction, whereas white matter area was comparatively preserved. The combined pattern of vermian atrophy, supratentorial white matter diffusivity abnormalities, and spinal cord gray matter loss delineated a distinct structural signature of SCA27B.

Conclusion: SCA27B is associated with a structural phenotype extending beyond the cerebellum, involving subcortical white matter microstructure and spinal cord gray matter.

Funding Sources: This work was funded by Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP, São Paulo, Brazil, grant number #2013/07559-3.

B20. Disentangling Pontine Fiber Geometry and Microstructure in ARSACS Using Advanced MRI

3:12 PM - 3:30 PM

Ilana R. Leppert¹; Aziz Benbachir²; Jennifer S. Campbell³; Santiago Coelho⁴; Sajjad Feizollah³; Mark C. Nelson³; Bernard Brais⁵; Sirio Cocozza⁶; G B. Pike⁷; Roberta La Piana⁸; Christine L. Tardif³

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Background: Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a genetic disease characterized by spasticity and ataxia which reflects involvement of the corticospinal tracts (CST) and cerebellum. The primary involvement of the middle cerebellar peduncles (MCP) and transverse pontine fibers (TPF) at the crossing with the CST, and their role in the pathophysiology of the disease, is currently debated.

Objectives: Advanced MRI techniques capable of isolating sub-voxel microstructural parameters can test the hypothesis that the MCP and TPF are abnormally large, compressing the CST at their crossing, and potentially impairing CST development.

Methods: Tract macro- and micro-structural properties, including axon and tract caliber, axon density and geometry, and myelin content were estimated from diffusion-relaxometry and magnetization transfer imaging. These features were analyzed along segments of the CST, MCP, and TPF of 9 patients and 9 age-matched controls.

Results: While the CST showed significant decreases in tract size, axon caliber, and myelination throughout its length compared to controls ($p < 0.01$), the MCP and TPF were relatively unaffected. In our group, neither the MCP nor the pons were enlarged. The proximal MCP showed an increase in axon caliber.

Conclusions: The increase in fractional anisotropy and axon density towards the center of the TPF could be driven by geometric confounds related to differences in the relative sizes of the CST and TPF compared to controls. This highlights the importance of investigating tract-specific microstructural profiles, particularly in regions of geometric complexity. The findings confirm the involvement of the CST, with a relatively limited involvement of the MCP and TPF.

Funding Sources: Financial support was provided by the Ataxia Charlevoix-Saguenay Foundation (RLP, CLT), Brain Canada, Fonds de recherche du Québec - Santé (FRQS) Research Scholars (CLT, RLP), Killam Trusts (CLT), and Natural Sciences and Engineering Research Council (NSERC) Discovery Grant (CLT).

Natural History, Clinical Research and Outcome Assessments

Thursday, November 12, 4:00 PM-5:30 PM

C23. The Friedreich's Ataxia-Health Index (FA-HI): Association Between Patient-Reported Disease Burden and Clinical Outcomes in the UNIFAI Study

4:00 PM - 4:18 PM

Anika Varma¹; Benson Chen²; Jennifer Farmer³; Christian Rummey⁴; Chad Heatwole¹

1. University of Rochester, 2. Auckland City Hospital and University of Auckland, 3. Friedreich's Ataxia Research Alliance, Downingtown, PA, 4. Clinical Data Science GmbH, Basel, Basel-Stadt

Background & Objectives: The Friedreich's Ataxia-Health Index (FA-HI) is a disease-specific, patient-reported outcome measure (PRO) that quantifies point-in-time disease burden in 18 symptomatic domains in FA patients. UNIFAI is an ongoing longitudinal observational study of FA patients that uses the FA-HI, alongside other clinical outcome assessments, to evaluate disease burden and progression over time. In this cross-sectional analysis, we evaluate the known groups validity of the FA-HI and its association with clinical function, using baseline data from UNIFAI.

Methods: Participants enrolled in the UNIFAI study provided their demographic and clinical information and completed outcome measures, including the FA-HI, FA-Activities of Daily Living (FA-ADL), Modified FA Rating Scale (mFARS), and Short Form-36 (SF-36). We conducted known groups analyses using unpaired t-tests and association analyses using Spearman's correlation.

Results: A total of 543 participants (50.5% female, mean age 29.7 years) were included, with mean disease duration 16.8 years. Mean±SD scores for each outcome measure were 39.3±20.4 (FA-HI), 16.0±7.2 (FA-ADL), 52.2±17.3 (mFARS), and 41.0±21.6 (SF-36). The FA-HI total score demonstrated strong significant associations with the FA-ADL ($p = 0.714$), mFARS ($p = 0.629$), and FDS ($p = 0.624$); while showing a weak association with the SF-36 ($p = -0.358$), all $p < 0.0001$. The FA-HI total and subscale scores showed significant and robust differences between groups based on demographics, genetics, disease duration, and disease severity.

Discussion & Conclusion: The regulatory-grade FA-HI exhibits strong associations with disease-specific outcome measures, remaining clearly distinct from less sensitive generic quality-of-life instruments. This study provides evidence for the FA-HI's convergent and discriminant validity as well as its sensitivity in detecting differences in symptomatic burden based on functional status. These results support the use and understanding of the FA-HI as a sensitive marker of clinically meaningful disease burden for routine clinical use and in observational and therapeutic trials.

Funding Sources: Friedreich's Ataxia Research Alliance (FARA)

C27. The SARA Qualification Program: Concept and Current Status

4:18 PM - 4:36 PM

Thomas Klockgether¹; Marcus Grobe-Einsler²; Holm Graessner³; Tamara Schaprian⁴; Sorina Gorcenco⁵; Lisa H. Graf⁴; Michele Potashman⁶; Celeste Stuart⁷

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Background and Objectives. SARA is a widely used clinical scale to measure severity of ataxia. However, there are a number of unsolved issues regarding its (1) content validity and patient relevance, (2) measurement properties, and (3) applicability. To address these issues and gain regulatory acceptance of SARA, we initiated the SARA Qualification Program.

Methods. (1) To establish content validity and patient relevance, we use data of 1.512 patients from five surveys. We make a selection of symptoms based on reported relevance and match them to ICF codes. We then analyse the frequency with which the ICF codes are mentioned and map them on SARA items. (2) To assess measurement properties, we analyse longitudinal data of SCA1 and SCA3 mutation carriers and MSA-C patients from six cohorts. Sensitivity to change ratio (SCS) is determined for each disease and disability stage (0: no gait difficulties 1: gait difficulties; 2: walking aid; 3: wheelchair). (3) To improve applicability, we established a consensus-building process among clinical experts, patients, and industry representatives that forms the basis for the SARA User Manual.

Results. (1) Matching of reported symptoms yielded a set of 160 ICF codes from four categories. Further analysis and mapping on SARA items are ongoing. (2) In SCA3, sensitivity of SARA was high throughout the entire disease course (SCS = 1.48), in stage 1 (1.57), stage 2 (1.92), and stage 3 (1.18), but low in stage 0 (0.19). SCS values of SARA items ranged between 0.77 (nose-finger) and 1.20 (gait). Further analyses are ongoing. (3) The SARA User Manual was finally approved by the consensus group. It contains detailed item and scoring instructions.

Discussion and Conclusion. The analyses conducted as part of the SARA Qualification Program yielded important insights into properties of SARA. In a pre-qualification meeting, we received positive responses from the EMA.

C99. Learning Submovement Sequence Patterns from At-Home Wearables Improves Detection of Motor Decline in Ataxia-Telangiectasia

4:36 PM - 4:54 PM

Ryan Anthony J. de Belen¹; Annika R. Toivonen²; Anna Luddy³; Kaitlyn Arrambide²; Nathan S. Zhang²; Yi Zheng³; Faye Yang³; Franziska Hoche³; Anoopum Gupta³

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Sensitive and scalable measures of motor function are critical for detecting subtle disease progression and assessing therapeutic response in ataxia-telangiectasia (A-T) clinical trials. While kinematic features of submovements derived from wearable sensors have shown promise as digital measures of motor behavior, they incompletely capture the temporal dynamics of real-world movement. Here, we leverage self-supervised learning to derive richer movement representations and evaluate their ability to quantify motor impairment and track disease progression in A-T.

A total of 266 individuals (54 A-T, 68 SCAs, 43 FA, 11 other rare ataxias and 90 controls) continuously wore ankle inertial sensors for one week per session, repeated every 6–12 months. Recordings were automatically segmented into activity bouts (sequences of submovements characterized by 12 kinematic features) separated by period of inactivity, yielding approximately 4.5 million activity bouts. A dilated transformer-based encoder that discretizes each feature was trained to predict masked kinematic features and generate learned submovement embeddings that capture sequential context. Kinematic features and learned embeddings from 54 A-T individuals and 56 controls were independently aggregated over one-hour windows, and separate linear regression models were trained to predict BARS total scores.

Learned embedding-based scores consistently outperformed kinematic feature-based scores across five evaluation criteria: sensitivity to disease progression (MSDR: 0.75 ± 0.07 vs. 0.52 ± 0.08), surpassing clinician-administered BARS (MSDR: 0.62); group discrimination (Cohen's $d = -2.69 \pm 0.08$ vs. -2.06 ± 0.67); association with BARS ($r = 0.71$ vs. 0.68); prediction error variability over one-week recording (RMSE: 5.15 ± 0.23 vs. 5.89 ± 0.59). Test-retest reliability was comparable (ICC: 0.973 ± 0.00 vs. 0.967 ± 0.01).

By learning the temporal patterns of real-world movement, the self-supervised movement representations generated more sensitive measures of motor impairment than kinematic features while maintaining within-session prediction consistency. Future work will extend this framework to larger and more diverse datasets, broader neurological populations, and end-to-end learning directly from raw sensor signals.

Funding Sources: NIH R01NS134597, NIH R01NS117826, A-T Children's Project, FARA

C84. Multisystemic and repeat length-associated evolution of RFC1-disease: first results of 206 patients from the international RFC1 natural history study

4:54 PM - 5:12 PM

Andreas Traschütz¹; Thomas Wirth²; David Szmulewicz³; Kathy H. Wu⁴; Berkan Koyak⁵; Rachael Taylor⁶; Ivana Ricca⁷; Max Borsche⁸; José L. Pedroso⁹; Björn H. Falkenburger¹⁰; Astrid D. Adarmes Gómez¹¹; Chiara Pane¹²; Atay Vural¹³; Marcondes C. França¹⁴; Jonathan Baets¹⁵; Thomas Klopstock¹⁶; Dagmar Timmann¹⁷; Riccardo Curro¹⁸; Andrea Cortese¹⁹; Norbert Brüggemann²⁰; Mathieu Anheim²¹; FILIPPO M. SANTORELLI²²; Richard H. Roxburgh²³; Jennifer Faber²⁴; Ian H. Harding²⁵; Matthias Synofzik²⁶

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Background and objectives: RFC1-disease is predominantly caused by biallelic (AAGGG)exp repeat expansions in RFC1 and typically manifests with cerebellar ataxia, neuropathy and vestibular areflexia syndrome (CANVAS), but its multisystemic evolution within and beyond CANVAS is unclear.

Methods: Cross-sectional analysis of 206 genetically confirmed RFC1-patients prospectively recruited into the international multi-modal RFC1 natural history study (ClinicalTrials.gov: NCT05177809; 16 sites/8 countries). Patients with unequivocal phenotypes were stratified as presymptomatic (n=5), isolated neuropathy (n=20), neuropathy+vestibulopathy (n=17; clinical and/or, if available, video head-impulse-test [vHIT, gain<0.6]), neuropathy+cerebellar ataxia (n=14; ≥2 cerebellar signs), and complete CANVAS (n=121).

Results: Assessment by SARA, activities of daily living (FARS-ADL) and global mobility (FARS-stage) captured stepwise worsening of severity from isolated neuropathy to additional vestibulopathy to CANVAS (SARA: 3 [IQR: 2-5.5], 6 [4.5-7] and 13 [9-17.5], ANOVA, p<0.001). Cross-sectional progression was 0.63 SARA points/year of disease duration, defined by gait/balance problems (95%CI: 0.56-0.71). Duration was highly variable, overall shorter in isolated neuropathy than CANVAS (6.2±7.7 vs. 12.9±8.9 years, p=0.003, ANOVA), but not different in incomplete CANVAS phenotypes. Rather, compared to isolated neuropathy, vestibulopathy and cerebellar ataxia were associated with higher repeat-lengths on the shorter (IQR: 500-850 vs. 800-1250) and longer allele (750-1000 vs. 950-1400; n=57; Kruskal-Wallis, Dunn-corrected, all p<0.017); and the longer allele correlated with vestibular impairment (vHIT gain: rho=-0.45, p=0.010). Cognitive impairment, measured by CCAS scale, was more common and severe in 119 RFC1-patients than 66 age-, sex- and education-matched controls (‘definite CCAS’: 64% vs. 24%; CCAS score: 82.8±20.2 vs. 98.1±12.0, t-test, p<0.001), and correlated more with ataxia severity (SARA, rho=-0.60, p<0.001) than merely disease duration (rho=-0.19, p=0.047). Severity of chronic cough (Leicester Cough Questionnaire) correlated only with severity of dysautonomia (COMPASS-31; n=94; rho=-0.31, p=0.002).

Discussion and Conclusion: Multisystemic involvement gradually increases RFC1-disease severity, depends on bi-allelic repeat-lengths, and evolves in non-uniform patterns not explained by disease duration alone.

Funding Sources: Funding sources include, but are not limited to, Clinician Scientist program “PRECISE.net” funded by the Else Kröner-Fresenius-Stiftung; project European Rare Disease Research Alliance (ERDERA),

funded by the European Union; Federal Ministry of Research, Technology and Space (BMFTR); Connaître les Syndromes Cérébelleux; and Australian National Health and Medical Research Council.

C41. Digital Speech Assessment in Friedreich Ataxia: The Relationship to Genetic Factors and Clinical Outcomes

5:12 PM - 5:30 PM

Christian Rummey¹; Louise Corben²; Sub Subramony³; Gustavo Noffs⁴; Chad Hoyle⁵; Katherine D. Mathews⁶; Trevor Hawkins⁷; Richard Finkel⁸; Antoine Duquette⁹; Lucie Stovickova¹⁰; Claudia Yang Santos¹¹; Syed Syed Farooq¹¹; Jennifer Farmer¹²; Martin Delatycki¹³; David Lynch¹⁴; Adam Vogel¹⁵

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Background and objectives: Speech impairment, a core feature of Friedreich ataxia (FA), can't be fully assessed through the gold standard of ataxia progression, the rating scales. Based on data from the global natural history study UNIFAI, we established the cross-sectional relationship of REDENLAB speech outcomes (Naturalness, Intelligibility, Diadochokinesis) with neurological outcomes, and how they are impacted by genetic and clinical severity.

Methods: Speech observations from 340 individuals with FA were related to GAA1repeat length, disease duration, ambulatory status, mFARS, USS, the FA-ADL, and disability stages, both through correlation analysis (Pearson/Spearman r , as appropriate) and multivariable modeling. Assessments collected after omaveloxolone exposure were excluded.

Results: Speech outcomes correlated with neurological function (mFARS $r = -0.66, -0.65, -0.51$ for intelligibility, naturalness, diadochokinesis) but followed separate trajectories, when assessed by disease stage. Speech outcomes of naturalness and intelligibility correlated best with FDS. At early FDS 0-1, naturalness of speech was impacted more than intelligibility (median of 128 and 150 respectively on a scale of 0-200, higher = better). 10% of patients preserved naturalness and 26% preserved intelligibility. Intelligibility fell at intermediate stages (median 154 to 119), with naturalness staying at its lower baseline; diadochokinesis declined steadily across all stages (median 3.3 to 2.2 syllables/s); both intelligibility and naturalness declined markedly only at FDS 5-6 (non-ambulatory, medians 97 and 85). In multivariable models, disease duration and ambulatory status independently predicted all three speech outcomes (all $p < 0.005$), while GAA1 did not ($p \geq 0.38$), unlike mFARS and USS where GAA1 was a strong independent predictor. Speech models explained 11-34% of cross-sectional variance.

Discussion and conclusion: Automated speech metrics track clinically meaningful aspects of FA disease burden, correlating with neurological assessments, and follow related, yet independent trajectories. Repeat length showed little impact. Disease duration is the dominant cross-sectional driver, motivating the longitudinal companion

Funding Sources: Biogen, FARA, Redenlab

Clinical Trials

Friday, November 13, 9:50 AM-11:10 AM

EC7. Positive Results from a Global Phase 3 Trial (IB1001-303) Evaluating Levacetylleucine in Ataxia-Telangiectasia

9:50 AM - 10:08 AM

Janelle Raymond¹; Franziska Hoche²; Jeremy D. Schmahmann³; Nivedita Thakur⁴; Susan Perlman⁵; Marc C. Patterson¹; Tatiana Bremova-Ertl⁶; Michael Strupp⁷; Bethany Zanrucha⁸; Jorgji Kerthi⁹; Taylor Fields⁸; Ian Billington⁸; Kyriakos Martakis¹⁰

1. IntraBio Inc, 2. Massachusetts General Hospital, 3. Massachusetts General Hospital, Waban, MA, 4. UTHHealth Houston, Houston, Texas, USA, 5. University of California - Los Angeles, 6. Department of Neurology and Center for Rare Diseases, University Hospital Inselspital Bern, Bern, Switzerland, 7. Department of Neurology, LMU University Hospital, Munich, Germany, 8. IntraBio Inc., Austin, Texas, USA, 9. IntraBio Inc, Austin, TX, 10. Department of Neuropediatrics, Justus Liebig University, Giessen, Germany

Background and Objectives: Ataxia-telangiectasia (A-T) is a rare, inherited cerebellar ataxia. Levacetylleucine is a modified amino acid that enters enzyme-controlled pathways to correct metabolic dysfunction and restore cellular communication. The Phase 3, randomized, double-blind, placebo-controlled trial (IB1001-303) evaluated the safety and efficacy of levacetylleucine in people with A-T.

Methods: IB1001-303 enrolled A-T participants aged ≥ 4 years across 10 multinational trial sites. The primary efficacy endpoint was the Scale for the Assessment and Rating of Ataxia (SARA). Secondary endpoints included Spinocerebellar Ataxia Function Index, International Cooperative Ataxia Rating Scale (ICARS), Neurology Quality of Life-Upper Extremity Function, quality of life, and investigator, caregiver, and patient Clinical Global Impression of Improvement (CGI-I). Safety assessment included adverse event incidence and severity.

Results: Seventy-three participants aged 4-50 were enrolled; 47 pediatric (<18 years) and 26 adult participants (≥ 18 years). The primary endpoint was met; mean change in the SARA total score with levacetylleucine was -1.92 (SD=2.81) and -0.14 (SD=2.38) with placebo; Linear Mixed Model (LMM) treatment effect -1.88 (SE=0.41) (95% CI -2.70, -1.06; $P<0.001$) after 12 weeks of treatment. The trial met key secondary endpoints, including ICARS (95% CI: -4.87, -0.81; $P=0.003$) and investigator's CGI-I (95% CI -0.7, 0.0; $P=0.02$). Subgroup analyses showed improvement in neurological status across all demographics of participants i.e., age of neurological disease onset, disease severity, and others. The frequency of adverse events was similar between the two groups and no serious adverse events or deaths occurred in either group.

Discussion: Levacetylleucine demonstrated a statistically significant benefit versus placebo, clinically meaningful improvements in neurological manifestations of A-T, functioning, and quality of life, and a favorable benefit-risk profile for the treatment of A-T. Levacetylleucine was observed to be safe and well-tolerated, consistent with its established safety profile.

Conclusion: Meaningful neurological and functional benefits of levacetylleucine over placebo support its therapeutic value in A-T.

Funding Sources: This trial was funded by IntraBio Inc.

EC10. Small Molecule, DT-216P2, Shows Dose-Dependent Clinical Improvements and Frataxin Increases in Friedreich Ataxia: RESTORE-FA 4-week IV Results

10:08 AM - 10:26 AM

David Lynch¹; Martin Delatycki²; Christopher Argent³; Matt Kurth⁴; James Kerr⁵; Chengzhi Zhang⁵; Doane Chilcoat⁵; Sean Jeffries⁶; Pratik Shah⁵; Chris Storgard⁶

1. CHOP, Philadelphia, PA, 2. Murdoch Children's Research Institute, Hawthorn, 3. Scientia Clinical Research, 4. Design Therapeutics, Inc., 5. Design Therapeutics, Inc., 6. Design Therapeutics

Background and objectives: Friedreich ataxia (FA) is an autosomal recessive neurodegenerative disease caused by a GAA trinucleotide repeat expansion in the FXN gene, resulting in reduced frataxin (FXN) expression. DT-216P2 is a gene-targeted small molecule designed to recognize the expanded GAA repeat region of the FXN gene, engage the transcriptional machinery and restore endogenous FXN expression, addressing the root cause of FA. Nonclinical studies demonstrated wide tissue distribution including CNS.

Methods: DTX-216P2-101 (RESTORE-FA) is a multi-part open-label, multiple-ascending-dose Phase 1/2 study in participants with FA. A total of 16 participants received 4-weekly IV doses at 0.1, 0.3, 0.6 and 1.0 mg/kg (n=4/cohort). Clinical endpoints included mFARS, Upright Stability Score (USS), and PROMIS Fatigue T-score. FXN mRNA and protein were measured in whole blood and skeletal muscle.

Results: Dose-dependent improvements in clinical endpoints and FXN biomarkers were observed across all IV cohorts. At 1.0 mg/kg, participants demonstrated a mean improvement from baseline of -6.4 points in mFARS. A dose-dependent improvement in USS was observed, reaching -2.7 points at 1.0 mg/kg. On PROMIS Fatigue, a >6-point improvement was observed at 1.0 mg/kg, with 75% achieving a ≥5-point response. At 1.0 mg/kg, whole blood FXN mRNA increased 65% from baseline (p<0.001) and skeletal muscle FXN mRNA increased 42% (p=0.015). Whole blood FXN protein levels increased 22–27% at 2 weeks post-final dose (p<0.001), consistent with the known half-life of endogenous FXN protein and confirming translation of mRNA increases into protein. DT-216P2 was generally well tolerated with no serious adverse events and no discontinuations; all adverse events were mild or moderate.

Conclusion: DT-216P2 demonstrated rapid, dose-dependent, and clinically meaningful improvements in mFARS, USS and fatigue in FA, accompanied by dose-dependent statistically significant increases in FXN mRNA and protein. These data support accelerated development of DT-216P2 as a potentially disease-modifying therapy for Friedreich ataxia.

Funding Sources: Design Therapeutics, Inc.

EC5. Real-world Effectiveness of Omaveloxolone in Friedreich Ataxia: Experience in the UNIFAI Natural-history Study

10:26 AM - 10:44 AM

Christian Rummey¹; Adrian Coles²; Tomas Andriotti²; Shobhana Natarajan³; David Lynch⁴

1. Clinical Data Science GmbH, Basel, Basel-Stadt, 2. Biogen Inc., Cambridge, MA, USA, 3. Biogen Inc., Cambridge, MA, USA, Boston, MA, 4. CHOP, Philadelphia, PA

Background and Objectives Omaveloxolone (SKYCLARYS) received FDA approval in early 2023 for Friedreich ataxia in patients aged ≥ 16 , based on the MOXIe Part 2 trial in mostly ambulatory adolescents and adults. Real-world effectiveness in broader populations — including non-ambulatory subjects — is unknown. We compared the difference in disease progression between patients using omaveloxolone and untreated individuals in the UNIFAI Natural History Study (NCT06016946).

Methods UNIFAI participants were eligible when enrolled at a US center, aged ≥ 16 , with mFARS ≥ 20 at first visit. Investigational omaveloxolone exposure (clinical trial settings) were excluded. Treatment associations at 1 year were estimated using a linear mixed effect model including time, time-varying treatment, and their interaction. The model also included a random slope to accommodate patient-level heterogeneity in disease progression over time, and age, sex and baseline mFARS as covariates. Primary outcome was disease progression as measured by mFARS, with USS, FARS B, and FARS C as secondary outcomes.

Results 690 eligible participants contributed 4,146 mFARS assessments: 515 were never treated, 162 initiated commercial omaveloxolone post-approval, and 13 enrolled in UNIFAI at treatment initiation. At 1 year, omaveloxolone-treated patients' mFARS was 1.79 points lower than the non-treated group [95% CI -2.75, -0.82], $p=0.0003$. The association with omaveloxolone treatment was driven by improvements in upper- (FARS B -1.54 [-2.18, -0.90], $p<0.0001$) and lower limb coordination, (FARS C -0.53 [-0.95, -0.11], $p=0.014$). No change was seen on the balance and gait outcome (USS +0.03 [-0.46, +0.52], $p=0.91$).

Discussion and Conclusion Omaveloxolone used in routine US practice is associated with slower mFARS progression relative to expected natural history over the first year of treatment. Treated individuals predominantly experienced improved upper- and lower-limb function, potentially reflecting the broader real-world scenario (including non-ambulatory subjects). A comprehensive assessment will require longer follow-up data. Limitations were the observational design and 1-year follow-up data.

Funding Sources: This work was funded by Biogen.

EC21. Upright stability in Friedreich ataxia after 5 years of omaveloxolone treatment: propensity-matched analysis versus natural history controls

10:44 AM - 11:02 AM

Christian Rumme¹; David Lynch²; Theresa Zesiewicz³; Richard Lawson⁴; Claudia Yang Santos⁵; Syed Syed Farooq⁵; Shobhana Natarajan⁶; Jonathan Smith⁷; Nicolas Folschweiller⁸

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Background and objectives: Friedreich ataxia (FA) is a progressive neurodegenerative disease characterized by impaired balance, gait dysfunction, and eventual loss of ambulation. The upright stability score (USS or FARS E), a subscale of the modified Friedreich Ataxia Rating Scale (mFARS), assesses axial function, balance, and postural stability (0-36, higher scores indicate greater impairment), and can predict future risk of loss of ambulation. Omaveloxolone is approved for treating FA in patients aged ≥ 16 years and was evaluated in the MOXIe clinical trial program (NCT02255435). This post hoc analysis evaluated the long-term effect of omaveloxolone on USS progression versus matched untreated natural history controls.

Methods: Ambulatory patients (USS gait score < 5 at baseline) aged 16-41 with mFARS scores 20-80 initially randomized to omaveloxolone in MOXIe Part 2 and continuing in the open-label extension (OLE; $n=39$) were propensity matched 1:2 with Friedreich's Ataxia Clinical Outcome Measures Study (FACOMS) controls ($n=78$) based on age at FA onset; sex; and baseline age, gait score, and USS. Annual USS progression rates were compared using mixed-model slope analysis. Change from baseline (MOXIe Part 2 Day 1) through OLE Year 4 (≈ 5 years total) was also assessed using mixed-model repeated measures (MMRM).

Results: Annual USS progression was significantly slower with omaveloxolone than matched FACOMS controls (0.67 versus 1.18 points/year; difference: -0.51 points/year; $p=0.0063$). MMRM analysis showed slower USS progression after ≈ 5 years of omaveloxolone treatment (mean change from baseline: 3.23 versus 6.05 points; difference: -2.82 points; nominal $p=0.0007$).

Discussion and conclusion: Long-term omaveloxolone treatment was associated with slower progression in USS versus matched untreated natural history controls. Findings should be interpreted in the context of the post hoc design and relatively small matched sample.

Funding Sources: Funding: Biogen

Natural History, Clinical Research and Outcome Assessments II

Friday, November 13, 11:30 AM-1:00 PM

C11. Prevalence and progression of non-ataxic signs and symptoms in spinocerebellar ataxias with CAG repeat expansions

11:30 AM - 11:48 AM

Emilien Petit¹; Thomas Klockgether²; Quentin Thomas³; Heike Jacobi⁴; Tanja Schmitz-Hübsch⁵; Tetsuo Ashizawa⁶; Alexandra Durr⁷; Sophie Tezenas du Montcel³; Giulia Coarelli⁸

1. Paris Brain Institute, Paris, 2. German Center for Neurodegenerative Diseases (DZNE), Bonn, 3. Sorbonne Université, Institut du Cerveau-Paris Brain Institute-ICM, CNRS, Inria, Inserm, AP-HP, Groupe Hospitalier Sorbonne Université, Paris, France, 4. Department of Neurology, University Hospital Heidelberg, Heidelberg, Germany, Heidelberg, 5. Experimental and Clinical Research Center, Max Delbrueck Center for Molecular Medicine, Charité-University Hospital Berlin, 10117, Berlin, Germany, Berlin, Berlin, 6. Stanley H. Appel Department of Neurology, Houston Methodist Research Institute and Weill Cornell Medicine, Houston, TX, USA., Seabrook, TX, 7. Sorbonne University, Paris, 8. ICM - Paris Brain Institute, PARIS

Background and Objectives: The Inventory of Non-Ataxia Signs (INAS) is frequently used to report extracerebellar manifestations in spinocerebellar ataxias (SCAs). We aim to provide a comprehensive analysis of non-ataxic signs and symptoms in polyglutamine SCAs upon disease progression.

Methods: We analyzed data from 1,101 individuals carrying CAG repeat expansions in ATXN1/SCA1, ATXN2/SCA2, ATXN3/SCA3, CACNA1A/SCA6, and ATXN7/SCA7, enrolled in six natural history studies (EUROSCA, RISCA, READISCA, BIOMOV, CERMOI, and SPATAX). Cross-sectional signs prevalence was assessed across disease stages (preataxic, mild, moderate, advanced) and SCA subtypes. CAG repeat length impact on INAS was assessed with logistic models adjusted for genotype and SARA. The link between estimated time to onset and INAS prevalence was modeled longitudinally with ordinal mixed-models.

Results: There were 244 SCA1, 260 SCA2, 381 SCA3, 128 SCA6, and 88 SCA7 carriers, with a mean follow-up duration of 4.0±2.5 years (n=884), including 269 (24%) preataxic individuals. More than half of SCA7 individuals already exhibited extracerebellar signs at preataxic stages: hypopallesthesia at ankles in 64%, (compared to 33% in SCA6 and 31% in SCA1), and hyperreflexia in 57%. SCA6 showed no reflex abnormalities at any disease stage but was characterized by frequent saccadic pursuit (>50%) in preataxic individuals, detectable up to 10 years before onset. SCA2 showed a rapid increase of slowed saccade prevalence during the 10 years before onset, from <10% to 80% around conversion. At the ataxic stage, each SCA showed a unique profile, but dysphagia and urinary dysfunction were consistently observed across all subtypes. In SCA3, longer CAG was associated with higher frequency of spasticity and brisk reflexes in both upper and lower limbs.

Discussion and Conclusion: This study provides unique SCA profiles of extracerebellar manifestations and their progression from preataxic to advanced stages. Their high prevalence in preataxic stages make them a privileged readout for therapeutic approaches before

C81. Longitudinal multi-modal evaluation of brain/cardiac mitochondrial complex 1 expression, transcriptomics and ethomics in Friedreich's Ataxia using AI

11:48 AM - 12:06 PM

Ilan Rabiner¹; Sandeep Golla²; Frans Van Den Berg¹; Alex Whittington¹; Leon Fonville¹; Yvonne Lewis¹; Julius Labao²; Cristian Salinas³; Balasundaram Kadirvelu²; Daolong Chen²; Jackson Chan²; Paola Giunti⁴; Aldo Faisal²; Richard Festenstein⁵

1. Perceptive, 2. Imperial College, 3. Takeda, 4. University College London, London, 5. Imperial College, London

Aim/Introduction: We previously used PET with the mitochondrial complex I (MC1) radioligand BCPP-EF to demonstrate reduced cardiac MC1 expression in Friedreich's Ataxia (FA), together with decreases in selected brain regions. We also showed that AI-based motion-capture analysis (ethomics) outperforms conventional clinical scales in monitoring disease progression. Here, we evaluated longitudinal changes in cardiac and brain MC1 expression over approximately 12 months, alongside SARA and SCAFI assessments, PBMC transcriptomics, and AI-derived motion-capture predictions. Exploratory diffusion tensor imaging (DTI) and quantitative susceptibility mapping (QSM) analyses were also performed.

Methods: Ten FA patients underwent BCPP-EF PET imaging of the brain and heart and 3T MRI on two occasions (mean interval 387 days; range 317–491). PET data were analysed as previously described to derive cardiac SUVR and brain VT/DVR-1 parameters. Results were compared with age- and sex-matched healthy volunteers (HV). Regional DTI and QSM analyses were also conducted. AI methods will be presented.

Results: Cardiac BCPP-EF SUVR was significantly reduced in FA versus HV at baseline (89 ± 32 vs 251 ± 89). FA patients showed lower fractional anisotropy and increased mean and radial diffusivity in the dentate nucleus and superior cerebellar peduncle. No significant differences in brain BCPP-EF binding were observed between groups. Longitudinally, no significant changes were detected in cardiac or brain imaging parameters over 12 months; follow-up cardiac SUVR was 98 ± 30 . AI-based predictions of MC1 expression using transcriptomic and motion-capture features will be presented.

Conclusion: FA patients showed markedly reduced cardiac MC1 expression and altered regional DTI measures, while imaging parameters remained stable over 12 months. Detecting a 25% improvement in cardiac MC1 expression would require 12 participants per group (80% power). The inclusion of transcriptomic and ethomic features enables multimodal AI prediction of disease progression. ****References:**** 1) jnumed.124.268698. 2) doi:10.1038/s41591-022-02159-6.

Funding Sources: Takeda

C96. Acoustic and linguistic features of speech during reading capture early changes, progression, and function in ataxias

12:06 PM - 12:24 PM

Ryan Anthony J. de Belen¹; Yi Zheng²; Matthew B. Walsh²; Franziska Hoche²; Chih-Chun Lin²; Christopher D. Stephen³; Jeremy D. Schmahmann⁴; Hasnaa Ouadoudi Belabzioui⁵; Divya D. Kulkarni⁶; Siddharth Patel⁷; Lawrence White²; Anoopum Gupta²

1. Massachusetts General Hospital/Harvard Medical School, 2. Massachusetts General Hospital, 3. Massachusetts General Hospital, Boston, MA, 4. Massachusetts General Hospital, Waban, MA, 5. Harvard University, 6. Massachusetts General Hospital - Harvard Medical School, 7. Neurology, Massachusetts General Hospital, Harvard Medical School

A major obstacle for clinical trials is the lack of objective, sensitive, and reliable measures that can detect modest changes in disease progression. Here, we determine whether acoustic and linguistic digital speech measures automatically obtained during a functionally relevant passage-reading task capture multiple dimensions of disease in ataxia, including functional communication impairment, subclinical cerebellar dysfunction, and disease progression.

A total of 157 individuals with ataxia and 84 controls contributed cross-sectional data, and 54 individuals with ataxia and 43 controls contributed longitudinal data in Neurobooth. Participants completed standardized speech recordings, patient-reported outcome measures (PROMs), and neurologist-rated clinical evaluations. A novel speech processing pipeline was developed to automatically transcribe audio recordings, identify word boundaries, and extract a predefined set of linguistic and within-word acoustic features.

Individuals with ataxia exhibited slowed speech ($d=1.23$), prolonged inter-word pauses ($d=-0.91$), higher/more variable vocal intensity ($|d|=0.43-0.51$) and altered spectral content ($|d|=0.43-0.79$) compared to healthy controls. Linguistic features (e.g., speaking rate and within-word pause duration) showed strong associations with clinician-rated severity and PROMs ($|r| = 0.23-0.68$). In contrast, acoustic features (e.g., cepstral) captured subtle speech abnormalities, differentiating not only individuals with ataxia ($d=0.65$) but also pre-ataxic individuals ($d=0.56$), and those without clinically evident dysarthria ($d=0.45$), from controls. Several acoustic measures (e.g., cepstral) were sensitive to disease progression even in cases where clinical scales showed no detectable change ($MSDR=0.19-0.68$).

Acoustic and linguistic measures during reading provide complementary but different clinical information in ataxia. Linguistic measures primarily reflect downstream functional consequences of ataxic dysarthria, whereas acoustic measures provide sensitive indicators of subclinical cerebellar motor dysfunction before overt clinical speech impairment emerges. These findings demonstrate that natural speech analysis can produce digital measures for detecting subclinical disease, quantifying functional impairment, monitoring progression in ataxia, with strong potential for application in clinical trials and remote monitoring.

Funding Sources: Massachusetts Life Sciences Center, U.S. Department of Defense, NIH R01NS117826, Biogen, Broad Institute, Duke Family Foundation

C39. Gene Therapy Awareness, Attitudes, and Trial Willingness in Genetic Ataxias: An International Patient Survey

12:24 PM - 12:42 PM

Christian Rummey¹; Emily Cutting²; Julie Greenfield³; Celeste Suart⁴; Andreas Nadke⁵; Chantal Gobeil⁶; Shandra J. Trantham⁷; Bernard Brais⁸; Manuela Corti⁹; Matthias Synofzik¹⁰

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Background & Objectives: Gene and RNA therapies (GRT) is under active development for several genetic ataxias, yet patient-level perspectives on GRT awareness, risk tolerance, and trial willingness remain uncharacterized. We surveyed individuals with genetic ataxia to quantify GRT knowledge, clinical-trial (CT) attitudes, and participation preferences.

Methods: Cross-sectional online survey distributed in English, French and German by Ataxia UK, the National Ataxia Foundation, the ARSACS Foundation, DHAG and AFAF. Domains: demographics, disease stage, symptom-treatment priorities, CT and GRT awareness, information sources, attitudes (Likert), risk tolerance, and willingness to join four trial modalities.

Results: Of 493 respondents, 55% were female, median age 58 years (IQR 45-68.5), across diverse genotypes. The most frequent ones were SCA6 (n = 78), SCA3/MJD (n=62), FA (n=57), SCA27B (n=47), RFC1/CANVAS (n=39), SCA1 (n=26), SCA2 (n=24) and ARSACS (n=20). Support for CTs was near-universal (95%) but based on unequal information access: only 21% felt informed about upcoming trials by clinicians, versus 61% via internet and 55% via patient organizations. Willingness was 71% for experimental drugs/non-permanent GRT, 55% for permanent GRT, and 36% for GRT excluding future trial eligibility; 65% would accept lumbar puncture and 52% brain surgery. Gait/balance was the top treatment priority (71%); GRT awareness was 70%, ASO awareness 14%, prior CT participation 14%. Post-survey, 61% reported improved GT understanding.

Discussion & Conclusion: Patients express strong, pragmatic support for GRT research, and recognize the importance of CTs. However, only up to two-thirds tolerate invasive procedures and only little more than half of them permanent GRT. A dominant finding is a systematic information gap: only 21% feel adequately informed by their clinician, while patient organizations and the internet are the primary CT channels (though related to survey's distribution routes). Awareness was high for gene therapy but low for ASOs. Closing communication deficits remains essential for future GRT research.

Funding Sources: The TREAT-ARCA Project, funded by European Joint Programme for Rare Diseases (EJP-RD) and UCB. Contribution by M.S. was supported by European Rare Disease Research Alliance (ERDERA), GA n°101156595, funded under call HORIZON-HLTH-2023-DISEASE-07.

C64. Trial Ready Natural History of Spinocerebellar Ataxia Type 31

12:42 PM - 1:00 PM

Kinya Ishikawa¹; Hanako Aoki²; Miwa Higashi²; Akiko Amano³; Reiko Yajima¹; Takaaki Aratake¹; Taro Ishiguro³; Masaki Oyagi³; Hiromasa Sato¹; Akihiro Hirakawa¹; Sonoko Misawa¹

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Background and Objectives: Spinocerebellar ataxia type 31 (SCA31) is characterized by a late-onset slowly progressive cerebellar ataxia, caused by a penta-nucleotide expansion containing (TGGAA/TTCCA)_n in an intron of BEAN1 and TK2 (Am J Hum Genet 2009; 85: 544-57). In order to treat this disease, it became essential for us to understand progression rates as well as biomarkers in SCA31. We undertook this study to understand the natural history of SCA31.

Methods: We designed a three-day clinical study consisting of SARA, International Cooperative Ataxia Rating Scale (ICARS), 6 minutes-walk (6MW), 10 meter walking speed (10mWS), 25-foot speed walking (25FWS) and 9HPT. Cerebrospinal fluid, blood and urine samples were also collected. By repeating this observation every 48 weeks in each participant, we will be able to collect annual clinical progressions in all parameters.

Results: The mean age of onset at entry was 66.7 ± 6.9 (SD) years. By the time of the abstract submission, 33 patients completed the baseline observation. Among these, 27 patients had the second observation at 48 weeks, 20 patients had been further checked at 96 week, and 9 of the 33 patients completed 144 week observations. Our latest analysis disclosed that SCA31 patients show SARA score increase around 1.3 points/48 weeks in average, and the 6MW distance was shortened for 23.9 meters/48 weeks in average. Other parameters such as ICARS, 10mWS, or 9HPT also showed statistically significant changes in 48 weeks. Despite that our program is more comprehensive than other programs such as Ataxia Functional Composite Scale (Mov Disord 2021;36(2):283-297), no significant concerns were encountered.

Discussion and Conclusion: We are considering to use these parameters in our first-in-human clinical trial in SCA31.

Funding Sources: Japan Agency for Medical Research and Development. Research Group for the Improvement of Medical Standard and Quality of Life of Patients with Ataxia, the Ministry of Health, Labour, and Welfare, Japan.

Late Breaking Research

Friday, November 13, 1:00 PM-2:15 PM

EP55. Prime editing corrects pathogenic trinucleotide repeat expansions in Friedreich's ataxia and Huntington's disease models

1:00 PM - 1:18 PM

Zaneta Matuszek¹

1. Harvard University

Trinucleotide repeat (TNR) expansions cause at least 40 genetic disorders, including Huntington's disease (HD) and Friedreich's ataxia (FRDA). Current therapeutic approaches do not directly correct the underlying repeat expansions. We sought to develop prime editing strategies to precisely remove pathogenic CAG and GAA repeat expansions in cellular and mouse models of HD and FRDA, respectively.

We developed prime editing strategies to replace expanded HTT CAG repeats with shorter, non-pathogenic tracts and to excise expanded GAA repeats from FXN while preserving intronic regulatory elements. We evaluated editing efficiency and molecular rescue in patient-derived cells and differentiated neurons, and, for FRDA, also in vivo in YG8s mice following dual-AAV9 delivery.

In HD patient-derived iPSCs, prime editing achieved an average HTT correction efficiency of 24%. Medium spiny neurons derived from corrected iPSCs exhibited near-complete elimination of huntingtin inclusions to levels indistinguishable from healthy controls. In FRDA patient-derived iPSCs and sensory neurons, GAA repeat excision reduced methylation at repressive FXN CpG sites by up to 20% and increased FXN mRNA and protein expression by ≥ 1.8 -fold. In vivo, prime editing achieved up to 26% GAA repeat excision in the cortex of YG8s mice, reduced repressive CpG methylation across multiple tissues, and increased FXN expression in the cortex and liver. Edited alleles and epigenetic rescue persisted for at least 24 weeks without an observed increase in off-target editing.

These findings establish prime editing as a strategy for precise correction of pathogenic TNR expansions and demonstrate molecular and disease-relevant phenotypic rescue across HD and FRDA models. This approach may provide a framework for therapeutically addressing the underlying genetic cause of repeat expansion disorders.

Funding Sources: Friedreich Ataxia Research Alliance; US NIH U19NS132304

A23. SCA27B-linked FGF14 AAG repeat expansions are common and associated with specific motor impairment in an unselected aging cohort from Germany

1:18 PM - 1:36 PM

Sophie Kipka¹; Thomas W. Mühleisen²; Patrick Nysten²; Clara Rentz³; Nora Bittner²; Christiane Jockwitz²; Andreas Forstner²; Per Hoffmann⁴; Katrin Amunts⁵; Svenja Caspers²; Sven Cichon⁶; Margit Burmeister⁷; Martina Minnerop⁸

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Spinocerebellar ataxia type 27B (SCA27B) is a late-onset disorder caused by an intronic AAG (equivalent to GAA) repeat expansion in the fibroblast growth factor gene FGF14. While expanded repeat alleles occur in the general population, their relevance outside the SCA27B phenotypic spectrum remains unknown, particularly whether large allele carriers without diagnosed ataxia exhibit subtle motor deficits. We therefore investigated allele frequencies and their association with motor performance in 1000BRAINS, a population-based German cohort, unselected for neurological disorders.

We genotyped the cohort by PCR (588 males/480 females; age 63±12). Motor performance of probands carrying ≥250 repeats was compared with age-, sex-, and education-matched probands with ≤30 repeats (Wilcoxon rank-sum tests, Bonferroni correction, pFWE<0.05). Large allele carriers were re-sequenced for repeat structure by Nanopore. Repeat length and structure were analysed using an in-house Python pipeline and independently cross-checked by HMMSTR.

We identified 42 probands (4%) carrying an expansion (≥250 repeats). Matched comparison with probands from the normal allele range (≤30 repeats) revealed that expansion carriers had poorer performance in verbal dual-task walking (36 pairs, pFWE=0.008) and reactive postural control on a balance platform (34 pairs, pFWE=0.03); none of the expansion carriers reported a diagnosis of ataxia. In the re-sequenced subgroup (n = 39/42), 13 showed pure triplets (33%), while 26 had non-pathogenic interrupted triplets or hexameric (AAGGAG) repeats (67%). Compared with matched controls, carriers with pure triplets showed worse verbal dual-task walking and worse reactive postural control (11 and 10 pairs, p=0.04 uncorrected).

Our results suggest that pathogenic variation at the FGF14repeat locus is relatively common in the general population from Germany and may be associated with subtle impairments in gait and postural control, even in individuals without a diagnosis of ataxia. These findings raise the possibility that FGF14repeat expansions may already contribute to subtle motor impairment during normal aging.

Funding Sources: MB received funding from the Humboldt Foundation (nanopore sequencing). SK received funding from the Jürgen-Manchot-Foundation to support her doctoral research.

DR53. Transcription within Repressive Chromatin: A Mechanistic Strategy for desilencing FXN Expression in Friedreich's Ataxia

1:36 PM - 1:54 PM

Homa Rezaei¹; Aseem Z. Ansari²; Ashraf Mohammed³

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Background and Objectives: Friedreich's ataxia (FRDA) is caused by pathogenic expansion of GAA trinucleotide repeats within the first intron of the FXN gene. These expanded repeats promote the formation of repressive chromatin, including enrichment of H3K9me3 and heterochromatin protein 1 (HP1), resulting in impaired transcriptional elongation and production of frataxin. Although transcriptional reactivation is a promising therapeutic strategy, the mechanisms that enable transcription through disease-associated repressive chromatin remain poorly understood. We sought to define how SynGR1, a sequence-specific DNA-binding molecule designed to recruit the transcriptional regulator BRD4 to pathogenic GAA repeats, restores FXN transcription in FRDA cells and to determine whether transcriptional activation requires removal of repressive chromatin.

Methods: We applied SynGR1 to patient derived FRDA cells and examined the relationship between FXN transcription, H3K9me3, HP1, and BRD4 via chromatin profiling, gene-expression analysis, imaging, genetic perturbation, and reconstituted biochemical systems.

Results: Unexpectedly, SynGR1-mediated activation of FXN did not require erasure or replacement of repressive H3K9me3. Instead, active FXN transcription was associated with persistent—and in some conditions increased—H3K9me3 and HP1 across the GAA-repeat region. SynGR1 selectively recruited BRD4 to the repressive GAA-repeat region and enabled RNA polymerase II transcriptional elongation through this chromatin environment. Consistent with this mechanism, inhibition or degradation of BRD4 reduced FXN expression. Furthermore, BRD4 was found to partition into HP1-containing condensates in vitro and to colocalize with HP1-rich nuclear structures in cells, challenging the prevailing view that BRD4-mediated activation and HP1-associated repression are mutually exclusive. Combined treatment with SynGR1 and the epigenetic modulator RGFP109 further enhanced FXN expression, while washout and retreatment experiments demonstrated that the repressive chromatin state remained dynamic and reversible.

Conclusions: These findings establish a new mechanism of FXN desilencing in which BRD4 enables transcription through H3K9me3/HP1 enriched chromatin, revising the conventional model that gene activation must involve requires the erasure of repressive chromatin. More broadly, the study provides a mechanistic framework for developing locus-directed transcriptional therapies that activate disease-associated genes while preserving the underlying chromatin architecture.

Funding Sources: ALSAC and the National Cancer Institute grant P30CA021765

DD46. The SCA3 disease protein ATXN3 regulates lysosomal integrity and autophagic-lysosomal flux

1:54 PM - 2:12 PM

Anushka Chakravorty¹; Dan Lubicki²; Katie J. Lam³; Brynn Kroke⁴; Ashley Haralson²; Ilkim Erturk²; Claire Clelland⁴; Henry L. Paulson⁵

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Background: Spinocerebellar Ataxia Type 3 (SCA3) is caused by a CAG repeat expansion in the ATXN3 gene, resulting in an expanded polyglutamine (polyQ) tract in the disease protein ATXN3, a deubiquitinase (DUB). ATXN3 functions in many cellular processes including the ubiquitin–proteasome system, lysosomal pathways, endoplasmic reticulum–associated degradation, and DNA repair. Precisely how mutant ATXN3 alters ubiquitin-dependent pathways remains poorly understood. To begin addressing this question, we performed quantitative proteomics in SCA3 human embryonic stem cells and isogenic control lines.

Methods: In a series of CRISPR-engineered human embryonic stem cell lines, mass spectrometry identified differentially expressed proteins in various pathways of cellular proteostasis. To query changes in autophagic and lysosomal function, we employed dynamic immunofluorescence together with various functional assays.

Results: Compared to wild type cells, SCA3 and ATXN3 knockout stem cells display diametrically opposed proteomic signatures, supporting a gain-of-function mechanism in SCA3. Many altered proteins are linked to ubiquitin-dependent processes, including lysosomal pathways, prompting further investigation into the role of ATXN3 in the autophagy-lysosomal system. We find that mutant ATXN3 compromises lysosomal function, as evidenced by reduced fluorescence of the lysosome protease reporter DQ-BSA. In addition, wild-type ATXN3 rapidly translocates to damaged lysosomes, supporting a normal role in lysosomal repair. Moreover, loss of ATXN3 enhances autophagic degradation, suggesting that wild type ATXN3 normally impedes autophagic flux. Intriguingly, mutant ATXN3 impairs autophagic flux to a greater extent. Ongoing studies seek to define the ubiquitin-dependent processes by which ATXN3 regulates autophagic-lysosomal flux and lysosomal integrity.

Discussion and Conclusions: Together, these findings identify ATXN3 as a regulator of multiple steps in the autophagy-lysosomal system and support the view that mutant ATXN3 contributes to dysfunction in ubiquitin-dependent proteostasis.

Funding Sources: R35NS122302 (Paulson, PI)

Posters

Advances in genetics and diagnostics

A1. CGG repeat expansion in GIPC1 is associated with childhood-onset hereditary ataxia

Cuijie Wei¹; Ying Wu¹; Jianwen Deng²

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Background: Hereditary ataxias are genetically heterogeneous; however, despite major advances in next-generation sequencing technologies, 20%–54% of childhood-onset cases remain genetically undiagnosed.

Objective: To elucidate the genetic etiology of childhood-onset hereditary ataxia.

Methods: Oxford Nanopore long-read genome sequencing (LRS) was performed in two unrelated Chinese patients with clinically suspected childhood-onset hereditary ataxia.

Results: Both patients presented with childhood-onset, slowly progressive ataxia, accompanied by mild cognitive impairment. Brain MRI demonstrated cerebellar atrophy, and electromyography showed neurogenic damage. Muscle biopsy revealed fiber-type grouping, indicative of neurogenic changes, with no evidence of primary myopathy. LRS detected pathogenic-length CGG repeat expansions (>100 repeats) in GIPC1, which were validated by repeat-primed PCR.

Conclusions: These findings expand the phenotypic spectrum associated with GIPC1 CGG repeat expansion and define a novel subtype of childhood-onset hereditary ataxia accompanied by mild cognitive impairment and neurogenic involvement.

Funding Sources: National Natural Science Foundations of China (Grants 82402177, 82171846, 82422025); The Beijing High-Level Innovation and Entrepreneurship Talent Support Program-Healthcare Platform young backbone talent projects (G202531202)

A2. SAMD9L, an underdiagnosed cause of dominant cerebellar ataxia

Quentin Thomas¹; Indr Singh²; Lise Larcher³; Jean-Loup MÉREAUX⁴; Faruq Mohd⁵; Mathieu Anheim⁶; Claire Ewencyk⁷; Giulia Coarelli⁸; Ange-Line Bruel⁹; Marie De Tersant¹⁰; Alexis Brice²; Jean Soulier¹⁰; Alexandra Durr¹¹

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Background and Objectives: SAMD9L gain-of-function (GOF) variants cause Ataxia-Pancytopenia Syndrome (ATXPC), a disorder that combines cerebellar ataxia with a large spectrum of hematological disorders. Individuals with SAMD9L GOF variants survive such complications via "somatic genetic rescue" (SGR): a secondary loss-of-function variant that neutralizes the GOF effect in hematopoietic cells. Reaching a diagnosis of ATXPC is often challenging: SGR may erase GOF variants from the blood, in silico scores struggle to correctly evaluate their pathogenicity and should SGR happen early enough, patients may have neurological symptoms without blood disorders. Consequently, SAMD9L is likely overlooked in adult ataxia cohorts lacking hematological history. We aimed to investigate the prevalence of SAMD9L variants in a large cohort of ataxia patients, hypothesizing that GOF variants represent an underdiagnosed cause of isolated cerebellar ataxia. **Methods:** We screened 3754 exomes/genomes from ataxia/spastic paraplegia cohorts (Paris, Dijon, PFMG2025, New Delhi). We extracted SAMD9L variants, assessing pathogenicity via frequency, segregation, specialized in silico tools, and 3D protein modeling. Control cohorts included 20016 exomes from other neurological disorders and healthy controls. **Results:** We identified 13 individuals with rare SAMD9L missense variants. Crucially, three carried pathogenic variants presenting with adult-onset cerebellar ataxia without any hematological history. One family showed evidence of SGR (mosaic stop-gain variant). Ten additional patients harbored candidate variants (ACMG class 3) with overlapping phenotypes. These 13 individuals accounted for 0.02% of Paris's ataxia cohort unsolved cases, which was more than well recognized ataxia genes. No association was found with non-ataxic phenotypes. **Discussion and Conclusion:** SAMD9L is a significant cause of dominant adult-onset cerebellar ataxia that can present without hematological involvement, likely due to SGR or variable expressivity. The poor performance of standard prediction algorithms for GOF effects further hinders diagnosis. Clinicians must consider SAMD9L in unsolved ataxia cases regardless of hematological history to ensure accurate genetic counselling.

A3. The new X-linked spastic ataxia related to CD99L2 variants can affect females and males alike

Jean-Loup MÉREAUX¹; Quentin Thomas²; Fabienne Oy-Magne³; Guillaume Banneau⁴; Claire Ewencyk⁵; Anna Heinzmann²; Chloé Laurencin⁶; Isabelle Le Ber⁷; Mathieu Georget⁸; Ange-Line Bruel⁹; Claire-Sophie Davoine¹⁰; Thomas Wirth¹¹; Frederic Tran-Mau-Them¹²; Francis Ramond¹³; Giulia Coarelli¹⁴; Alexandra Durr¹⁵

1. Paris Brain Institute, PARIS, 2. Sorbonne Université, Institut du Cerveau-Paris Brain Institute-ICM, CNRS, Inria, Inserm, AP-HP, Groupe Hospitalier Sorbonne Université, Paris, France, 3. Purpan Hospital, CHU de Toulouse, Toulouse, 4. CHU de Toulouse, Toulouse, 5. Sorbonne University, ICM, INSERM, CNRS, Pitié-Salpêtrière Hospital, APHP, Paris, Paris, 6. CHU de Lyon, Lyon, 7. Sorbonne Université, Institut du Cerveau - Paris Brain Institute- ICM, Inserm, CNRS, APHP, University Hospital Pitié-Salpêtrière, Paris, Paris, 8. Hôpital Pitié Salpêtrière, Paris, Paris, 9. Université Bourgogne Europe, CHU Dijon Bourgogne, Service Neurologie, Centre de Référence Maladies Rares Neurogénétiques, Inserm, CTM UMR 1231, équipe GAD, 21 000 Dijon, France, Dijon, 10. Paris Brain Institute, PARIS, Paris, 11. CHU de Strasbourg / Université de Strasbourg, Strasbourg, 12. Plan France Médecine Génomique, Lyon, 13. Plan France Médecine Génomique & Department of Medical Genetics, Lyon, 14. ICM - Paris Brain Institute, PARIS, 15. Sorbonne University, Paris

Background and Objectives: Despite the identification of hundreds of genes causing spinocerebellar degenerations, around half of the families still lacks a molecular diagnosis. Loss-of-function variants in CD99L2 located on the X chromosome were recently identified as responsible for less than 1% of cases with spinocerebellar degeneration in a German movement disorders cohort. We wanted to confirm CD99L2 as a gene associated with spastic ataxia in a French cohort.

Methods: We screened five French cohorts gathering individuals with spinocerebellar degeneration sequenced by short-read exome or genome. We searched for loss-of-function single nucleotide and structural variants in CD99L2 and correlated clinical findings.

Results: We identified six index cases (five males, one female) carrying a loss-of-function variant in CD99L2 among the 2539 probands screened (0.24%). This cohort included 1759 probands with cerebellar ataxia, while the remaining 780 had only spastic paraplegia. Amongst the six different variants, four were novel and included one structural variant with exons deletion. The family history was consistent with X-linked transmission with female carriers exhibiting minimal or mild symptoms. The clinical presentation ranged from pure cerebellar ataxia or spastic paraplegia, to complex spastic ataxia. The median age at onset was 48.5 years. Interestingly, one of the proband was a female starting at 54 years. We did not find a X-inactivation bias to explain this earlier severe presentation in a female. Cerebellar atrophy in brain MRI was present in half of cases.

Discussion and Conclusion: CD99L2 loss-of-function variants are a rare cause of X-linked spastic ataxia. The phenotype ranges from pure cerebellar ataxia to spastic ataxia. Females can also be affected. X-inactivation bias restricted to the brain and genetic modifiers could contribute to phenotypic variability in female carriers and exceptional very early onset cases.

Funding Sources: Paris Brain Institute, SPATAX and BIOMOV studies, Inserm, APHP.

A4. CRISPR/Cas9 Customization of Electronic Genome Mapping Repeat Expansion Assays

Kasra Tabatabaei¹; Syndi Koltz²; Lindsay Opsahl²; Wentao Huang²; Dong Zhang²; Xu Tan²; Shuk Shukor³; Mike Kaiser²; John F. Thompson²

1. Nabsys, Providence, RI, 2. Nabsys, 3. Hitachi

Background and Objectives: Many genetic disorders are caused by simple sequence repeat expansions, including Fragile X syndrome (FXS) and Friedreich's ataxia (FA). Accurate sizing of these expansions is critical for determining pathogenicity. Conventional PCR and sequencing methods are unreliable for long repeat regions.

Electronic genome mapping (EGM) uses solid-state nanodetectors to survey long DNA molecules and detect structural variants (SVs), including those caused by repeat expansions.

Methods: We assessed EGM's ability to detect FMR1, FXN, DMPK, and c9orf72 repeat expansions using Coriell cell lines. Single injections and replicates were analyzed for concordance with published repeat lengths using the custom RepX™ Repeat Expansion Analysis (RepX) pipeline.

Results: Multiple replicates of multiple samples were concordant with expectations across all FMR1 cell lines, but some FXN cell lines did not match the original published findings. Because FXN has been shown to be an unstable repeat, with the same cell line yielding different results, we analyzed the FXN cell lines by PCR and EGM. Some cell lines were found to have multiple repeat alleles via PCR, and these patterns were mirrored by the single-molecule EGM results.

Discussion and Conclusion: These findings confirm the accuracy and robustness of EGM for repeat expansions that are challenging to analyze by standard techniques. In addition, we have customized the FMR1 and FXN assays by including specific nicks and blocks to optimize the interval size for calling premutation alleles. The impact of the simple CRISPR customization will be shown for these repeat expansion assays. The pipeline likely can detect many other repeat expansions.

EGM offers integrated workflows for high-resolution analysis of repeat regions in either whole-genome or gene-specific mode. This approach enables efficient, scalable detection of repeat expansions.

Funding Sources: Funding was provided by Nabsys, the employer of all authors. No outside sources funded this project.

A5. Sequence-dependent phenotypic consequence of expanded composite alleles in Friedreich ataxia

Morgan C. Devore¹; Christian Rummey²; Christina Lam³; Courtney PArk⁴; David Lynch⁵; Sanjay Bidichandani

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Background and objectives: Most people with Friedreich ataxia (FRDA) inherit abnormal expansions of a GAA triplet-repeat sequence in intron 1 of both copies of the FXN gene. However, 20% have at least one expanded composite allele, containing substantial amounts of non-GAA sequence. Pathogenic composite alleles were assessed for their phenotypic effects.

Methods: Composite and pure GAA alleles were characterized by longread sequencing.

Results: Four hierarchical subclasses of composite alleles were identified, most of which are defined by tracks of tandem GGA triplets, with/without GAAGGA hexanucleotides, with a range of non-GAA content of 2.6 - 33.5%. All pathogenic composite alleles share the same architecture; the non-GAA component starts close to the 5' end (after ~24 GAA triplets) and occupies the 5' half of the expanded repeat, and all end with long tracks of GAA triplets. Haplotype analysis and flanking rare variants indicate that composite alleles originated multiple times. Most FRDA patients with composite alleles are compound heterozygous, i.e., they also have an expanded pure GAA allele, and the composite allele is typically larger (median +335 triplets). In such individuals, the "GAA content" of the composite allele (rather than its full length), along with the length of the pure GAA allele, significantly predicted residual frataxin level, age of onset, and time to loss-of-ambulation. Each additional 100 GAA triplets of total GAA content in the pure and composite alleles shortened the time to loss-of-ambulation by ~6%. This association was stronger than with the pure GAA allele alone.

Discussion and Conclusions: In FRDA individuals who are compound heterozygous for an expanded composite allele and an expanded pure GAA allele, the combined GAA burden of both expanded alleles is predictive of the phenotype. The sequence-dependent phenotypic effect of composite alleles suggests that the non-GAA sequence partially mitigates the pathogenicity of composite alleles.

Funding Sources: This work was supported by a FARA Graduate Research Fellowship (MCD), Friedreich Ataxia Research Alliance Grant (SIB), US Department of Defense grant (USAMRAA) HT94252510541(SIB), US Department of Defense grant (USAMRAA) HT94252510542(DRL).

A6. Accurate Characterization of the FGF14 Repeat Locus Using PacBio PureTarget Long-Read Sequencing

David Pellerin¹; Matt C Danzi¹; Egor Dolzhenko²; Pablo Iruzubieta³; Marie-Josée Dicaire⁴; Michael Eberle²; Sarah Kingan²; Sanjay Bidichandani; Bernard Brais⁵; Stephan Zuchner⁶

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Background and Objectives: SCA27B, caused by a GAA repeat expansion in intron 1 of FGF14, is one of the most frequent causes of late-onset cerebellar ataxia (LOCA). Diagnosis requires resolution of both allele length and purity, as the length of uninterrupted GAA segment determines pathogenicity. PCR-based approaches may be inadequate to characterize very large or interrupted alleles. Here, we evaluated PacBio PureTarget sequencing for identifying SCA27B alongside other repeat expansion disorders.

Methods: Thirty-eight individuals were sequenced on a PacBio Revio system using the PureTarget panel v2.0 (assessing 38 repeat loci), multiplexed on a single SMRT Cell, comprising: 24 with SCA27B (some carrying large or interrupted expansions), 10 with LOCA in whom FGF14 status was unresolvable by PCR, 3 with Friedreich ataxia compound heterozygous for GAA and composite GAA-GGA expansions, and 1 with RFC1-related CANVAS. We developed a novel algorithm to distinguish genuine interruptions from sequencing artefacts.

Results: Median overall target coverage was 645x. Coverage varied by motif: hexanucleotide loci showed highest enrichment (median 737x) and CAG/CTG loci performed well (726x), while GAA-motif loci were lower (608x), with FGF14 specifically at 476x and FXN at 615x. For FGF14, length concordance was excellent (Pearson $r = 0.976$, $p < 0.0001$) between PCR and PureTarget allele measurements. The novel algorithm reliably differentiated genuine interruptions from artefacts in all FGF14 alleles and characterized FGF14 genotypes in all 10 PCR-indeterminate LOCA cases. However, coverage was lower for large RFC1 and FXN alleles, and the tool could not reliably distinguish interruptions from sequencing artefacts.

Discussion and Conclusion: PureTarget sequencing resolves FGF14 allele length and purity, and characterized FGF14 genotypes where PCR had failed. The novel pipeline accurately discriminated true interruptions from sequencing artefacts. Limitations persist for structurally complex loci, such as RFC1 and FXN. Overall, these findings support PureTarget as a scalable platform for clinical SCA27B testing.

Funding Sources: Canadian Institutes of Health Research, NINDS, Ataxia UK, National Ataxia Foundation, FARA

A8. Complex cerebellar ataxia, diagnosed using ONT, extending the phenotypic spectrum of SPG83

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Background and objectives: Despite major advances in next-generation sequencing, the genetic cause of cerebellar ataxia (CA) remains unresolved in ~60% of patients, particularly adult-onset and sporadic cases. Incorporation of long-read sequencing technologies such as Oxford Nanopore Technologies (ONT) into the diagnostic workflow is increasingly warranted. Methods: Staged workup of patient with complex CA phenotype included an exome based NGS panel, segregation studies and targeted ONT. Results: 45 year old male of non consanguineous family of Ashkenazi Jewish ancestry presenting with chronic progressive combined cerebellar ataxia, spastic paraparesis, parkinsonism and myoclonus. Symptoms presented at 34 years of age. Mild cognitive decline, depression and autonomic involvement have recently evolved. Family history was non informative. Three consecutive brain MRI (2014-2023) demonstrated progressive atrophy of cerebellum and spinal cord. 18F DOPA PET CT scan demonstrated symmetrical decreased uptake. Patient is responsive to levodopa and partial response to deep brain stimulation (DBS) which gradually ceased. Exome based NGS panel revealed two HPDL missense variants; a pathogenic variant (c.847C>T;p.(Pro283Ser)), and a variant of unknown significance (VUS:c.694G>C;p.(Glu232Lys)). Segregation studies demonstrated the pathogenic variant to be maternally inherited. Targeted ONT was required to complete allele phasing and upgrade the VUS to likely pathogenic, confirming diagnosis of autosomal recessive spastic paraplegia type 83 (OMIM 619027). Discussion: This unique case demonstrates the rare multidimensional HPDL-associated spectrum of neurodegenerative disorders and expanding the phenotype to include both cerebellar and Parkinsonian features. The case emphasizes the need for comprehensive molecular work up amongst patients with early onset complex cerebellar ataxia, especially prior to DBS. Long read studies performed at the initial stages of investigation enable allele phasing, as illustrated by this case, along with characterization of repeat expansions, methylation signatures, structural variants, and variants in difficult-to-sequence genomic regions within a single assay. Such testing offers a higher diagnostic yield at a shorter turn

Funding Sources: ONT was funded by Dr Achinoma Socher TLVMC resurch fund.

A9. FGF14-SCA27B: Clinical, Genetic, and Imaging Characteristics of the Multicentric Italian Cohort

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Background and objectives: Since its identification in 2023, GAA repeat expansion (GAARE) in FGF14 has emerged as the most common cause of late onset ataxia. Multiple questions remain unresolved, including the pathogenicity of intermediate expansions, the factors influencing repeat instability, and penetrance. In addition, important clinical and imaging issues remain open, including the extent of the response to 4-aminopyridine therapy.

Methods: In a 3-year multicenter Italian study, we analyzed for GAARE in FGF14 in a single molecular laboratory in Pisa. Deep phenotyping was performed in all GAA>250 patients, whereas alternative diagnoses were excluded in GAA200–249 cases. Brain MRI scans are being collected for centralized cerebellar and cerebral volumetric analyses. Furthermore, the gene and the flanking sequence of the expansion were investigated to assess their effect on the instability of the repeats during intergenerational transmission.

Results: Among 481 samples, 127 harbored ≥250 GAA repeats (26%), and 36 (33%) had 240–249 repeats. We identified 21 alleles with interrupted repeat tracts. Intergenerational transmission data were available for 40 families, and in all cases parent-of-origin influenced repeat instability: maternal transmission was associated with expansion in the offspring (median change: +31.5 GAA repeats; $p = 0.0001$), whereas paternal transmission led to contraction (median change: -124 GAA repeats; $p = 0.0002$). Offspring repeat size also tended to increase with maternal age at conception ($p = 0.018$). Whilst clinical data collection is ongoing, we have reviewed records from 93 patients (38% females) who presented a median age of 88 years, a mean age at onset of 60, a mean SARA score of 8 (IQ 0–28.5; estimated yearly progression rate of 0.75 points).

Discussion and Conclusion: These population-based data provide insights into the molecular architecture and intergenerational instability of the GAARE, support a clear parent-of-origin effect, and contribute to refining the diagnostic boundaries of SCA27B.

Funding Sources: Ricerca Corrente 2025-2026-Italian Ministry of Health

A10. Development of an Artificial Intelligence-Based Neuroimaging Model for the Optimization of Hereditary Cerebellar Ataxia Diagnosis

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Background and Objectives: Hereditary cerebellar ataxias (HCA) are a group of rare diseases characterized by cerebellar ataxia. Diagnosis is challenging due to overlapping clinical symptoms, resulting in diagnostic delays and difficulties in clinical trials, which rely on low-sensitivity clinical scales. Notably, neuroimaging techniques have been successfully applied to characterize and clinically distinguish neurodegenerative diseases. Therefore, this study aims to develop an artificial intelligence tool that utilizes brain T1-weighted MRI to identify the main HCAs, including Friedreich's ataxia (FRDA) and spinocerebellar ataxia type 3 (SCA3), to improve patient screening and diagnostic support.

Methods: Brain T1-weighted MRI scans from the Campinas cohort (FRDA=79, SCA1=31, SCA2=11, SCA3=88, SCA6=16, and age- and sex-matched controls=57) were processed using FastSurfer, CerebNet, FreeSurfer, and Spinal Cord Toolbox to extract cortical, subcortical, cerebellar, brainstem, and spinal cord features. A multiclass machine learning model was trained for diagnostic classification, including K-nearest neighbors, K-means clustering, and random forest and logistic regression, and externally validated in the independent TRACK-FA (n=272) and READISCA (n=20) cohorts.

Results: Among the evaluated models, logistic regression achieved the highest multiclass classification performance, reaching a weighted F1score of 87.4% in the hold-out test set. External validation demonstrated robust generalizability, F1of 94.8% in the TRACK-FA cohort. SCA3classification in the READISCA cohort achieved F1of 73.7%.

Discussion and Conclusion: Our findings support the potential of neuroimaging to improve patient screening and diagnostic support, particularly in regions with limited access to genetic testing. Notably, the strong performance observed in the external TRACK-FA and READISCA cohorts, which include early-stage and pre-symptomatic individuals, suggests good real-world sensitivity and applicability. Increasing cohort size is expected to further improve model robustness and generalizability across additional HCA subtypes, particularly SCA1, SCA2, and SCA6.

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A11. Intrafamilial Molecular and Phenotypic Heterogeneity in SCA27B aka Nye/Darby ataxia

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Background/ Methods: In families with adult-onset ataxia, we identified expansion of the GAA repeat in FGF14 indicative of SCA27B. We used PCR based screening followed by precise size determination as described (HMMSTR, NAR 2025).

Results: Consistent with Pellerin (NEJM 2023), repeats expanded in the female and contracted in the male germline. We also observed this pattern in unaffecteds with 30-250 repeats. Consistent with Pellerin (Nat Gen 2024), sequences <30 repeats carried a stabilizing sequence, while cases with >30 repeats had lost that sequence. Two cases of large repeat loss (60 and 84 repeats) were observed, the latter being unaffected, which could lead to skipping of generations. A Website created by a genetic counselor, Leah Hardy, is available to help families understand this complex inheritance: <https://sites.google.com/umich.edu/sca27b/home?authuser=0>

We asked whether birth order affects intergenerational expansions/contractions of repeats. In 12 pairs of siblings, half inheriting the repeat through the mother, the other half through the father, there was no consistent pattern by birth order. The one case with a large repeat loss was flanked in age by younger and older affected siblings who had not lost many repeats. Our results suggest no effect of parental age or birth order on size of the expansion/ contraction of repeats.

Phenotypically, there was not much correlation between expansion size and age of onset or severity. Rather, we observed many pairs of even close relatives in the same family in which the person with the larger expansion had milder symptoms than the close relative with the less expanded repeat.

Conclusion: In summary we confirm the unusual inheritance pattern of SCA27B and added the novel finding of lack of correlation with birth order/ parental age within families. In addition, even in close relative pairs, we saw no strong correlation between severity and length of the repeats. Clearly, more work on both lifestyle and genetic modifiers is needed.

Funding Sources: National Ataxia Foundation, Raynor Cerebellum Project

A12. DNA repair pathways variants are enriched in individuals with biallelic AAGGG CANVAS and RFC1-related disease

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Background and objectives: Cerebellar ataxia, neuropathy and vestibular areflexia syndrome (CANVAS) is most commonly caused by biallelic AAGGG repeat expansions in RFC1. The substantially higher population frequency of this expansion (0.7%-6.5%) relative to the prevalence of CANVAS (0.005%) suggests incomplete penetrance. Therefore, we tested if additional genetic modifiers influence disease manifestation.

Methods: biallelic RFC1AAGGG status was profiled in Genomics England (GEL) and the UK Biobank (UKB) discovery cohorts using computational methods, and by PCR in an Australian ataxia cohort (AAC). Polygenic scores (PGS) were calculated using variants previously associated with Huntington's Disease age at onset.

Results: We identified 56 (GEL) and 18 (UKB) biallelic individuals with ataxia and/or neuropathy, and 26 (GEL) and 207 (UKB) age-matched phenotypically unaffected biallelic carriers. Meta-analysis of GEL and UKB identified elevated PGS in biallelic affected individuals (OR = 1.43, $p = 2.2 \times 10^{-3}$) compared to controls, but not in the biallelic carriers (OR = 1.01, $P = 0.92$). The variant rs245100, upstream MSH3, was identified as the strongest contributor to the elevated PGS burden (OR = 2.35, adjusted $p = 0.02$). Replication in the AAC confirmed elevated PGS in individuals with molecular confirmation of CANVAS or RFC1-related disease ($n=54$) (OR = 1.93, $P = 4.4 \times 10^{-5}$), and enrichment of rs245100 (OR = 4.24, $p = 1.0 \times 10^{-4}$).

Discussion and conclusion: These findings support a role for polygenic modifier burden in influencing penetrance and clinical manifestation in RFC1-related disease.

Funding Sources: Australian Government National Health and Medical Research Council grants (MRFF2007677, MRF2024989, GNT2001513 and GNT2028681).

A13. Healthy Older Adult Controls Demonstrate Disease Risk Below the Current SCA27B Pathogenic Threshold

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Background/objectives: SCA27B is a late-onset cerebellar ataxia caused by an intronic repeat expansion in FGF14. Current VUS and pathogenic thresholds (200–249 and ≥ 250 repeats, respectively) were derived primarily from population control cohorts of unknown neurological status. None of these were comprised of neurologically healthy individuals older than the median SCA27B onset age. We characterised FGF14 repeat variation in healthy older adults to establish a robust non-pathogenic reference range and reassess pathogenic thresholds.

Methods: FGF14 repeat size and motif composition were assessed using long-range and repeat-primed PCR in 1,976 healthy older controls (≥ 70 years) from the ASPREE cohort (median age 87.3 years, IQR 85.4–90.0) and 639 individuals with CA. Non-GAA alleles >100 repeats were excluded, yielding 3,898 control and 1,253 CA alleles. Allele distributions were compared using Fisher's exact test and odds ratios for 25-repeat intervals. Kaplan–Meier (KM) analysis assessed age-dependent risk across groups.

Results: Most control alleles were <200 repeats (98.6%), with no enrichment in the 175–199 repeat range (OR 1.1 [95% CI 0.62–1.89]; $p=0.77$). Alleles in the 200–224 and 225–249 repeat ranges were significantly enriched in CA (OR 2.35 [95% CI 1.32–4.04]; $p=0.005$ and OR 4.72 [95% CI 2.22–9.57]; $p<0.0001$, respectively). Ten (of 19) affected individuals with a VUS were in the 225–249 range. KM analysis demonstrated that disease-free survival decreased with increasing repeat length, with a distinct separation between 225–249 and <200 repeats ($p<0.0002$).

Discussion and Conclusion: This is the first large-scale assessment of FGF14GAA repeat size in neurologically healthy individuals older than the median SCA27B onset age. Progressive increases in disease association and age-dependent risk demonstrate that clinically relevant disease risk extends below the current pathogenic threshold. These findings support 225–249 alleles as reduced-penetrance pathogenic, improving molecular diagnosis, reducing inconclusive VUS results and enabling access to genetic counselling and emerging clinical trials.

Funding Sources: Australian Government National Health and Medical Research Council grants (MRF2024989 and GNT2028681)

A15. Exploring the Role of Genetic Variation to explain Functional Heterogeneity in Anti-CASPR2 Encephalitis Using Human Neuronal Networks

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Background and Objectives: Immune-mediated ataxias are increasingly recognized as a spectrum of autoimmune neurological disorders in which autoantibodies disrupt cerebellar function and associated neural pathways. Anti-CASPR2 encephalitis is an autoantibody-mediated disorder with heterogeneous clinical features involving the central, peripheral, and autonomic nervous systems. Patients show considerable phenotypic variability, and not all develop ataxia. However, the effects of anti-CASPR2IgG on human neuronal networks and the contribution of genetic factors to this variability remain unclear. We hypothesized that anti-CASPR2IgG disrupts CASPR2-mediated protein interactions, impairing voltage-gated potassium channel (VGKC) function and increasing neuronal excitability, reflected by elevated mean firing rate (MFR) and network burst rate (NBR). **Methods:** Human iPSC-derived control neurons were co-cultured with rat astrocytes and matured on micro-electrode arrays (MEAs) to assess network activity. After 9 weeks of maturation, neurons were exposed to purified anti-CASPR2IgG from four patients and neuronal activity was monitored for 24 hours.

Results: Anti-CASPR2IgG from two out of four patients induced an acute dose-dependent increase in MFR and NBR in one out of two control lines, whereas IgG from the remaining patients showed no effect. In addition to enhanced neuronal activity, exposure to the same two IgG samples induced a dose-dependent increase in spike train correlation, indicating enhanced network synchrony.

Discussion and Conclusion: Anti-CASPR2IgG from two patients induced robust, dose-dependent neuronal hyperexcitability in human iPSC-derived networks. This variability occurred independently of ataxia status, highlighting functional heterogeneity among patient-derived IgG samples. As CACNA1A loss-of-function mechanisms may modulate VGKC function, ongoing experiments in CACNA1A patient and CRISPR lines aim to determine whether genetic modifiers contribute to this variability. Given that Cav2.1 deficiency has been shown to disrupt synchrony, we hypothesize that Cav2.1 dysfunction may either attenuate the anti-CASPR2IgG-induced increase in synchrony or destabilize network bursting patterns.

Funding Sources: ACT-MD consortium (project 09120232310026) funded by ZonMw.

A17. SCA4 as an RFC1-negative CANVAS mimic: diagnostic lessons from the first UK family

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Background and Objectives: Spinocerebellar ataxia type 4 (SCA4) is a rare autosomal dominant disorder characterised by progressive cerebellar ataxia, sensory neuropathy, dysautonomia and motor neuron involvement. In 2024, an exonic GGC-repeat expansion in ZFH3 was identified as the cause of SCA4 in families of Scandinavian ancestry. SCA4 can mimic cerebellar ataxia, neuropathy and vestibular areflexia syndrome (CANVAS), creating diagnostic challenges. We describe the first molecularly confirmed UK family with SCA4 and highlight lessons regarding variant interpretation and differential diagnosis.

Methods: A father and son were investigated at the National Hospital of Neurology and Neurosurgery, Queen Square. Both underwent clinical, neurophysiological and neuroimaging assessments. Standard genetic investigations included hereditary ataxia and neuropathy panels, repeat expansion testing and mitochondrial DNA sequencing. Research analysis of whole-genome sequencing (WGS) was performed using ExpansionHunter, followed by targeted long-read sequencing (LRS) through adaptive sampling.

Results: The father presented in his 30s with progressive cerebellar ataxia, upper limb-predominant sensorimotor neuropathy, dystonic tremor and dysautonomia; MRI demonstrated cerebellar and medullary atrophy. Previous testing identified a heterozygous PRKCG variant of uncertain significance (c.1372G>A, p.Ala458Thr); however, the phenotype was considered atypical for SCA4 and the variant did not segregate in the family. His son developed predominantly sensory ataxia in his 40s with cough, mild vestibular failure and neuropathy/neuronopathy on nerve conduction studies, closely resembling CANVAS. Inflammatory, metabolic, paraneoplastic and genetic causes including RFC1 expansions were excluded. ExpansionHunter analysis of WGS flagged a candidate ZFH3 expansion, subsequently confirmed by targeted LRS as a heterozygous 55-repeat GGC expansion in both individuals, establishing a diagnosis of SCA4.

Conclusions: This report highlights the importance of genomic reanalysis when the phenotype does not fully align with identified genetic variants. Furthermore, ZFH3 testing should be considered in RFC1-negative CANVAS-like presentations, particularly when there is a dominant family history, and should not be restricted to Scandinavian populations.

Funding Sources: Charcot-Marie-Tooth Association (SR-202504). AFM-Telethon (28813). National Ataxia Foundation

A18. Genome-wide identification of genetic modifiers of age at onset and repeat instability in spinocerebellar ataxias

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Background: Spinocerebellar ataxias (SCAs) are a group of inherited neurodegenerative disorders caused by expanded CAG repeats in different genes. Although repeat length is the major determinant of age at onset (AAO), considerable variability remains among individuals carrying similar repeat sizes, indicating the presence of additional genetic modifiers. Previous studies have implicated DNA repair pathways in modifying disease onset through effects on repeat instability, but the broader genetic architecture underlying AAO in SCAs remains incompletely understood. **Methods:** We assembled a worldwide cohort of 6,246 individuals with SCA1, SCA2, SCA3, SCA7, and SCA12. To date, 4,672 individuals have been genotyped, including 850 SCA1, 1,356 SCA2, 1,891 SCA3, 276 SCA7, and 299 SCA12 cases. Participants have molecular confirmation of the causative repeat expansion and detailed phenotypic information, including age at onset and repeat sizing. Samples were genotyped using a custom Illumina array enriched for DNA repair genes and imputed using the TOPMed reference panel. Genome-wide association studies (GWAS) is being performed using residual AAO after correction for repeat length and repeat sequence composition. Association testing conducted using mixed-model approaches accounting for ancestry and relatedness. In parallel, MiSeq deep sequencing is used to characterise repeat interruptions and somatic instability, enabling investigation of their contribution to disease onset. **Results:** Initial analyses confirm that repeat length explains a substantial proportion of AAO variance, but significant residual variation remains across all disease groups. Ongoing GWAS analyses are evaluating established and novel modifier loci, with particular focus on DNA damage repair pathways previously implicated in Huntington's disease and SCAs. Deep sequencing data is ongoing to determine repeat sequence composition and somatic instability. **Conclusions:** This study represents one of the largest international genetic studies of CAG-repeat SCAs to date. By integrating GWAS with high-resolution repeat sequencing, we aim to identify novel genetic modifiers of AAO and clarify disease pathogenesis

Funding Sources: UKDRI, UKRI

A20. Genome-wide association study of genetic modifiers of age at onset in spinocerebellar ataxia 27B (SCA27B): study design and cohort

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Background: Spinocerebellar ataxia 27B (SCA27B) is a recently described and frequent autosomal dominant late-onset cerebellar ataxia caused by an intronic GAA•TTC repeat expansion in FGF14. Unlike polyglutamine SCAs, repeat length is only weakly correlated with age at onset (AAO), explaining only 10-30% of its variance, implying additional genetic modifiers. Variants in DNA repair pathways modulate AAO in other repeat-expansion disorders but have not been investigated in SCA27B.

Methods: Through the SCA27B Study Group, we have recruited 1,476 patients with molecularly confirmed SCA27B (≥ 250 uninterrupted GAA•TTC repeats) from international centres, and we are collecting standardized clinical data (sex, age at onset, episodic symptoms, walking-aid use, and SARA scores where available). The FGF14 locus is being re-genotyped by long-range and bidirectional repeat-primed PCR to resolve repeat size and interruptions, as well as standardize measurement. Samples will be genotyped on a custom Illumina Infinium Global Screening Array enriched for DNA repair genes, with quality control in PLINK and imputation via TOPMed. Residual AAO, after regression on repeat size and interruptions will serve as the primary phenotype, with disease progression (walking-aid use and SARA) as the secondary phenotype. Association testing will use mixed models correcting for relatedness and population stratification, followed by fine-mapping and MAGMA pathway analysis prioritizing DNA repair.

Results: Recruitment is now complete ($n=1,476$ patients) and genotyping is about to begin. At this sample size we project $>90\%$ power to detect modifier effects explaining $\sim 4.5\%$ (common variants) of AAO variance at genome-wide significance ($p=5 \times 10^{-8}$).

Discussion and Conclusions: This will be the first GWAS of genetic modifiers in SCA27B. Identifying modifier loci, particularly within DNA repair pathways, may refine prognostic prediction and reveal druggable targets, with comparison to the ongoing SCA1/2/3 GWAS highlighting shared disease mechanisms.

Funding Sources: Canadian Institutes of Health Research, Ataxia UK, National Ataxia Foundation, UKDRI

A21. Adaptive Sampling Nanopore Sequencing as a Single-Test Strategy for Genetic Ataxias and Related Hereditary Movement Disorders

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Background and Objective: Hereditary ataxias, like other rare diseases, pose a significant diagnostic challenge due to their heterogeneity and genetic complexity, which includes repeat expansions, single nucleotide variants, insertions, deletions, and duplications. Despite extensive phenotypic characterization and numerous panels and protocols, the global diagnostic yield is still only around 50%, which is often constrained by economic barriers. A single test capturing the major causes of ataxias could reduce sequential testing and diagnostic odyssey. We aimed to optimize a low-cost Nanopore adaptive sampling workflow as a single-test strategy for hereditary ataxias and related movement disorders. **Methods:** We developed a Nanopore adaptive sampling workflow integrating custom target catalog design, sequencing optimization, and downstream variant analysis. The catalog included 33 loci: 20 tandem repeat loci and 13 SNV-associated loci, with ± 50 kb flanking regions. The total target size was 11,077,540 bp, representing 0.37% of the genome. Performance was assessed by active pore number, target enrichment, coverage, and read length distribution. **Results:** Across 16 patient samples, mean target coverage increased with starting active pore number, rising from 5–40 $\times$ in lower-output runs to 80–100 $\times$ in higher-output runs. On-target reads peaked around 10–15 kb and extended to 50 kb. Most tandem repeat loci achieved median coverage of 50–70 $\times$, while SNV-associated loci showed more uniform coverage of 55–60 $\times$. The workflow detected a known RFC1 expansion carrier, with improved expanded-allele coverage and motif consistency compared with previous whole-genome Nanopore sequencing. FGF14 was the main low-coverage outlier, with coverage around 5–10 $\times$. **Conclusion:** We optimized an adaptable Nanopore adaptive sampling workflow for screening repeat expansions and SNV-associated loci relevant to hereditary ataxias and related movement disorders. This single-test strategy could reduce reliance on multiple costly sequential assays in resource-limited settings. Additional testing at 100 $\times$ target coverage is planned to improve read support in difficult-to-sequence regions, particularly FGF14.

Funding Sources: National Institutes of Health, NIH HPC Biowulf cluster, Global Parkinson's Genetics Program (GP2)

Biomarkers

B1. Associations between retinal OCT measures and regional brain structure in SCA3

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Background: Retinal neurodegeneration assessed by optical coherence tomography (OCT) is increasingly recognized in spinocerebellar ataxia type 3 (SCA3). Although OCT abnormalities have been linked to clinical severity, whether a composite retinal structural phenotype corresponds to selective regional brain vulnerability in SCA3 remains unclear.

Objective: To derive a composite OCT phenotype in SCA3, identify the principal retinal component with the best discriminative performance, and evaluate its associations with regional brain volumes on structural MRI.

Methods: We studied 133 patients with SCA3 who underwent OCT and brain MRI. Principal component analysis (PCA) was performed on OCT measures to derive major components. Receiver operating characteristic (ROC) analysis was then used to compare the discriminative performance of the derived components for ocular impairment, and the direction-harmonized PC1 was selected as the primary composite OCT phenotype. Candidate regions of interest (ROIs) identified from prior OCT-ROI association analyses were subsequently entered into multivariable linear regression models, adjusting for age, sex, and total intracranial volume (TIV).

Results: PCA identified four OCT-derived components. After direction harmonization, PC1 showed the best discriminative performance in ROC analysis (AUC = 0.874) and was therefore selected for subsequent analyses. Multivariable regression identified 13 ROIs associated with OCT-PC1. These ROIs were predominantly distributed across the cerebellar hemispheres (8 ROIs), with additional involvement of vermis/brainstem regions (3 ROIs) and limbic-related areas (2 ROIs). This spatial pattern suggests that retinal structural abnormality in SCA3 is linked not only to cerebellar degeneration but also to broader selective brain involvement.

Conclusions: A PCA-derived composite OCT phenotype, particularly PC1, captures a major dimension of retinal structural abnormality in SCA3 and shows distributed associations with cerebellar, brainstem, and limbic regions. These findings support retina-brain structural coupling in SCA3 and suggest that OCT may serve as a practical peripheral imaging marker for characterizing disease-related brain involvement.

B2. Peripheral Blood Biomarkers and the TyG Index Predict Cognitive Impairment in SCA3: An Interpretable Machine Learning Study

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Background and objectives: Cognitive impairment is an important non-motor feature of spinocerebellar ataxia type 3 (SCA3), yet clinically accessible biomarkers for risk stratification remain limited. We aimed to develop an interpretable machine learning model based on peripheral blood biomarkers, with particular focus on the triglyceride-glucose (TyG) index, for predicting cognitive impairment in SCA3.

Methods: We enrolled 130 genetically confirmed patients with SCA3 from the Organization for Cerebellar Ataxia Research in South-East China between June 2022 and October 2024. After structured data cleaning, 113 patients were included. Cognitive status was defined using education-adjusted Montreal Cognitive Assessment scores. Candidate predictors underwent collinearity screening followed by recursive feature elimination. Eight machine learning models were compared using 10 repeats of 5-fold stratified cross-validation, and SHapley Additive exPlanations analysis was used for model interpretation.

Results: Of the 113 included patients, 63 had cognitive impairment and 50 had normal cognition. The final selected features were age, ALT, estimated glomerular filtration rate, LDL-C, and TyG. Random forest achieved the best overall performance, with an AUC of 0.781 (95% CI 0.758–0.804), average precision of 0.821, accuracy of 0.730, F1score of 0.773, and Brier score of 0.192. SHAP analysis showed that lower estimated glomerular filtration rate, older age, and higher TyG generally increased predicted risk.

Discussion: These findings suggest that accessible peripheral metabolic biomarkers, especially TyG, may support cognitive risk stratification in SCA3. The superior performance of random forest further indicates that cognitive risk in SCA3 may involve nonlinear and multidimensional clinical relationships.

Conclusion: We developed an interpretable peripheral blood biomarker-based model for predicting cognitive impairment in SCA3, with random forest showing the best performance. TyG may serve as a practical biomarker candidate for future cognitive risk assessment in SCA3.

B4. Multi-Omics Biomarker Discovery in Friedreich's Ataxia Cardiomyopathy

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Background and Objectives: Friedreich's ataxia (FA) is often accompanied by cardiomyopathy that is a leading cause of mortality. Conventional clinical tools primarily capture late consequences of disease rather than upstream molecular drivers. To address this, we initiated an integrated translational program combining detailed clinical phenotyping with multi-omics profiling to identify novel markers to predict early cardiac disease in FA.

Methods: We are conducting descriptive and integrative analysis of a deeply phenotyped cohort of FA patients and heterozygous carriers. Clinical status is assessed using FA questionnaires (FARS, SARA, and FADL), the Kansas City Cardiomyopathy Questionnaire-12, advanced cardiac imaging, clinical plasma biomarkers, and GAA repeat length. Research plasma profiling was performed using multiplex Luminex cytokine assays, targeted lipidomics by LC-MS/MS, and mass spec-based proteomics. Single-cell immune profiling using CITE-seq and genomic analyses are ongoing. Multivariate modeling approaches, including principal component analysis and elastic net-based regression, are applied to identify candidate predictive molecular features associated with FA cardiac involvement.

Results: Cytokine analyses demonstrated alterations in circulating inflammatory and remodeling markers; however, predictive modeling indicated that these alone had limited discriminatory performance. Model accuracy improved when variables were integrated with clinical and imaging metrics, suggesting a subset of circulating proteins contributes to disease pathways. Lipidomic profiling revealed distinct remodeling of inflammatory lipid mediator pathways, with clear separation between FA and control driven by coordinated pathway-level changes. Mass spec proteomics and early single-cell immune profiling further identified trends consistent with mitochondrial stress, immune activation, and fibro-inflammatory signaling.

Discussion and Conclusions: These preliminary findings support integrating clinical phenotyping with genomic, proteomic, lipidomic, and immune datasets aim to refine molecular signatures associated with cardiac disease risk, progression, and therapeutic response. This work provides a translational framework for developing blood-based biomarker panels to improve early detection and monitoring of cardiomyopathy in Friedreich's ataxia.

Funding Sources: DOD funded project: HT9425-23-1-0643

B5. Eyeblink Conditioning as an Accessible Digital Biomarker in Ataxia: A Pilot Study using BlinkLab

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Background and Objectives: Patients with spinocerebellar ataxia (SCA) experience unsteady gait, balance, and coordination due to cerebellar degeneration. Eyeblink conditioning (EBC) is a form of classical conditioning that relies on cerebellar motor learning. The conditioned response (CR) amplitude demonstrates how well participants learn to blink in response to a conditioned stimulus. EBC response acquisition is hypothesized to be impaired in SCA. EBC testing normally requires expensive equipment and frequent in-person visits, limiting practicality. An innovative iOS mobile application “BlinkLab” enables remote, affordable EBC testing. This study aims to assess whether EBC via BlinkLab can be used as an accessible marker of SCA progression.

Methods: This cross-sectional study compares EBC in SCA and age- and sex-matched control participants. Patients with SCA were recruited. Participants watched one 10-minute audio-normalized nature documentary per day on the BlinkLab application for five consecutive days. CR amplitude was measured after each session. After five sessions, descriptive statistics and linear mixed effects model were performed to compare the CR amplitude between SCA and control groups.

Results: We recruited 21 SCA patients and 21 age- and sex-matched controls. Of these, only three participants were excluded due to setup issues. Controls showed greater improvement in CR amplitude compared to participants with SCA. Mean CR amplitudes in controls increased steadily from 0.021 to 0.073, whereas SCA participants showed a smaller rise from 0.016 to 0.044 then decline to 0.020. For CR amplitude, session number was significant ($p = 0.033$), session number with diagnosis was significant ($p = 0.0074$), but diagnosis alone was not ($p = 0.70$).

Discussion and Conclusion: These pilot findings suggest EBC response acquisition over time is impaired in SCA patients and is feasible to use remotely. EBC may present an accessible way to monitor ataxia progression or the impact of disease-modifying treatments.

**B6. Early and Extensive Degeneration of Sensory Neurons in Spinocerebellar Ataxia Type 3:
Integrating Clinical, Electrophysiological, and Imaging Findings**

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Background and Objectives: Post mortem studies have shown significant degeneration of the peripheral nervous system (PNS) in spinocerebellar ataxia type 3 (SCA3). Although PNS involvement potentially accelerates overall ataxia progression, PNS biomarkers for therapeutic trials are lacking and our mechanistic understanding of related clinical manifestations remains incomplete. We employed a multimodal approach to comprehensively characterize and quantify peripheral sensory nervous system degeneration throughout the course of SCA3.

Methods: SCA3mutation carriers without a medical history of additional conditions known to predispose to neuro(no)pathy were enrolled. Detailed clinical assessments of ataxia and neuropathy severity were combined with high-resolution nerve ultrasound and electrophysiological examinations to evaluate cross-sectional areas (CSAs) and sensory action potential amplitudes of upper and lower limb nerves.

Results: Forty SCA3mutation carriers, including ten pre-ataxic subjects, participated in this study. One or more clinical signs indicating PNS involvement were encountered in the vast majority of participants, sometimes already in the absence of ataxia. Sensory axonal loss in at least one nerve was found in 97% of ataxic and 50% of pre-ataxic individuals. Ultrasound examinations showed CSA reductions at one or more measurement sites in at least two nerves in all participants. Upper limb nerves were more commonly affected than the sural nerve, which supports a widespread, non-length-dependent pattern of axonal damage. The degree of sensory axonal loss was associated with ataxia severity, estimated disease duration, and patient-reported ability to perform activities of daily living.

Discussion and Conclusion: Abnormalities of the peripheral sensory nervous system are highly prevalent in SCA3, emerge before ataxia onset, and reflect disease severity. The combination of electrophysiological and ultrasound profiles suggests early involvement of the dorsal root ganglia, highlighting an underestimated source of (sensory) ataxia in SCA3. Longitudinal studies are warranted to validate the biomarker potential of these measures.

Funding Sources: This study was funded by the National Ataxia Foundation and Ataxia UK.

B7. Serial assessment of echocardiographic measures in Friedreich Ataxia

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Background and Objectives. Cardiomyopathy is the most common cause of death in Friedreich ataxia (FRDA), but the relationship between genetic factors, disease course, echocardiographic measures, and clinical outcomes is not completely defined.

Methods. Data on echocardiographic results and disease status were obtained from records from a large cohort of subjects followed at the Children's Hospital of Philadelphia (CHOP) or other locations. We compared clinical features with long term clinical outcomes and echocardiographic markers of cardiomyopathy using correlation and linear regression.

Results. Overall, FRDA hearts initially showed mild hypertrophy with normal systolic function, and a decrease in systolic function up to 30 years later in the disease course. Markers of maximal hypertrophy, in particular septal wall thickness (IVSTd), moderately correlated with genetic severity (GAA1). GAA1 values predicted later stage cardiac disease manifestations in FRDA (presence of arrhythmias, decreases in ejection fraction to less than 50%). Amongst echocardiographic parameters, early elevations in septal and posterior wall thickness and a hyperdynamic ejection fraction predicted later adverse outcomes including arrhythmias, decreases in ejection fraction, and death.

Discussion and Conclusions. The present data are consistent with the prevailing pathophysiological hypothesis that decreased levels of frataxin lead to early hypertrophy, with later cardiac problems manifesting as decreased systolic function, arrhythmias, and death.

Funding Sources: FARA COE to DRL and KYL

B8. OCT of the retina in FRDA

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Background and Objectives: Optic neuropathy is a common (though sometimes subclinical) feature of Friedreich Ataxia (FRDA). In previous studies, retinal nerve fiber layer (RNFL) thickness in FRDA decreased in a manner predicted by subject age and genetic severity (GAA1). RNFL was also decreased in young children with FA, consistent with hypoplasia as well as degeneration. The present study assessed the RNFL and macular ganglion cell layers cross sectionally and longitudinally in a large cohort.

Methods: 410 patients with FRDA (along with controls/carriers) were assessed for RNFL and macular ganglion cell thicknesses by OCT. Values were analyzed in the context of disease features and longitudinal change.

Results: RNFL levels were symmetric between the 2 eyes in FRDA patients and markedly lower than control values. FRDA eyes had the greatest thinning in the superior and inferior quadrants, consistent with the normal distribution of thickness in control retinas. FRDA also affected the macular ganglion cell layer in both eyes and across all sectors. RNFL and ganglion cell thicknesses correlated with visual function (visual acuity, low contrast vision), disease duration, and age, and were predicted in linear regression models by age and genetic severity (GAA1repeat length). Correlations with visual dysfunction were substantially higher for RNFL values than ganglion cell values, suggesting that FRDA affects axons more severely than ganglion cells. Furthermore, RNFL levels correlated ($r=0.64$) with neurologic mFARS scores, showing that RNFL provides a marker of neurological progression in FRDA. Finally, RNFL values worsened longitudinally, with faster progression in patients with more severe genetic disease.

Discussion and Conclusions: The present study demonstrates that RNFL thickness provides a surrogate marker of progression in FRDA for ongoing clinical trials. The present data also suggest that the primary pathophysiology is a dying back optic neuropathy analogous to findings in other locations of progression in FRDA.

Funding Sources: FARA to DRL.

B9. Frataxin levels in blood of FRDA patients with Non-GAA repeats

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Background and objectives: Friedreich ataxia is caused by biallelic mutations in the FXN gene, 96% of which are expanded GAAs in intron1 that silence the gene and block frataxin production. Here, we assessed how the remaining 4 % of alleles alter frataxin levels in blood.

Methods: Blood samples from FRDA patients carrying non GAA- mutations were assessed for levels of FXN-M and FXN-E and compared with similar samples from individuals with biallelic GAAs.

Results: Mean GAA1length for the single expanded allele in compound heterozygote patients was 680 + 264. Among individuals carrying non-GAA mutations, mean frataxin E was 19.0 + 20.5 ng/ml. while frataxin M was 3.1 + 2.4 ng/ml. Both FXN-M ($r=-0.61$) and FXN E ($r=-0.32$) correlated with GAA1length and with each other ($r=0.46$). However, there were mutation specific features. Missense mutations associated with potential partial sparing of FXN protein had higher FXN-M levels than deletions or severe frameshift mutations. This could be explained by shorter GAA1length in the missense mutations. In addition, FXN-E (but not FXN-M) levels were markedly elevated in people with mutations in the first or second exons. However, levels of FXN-M and FXN-E were low in people with G130V mutations, a missense mutation associated with mild phenotype, and high in a subject with R165C, associated with a severe but paradoxical phenotype.

Discussion and Conclusions. Levels of FXN-E and FXN-M in blood, as detected by an assay to the middle portion of the protein, did not explain the paradoxical phenotype of selected missense mutations. The persistence of FXN-E in individuals with mutations in the first exon, consistent with the differential splicing of this region. Overall, this suggests that other explanations are needed to explain the atypical phenotypes associated with selected missense mutations.

Funding Sources: FARA

B11. Mechanism-Based Lipid Mediator Profiling in Friedreich's Ataxia: Bioactive lipid signatures of Inflammation, Resolution Failure and Cardiac Remodeling

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Background and objectives: Friedreich's ataxia (FA) is a rare inherited disease caused by GAA repeat expansion in the FXN gene, leading to frataxin deficiency, mitochondrial dysfunction, and progressive neurological and cardiac disease. Although cardiomyopathy is the leading cause of death in FA, we lack blood biomarkers that can detect early cardiac inflammation before irreversible damage occurs. Bioactive lipid mediators generated from Omega-6 and omega-3 fatty acids critically control inflammation, but are largely unexplored in FA biology. We hypothesized that mitochondrial dysfunction in FA interferes with this balance, thereby promoting chronic, unresolved inflammation leading to cardiac injury and fibrosis.

Methods: We used mass spectrometry to detect targeted lipid mediators in serum samples taken from a clinical cohort consisting of FA patients, heterozygous carriers, and age-matched healthy controls.

Results: FA serum showed elevations in short-chain fatty acids, including propionic acid, isobutyric acid, and 4-methyl-valeric acid, supporting broader metabolic disruption linked to mitochondrial dysfunction. Strong activation of inflammatory arachidonic acid pathways was observed, with significant increases in 12-HETE, 5-HETE, TXB2, LTB4, and PGF2 α . In contrast, the pro-resolving mediator lipoxin A4 was significantly reduced, suggesting that FA results in a failure to appropriately resolve inflammation.

Multivariate analysis demonstrated clear separation between FA and control samples, indicating coordinated remodeling of inflammatory lipid networks rather than isolated metabolite changes.

Discussion and Conclusion: To establish biological relevance, we will test these pathways in frataxin-deficient iPSC-derived cardiomyocytes and fibroblasts. These studies will determine whether restoring pro-resolving lipid signaling can reduce inflammatory and fibrotic pathways, while improving mitochondrial function.

Together, these findings point to a previously underappreciated inflammatory lipid imbalance in FA and identify oxylipin pathways as both promising blood biomarkers and potential therapeutic targets. By linking mitochondrial dysfunction to chronic inflammatory remodeling, this work aims to open new opportunities for biomarker development and anti-inflammatory therapies in Friedreich's ataxia.

Funding Sources: The funding for this project is from the CDMRP - Congressionally Directed Medical Research Program (DoD)

B12. Evaluation of Cardiac Markers and Clinical Metrics in Friedreich's Ataxia

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Objective: To evaluate cardiac specific markers and clinical metrics associated with Friedreich's Ataxia (FA) and FA cardiomyopathy severity and progression.

Background: Friedreich's Ataxia is a rare recessive neuromuscular disorder typified by progressive ataxia caused by extended GAA repeats in the frataxin gene. In addition to neurologic features, cardiomyopathy is a common finding in FA contributing to increased mortality. Research has focused on neuromuscular symptoms and indicators, however identifying effective cardiac markers and associated clinical metrics may help predict who will develop cardiac disease and benefit from early treatment.

Methods: As part of a multi-year cohort, FA patients and related heterozygous carriers had history, blood markers, and cardiac diagnostics evaluated. Neurologic and cardiac clinical status were evaluated via the FA Functional staging score, FA Rating Score- Activities of Daily Living (FARS-ADL), Scale for the Assessment and Rating of Ataxia, and the Kansas City Cardiomyopathy Questionnaire-12 (KCCQ-12).

Results: KCCQ-12 scores were lower in the FA group versus heterozygous carriers. Low KCCQ values correlated with a high functional staging score (Spearman's $\rho=-0.62$, $p<0.001$) and FARS-ADL (Spearman's $\rho=-0.67$, $p<0.001$) indicating advanced neurologic disease, however there was variability when compared to other neurologic metrics. In the FA group, low KCCQ-12 scores was associated with abnormal cardiac parameters including left ventricular volume and chamber size.

Conclusion: These results suggest that the KCCQ-12 may be a viable metric for FA cardiac disease however, further evaluation is needed as the contribution of significant neurologic dysfunction to the overall score is not known. With robust clinical, translational and molecular data points, this study provides the foundation for creation of a multi-measurement, integrated cardiac specific metric in FA to identify early heart disease and monitor progression. Future research will focus on evaluating longitudinal data and clarifying associations with both neurologic and cardiac clinical metric assessments with imaging and blood biomarkers.

Funding Sources: DOD "Mechanisms, Markers, and Risk Factors in Friedreich's Ataxia Heart Disease (FA-HD)"

B13. CeMoQu: A Multimodal Platform for Accessible and Quantitative Assessment of Ataxia

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Background and Objectives: The Scale for the Assessment and Rating of Ataxia (SARA) is widely used as a clinical rating scale to quantify ataxic motor impairment in movement disorders. However, SARA relies on examiner observation, which can introduce variability and limit reproducibility. Furthermore, the low prevalence of ataxia limits clinical data availability, posing challenges for longitudinal monitoring and data-driven research. To address these limitations, we developed Cerebellar Motor Quantification (CeMoQu), a web-based assessment platform.

Methods: CeMoQu implements key SARA-related tasks, including Nose-Finger, Finger Chase, Sitting, and Speech Disturbance, using MediaPipe-based motion and pose tracking, calibrated target-touch tasks, line-drawing analysis, and browser-based acoustic analysis. These modules evaluate upper-limb motor function, postural stability, and speech. Unlike observational scoring, CeMoQu converts task performance into continuous quantitative time-series data for longitudinal monitoring and computational analysis. A controlled pilot used predefined task conditions to evaluate scoring consistency and clinically interpretable outputs across SARA-based score categories.

Results: CeMoQu consistently generated scores aligned with predefined SARA-based scoring criteria. In controlled trials, all 30 trials per task matched their intended classifications. Across modules, upper-limb tasks captured trajectory deviation, discontinuity, turn characteristics, target error, reaction time, contact duration, tremor amplitude, and movement smoothness; the Sitting module captured postural sway and support-related metrics; and the Speech Disturbance module captured acoustic variability in loudness and pitch. CeMoQu's web-based design and compatibility with accessible, non-specialized consumer devices support its potential for regular longitudinal testing and broader data collection.

Discussion and Conclusion: These findings suggest that CeMoQu can extend observer-dependent SARA-based assessment toward structured digital measures and candidate digital biomarkers of ataxia-related motor and speech impairment. Additional SARA-based tasks, including Gait, Stance, and Fast Alternating Hand Movements, are currently being integrated. Future work will involve clinician-guided validation to map these measures onto clinically meaningful scoring frameworks.

Funding Sources: This research has been supported by the Life and Research Center of Voice of Calling NPO since 2018. A early version of this work was selected as a Top 5 Finalist for MIT Solve–The Amgen Prize, whose support enabled its presentation at the Concordia Annual Summit during UNGA 79.

B14. Quantifying (mutant) ataxin-1 protein levels in human biofluids: mission impossible?

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Background and objectives: Spinocerebellar ataxia type 1 (SCA1) is a neurodegenerative disorder caused by an abnormal expansion of a CAG repeat in the ATXN1 gene, resulting in the production of toxic mutant ataxin-1 protein with an elongated polyglutamine tract. Over time, this causes progressive motor impairment, dysarthria, and often early death. RNA-based therapies to lower toxic ataxin-1 levels require biomarker assays to provide early molecular evidence of drug action. However, currently no adequate assay exists to measure mutant ataxin-1 in cerebrospinal fluid (CSF) or blood.

Methods: We describe an effort to develop immunoassays for both total and pathogenic ataxin-1 using multiple detection platforms: Meso Scale Discovery (MSD), Single Molecule Counting (SMC), Simoa and Nucleic acid Linked Immuno-Sandwich Assay (NULISA). Multiple antibody combinations were evaluated for sensitivity and specificity on each immunoassay platform, and the best combinations were assessed in SCA1 patient derived CSF, plasma, and tear fluid samples. As an alternative approach, we explored the use of Liquid Chromatography–Mass Spectrometry (LC-MS).

Results: Despite good assay performance on recombinant protein, none of the immunoassays were sensitive enough to reliably detect ataxin-1 in SCA1 CSF. In plasma and tear fluid, total ataxin-1 levels were higher, but no difference in mutant ataxin-1 was seen between SCA1 and healthy donor samples. Using LC-MS, one potential ataxin-1-derived peptide was detected, but its source was not confirmed.

Discussion and Conclusion: CSF concentrations of ataxin-1 are below the detection threshold of current technologies. The apparent lack of specificity for mutant ataxin-1 in plasma and tear fluid may be explained by the relatively long polyQ repeat in unexpanded ataxin-1, combined with the possibility of mutant ataxin-1 becoming insoluble and undetectable in biofluids. Ataxin-1 detection in biofluids remains a major challenge for SCA1 clinical trials that need to measure ataxin-1 as a drug target engagement biomarker.

Funding Sources: Most of the work was funded by Vico Therapeutics, but the development on SMC was a collaboration between Evotec and Vico: In return for a reduced fee, Evotec can use assay details for third parties.

B15. Longitudinal measures of ferroptosis-related molecules in blood of patients with Friedreich's ataxia: 1-year longitudinal study

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Background and objectives: Ferroptosis is a non-apoptotic form of regulated cell death driven by iron-dependent lipid peroxidation, which has been recognized to have a critical role in the pathogenesis of Friedreich's ataxia (FRDA). In FRDA, markers of redox imbalance are well documented in animal and cellular disease models, while studies on patient blood are relatively few. In this study, we analysed a panel of ferroptosis-related molecules in adults and children with FRDA, with the aim of identify peripheral markers related to disease pathology.

Methods: We measured blood concentration of 4-hydroxyl-2-nonenals (4-HNE), hydroxyeicosatetraenoic acid (15-HETE), glutathione (GSH), oxidised glutathione (GSSG), and mRNA levels of major targets of nuclear factor erythroid 2-related factor 2 (NRF2), in 63 FRDA patients (51 adults and 12 children) and 63 age matched healthy controls. Biochemical values were correlated with clinical data (age, disease duration, and scores for ataxia severity scale). Blood and clinical assessments were repeated at 1-year follow up.

Results: At baseline, FRDA patients had significantly increased peripheral levels of 4-HNE and GSSG and decreased levels of GSH, compared to controls. Measurement of mRNA concentration of Nrf2-target proteins showed that children with FRDA had lower levels of glutathione peroxidase 4 (GPX4) compared to adult patients. At 1-year follow up, we observed a significant increase of 4-HNE, and of GPX4mRNA blood levels in patients. GPX4 correlated with age, disease duration, and clinical scores.

Discussion and conclusion: Findings from the present work confirm in a large cohort of patients that oxidative stress and lipid peroxidation are central processes in FRDA pathophysiology, and offer easily measurable potential markers for disease progression and drug response monitoring. Blood concentrations of ferroptosis-related molecules can effectively discriminate patients from controls. Levels of GPX4mRNA expression enable to trace metabolic defects during disease progression in patients with FRDA.

Funding Sources: Italian Ministry of Health (RC)

B16. Mature Frataxin Beyond Mitochondria in Friedreich's Ataxia

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Background and Objectives: Friedreich's ataxia (FRDA) is widely viewed as a mitochondrial disease caused by deficiency of frataxin, a nuclear-encoded protein imported into mitochondria and processed to mature mitochondrial frataxin (FXN-M). However, unappreciated prior studies in human cells and mouse models and our recent data suggest that this model is incomplete. We investigated whether canonical FXN-M is also present in the cytosol of disease-relevant mammalian cells and tissues. Previous studies have shown that cytosolic FXN can protect against ferroptotic stress.

Methods: Subcellular fractionation was performed in SH-SY5Y neuronal cells and mammalian heart. Cytosolic and mitochondrial fractions were analyzed by western blotting, immunoprecipitation, and targeted LC-MS/MS. Mitochondrial contamination was assessed using citrate synthase, VDAC1, and other mitochondrial markers. Proteoform identity was evaluated by detection of FXN-M N-terminal and internal peptides.

Results: Canonical mature frataxin was detected in cytosolic fractions from human SH-SY5Y cells, heart, and cerebellum. Cytosolic FXN-M was more abundant in heart and cerebellum than mitochondrial FXN-M under conditions where mitochondrial marker contamination was minimal. LC-MS/MS was used to confirm the presence of N-terminal and internal FXN-M peptides in both cytosolic and mitochondrial fractions.

Discussion: These findings challenge the prevailing view that mature FXN functions exclusively within mitochondria. Because FXN-M is generated by mitochondrial processing, its presence in cytosol implies an unrecognized pathway by which mature FXN reaches or persists outside mitochondria. This is analogous to HSP 60 which is processed in a similar manner in mitochondria but appears in both cytosolic and mitochondrial compartments. Therefore, a cytosolic pool FXN-M may contribute to cytosolic iron-sulfur protein maintenance as well as protection from ferroptotic injury.

Conclusion: Mature mitochondrial FXN forms an abundant cytosolic pool in disease-relevant mammalian tissues. FRDA may therefore involve deficiency of both mitochondrial and cytosolic frataxin, with important implications for pathogenesis, biomarkers, and therapy.

Funding Sources: Penn Institute on Aging

B17. An Anatomical Guideline for Upper Cervical Spinal Cord Vertebral Level Identification on Brain MRI

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Background and Objectives: Accurate spinal level identification in upper cervical spinal cord MRI is essential for quantitative analysis but remains challenging due to the lack of an intervertebral disc at C1-C2 levels to serve as an anchor point for segmentation. Standard guidelines to reliably identify C1 and C2 spinal levels are lacking and intrinsic anatomical variability of these vertebrae limits the performance of current automated labeling methods. Therefore, this study aimed to develop and evaluate an anatomical guideline for identifying upper cervical spinal cord spinal levels on brain MR images.

Methods: A manual annotation guideline based on anatomical landmarks was developed to define C1 and C2 spinal boundaries on routine 3D T1-weighted brain MRI. Reliability was assessed across annotators, participants, and longitudinal scans, using data from 20 randomly selected READISCA participants. Spatial concordance of C1–C3 labels was evaluated in participants with 5 longitudinal MRI datasets exhibiting varying spinal cord angulations and image quality. Performance metrics included intraclass correlation coefficients (ICC) and coefficients of variation (CV).

Results: The proposed guideline enabled consistent identification of upper cervical spinal levels across annotators (inter-rater mean ICC: 0.99) and longitudinal scans (intra-rater mean ICC: 0.99). C1–C3 labels demonstrated high spatial concordance and lower inter-participant variability for C1 and C2 labeling despite differences in cord angulation when compared to the leading automated tool (mean CV for C1: $2.4 \pm 0.9\%$ vs $4.7 \pm 3.3\%$, $p=0.034$; C2: $4.2 \pm 1.8\%$ vs $5.1 \pm 2.2\%$, $p=0.008$; C3: $5.6 \pm 3.9\%$ vs $6.3 \pm 5.4\%$, $p=0.475$).

Discussion and Conclusion: Standardized C1–C2 spinal landmarking on routine brain MRI enables reproducible localization of the upper cervical spinal cord spinal levels. This approach is expected to facilitate multicenter studies and to enable retrospective upper spinal cord analysis in large neuroimaging cohorts with existing T1-weighted brain MRI data, in the absence of dedicated spinal cord imaging.

Funding Sources: This work was supported by the National Institute of Neurological Disorders and Stroke (NINDS) grant U01NS104326 and São Paulo Research Foundation (Fapesp, grant #25/001864).

B18. Delayed clinical response to MRI changes in SCA1 and SCA3

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Background and Objectives: Disease-modifying therapies are under development for polyglutamine SCAs, but clinical trials remain challenging due to low longitudinal sensitivity of clinical outcome measures, especially at early stages. MRI biomarkers are emerging as potential surrogate endpoints, although their clinical relevance requires further validation. We aimed to determine if short-term change in MRI outcomes predicts subsequent clinical progression in SCA1 and SCA3.

Methods: READISCA MRI participants who were ataxic (SARA $\geq$ 3) or who were preataxic at baseline and phenoconverted (SARA $\geq$ 3 at one follow-up visit), and who had at least two SARA assessments after two MRI visits were included. Annualized changes in the top ten MRI biomarkers most sensitive to progression (Rezende et al, 2026 ACTN) were obtained from the first two MRI visits (mean MRI interval 0.85 years) and evaluated as predictors of subsequent clinical worsening using linear mixed-effects models and Spearman correlations (Holm adjusted). The impact of genotype, preataxic status at baseline, MRI-interval and SARA follow-up duration on the associations was assessed.

Results: Fifty participants were included (14 SCA1, 36 SCA3, 10 preataxic, mean baseline SARA=6 $\pm$ 3, mean age=46 $\pm$ 9 years). Annualized pons and whole brainstem atrophy correlated with subsequent SARA progression in the pooled cohort (pons: ρ =-0.56, p =0.0004; whole brainstem: ρ =-0.48, p =0.004). Pons volume change was associated with SARA change in each SCA, with a stronger correlation in SCA1 (SCA1: ρ =-0.64, p =0.017; SCA3: ρ =-0.49, p =0.0029). However, none of the tested covariates showed a significant interaction with pontine change.

Discussion and Conclusions: Slower brainstem atrophy was associated with subsequent slower clinical progression in SCA1 and SCA3, supporting the potential clinical relevance of MRI biomarkers as surrogate endpoints in therapeutic trials. The temporal sequence between imaging and clinical changes suggests that treatment-related neurodegenerative slowing may precede detectable clinical benefits, with important implications for the design and duration of future clinical trials.

Funding Sources: This work was supported by the National Institute of Neurological Disorders and Stroke (NINDS) grant U01NS104326 and São Paulo Research Foundation (Fapesp, grant #25/001864).

B19. Cross-sectional and longitudinal analysis of posture and gait in RFC1-related disorder using OPAL sensors

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Background and Objectives: Quantitative biomarkers able to capture disease severity and progression in slowly progressive ataxias are urgently needed. Inertial motion sensors emerge as promising candidates but have not yet been explored in RFC1-related disorder, a frequent cause of late-onset ataxia.

Methods: We used the OPAL® sensors placed in 6 different body regions to capture posture and gait performance in 31 RFC1patients and 24 age and sex-matched healthy controls. Thirteen patients were reassessed one year thereafter. Between-group comparisons (patients vs controls) were performed using the Mann–Whitney U test. Correlations with SARA scores were assessed using Spearman's rank correlation coefficient. Longitudinal within-patient analyses were performed using the Wilcoxon signed-rank test. Effect-sizes (ES) were calculated using Cohen's d for cross-sectional analyses and standardized response mean (SRM) for longitudinal analyses.

Results: Mean age was 63.3 ± 11.2 years and mean SARA score was 9.5 ± 6.1 in this cohort. Postural parameters that best separated patients and controls were postural sway sagittal acceleration centroidal frequency with feet together (ES=1.68), postural sway sagittal acceleration path length with feet together (ES=1.64), and postural sway acceleration centroidal frequency with eyes closed (ES=1.49). Considering gait parameters, the best parameters were toe-off angle (ES=-1.42) and lower limb elevation at midswing (ES=1.41). SARA scores correlated with sagittal acceleration path length ($R=0.74$, $p=0.003$), toe-off angle ($R=-0.73$, $p<0.001$), and elevation at midswing ($R=0.71$, $p<0.001$). In the longitudinal analysis, the highest SRM values were observed for upper limb arm swing velocity (SRM=0.74), Δ sway acceleration centroidal frequency (SRM=-0.68), and turn velocity (SRM=0.66).

Discussion and Conclusion: Inertial motion sensors are able to capture disease severity and progression in RFC1-related disorder, with several parameters showing large cross-sectional and longitudinal ES. These are potential biomarkers to be included in clinical trials for the condition.

Funding Sources: This work was funded by Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP, São Paulo, Brazil, grant number #2013/07559-3.

B23. Comparative Evaluation of Cerebellar Segmentation Pipelines for Generating Reliable and Sensitive MRI Biomarkers in Degenerative Cerebellar Diseases

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BACKGROUND: Reliable segmentation of the cerebellum and its anatomical subregions (lobules) from MRI enables detailed spatial descriptions, accurate longitudinal tracking, and robust biomarker validation of volumetric changes in people with cerebellar diseases. In this study, we compare the relative reliability and sensitivity of contemporary automated cerebellar parcellation algorithms applied to structural MRI from people with Friedreich ataxia (FRDA) and SCA.

METHODS: We evaluated three segmentation (ACAPULCO, CerebNet, DeepCERES) and three atlas registration (ANTs, CAT12, SUIT) approaches for whole cerebellum volume and lobule-level volume estimation across three performance metrics:

- test-retest reliability: mean within-subject coefficient of variance (wCV) in 67 SCA2, SCA3, FRDA & Controls scanned twice within two weeks
- cross-sectional sensitivity: Cohen's d in 160 FRDA vs. 86 controls and 53 SCA3 vs. 14 control
- longitudinal sensitivity: Standardised Response Mean (SRM) in 136 FRDA and 33 SCA3 scanned twice with 1-year interval

RESULTS: Test-retest reliability, wCV:

- Whole cerebellum: DeepCERES (0.53%) < ACAPULCO (0.74%) < CAT12 (0.95%) < ANTs (0.99%) < CerebNet (1.2%) < SUIT (1.5%).
- Average lobule-level: DeepCERES (2.0%) ≈ CAT12 (2.0%) < SUIT (2.2%) < ANTs (2.4%) < CerebNet (2.6%) < ACAPULCO (3.0%)

Cross-sectional sensitivity, Cohen's d relative ranking:

- Whole cerebellum: DeepCERES ≈ CerebNet > ACAPULCO > CAT12 > ANTs > SUIT
- Average lobule-level: DeepCERES ≈ CAT12 > SUIT > CerebNet ≈ ANTs > ACAPULCO

Longitudinal sensitivity, SRM relative ranking:

- Whole cerebellum: DeepCERES ≈ CerebNet > ACAPULCO ≈ CAT12 > SUIT
- Average lobule-level: DeepCERES ≈ CerebNet ≈ SUIT > ACAPULCO ≈ CAT12

CONCLUSION: Segmentation typically outperforms atlas registration. DeepCERES is consistently the top-performing algorithm and CerebNet provides comparable cross-sectional and longitudinal disease sensitivity. Results are similar between independent FRDA and SCA3 datasets, supporting generalisability. These findings provide practical, empirically grounded guidance for analytical pipeline selection in ataxia research.

Funding Sources: TRACK-FA: FARA, Takeda Pharmaceuticals Company, Novartis Gene Therapies, IXICO and PTC Therapeutics, READISCA: National Institute of Neurological Disorders and Stroke (NINDS). BRP: NIH Bioengineering Research Partnerships

Investigator Support: Australian National Health and Medical Research Council.

B24. The Use of Sensor-Insoles for Quantifying Upright Stability during Stance in Patients with Friedreich's Ataxia

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Background/Objectives: Friedreich's ataxia (FRDA) is a rare neurodegenerative disorder with ataxia being a hallmark symptom. FRDA is primarily assessed clinically using the Scale for the Assessment and Rating of Ataxia (SARA) and modified Friedreich's Ataxia Scale (mFARS). However, quantifiable measures with higher sensitivity to capture subtle changes in disease progression are necessary for future therapy evaluation. This study examines digital markers utilizing sensor-based insoles during stance.

Methods: 28 patients with FRDA and 28 controls (53% male, mean age: 28.8 and 26.8 years, respectively) were examined. Four stance tasks (mFARS sub-item E), feet apart (FA) and together (FT) with eyes open (EO) and eyes closed (EC) conditions, were recorded for minimum 10 seconds using pressure-sensor equipped insoles inserted into participant's shoes. Features were extracted for each foot's center of pressure individually and averaged. Group-wise statistical differences were assessed applying the Bonferroni correction for $\alpha = 0.05$.

Results: Completion rates varied per test for patients with FRDA (100% FA-EO, 42.9% FA-EC, 64.3% FT-EO, 14.3% FT-EC). Preliminary results show that 102/247 (41.3%) features were significantly different ($p < 0.001$) between patients with FRDA and controls, with 101/102 (99%) features exhibiting consistently large effect sizes (Cohen $d > 0.8$). Compared to controls in the FA-EO, FA-EC and FT-EO tasks, patients with FRDA had higher sway speed and longer, broader sway paths. The sway velocity and acceleration signals were more variable, and their mean frequencies were lower. The FA-EC task exhibited the greatest quantity of statistically significant differences (93/247 features). All significant features for FA-EO and FT-EO correlated with SARA stance scores with Pearson r -values of (0.34-0.85) and (0.50-0.73), respectively.

Discussion/Conclusion: Features from sensor-insoles are strong candidates for monitoring disease progression in clinical trials. The current findings are limited to linear, cross-sectional relationships; however, they lay the foundation for longitudinal feature investigation.

Funding Sources: This work was supported by a grant from the Interdisciplinary Center for Clinical Research within the faculty of Medicine at RWTH Aachen University, Germany (OC2).

B25. Large Scale Plasma Proteomics Profiling in Friedreich's Ataxia Using the FACOMS Natural History Cohort

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Background: Friedreich's ataxia (FA) is a rare, progressive, debilitating, and fatal multi-organ disease characterized by progressive neurological dysfunction, cardiomyopathy, and multisystem involvement. While the genetic etiology is well defined, there remains a need for robust and minimally invasive fluid-based biomarkers that can easily be measured longitudinally to monitor disease severity and progression, and which can be developed and verified as pharmacodynamic response biomarkers upon treatment.

Objective: To identify and characterize plasma protein biomarkers associated with disease onset, severity, and progression in FA clinical samples using high-throughput proteomic profiling integrated with longitudinal clinical data.

Method: Plasma samples were obtained from FA patients (n=42) enrolled in the FACOMS natural history study and from healthy controls (n=40). For FA patients, three serial plasma samples were collected at regular intervals over the course of 5 to 8 years. Proteomic profiling was performed using the Olink Explore HT panel. Supporting data included demographic information, age of onset, type of mutation and functional status as assessed by the modified Friedreich's Ataxia Rating Scale (mFARS). Orthogonal methods were used to validate the O-Link proteomic findings.

Results: Olink HT Explore panel readouts were used to describe and analyze longitudinal changes in the FA proteome compared to healthy controls. Analysis included the stratification of proteomics data by age of onset and by disease severity. The resulting dataset may enable evaluation of FA-related proteomic signatures, pathway perturbations, and longitudinal biomarker trends, supporting translational biomarker discovery and informing future clinical research and therapeutic development.

Funding Sources: Solid Biosciences sponsored this work.

B26. A Novel Composite Digital Gait and Balance Score for Spinocerebellar Ataxia

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Background and Objectives: Clinical trials in spinocerebellar ataxia (SCA) require large sample sizes, and although digital gait measures capture disease features, their utility as endpoints is not fully established. We aimed to develop and validate a composite digital gait and balance measure, the Score of Integrated Balance in Ataxia (SIBA), using wearable sensors.

Methods: SIBA was developed from retrospective cross-sectional data and validated in an independent cohort from the IDEA longitudinal study (NCT04268147), including adults aged 18–75 years with genetically confirmed SCA types 1, 2, 3, or 6 across five sites. Gait and balance were assessed using six wearable inertial sensors during a 2-minute walk and standardized standing tasks. A multiple criteria decision analysis framework guided development, incorporating discrimination, validity, reliability, progression, and clinical relevance. Participants were assessed at baseline, 6 months, and 12 months. Effect size, defined as the ratio of the group-average slope to its standard error, quantified sensitivity to progression. Performance was compared with the Scale for the Assessment and Rating of Ataxia (SARA).

Results: The development cohort included 258 participants with SCA and 100 controls; the validation cohort included 53 participants and 24 controls. The final score combined two gait variables and two balance variables. SIBA demonstrated strong concurrent validity with SARA ($r=0.736$) and excellent test-retest reliability ($ICC=0.970$). It effectively differentiated patients from controls ($AUROC=0.956$) and was associated with fall risk ($AUROC=0.760$). Over one year, SIBA showed greater sensitivity to progression than SARA (effect size 0.59 vs 0.11). Estimated sample sizes for clinical trials were reduced by 88% using SIBA.

Discussion and Conclusion: SIBA is a reliable and sensitive digital outcome capturing static and dynamic balance in SCA compared to SARA. Further studies are warranted to confirm responsiveness to therapeutic interventions in larger cohorts.

Funding Sources: This IDEA (Instrumented Data Exchange for Ataxia) study was sponsored by Biogen and Pfizer.

B27. The Ataxia Instrumented Measurement System (AIMs) predict TRACK-FA MRI-derived biomarkers in Friedreich Ataxia.

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Background and objectives. MRI can track disease progression in Friedreich ataxia (FRDA) but cost and participant burden limit MRI scanning in long-term monitoring. This cross-sectional study investigated whether movement features derived from inertial and force signals from the Ataxia Instrumented Measurement System (AIMs) can be used to build models that predict MRI changes in FRDA, thus serving as an alternate endpoint in clinical trials.

Methods. Sensor signals were recorded from 17 participants with FRDA (34 visits) during simulated feeding (AIMs-Spoon), drinking (AIMs-Cup) and standing (AIMs-Pendant). These data were paired with MRI data obtained in a concurrent study (TRACK-FA), including structural and diffusion features of the cerebellum and spinal cord. Using leave-one-participant-out cross-validation and a model-based residual bootstrap test with false discovery rate (FDR) correction, machine learning models using AIMs derived movement features were trained on MRI features and evaluated for their ability to predict MRI changes.

Results. The strongest predictions by models using the AIMs were the superior cerebellar peduncle (SCP) diffusivity, dentate nucleus (DN) volume and susceptibility and cervical spinal cord (CSA) diffusion metrics. Features from the AIMs-Cup predicted SCP fractional anisotropy ($r = 0.88$, $pFDR < 0.001$) and SCP volume ($r = 0.70$, $pFDR = 0.018$). The AIMs-Pendant predicted CSA ($r = 0.76$, $pFDR = 0.022$) and SCP axial diffusivity ($r = 0.8$, $pFDR = 0.011$). Comparison of movement segments revealed that the AIMs_Spoon returned the best predictive segments, providing the strongest predictions for SCP radial diffusivity ($r = 0.90$, $pFDR = 0.028$), DN volume ($r = 0.85$, $pFDR = 0.042$) and DN susceptibility ($r = 0.84$, $pFDR = 0.042$).

Discussion and Conclusion. This study demonstrated that data derived from the AIMs devices predict important FRDA neuroanatomical changes as measured by MRI. Low cost and low burden AIMs assessments can be completed in the clinic or home and complement annual neuroimaging as a robust outcome measures of efficacy in clinical trials.

Funding Sources: TRACK-FA is funded by grants from the Friedreich's Ataxia Research Alliance (FARA, USA) to each of the academic sites and IXICO with financial support from Takeda Pharmaceuticals Company, Novartis Gene Therapies, IXICO, Larimar Therapeutics Inc., and PTC Therapeutics.

B29. A Web-Based Video Framework for Objective Finger Chase Assessment in Ataxia

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Background and Objectives : Ataxia refers to a neurological disorder marked by motor impairments and lack of limb coordination. The finger chase test is a clinical scale used to assess upper-limb motor performance. However, traditional methods rely on ataxia specialists to visually score patients' movement quality, which is subjective and poorly accessible. This study presents a novel web-based video framework for finger chase motor assessment. The framework allows for intuitive interaction and cross-platform accessibility without the need for specialized hardware.

Methods: A proof-of concept study was conducted involving six individuals with ataxia and four age-matched healthy controls. Temporal (execution time) and spatial (distance ratio) metrics were extracted to assess motor performance.

Results: Results showed that individuals with ataxia exhibited significantly longer execution times ($p < 0.05$) and higher distance ratios ($p < 0.05$) compared to healthy controls.

Discussion and conclusion: These findings suggest that the proposed approach effectively identifies differences between ataxia and healthy groups, offering a rapid, objective, and scalable tool for ataxia assessment that could facilitate both in-clinic and remote evaluations.

Funding Sources: This work was supported by the National Natural Science Foundation of China under Grant W2441018

B30. Volumetric Analysis of Cerebellar Lobules in Spinocerebellar Ataxia 27B (SCA27B)

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Background and Objectives. Spinocerebellar ataxia 27B (SCA27B) is an autosomal dominant, late-onset cerebellar ataxia caused by an intronic FGF14 GAA•TTC repeat expansion and frequently associated with cerebellar atrophy. In this study, we aimed to quantify the pattern of cerebellar atrophy in SCA27B as well as investigate whether it is more evident in some sub-cerebellar regions using automated volumetric analysis in clinical MR images.

Methods: We analyzed 3D and sagittal T1-weighted MRI scans from 13 subjects with genetically confirmed SCA27B (median age: 67 years, IQR: 9 years, 2 females; median GAA size 383) and 52 age- and sex-matched controls. Cerebellar segmentation was performed using the DeepCERES pipeline. For each region of interest, we calculated mean volume, standard deviation, and relative volume differences between groups. Analysis of covariance (ANCOVA) was used to test the effect of disease status on the volumes while adjusting for age and sex.

Results: The volume was significantly reduced in all regions of interest, except the whole white matter, lobules I–II and IX, with a percentage of volume reduction of at least -10% when compared to controls. The greatest relative volume difference was seen in lobules IV (-25%), VI (-18%), and X (-18%).

Discussion and Conclusion: SCA27B is associated with diffuse cerebellar atrophy with relatively important involvement of lobules IV and X. Automated segmentation tools such as DeepCERES enable reliable volumetric characterization from clinical MRI and may support future comparative studies of SCA27B and other forms of late-onset ataxias.

Funding Sources: Ataxia Canada

B31. Assessment of Dorsal Root Ganglia Involvement in SCA27B Using Lumbar Spine MRI

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Background and Objectives: SCA27B is a recently recognized late-onset autosomal dominant ataxia characterized by progressive cerebellar ataxia and oculomotor abnormalities. Sensory neuropathy may also occur, although it is typically mild and nonspecific. Clinically, SCA27B may overlap with CANVAS, a disorder in which sensory symptoms result from dorsal root ganglia (DRG) degeneration, a core pathological hallmark of the disease. However, the structural involvement of DRG in SCA27B remains poorly understood. Therefore, this study aimed to investigate DRG involvement in patients with SCA27B.

Methods: In this cross-sectional study, genetically confirmed patients with SCA27B and healthy controls underwent standardized 3T Philips MRI acquisition. Dorsal root ganglia (DRG) assessment of the lumbar spine was performed using a coronal isotropic PROSET T2-weighted sequence. Manual DRG segmentation was conducted using ITK-SNAP software, focusing on bilateral L2–L5 levels. Disease severity in SCA27B patients was assessed using the Scale for the Assessment and Rating of Ataxia (SARA).

Results: Twelve participants were included, comprising 6 patients with SCA27B (4 males; median age: 64.5 years [62–73]) and 6 healthy controls (3 males; median age: 50.5 years [40–64]). No statistically significant differences in DRG volumes were observed between groups across any lumbar level analyzed (all $p > 0.05$). However, effect size analysis demonstrated moderate-to-large Cohen's d values at bilateral L2 (left: $d = 0.97$; right: $d = 0.68$) and right L3 ($d = 1.00$), with lower DRG volumes observed in patients with SCA27B compared with controls.

Discussion and Conclusion: These preliminary findings suggest that marked structural ganglionopathy may not represent a major pathological feature of SCA27B, despite moderate-to-large effect sizes observed at selected lumbar levels. Hence, DRG imaging may represent a promising biomarker to help differentiate SCA27B from CANVAS and improve the characterization of sensory involvement in late-onset hereditary ataxias.

Funding Sources: This work was supported by the São Paulo Research Foundation (Fapesp, grants #25/00186-4, 2025/24347-7).

B32. Erythrocyte lipofuscin-like pigments mark oxidative stress and decrease after omaveloxolone in patients with Friedreich ataxia

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Background and objectives: Oxidative stress (OS) driven by mitochondrial dysfunction is a major contributor to neuronal injury in Friedreich ataxia (FA), but validated OS biomarkers in FA are lacking. Lipofuscin-like pigments (LFPs) are naturally fluorescent end-products of lipid peroxidation and may reflect cumulative oxidative damage. Omaveloxolone, the first approved FA-specific treatment, activates the Nrf2 antioxidant pathway and increases resistance to oxidative injury in preclinical models. We aimed to assess whether erythrocyte LFPs are increased in FA and whether they decrease after omaveloxolone treatment.

Methods: Erythrocytes were obtained from FA patients at baseline and after 6 and 12 months of omaveloxolone treatment, and from healthy controls (HC). LFPs were extracted and analyzed using complex fluorimetric profiling, including 3D excitation-emission and synchronous fluorescence spectra. Baseline FA-HC differences and longitudinal changes during treatment were evaluated. Statistical comparisons were corrected for multiple testing.

Results: Ten FA patients and 19 HC were included. At baseline, FA patients showed higher LFP fluorescence than HC across 9 fluorescence maxima (excitation/emission), indicating increased lipid peroxidation-derived OS (all $p < 0.05$). Four of these maxima fully discriminated FA from HC in this cohort, with 100% sensitivity and specificity and no overlap in fluorescence intensity. These 4 maxima showed a slight, non-significant decrease after 6 months of omaveloxolone and a significant decrease after 12 months: 290/350 nm by 30.3% versus baseline, $p = 0.044$; 333/358 nm by 27.9%, $p = 0.004$; 285/335 nm by 29.1%, $p = 0.016$; and 310/360 nm by 23.7%, $p = 0.034$.

Discussion and conclusion: Analysis of erythrocyte LFPs identified increased OS in FA patients and showed a gradual reduction during omaveloxolone treatment, with a significant effect after 12 months. These data support LFPs as candidate biomarkers of OS and treatment response in FA, and provide preliminary clinical evidence consistent with omaveloxolone-mediated modulation of OS. Further studies are needed to establish their biological and clinical relevance.

Funding Sources: Supported by Biogen through an investigator-initiated trial grant, ID CZ-SKY-12330. The study was investigator-initiated and conducted independently of the funder.

B33. Automated Deep Gray Matter Nuclei Segmentation in Friedreich Ataxia – Proof of concept and preliminary results

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Background and Objectives: Deep gray matter (DGM) structures play a central role in the pathophysiology of Friedreich ataxia (FRDA), particularly within cerebello-thalamo-rubral pathways involved in motor dysfunction and neurodegeneration. Quantitative susceptibility mapping (QSM) enables in vivo assessment of iron-sensitive changes in these regions, but a reliable segmentation of DGM nuclei remains challenging due to their relatively small size and complex anatomy, as well as limited visibility on conventional MRI. This study aims to establish expert-defined reference standard annotations for basal ganglia structures on QSM images as a prerequisite for developing a dedicated automated segmentation pipeline.

Methods: In this proof-of-concept study, QSM datasets from 3 subjects (2 healthy controls and 1 FRDA patient) were retrospectively analyzed. An expert neuroradiologist manually annotated seven bilateral structures: caudate nucleus, putamen, globus pallidus externus, globus pallidus internus, thalamus, substantia nigra, and red nucleus. The same images were independently processed using MRICloud, serving as the baseline for the current automated method. Spatial overlap between expert reference masks and MRICloud outputs was quantified using the Dice similarity coefficient (DSC).

Results: Comparison with MRICloud yielded a mean DSC of 0.73 ± 0.10 across all annotated structures. The highest agreement was observed for the putamen (DSC: 0.81 ± 0.01), while globus pallidus internus showed the lowest DSC (0.64 ± 0.15). Qualitative assessment demonstrated better boundary definition and spatial consistency of the expert annotations relative to the atlas-based outputs, which often failed to accurately delineate subregional anatomy.

Discussion and Conclusion: Preliminary results highlight the limitations of atlas-based tools, supporting the rationale for refined segmentation approaches. Defining robust annotations performed by experts is a mandatory first step toward training deep learning models. Accurate automated segmentation of these structures will facilitate large-scale investigations of iron-sensitive biomarkers in FRDA and other neurodegenerative ataxias, supporting future longitudinal and multicenter studies.

Funding Sources: Research was supported by a FARA (USA) grant, and the following agencies: FAPESP and CAPES (Brazil), and NHMRC (Australia). TRACK-FA is funded by grants from FARA grants to each of the academic sites and IXICO, with financial support from Takeda, Novartis Gene Therapies, IXICO, Larimar Therapeutics, and PTC Therapeutics.

B34. A deep learning framework for automated cervical spinal cord vertebral labeling and morphometrics from brain MRI with multicenter validation

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Background and Objectives: Accurate identification of upper cervical spinal cord (SC) vertebral levels on MRI is essential for quantitative SC analysis, especially in hereditary cerebellar ataxias, but remains challenging in routine brain imaging. This study aimed to develop and validate a fully automated pipeline for C1–C3 vertebral level identification in T1-weighted brain MRI, including an integrated quality control (QC) module for automated assessment of generated masks.

Methods: MRI data from 998 controls and 879 individuals with hereditary ataxias acquired across 32 sites (age range: 5–85 years; 46% male) were used to train deep learning models, including nnU-Net, U-Net with deep supervision, and SwinUNETR, for automated C1–C3 vertebral labeling. A secondary QC model based on VGG, ResNet, and EfficientNet architectures was trained to detect vertebral labeling errors. The pipeline also generated image quality metrics, including signal-to-noise ratio, contrast-to-noise ratio, coefficient of joint variation, and entropy focus criterion. External validation was performed in 2,737 MRIs from four public datasets, and clinical utility was assessed in a longitudinal cohort of 51 individuals with multiple sclerosis.

Results: The nnU-Net achieved the highest performance (Dice coefficients: C1= 0.85, C2= 0.89, C3= 0.89) and correctly identified 95.8% of mislabeled cases generated by the Spinal Cord Toolbox. The VGG-based QC classifier achieved 97.9% accuracy and 98.7% sensitivity. External validation demonstrated labeling accuracies above 92% in four of five datasets and sensitivity to longitudinal SC atrophy in multiple sclerosis.

Discussion and Conclusion: This automated pipeline enables scalable and reliable upper cervical SC labeling and morphometric extraction for multicenter studies, supporting biomarker development in neurodegenerative diseases.

Funding Sources: Research was supported by a FARA (USA) grant, and the following agencies: FAPESP and CAPES (Brazil), and NHMRC (Australia). TRACK-FA is funded by grants from FARA grants to each of the academic sites and IXICO, with financial support from Takeda, Novartis Gene Therapies, IXICO, Larimar Therapeutics, and PTC Therapeutics.

B35. Evaluating Glymphatic Dysfunction in Spinocerebellar Ataxias and Friedreich's Ataxia Using the DTI-ALPS Index: A Comparative Cross-Sectional Study

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Background and objectives: Spinocerebellar ataxias (SCAs) and Friedreich's ataxia (FRDA) are chronic neurodegenerative disorders characterized by progressive ataxia and extensive microstructural white matter damage. Emerging evidence highlights the role of glymphatic system impairment in the pathogenesis of various neurodegenerative diseases. We aimed to evaluate glymphatic function across patients with SCA1, SCA2, SCA3, SCA6, and FRDA compared to healthy controls using the non-invasive Diffusion Tensor Image Analysis Along the Perivascular Space (DTI-ALPS) index.

Methods: We conducted a cross-sectional neuroimaging study evaluating 135 participants (mean age 28±13 years; 63% female): 17 healthy controls and 118 patients with hereditary ataxias (SCA1: n=27, SCA2: n=17, SCA3: n=35, SCA6: n=18, FRDA: n=21). All subjects underwent 3T MRI (Philips Achieva) using a standard single-shell DTI protocol (b1= 1000 s/mm², 2-mm isotropic resolution, 32-channel head coil). Diffusion processing and automated DTI-ALPS index calculation were performed utilizing FSL pipelines and a dedicated 3D Slicer module. DTI-ALPS indices were statistically compared between patients and healthy controls.

Results: The DTI-ALPS index was significantly decreased in all ataxia cohorts compared to healthy controls. Mean values and statistical significance for each cohort were as follows: SCA1(p = 3.31x10⁻¹³), SCA2(p = 5.49x10⁻⁷), SCA3(p = 4.89x10⁻¹⁴), SCA6(p = 3.46x10⁻⁷), and FRDA (p = 2.55x10⁻⁹). Notably, the SCA3 cohort demonstrated the most pronounced reduction and highest statistical significance in perivascular diffusivity compared to controls.

Discussion and Conclusion: Our findings demonstrate robust, widespread impairment of the glymphatic system across multiple hereditary ataxias. These results complement previous evidence establishing extensive microstructural white matter damage and widespread neurodegeneration in SCA3, suggesting that perivascular clearance deficits may play a key parallel role in disease pathogenesis. The DTI-ALPS index serves as a sensitive, non-invasive imaging biomarker that could improve diagnostic classification and track disease progression or therapeutic efficacy.

Funding Sources: This work was supported by the São Paulo Research Foundation (FAPESP), grant #2013/07559-3

B38. Quantifying progression of speech abnormalities in spinocerebellar ataxia type 1 – a longitudinal study

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Background and objectives: Dysarthria in spinocerebellar ataxia type 1 (SCA1) is characterized by abnormalities in speech timing, diadochokinetic rate, naturalness and intelligibility. Acoustic and perceptual speech outcomes might serve as patient-relevant measures for clinical trials, but their sensitivity to capture progression remains unclear. We aimed to evaluate and compare the performance of clinician-based ratings, perceptual ratings by speech therapists, and automated acoustic analyses to detect progression of speech abnormalities in SCA1.

Methods: Thirty-three SCA1 individuals and 21 healthy controls participated in a longitudinal natural history and biomarker study. Acoustic and perceptual speech ratings, as well as clinical disease severity assessments, were conducted at baseline, one (Y1) and two years (Y2) of follow-up. Speech was rated by trained speech therapists and by a clinician. Automated acoustic analysis generated outcomes related to speech timing and machine-learning (ML)–derived composite measures of speech naturalness and intelligibility.

Results: At Y1 and Y2, the clinician-reported SARA speech item demonstrated significant progression. ML-derived composites of naturalness and intelligibility showed significant deterioration, with ML-derived naturalness detecting change after Y1 and both measures after Y2. Regarding responsiveness, ML-derived naturalness slightly outperformed the SARA speech item; however, it did not exceed overall disease progression reflected by the total SARA score. Non-machine-learning acoustic measures showed inconsistent changes at the group level. Among therapist-rated perceptual measures, only dysarthria severity worsened significantly at Y2, whereas ratings of naturalness and intelligibility did not show significant longitudinal decline.

Conclusion and discussion: Machine learning–based composite measures of speech naturalness and intelligibility, as well as perceptual speech assessments, can detect longitudinal changes in dysarthria severity in SCA1. ML-derived naturalness and the clinician-rated SARA speech item were the most sensitive measures, capturing disease progression after one year. In contrast, acoustic timing measures revealed only small and inconsistent changes, likely reflecting variability in speech performance.

Funding Sources: The study was supported by a grant from ZonMW (40-44600-98-606).

B39. Complementary platelet and lymphocyte mitochondrial signatures in Friedreich's ataxia: MEABET phase 1

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Background and objectives: Peripheral mitochondrial biomarkers are increasingly evaluated in Friedreich's ataxia (FA), but platelet and lymphocyte oxidative phosphorylation (OXPHOS) enzyme activities and coenzyme Q10 readouts remain insufficiently characterized. We compared omaveloxolone-naïve platelet and lymphocyte enzyme profiles and coenzyme Q10 blood levels to define the clearest blood-cell signature and assess whether the two compartments provide complementary pathophysiological information.

Methods: We analyzed the first-available sample per subject from the harmonized UNIFAI dataset, including 31 FA patients and 26 healthy controls (collected 2021-2025). Platelet and lymphocyte raw activities of citrate synthase (CS), a surrogate marker of mitochondrial content, and OXPHOS Complexes (C_I to C_IV), CS-normalized ratios, and plasma Q10 levels were compared using Mann-Whitney tests with false-discovery-rate (FDR) correction.

Results: Platelets showed the strongest FA-control separation. Raw platelet C_IV was markedly lower in FA than controls ($p < 0.001$), and C_IV/CS was the strongest signal after CS-normalization ($p < 0.001$). Platelet C_II/CS was also reduced ($p < 0.001$), with smaller reductions in raw C_II, raw C_I, and C_I/CS ratio (all $p < 0.05$). Platelet C_III and C_III/CS were not significant. Lymphocytes showed a contrasting pattern: the only FDR-significant difference was higher raw C_III in FA ($p < 0.05$), whereas CS and raw Q10 were nominally higher ($p < 0.05$). CS-normalized OXPHOS ratios did not show significant FA-control differences after correction. Exploratory analyses suggested that lymphocyte measures, particularly lower C_II and lower C_I/CS, related more closely to INAS upper-limb findings and SARA severity. Paired analyses confirmed compartmental non-equivalence, with FA-control platelet-lymphocyte balance differences most evident for C_II and C_III.

Discussion and Conclusion: Baseline omaveloxolone-naïve FA shows a platelet-dominant OXPHOS deficit, especially C_IV/CS and C_II/CS ratios, indicating impaired respiratory-chain activity relative to mitochondrial content. Overall, platelets best discriminated FA from controls, whereas lymphocytes linked mitochondrial variation to neurological burden. These findings support dual-compartment blood-cell profiling in future FA biomarker studies.

Funding Sources: Grant Agency of Charles University (GAUK grants 2021-2022 No. 309121), EFACTS (NCT02069509), UNIFAI (NCT06016946), and the Friedreich's Ataxia Research Alliance (FARA; MEABET-FA project 2024-2026).

B40. Phase-dependent platelet mitochondrial remodeling after omaveloxolone initiation in Friedreich's ataxia

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Mitochondrial dysfunction is central to Friedreich's ataxia (FA). Our previous work identified reduced platelet oxidative phosphorylation (OXPHOS) enzyme activities, especially Complex IV and II, supporting their potential as treatment-monitoring biomarkers. Omaveloxolone, an Nrf2 activator and approved FA therapy, targets pathways linked to mitochondrial resilience and oxidative stress. MEABET-FA phase 2 assessed omaveloxolone-associated changes in platelet OXPHOS activities, citrate synthase (CS; marker of mitochondrial mass), and coenzyme Q10 (Q10).

We analyzed 18 patients initiating omaveloxolone during longitudinal follow-up. Platelet CS, OXPHOS Complexes I-IV, CS-normalized ratios, and plasma Q10 were assessed at pre-treatment baseline, 6-month/12-month post-treatment windows. Paired changes used Wilcoxon signed-rank tests. Annualized slopes were calculated as paired percent change per year; 12 healthy controls (HC) and 4 untreated FA patients provided natural-history context.

After omaveloxolone initiation, CS decreased at 6 months and rose by 12M, indicating mitochondrial-content remodeling. Q10 showed no consistent treatment-associated change. Platelet respiratory-chain activity increased, predominantly involving Complex IV. Raw Complex IV increased at 6M and remained elevated at 12M ($p \leq 0.001$), while ratio IV/CS also increased at 6M and 12M ($p = 0.011$ and $p = 0.025$), with attenuation by 12M. Raw Complex I and III increased mainly at 12M, whereas ratios I/CS and III/CS changed less clearly. Ratio II/CS showed an early signal that was not maintained. Annualized slopes suggested phase-dependent platelet remodeling rather than simple normalization, with early IV/CS ratio increase, later CS rebound, and later Complex III rise. Untreated FA trajectories also showed upward drift relative to HC for some markers, supporting biomarker sensitivity but limiting treatment-specific interpretation.

These results provide pilot pharmacodynamic evidence that omaveloxolone is associated with platelet OXPHOS remodeling in FA. Combined raw and CS-normalized readouts may help distinguish respiratory-chain activation from mitochondrial-content remodeling in treatment-monitoring studies in future trials. Larger cohorts are needed to validate treatment specificity and longitudinal robustness in controlled longitudinal settings.

Funding Sources: Grant Agency of Charles University (GAUK grants 2021-2022 No. 309121), EFACTS (NCT02069509), UNIFAI (NCT06016946), and the Friedreich's Ataxia Research Alliance (FARA; MEABET-FA project 2024-2026). Supported by Biogen through an investigator-initiated trial grant, ID CZ-SKY-12330, the study was conducted independently of the funder.

B41. Multiple system atrophy cerebellar type (MSA-C): Proof-of-principle longitudinal pilot study reveals monthly pontine and cerebellar volume loss

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Background and Objectives: The diagnosis of MSA-C remains challenging in its earliest stages because the syndrome has often not yet fully manifested and diagnosis relies on observer-dependent rating scales susceptible to inter-rater and intra-individual variability. This delays clinical management and entry into clinical trials. There is an unmet need to develop quantitative observer-independent biomarkers for early diagnosis of MSA-C and measurement of progression over short time frames. This proof-of-principle study tested whether MRI can detect progression over monthly intervals beyond measurement error and compared utility of patient self-report versus clinician-administered ataxia rating scales.

Methods: We performed imaging and clinical assessments monthly for 6 months in 3 MSA-C patients (2 Possible Prodromal) and 2 healthy controls. We studied three-dimensional T1-weighted volumetric MRI, diffusion weighted imaging (DWI) parameters of mean diffusivity (MD) and fractional anisotropy (FA), and single voxel 1H MR spectroscopy measuring N-acetylaspartate (NAA/Cr) and myo-inositol (mI/Cr) relative to creatine. Clinical outcome assessments (COAs) were also administered.

Results: At baseline, all patients exhibited small pons, midbrain, and cerebellar volumes, reduced cerebellar FA, elevated pons and medulla MD, reduced pons and cerebellum NAA/Cr, and elevated COA scores. Patients' pons atrophied $-0.89\% \pm 0.22\%$ monthly; measurement error 0.5%. Individual monthly atrophy rates were -0.64% , -1.03% , and -1.00% . Cerebellum atrophied $-0.62 \pm 0.12\%$ monthly; measurement error 0.35%. Qualitative observation suggested that FA decreased in cerebellar white matter and midbrain over time, and that MD, NAA/Cr, and mI/Cr did not change. PROM-Ataxia and UMSARS-historical scores increased, whereas clinician-administered COAs were unchanged.

Discussion and Conclusion: Monthly pons and cerebellum atrophy rates exceeded measurement error, demonstrating potential for facilitating clinical diagnosis and monitoring progression over short intervals. This was complemented by patient-reported COAs. Spectroscopy may be useful for early diagnosis, and DWI for diagnosis and progression. This proof-of-principle study warrants validation in larger cohorts over longer follow-up.

Funding Sources: This study was supported by an unrestricted grant from Biohaven Pharmaceuticals (no role in design, conduct, or interpretation) and by the National Ataxia, Great Oaks, and Once Upon A Time, and MINDLink Foundations.

B42. Microbiomic Changes in a Rat Model of Christianson Syndrome

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Background/Objectives: Christianson syndrome (CS) is a rare X-linked disorder affecting the nervous system of young children, causing developmental delays, intellectual disability, seizures, and ataxia. CS results from loss-of-function mutations in SLC9A6, which encodes the NHE6 protein, leading to neuronal dysfunction and cerebellar degeneration. Gene therapy replacing SLC9A6 shows promise in rat models, but systemic effects remain poorly understood. This study investigates microbiomic changes associated with ataxia and gene therapy outcomes in a rat model of CS.

Methods: Wistar Furth rats carrying the spontaneous shaker mutation received gene therapy at postnatal weeks 1, 5, 7, 10, or 12. Wildtype and untreated shaker rats served as controls. Ataxia was assessed via force plate actometer, and fecal samples were collected concurrently. Genomic DNA was extracted and analyzed using 16S V4 amplicon sequencing to assess microbiomic diversity and composition.

Results: Fecal biodiversity differed between wildtype and untreated animals over 28 postnatal weeks. Bray-Curtis PCoA revealed distinct separation between wildtype and early-stage untreated animals compared to late-stage untreated animals, with Shannon diversity index showing lower microbial diversity in untreated animals over time. As untreated animals progressed, ataxia scores worsened, and these higher scores correlated with greater microbial community dissimilarity compared to earlier time points. In the wildtype animals, where ataxia scores remained stable, there showed a more consistent community structure, suggesting disease development is associated with progressive microbiome shifts.

Discussion: Increasing ataxia severity correlates with greater microbiomic dissimilarity. Ongoing analyses to be presented will assess how gene replacement influences these changes and identify specific taxa driving these changes, with the goal of determining whether early microbial shifts can predict eventual therapeutic outcome.

Conclusions: Given the limited therapeutic knowledge surrounding CS, exploring the gut microbiome's role in disease progression and gene therapy response represents a meaningful step toward improved treatments for this rare ataxic disorder.

Funding Sources: Rare Village Foundation Research Grant 2025

B43. The Quantitative Visually Enhanced Vestibulo-Ocular Reflex (VVOR) in Spinocerebellar Ataxia Type 27B (SCA27B)

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Background and Objectives: Spinocerebellar ataxia type 27B (SCA27B) is a recently described disease caused by a pathogenic GAA repeat expansion in the FGF14 gene. It is estimated to account for approximately 20% of inherited cerebellar ataxias and is increasingly recognised as the most common genetic causes of cerebellar ataxia. The visually enhanced vestibulo-ocular reflex (VVOR) reflects integrated visual pursuit, vestibular and optokinetic function and serves as an important marker of vestibulo-cerebellar integrity. This study aimed to characterise quantitative VVOR (qVVOR) abnormalities in SCA27B.

Methods: Eight individuals with genetically confirmed SCA27B underwent oculomotor assessment including video head impulse testing (vHIT) and quantitative VVOR (qVVOR) evaluation. qVVOR responses were assessed across three stimulation frequencies. Quantitative VVOR and vestibulo-ocular reflex (VOR) parameters were compared with age, age at symptom onset, disease duration and FGF14 GAA repeat expansion size.

Results: All participants demonstrated abnormal qVVOR responses across the three testing frequencies. Quantitative analysis revealed substantial abnormalities of both VOR and qVVOR function, supporting the presence of vestibulo-cerebellar dysfunction in SCA27B. Two individuals exhibited abnormal qVVOR responses at frequencies below 1 Hz despite preserved horizontal canal VOR gain on vHIT. qVVOR abnormalities were reproducible and observed across a range of disease severities.

Discussion and Conclusion: The VVOR relies on the integrated function of visual pursuit, optokinetic and vestibular reflex pathways, all of which are vulnerable to cerebellar dysfunction. The prevalence of qVVOR abnormalities suggests that vestibulo-cerebellar impairment is a core feature of SCA27B. Furthermore, the dissociation between abnormal qVVOR responses and preserved VOR gain in 2 patients raises the possibility that qVVOR testing may detect early disease. As a simple, non-invasive assessment the qVVOR represents a promising biomarker for diagnosis, disease monitoring and future therapeutic trials in SCA27B.

B44. Utility of Quantitative Oculomotor measures in Machado–Joseph Disease (MJD)/Spinocerebellar Ataxia Type 3 (SCA3)

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Background and Objectives: Machado–Joseph disease (MJD)/Spinocerebellar Ataxia Type 3 (SCA3) is a common inherited ataxia and is characterised by progressive cerebellar, vestibular, somatosensory and brainstem dysfunction amongst others. Oculomotor abnormalities are common and may precede significant gait impairment. Quantitative oculomotor metrics offer the potential to objectively measure disease-related dysfunction and provide sensitive biomarkers of disease severity and progression. This study aimed to evaluate the utility of quantitative oculomotor parameters in patients with MJD/SCA3.

Methods: Thirty-eight individuals with genetically confirmed MJD/SCA3 underwent comprehensive neurological and quantitative oculomotor assessment. Oculomotor parameters included visual pursuit, saccadic metrics, gaze-evoked nystagmus, vestibulo-ocular reflex (VOR), VOR suppression, visually enhanced vestibulo-ocular reflex (VVOR) function and fixation stability. Oculomotor findings were correlated with demographic, genetic and clinical measures including age, disease duration, CAG repeat length and ataxia severity scores.

Results: Quantitative oculomotor abnormalities were observed across multiple eye movement domains. Visual pursuit impairment, saccadic dysmetria, gaze-holding abnormalities, impaired VOR suppression, and abnormalities of VOR and VVOR function were prevalent findings. Quantitative testing identified abnormalities not readily apparent on routine bedside examination and provided objective measures across a broad range of disease severities. Oculomotor dysfunction was evident even in individuals with relatively mild clinical impairment, supporting the sensitivity of these measures to early disease-related change.

Discussion and Conclusion: The widespread oculomotor abnormalities observed reflect the involvement of cerebellar, vestibular and brainstem pathways in MJD/SCA3. Quantitative video-oculography provides a rapid, non-invasive and objective assessment of these neural systems and may offer advantages over conventional clinical measures. The ability to detect subtle abnormalities and quantify disease burden supports the use of oculomotor parameters as biomarkers in natural history studies and therapeutic trials. Quantitative oculomotor measures show considerable promise as objective outcome measures for future disease-modifying treatment studies in MJD/SCA3.

Funding Sources: National Health and Medical Research Council, Medical Research Future Fund VESKI FAIR Fellowship, Perpetual's 2024 IMPACT Philanthropy (The Lionel & Yvonne Spencer Trust, Kevin Darrell Clarke Endowment)

B45. Quantification of Ataxia Severity in Machado–Joseph Disease (MJD)/Spinocerebellar Ataxia Type 3 (SCA3) Using a Second-Generation Digital Phenotyping P

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Background and Objectives: Machado–Joseph Disease (MJD)/Spinocerebellar Ataxia Type 3 (SCA3) is a progressive neurodegenerative disorder characterised by progressive multi-focal ataxia. Clinical rating scales remain the current standard for assessing disease severity but are inherently subjective and insensitive to subtle longitudinal change. In our earlier work, a first-generation instrumented platform demonstrated the feasibility of objective movement quantification in cerebellar disease. This study evaluated a second-generation digital phenotyping platform integrating wearable inertial measurement units (IMUs), cloud-based analytics and machine-learning approaches to provide objective quantification of ataxia severity.

Methods: Participants with genetically confirmed MJD/SCA3 underwent neurological assessment together with instrumented movement analysis using wearable technology. Sensors were applied to quantify gait, balance and limb coordination during a battery of standardised motor tasks. Temporal, spatial and kinematic parameters were extracted and analysed using advanced signal-processing and machine-learning techniques.

Results: The second-generation AIMS platform identified abnormalities across multiple motor domains, including gait variability, postural instability, movement smoothness and movement consistency. Quantitative measures demonstrated sensitivity across a broad spectrum of disease severity and captured abnormalities not readily detected by traditional means. Several digital metrics demonstrated strong associations with established clinical measures of ataxia severity, supporting their validity as objective indicators of neurological dysfunction. The platform generated reproducible multidimensional measures of motor performance that were readily obtained in both clinical and research settings.

Discussion and Conclusion: Unlike first-generation wearable systems focused primarily on activity monitoring or isolated gait metrics, the second-generation platform provides multidimensional quantification of cerebellar dysfunction. By combining wearable sensing, cloud-based processing and machine-learning analysis, the system generates high-resolution digital biomarkers capable of objectively characterising disease burden. Its portability allows deployment in specialist centres, community clinics and research sites. These features make the platform a valuable outcome measure for natural history studies and disease-modifying therapeutic trials.

Funding Sources: National Health and Medical Research Council, Medical Research Future Fund

VESKI FAIR Fellowship, Perpetual's 2024 IMPACT Philanthropy (The Lionel & Yvonne Spencer Trust, Kevin Darrell Clarke Endowment)

B46. Test-Retest Reliability and Clinical Validity of Digital Gait Biomarkers Across Different Movement Analysis Systems in Cerebellar Ataxia

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Background and Objectives: Although digital gait variables are increasingly recommended as biomarkers and study outcomes in cerebellar ataxia, comparative studies across multiple gait assessment technologies remain scarce. As large clinical trials require evidence-based variable selection, this study evaluated reliability and validity of digital gait variables across three methodologically distinct measurement systems.

Methods: Performance-based gait variables for normal and backward gait were derived from literature and AGI consensus recommendations using three systems: force plate, wearable sensor system (17 sensors), and smartphones. For each variable a composite score combined test-retest-reliability (Intraclass Correlation Coefficient, ICC,) and clinical relevance (correlation with disease severity, r). Among the highest-ranking variables, logistic regression was used to identify those best discriminating people with ataxia from healthy controls.

Results: Data from 44 people with ataxia (54.9 ± 12.4 y, 21 female), and 26 healthy controls (38.3 ± 17.4 y, 12 female) was analyzed. In ataxia, about 17% of variables (of $n=102$) met both criteria ($r \geq 0.3$ and $ICC \geq 0.6$), both in normal and in backward gait. Slightly more normal than backward gait variables were among the highest-ranking variables (57%), and slightly more arm-sensor- (53%) than foot-sensor-derived variables. The three highest-ranking variables were force plate-derived step width during normal gait, step width during backward gait, and wearable sensor-derived stride length during backward gait. Notably, both step width variables ranked among the ten most discriminative variables (fourth/fifth). The three strongest discriminative variables were sensor-derived and included variability of maximum foot height during backward gait, backward gait speed, and peak forearm acceleration during backward gait.

Discussion and Conclusion: Reliable and clinically relevant variables were identified across all three measurement systems; however, optimal outcome variables depend on the specific system employed. Backward gait, previously identified in our work as sensitive to change, and arm-derived variables provided complementary and often highly informative measures, supporting their complementary use in digital gait assessment. **Funding Sources:** Deutsche Heredo-Ataxie-Gesellschaft e. V. (DHAG): funding for patient compensation. European Union's Horizon Europe Programme: Grant Agreement No. 101147319 (EBRAINS 2.0 Project), HIBALL: Helmholtz International Lab grant agreement InterLabs-0015, Helmholtz Association joint lab "Supercomputing and Modeling for the Human Brain (SMHB)"

B47. Plasma neurofilament light chain, brain-derived tau, glial fibrillary acidic protein and ubiquitin carboxy-terminal hydrolase L1 in DRPLA

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Background and objectives: Dentatorubral-pallidoluysian atrophy (DRPLA) is an ultrarare genetic type of ataxia caused by a CAG repeat expansion in the ATN1 gene. Currently, there are not treatments that can halt the progression of the disease, although therapies with antisense oligonucleotides have been used in n-of-1 trials, and might enter phase I clinical trials in the near future. For those studies, valid outcome measures and biomarkers will be required. We aim to characterise plasma concentrations of neurofilament light-chain (NfL), brain-derived tau (BD-tau), glial fibrillary acidic protein (GFAP) and ubiquitin carboxy-terminal hydrolase L1 (UCHL1) in the cohort of participants enrolled in the DRPLA Natural History and Biomarkers Study (DRPLANHBS).

Methods: We recruited 55 DRPLA carriers and 32 healthy controls in 9 participating centres. We analysed a preliminary set of 25 plasma samples from carriers and 8 samples from controls, as well as 3 CSF samples from carriers and 1 from a control. Samples were analysed with the Neurology 4-Plex D Advantage Plus assay (Quanterix, Billerica, MA) in the Simoa HD-X platform.

Results: Median plasma NfL was statistically higher in carriers compared to controls (9.3 pg/mL vs 3.5 pg/mL, $p=0.002$), with CSF NfL in carriers being more than ten times the concentration in the control sample (19100 vs 290 pg/mL). Median BD-tau plasma concentrations were also higher in carriers compared to controls (9.2 vs 5.3 pg/mL, $p=0.005$), with CSF concentrations in carriers slightly above the control sample (450 vs 370 pg/mL). We could not find statistically significant differences in GFAP or UCHL1 concentrations between the groups.

Discussion and Conclusion: Plasma NfL and BD-tau concentrations were higher in DRPLA carriers compared to controls. Analysis of data from the whole cohort and correlation with clinical and genetic variables will clarify the role of these biomarkers in DRPLA.

Funding Sources: Ataxia UK and CureDRPLA

B48. A Deep Learning Framework for Cerebellar Peduncle Segmentation Using Diffusion MRI in Friedreich Ataxia

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Background and Objectives: Cerebellar peduncles (CPs) are key white matter pathways affected in Friedreich ataxia (FRDA) and represent promising imaging biomarkers. However, reliable segmentation remains challenging due to their small size, complex anatomy, and disease-related atrophy. We developed an automated deep learning (DL) framework to enable accurate, reproducible CP delineation for biomarker development in ataxias.

Methods: Using diffusion MRI from the multi-site TRACK-FA cohort (n=90), we trained an nnU-Net-based model with a biologically informed 14-channel feature set integrating diffusion tensor metrics, PCA-derived diffusion features, fiber orientation information, and structural edge maps. Ground truth labels were derived from expert manual and semi-manual segmentation on T1-weighted images registered to native diffusion space, guided by diffusion-derived edge, orientation maps and VBAAtlas priors. An active learning strategy minimised manual annotation burden. Performance was evaluated against atlas-based (VBAAtlas) and tract-specific DL (TractSeg) methods using Dice similarity coefficient (DSC), 95th percentile Hausdorff-distance (HD95), and volume similarity (VS).

Results: The proposed framework achieved high accuracy across all cerebellar peduncles (mean DSC >0.86; global DSC = 0.92), with strong boundary agreement (HD95≈1 mm) and volumetric consistency (VS>0.94). Importantly, the decussation of the superior cerebellar peduncle, a historically challenging region, was segmented with DSC = 0.77, substantially outperforming VBAAtlas (0.69) and TractSeg (0.07). Improvements were statistically significant across all regions ($q < 0.001$), with large effect sizes (Cohen's $d_z > 1$). Performance remained consistent across FRDA (0.93 ± 0.02) and healthy controls (HCs) (0.91 ± 0.03), demonstrating robustness to neurodegeneration.

Discussion and Conclusion: This framework provides state-of-the-art, automated segmentation of CPs, enabling sensitive and reproducible imaging biomarkers across both HCs and individuals with FRDA. By combining biologically informed features with active learning, it addresses key challenges in small-structure segmentation under atrophy. The approach is broadly applicable to other ataxias and neurodegenerative disorders, supporting cross-disease investigations and therapeutic monitoring.

Funding Sources: Friedreich's Ataxia Research Alliance (USA)

B49. Brain Geometric Features for Diagnosis, Structure-to-Function Prediction, and Disease Monitoring in Hereditary Cerebellar Ataxias

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Background and Objectives: Hereditary cerebellar ataxias (HCAs) are rare neurodegenerative disorders characterised by overlapping clinical phenotypes and limited progression-sensitive biomarkers. This proof-of-concept study investigated whether cortical geometric features derived from structural MRI (sMRI) could support differential diagnosis, predict functional brain signatures, and capture longitudinal disease-related changes across HCA types.

Methods: We analysed sMRI and task-based functional MRI (fMRI) data from individuals with Friedreich ataxia (FRDA), spinocerebellar ataxia type 1 (SCA1), type 3 (SCA3), and healthy controls, collected across three independent cohorts (Belgium, TRACK-FA, Campinas). Cortical geometric eigenmodes were used to decompose brain MRI data into intrinsic spatial patterns, generating structural and functional geometric signatures. Deep learning models were developed to perform diagnostic classification and to predict functional signatures from sMRI. Longitudinal sensitivity was assessed using one-year follow-up data from the TRACK-FA cohort.

Results: Structural geometric signatures differentiated FRDA from controls with area under the curve (AUCs) up to 0.88 [95% CI: 0.78-0.95] and distinguished FRDA from SCA1 and SCA3 with AUCs up to 0.81 [0.65-0.93]. Combining structural and functional signatures improved classification performance with AUC 0.93 [0.85-0.98]. Structure-to-function models predicted functional geometric signatures from sMRI with coefficient of determination up to 0.62 and correlation reaching 0.86 across functional features and enabled partial reconstruction of individual task-activation maps. Several structural and predicted functional geometric features demonstrated greater sensitivity to annual progression than conventional volumetric MRI biomarkers.

Discussion and Conclusion: Geometry-driven brain signatures capture multiscale alterations relevant to HCA subtype differentiation, structure-function relationships, and disease progression. The ability to estimate functional features from routine sMRI may support more scalable assessment in settings where fMRI is unavailable. These findings highlight potential for biomarker development, although further validation in larger and diverse cohorts is required to establish robustness and clinical applicability.

Funding Sources: Friedreich's Ataxia Research Alliance – Award for Innovative Mindset

B50. Machine Learning Identifies Distinct Neuroimaging Progression Subtypes in Friedreich Ataxia

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Background and Objectives: Friedreich ataxia (FRDA) is a rare neurodegenerative disorder with substantial heterogeneity in clinical presentation and progression, complicating prognosis and clinical trial design. We aimed to identify biologically meaningful FRDA progression subtypes using longitudinal multimodal MRI and assess their associations with demographic, genetic, and clinical variables.

Methods: Longitudinal structural and diffusion MRI data from 54 FRDA participants and 57 controls across two sites (Australia and Brazil) were analysed. Annualised progression rates of macrostructural (volumetric) and microstructural (diffusion) imaging features across cerebellar, brainstem, and spinal cord regions were clustered using Gaussian Mixture Models. Cluster robustness was evaluated using bootstrap-derived stability metrics. Random Forest classification examined predictors of cluster membership using demographic, genetic, disease-history, and clinical variables.

Results: Four clusters were identified, three of which were biologically interpretable. Two progression subtypes were observed: a microstructure-dominant subtype, characterised by widespread white matter changes with modest volumetric decline, and a macrostructure-dominant subtype, marked by pronounced tissue atrophy with limited microstructural change. These clusters were predominantly composed of FRDA participants (75.0% and 78.3%, respectively). In contrast, clusters enriched with controls (68.2% and 66.0%) reflected minimal change or noise-related patterns. Notably, 44% of FRDA participants were classified into these minimal-change or noisy subgroups, indicating limited or inconsistent MRI-detectable annual progression. Genetic burden (GAA repeat length) was the strongest predictor of subtype membership.

Discussion and Conclusion: These findings highlight heterogeneous neurodegenerative trajectories in FRDA, whereby different MRI measures capture longitudinal change in different subgroups. Imaging markers of white matter integrity and tissue loss appear to be differentially sensitive across these subgroups, while a substantial proportion shows limited detectable change. This heterogeneity has important implications for biomarker selection, patient stratification, and clinical trial design. Further validation in larger cohorts is required to establish the robustness and translational relevance of these findings.

B51. Age- and tissue-dependent ATN1CAG repeat instability in Dentatorubral-pallidoluysian atrophy

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Background and objectives. Dentatorubral-pallidoluysian atrophy (DRPLA) is a progressive neurodegenerative disorder caused by a somatically unstable CAG repeat expansion mutation in the ATN1 gene. Here, we aimed to provide preliminary evidence on ATN1 repeat somatic instability in peripheral tissues and explore genetic variants with potential modifying effects.

Methods. A case-control and correlational study was conducted in an international cohort of participants enrolled in the DRPLA Natural History and Biomarkers Study. DNA was extracted from peripheral blood, buccal swabs, and cultured primary skin fibroblasts. PCR amplifications, fluorescently tagged DNA fragment analysis, and high-throughput ultra-deep MiSeq amplicon-targeted DNA sequencing were conducted.

Results. A high correlation was obtained between the modal repeat lengths determined by fluorescently tagged DNA fragment analysis and MiSeq amplicon-targeted DNA sequencing. Most non-expanded and expanded alleles consisted of pure CAG repeats. The expansion index showed no correlation with CAG repeat length, but significant associations with age were noted in blood and buccal epithelia. There were significant differences across tissues for the expansion index, with buccal epithelia showing higher levels of somatic instability. The mutant FAN1(rs3512) and PMS2(rs1805323) alleles in homozygosis were found only among DRPLA patients, whilst the frequencies of mutant MSH3(rs71636247) and MLH1(rs1799977) alleles in homozygosis were increased in the patients. There were no significant differences in the expansion index across genotypes for DNA repair genes under the additive genetic model.

Discussion and Conclusion. Somatic instability of the ATN1CAG repeat is tissue-specific and increases with age in peripheral blood and buccal epithelial cells. ATN1 repeat length and configuration, and DNA repair gene variants, do not seem to significantly affect somatic instability. Longitudinal data would provide stronger evidence for somatic instability as a candidate progression biomarker and end-point in clinical trials evaluating therapies that suppress somatic expansion in patients with DRPLA.

Funding Sources: Ataxia UK and CureDRPLA grants

B52. Advanced neuroimaging biomarkers and clinical correlates in SCA10

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Background and objectives: Neuroimaging biomarkers have been extensively investigated in several spinocerebellar ataxias (SCAs), particularly types 1, 2, 3, and 6, revealing distinct disease-specific patterns. However, little is known about neuroimaging beyond cerebellar atrophy in SCA10: a rare subtype characterized by pure cerebellar ataxia and epilepsy, most commonly found in Latin America and east Asia. Therefore, this study aimed to investigate advanced neuroimaging biomarkers of brain and spinal cord (SC) in SCA10 and its clinical correlates.

Methods: Patients with SCA10 and healthy controls underwent 3T T1-weighted brain and upper cervical spine MRI (GE HealthCare). Automated segmentation was performed using FastSurfer (cortical thickness and subcortical structures), CerebNet (cerebellum), FreeSurfer (brainstem), and ENIGMA-SC (spinal cord). MRI measures were correlated with disease duration and severity, measured by the Scale of Assessment and Rating of Ataxia (SARA), Cerebellar Cognitive Affective Syndrome Scale (CCAS) and Friedreich's Ataxia-Activities of Daily Living (FA-ADL). Age and sex were included as covariates.

Results: Twenty-four subjects, 15 SCA10 patients (9 males, average age: 51y, average SARA: 11.5) and 9 healthy controls (4 males, average age: 55y), were included. SCA10 patients showed widespread atrophy involving the cerebellum, most prominently in lobules V ($p < 0.001$; $d = 3.11$) and VI ($p < 0.001$; $d = 3.52$), and brainstem (superior cerebellar peduncle: $p < 0.001$, $d = 2.22$; pons: $p = 0.007$, $d = 0.97$). Volume reductions were also observed in the thalamus and ventral diencephalon. No significant SC or cortical abnormalities were detected. Cerebellar atrophy correlated with SARA (Left Crus II $R = -0.74$; Right Crus II $R = -0.63$), CCAS (Right Lobule IX $R = -0.62$) and disease duration (Vermis VII $R = -0.55$).

Discussion and Conclusion: SCA10 is characterized by a distinct neuroimaging signature involving diffuse cerebellar and brainstem atrophy with selective deep gray matter involvement, while sparing cortical and SC structures. MRI measures correlated with disease severity and duration, supporting their potential as biomarkers for differentiation among SCA subtypes and assessing disease progression.

Funding Sources: FARA, FAPESP, CNPQ, and CETAC.

B53. Turns increase the impact of impaired eye movements on locomotion in Spinocerebellar Ataxia Type 27B

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BACKGROUND AND OBJECTIVES: Turning movements account for 35–45% of everyday walking steps and are considered more challenging than straight walking due to increased demands on anticipatory postural adjustments and trunk–limb coordination. In addition, certain types of degenerative cerebellar ataxias are associated with disturbances in eye movements, like downbeat nystagmus (DBN), particularly during head rotation and peripheral gaze, and may therefore affect turning more than straight walking. We compared turning behavior of SCA27B patients with DBN to those of SCA1/2/3/6 patients without nystagmus and investigated the influence of 4-aminopyridine (4AP) on the reduction of DBN during turning.

METHODS: We performed cross-sectional analysis of lab-based motion data collected by three wearable sensors from subjects with SCA1/2/3/6 (n=359, SARA=6.81) and SCA27B (n=49, SARA=7.0) during: a) walking along a 60m corridor b) a turning task involving repeated 90° turns in a T-junction corridor. Analysis included mean and peak angular velocity (MAV, PAV), turn duration (TD), number of steps during turning (NoS), and lateral velocity change (LVC), a dynamic balance measure sensitive to ataxia-specific changes in turning and correlated with self-reported balance confidence (ABC-score).

RESULTS: LVC revealed significantly greater impairments during 90° turning ($p=0.001$, $\delta=0.45$) in SCA27B patients with DBN (n=18) than in SCA1/2/3/6 patients without oculomotor impairment (n=359). Standard turn parameters showed small or no effects (e.g., PAV: $p=0.49$, $\delta=0.10$; TD: $p=0.30$, $\delta=-0.15$). In a single-subject analysis of one 4AP-treated SCA27B patient with prominent DBN, treatment reduced slow-phase velocity by 16.1% (right gaze) and 51.2% (left gaze), accompanied by significant LVC improvements during right (-85.3%) and left (-98.4%) turns, without changes in standard turn measures.

DISCUSSION: Ataxia-related oculomotor impairments may reinforce dynamic balance deficits during turning beyond compensatory strategies such as slowing down or taking smaller steps. Reduction of DBN with 4AP may improve turning performance and everyday mobility in SCA27B patients.

B54. Brain-Wide Network Dysfunction in STUB1/CHIP Mouse Models of Cerebellar Ataxia

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Background and Objectives: Pathogenic variants in STUB1, which encodes the co-chaperone and ubiquitin ligase CHIP, are associated with cerebellar ataxias characterized by neurodegeneration and motor dysfunction. Previous studies have demonstrated cerebellar atrophy and behavioral deficits in mouse models of CHIP deficiency; however, the impact of STUB1 dysfunction on brain-wide neural connectivity remains incompletely understood. We sought to characterize alterations in functional connectivity in CHIP knockout (CHIP-KO) and disease-associated STUB1T246M hypomorphic mice relative to wild-type controls.

Methods: Wild-type (WT), CHIP-KO, and T246M mice underwent structural MRI and resting-state functional magnetic resonance imaging (rs-fMRI) at 51 weeks. Region-of-interest (ROI)-based functional connectivity matrices were generated from rs-fMRI data and compared between genotypes. Connectivity differences were visualized using heatmaps, network representations, and a custom 3D brain connectivity visualization platform. **Results:** Qualitative assessment of structural MRI revealed cerebellar-predominant atrophy in both CHIP-KO and T246M mice relative to WT controls. Functional connectivity analyses identified widespread reductions in significant ROI-to-ROI connectivity ($p \leq 0.05$) in both mutant lines, with T246M mice exhibiting over 22-fold more significantly decreased connections than CHIP-KO animals. In CHIP-KO mice, the largest connectivity losses involved cerebellar-thalamic ($n = 32$) and cerebellar-hypothalamic ($n = 9$) networks. In contrast, T246M mice exhibited extensive disruption of cerebellar-isocortical ($n = 291$), cerebellar-thalamic ($n = 244$), and isocortical-isocortical ($n = 211$) networks.

Discussion and Conclusion: STUB1 dysfunction was associated with disruption of cerebellar-associated networks implicated in motor coordination and cognition. The broader connectivity deficits observed in T246M mice suggest that disease-associated STUB1 mutations may alter neural circuitry through mechanisms that differ from complete CHIP loss. One limitation is that more extensive structural degeneration in CHIP-KO mice may reduce identifiable ROIs, potentially contributing to between-model differences. These results support the use of structural MRI and functional connectivity analyses for characterizing network-level consequences of STUB1 dysfunction in cerebellar ataxia.

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B55. One-year Sensitivity of Volumetric MRI Biomarkers in SCA3

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Background and Objectives: Spinocerebellar ataxia type 3 (SCA3) represents the most prevalent autosomal dominant spinocerebellar ataxia worldwide. Driven by an ATXN3polyglutamine expansion, the disease manifests as progressive ataxia and premature mortality, with no treatment currently available. Consequently, as disease-modifying therapeutics for SCA3transition into clinical phases, the validation of robust, sensitive biomarkers for treatment monitoring has become critical.

Methods: We analyzed de-identified clinical and T1-weighted MRI data from 47 SCA3carriers (13 pre-ataxic) and 18 healthy controls in the ESMI cohort at baseline and 1-year follow-up. Volumetric segmentation of the cerebrum, cerebellum, brainstem, and intracranial volume was performed using FastSurfer, CerebNet, FreeSurfer, and SAMSEG. We quantified the annualized percentage of volume change and standardized response means (SRM). Linear mixed-effects models, adjusted for baseline age, sex, and CAG repeat length, were used to evaluate regional atrophy rates.

Results: For patients with early ataxia (SARA<10), annualized volume change was highest in the fourth ventricle, pons, whole brainstem, and cerebellar white matter (CWM). Patients with moderate ataxia (10≤SARA<20) exhibited similar patterns, except for the fourth ventricle but including the medulla. Large responsiveness (SRM≥0.8) occurred in the pons, cerebellar cortex, and fourth ventricle for early ataxia, extending to the whole brainstem, medulla, and CWM in moderate ataxia. Linear mixed-effects models demonstrated significant 1-year longitudinal atrophy, most prominently in the pons (Estimate=-0.20, p<0.001), whole brainstem (Estimate=-0.31, p<0.001), and cerebellar cortex (Estimate=-0.76, p<0.001).

Discussion and Conclusion: The pons emerged, in line with literature, as the most robust progression biomarker, demonstrating the highest volume change and responsiveness over a one-year period, and most stable atrophy rate in patients with early ataxia, the population that would most benefit from early interventional disease-modifying clinical trials.

B57. Submovements and pauses derived from computer mouse movements provide a promising basis for sequence modeling of behavior in ataxias

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The Hevelius computer mouse task assesses arm motor function by quantifying mouse movement trajectories and clicks. In this study, we aim to segment movement trajectories to enable multimodal sequence modelling of motor behavior in ataxias (e.g., mouse movements and eye movements) using transformer-based architectures. Eye and mouse movements of 89 control and 107 ataxia participants enrolled in the “Neurobooth” study were digitally recorded as they made sixty-four on-screen point-and-click movements (trials) across eight blocks with varying target size and displacement. Mouse trajectories were decomposed in horizontal and vertical directions, and segmented into pauses and submovements based on mouse velocity profiles. Submovements were described via kinematic and shape features. Features were aggregated over each trial and evaluated using Cohen’s d.

Non-linear two-dimensional projections showed that submovement duration, distance, peak velocity, and acceleration were discriminative of control and ataxia groups. Control submovements traversed longer distances ($d=0.25$) in shorter times ($d=-0.27$) reaching higher peak velocities ($d=0.50$) and accelerations ($d=0.51$). Participants with ataxia exhibited higher counts of submovements and pauses per trial, and longer mean pause durations ($d=-0.55$, -0.61 , -0.42 respectively). Submovement velocity and acceleration, and pause counts were more discriminative for task blocks with the largest distance between consecutive targets ($d=0.61$, 0.57 , -0.73). Finally, total duration of pauses in the second half of the trials was more discriminative of ataxia from controls than the first half ($d=0.52$ vs $d=0.26$) highlighting temporal differences in participant behavior. All $p<0.001$.

These results demonstrate the ability of submovements and pauses to capture aspects of the ataxia phenotype, supporting the use of these units in future models that account for the full sequence of behavior to more fully represent movement impairment in ataxias.

Funding Sources: Massachusetts Life Sciences Center, NIH R01NS117826, Biogen, Broad Institute, Duke Family Foundation

B58. Towards Sensitive Video-Based Gait Assessment in SCA: Validation of 3D Biomechanical Models Derived from 2D Videos Against Wearable Sensors

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OBJECTIVES: Body-worn inertial measurement units (IMUs) are currently the most efficient and reliable technology for gait assessment in clinical and real-world settings. Although advances in computer vision and deep learning have established markerless video analysis as a cost-effective alternative, existing approaches still lack sensitive, ataxia-specific gait measures in both the frontal and sagittal planes from single-view videos. Here, we aim to unravel machine-learning based biomechanical 3D-models, enabling the analysis of a wide range of videos from clinical examinations and large-scale, multicentre trials.

METHODS: We assessed gait of 11 subjects with SCA3(SARA:8.0±6.2) during laboratory-based walking using two synchronised systems: (1) three body-worn inertial sensors, and (2) a 3D biomechanical model based on frontal, single-view, 2D-RGB videos and machine learning (PosePipe, R.J Cotton, Shirley Ability Lab, Chicago). The movement analysis focused on measures of spatiotemporal variability in different directions, particularly lateral step deviation (LSD) and stride length variability (SLcv), which have already been shown to be longitudinally sensitive.

RESULTS: The gait events detected by the video-based biomechanical model were found to match those from the wearable sensor system with over 90% accuracy. The calculated stride lengths showed good agreement (mean relative error<10%). Cross-sectional analyses revealed a high correlation between ataxia severity (SARA) and gait measures in the frontal (LSD, $r>0.8$) and sagittal (SLcv, $r>0.6$) planes for both systems, with similar effect sizes.

DISCUSSION: The combination of wearable sensors and video-based analysis opens up new possibilities for future multicentre therapy studies: In an outpatient setting, simple, single-view smartphone videos can be captured, while wearable sensors can be used at home when video capture is not feasible. The next step is to analyse test-retest reliability and sensitivity to longitudinal changes in different types of SCA. This will involve setting up a unique dataset containing synchronous video and IMU data for SCA1/2/3/6 for longitudinal analysis.

B59. Longitudinal Evaluation of plasma Neurodegeneration Biomarkers in Spinocerebellar Ataxia Type 3 (SCA3): insights from ESMI cohort

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Background and Objectives: Understanding biology across the disease continuum with biofluid reliable biomarkers identification is essential to monitor and predict disease progression and support future SCA3 clinical trials. In this context, challenges associated with pre-analytical conditions, batch analyses could be sources of variability making it difficult. Neurofilament light chain (NfL) has emerged as a promising biomarker of neuroaxonal damage, while the value of additional markers reflecting distinct pathological processes remains less clear.

Methods: Plasma samples from European Spinocerebellar Ataxia Type 3/Machado-Joseph Disease Initiative (ESMI) cohort participants were analyzed, (12 healthy controls, 30 pre-ataxic SCA3 mutation carriers, and 119 ataxic patients). Samples were collected at three yearly visits, processed following a standardized protocol and analyzed in a single batch using the New Neurology Quanterix 4-Plex-D assay, quantifying NfL, glial fibrillary acidic protein (GFAP), brain-derived Tau (BD-Tau) and ubiquitin C-terminal hydrolase L1 (UCHL1). Biomarker concentrations were compared across groups and correlated with clinical measures of disease severity.

Results: Consistent with previous studies, plasma NfL concentrations were significantly elevated in ataxic participants compared with pre-ataxic carriers and controls, supporting its role as a marker of neurodegeneration in SCA3. However, GFAP and UCHL1 levels did not differ significantly between groups. BD-Tau demonstrated a modest trend toward increasing concentrations with disease severity, suggesting a potential relationship with ongoing neuronal damage. Biomarkers correlations with disease severity and longitudinal data are under investigation.

Discussion and Conclusions: Our results highlight the importance of controlling preanalytical conditions and batch analyses when interpreting biomarker data. Moreover, they support NfL as biomarker of interest in SCA3. While GFAP and UCHL1 remain stable over progression, the observed trend for BD-Tau and its potential as progression biomarker warrants further investigation. Combined biomarker approaches, incorporating biofluids and imaging, may improve the characterization of disease progression and the evaluation of emerging therapeutic interventions in SCA3.

Funding Sources: Several ESMI site PIs are members of the of the European Reference Network for Rare Neurological Diseases. Moreover, this work has been funded by Servier.

B60. From Coarse to Continuum: Leveraging AI-Informed Motion Capturing for Digital Video-Based Movement Analysis in Neurodegeneration

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Background and Objectives: Clinical assessment of motor impairment remains largely qualitative and coarse-grained, limiting sensitivity to subtle disease-related changes. Advances in computer vision and artificial intelligence (AI) offer the opportunity to transform standard video recordings into scalable, objective, and highly granular digital biomarkers. We investigated the potential of sensor-free, monocular camera-based motion capture to quantify disease-specific motor abnormalities in ataxia and distinguish them from healthy controls (HC) and Parkinson's disease (PD).

Methods: Video recordings were acquired using a simple monocular camera setup (Nataxia=165, NPD=30, NHC=45, Nlongitudinal=52, N@home=104). Spatiotemporal movement trajectories and higher-order kinematic features were extracted using pose-estimation frameworks and subjected to AI-guided feature selection. Associations with established clinical (SARA) and patient-reported outcome measures (ADL, PROM-Ataxia in a subset) were assessed. In a cross-disease approach, digital motor signatures of ataxic and pre-ataxic individuals were compared with HC, prodromal PD (isolated REM sleep behavior disorder), and manifest PD. Home-based recordings were analyzed to assess robustness and ecological validity.

Results: Digital movement biomarkers showed significant associations with SARA ($r=0.67$), but higher correlations with patient reported outcomes, such as falls, than SARA ($r_{\text{digital}}=0.36$, $r_{\text{SARA}}=0.01$). They remained robust in home-based recordings ($r_{\text{gait}}=0.78$). AI-guided feature selection identified disease-specific motor signatures that captured pathological movement patterns relevant for both cross-sectional disease severity and longitudinal progression. Movement patterns in ataxia could be distinguished from HC and PD. Notably, selected biomarkers demonstrated sensitivity to subtle motor alterations before clinical manifestation (F1PresympAtaxia 75.69; F1ProdromalPD 60.00).

Discussion and Conclusion: Sensor-free monocular video analysis extends traditional clinical observation by providing objective, continuous, and highly granular measures of motor function. AI-informed feature discovery identified subtle disease-specific alterations, including presymptomatic and longitudinal changes not captured by conventional clinical scales. These findings highlight the potential of easily deployable video-based digital biomarkers for early detection, patient-centered monitoring, and telemedicine applications.

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B61. Automatic Video Gaze Estimation for Detection of Cerebellar Oculomotor Abnormalities in Ataxias

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Oculomotor abnormalities have the potential to serve as the basis for sensitive digital measures of cerebellar dysfunction in ataxias. Clinical assessments of oculomotor function are subjective and imprecise, and quantitative assessments currently require expensive research-grade eye trackers, making it impractical to capture eye movement information frequently in low-resource settings, limiting the impact and feasibility of oculomotor-based measures for clinical trials and care. We developed a deep learning model to estimate gaze from smartphone video, trained using high-precision eye-tracker ground truth, to detect ataxia-specific oculomotor abnormalities. Participants underwent a comprehensive Neurobooth assessment, including oculomotor, cognitive, motor, and speech tasks with simultaneous data collection from a smartphone video and an eye tracker. A spatiotemporal deep learning architecture was designed to process 7-frame video sequences of the face to produce gaze estimates. The architecture included spatial encoder to extract per-frame eye features, followed by a Transformer-based temporal encoder to model gaze dynamics across frames. The model jointly optimized losses for gaze position, velocity, and acceleration to capture saccade kinematics. For preliminary evaluation, an earlier version of the model was trained on approximately 2.2million frames from 268 subjects. Preliminary results from this model achieved a 3.8° mean angular error (median 2.7°) on 64 held-out subjects, better than existing video-based methods (~4-5°), with strong tracking (Pearson r=0.83). The preliminary model was able to identify saccadic pursuit and dysmetric saccades on visual inspection. The preliminary model's performance supports its potential to detect cerebellar oculomotor abnormalities. We will evaluate the model's ability to detect ataxia-associated oculomotor patterns and its performance in conditions where eye trackers often fail, such as poor calibration from gaze holding difficulties, glasses reflections, or eyelid occlusions. These preliminary results suggest potential for accessible oculomotor assessment via smartphones; future development and validation in home settings are needed for remote assessments and decentralized clinical trial

Funding Sources: Massachusetts Life Sciences Center, U.S. Department of Defense, NIH R01NS117826, Biogen, Broad Institute, Dake Family Foundation

B63. Proteomic profiling of human CSF enables differentiation of MSA-C from CAUE

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Background and Objectives: Multiple system atrophy-cerebellar type (MSA-C) is a rapidly progressive disorder that remains difficult to distinguish early in the disease from cerebellar ataxia of unknown etiology (CAUE). Proteomic approaches have identified molecular signatures sensitive for atypical Parkinsonisms. Disease-specific CSF proteomic signatures that discriminate MSA-C from CAUE and controls remain undefined.

Methods: CSF samples from MSA-C (n=10), CAUE (n=10), and controls (n=10) were collected at five university centers. MSA-C participants met Gilman criteria for possible or probable MSA. CAUE participants had cerebellar ataxia for more than 5 years, negative genetic testing for common ataxic disorders (SCA1, 2, 3, 6, 7, 8, 10, 27B, FXN, and RFC1), and did not meet criteria for possible or probable MSA. Clinical evaluations and lumbar puncture were performed at a single visit. Data-independent acquisition proteomics (diaPASEF) was performed on a timsTOF Ultra 2. Differential expression analysis used Perseus with permutation-based FDR correction. Pathway enrichment was assessed via KEGG and Gene Ontology. Multivariate classification used support vector machine (SVM) modeling in MetaboAnalyst.

Results: We quantified 1,777 proteins. MSA-C vs. control identified 603 differentially expressed proteins (DEPs; 187 up, 416 down; $q < 0.05$). CAUE vs. control yielded only 2 DEPs. MSA-C vs. CAUE identified 418 DEPs (165 up, 253 down). In MSA-C, pathway enrichment showed upregulated complement and coagulation cascades and downregulated cell adhesion, axon guidance, and synapse organization pathways, suggestive of blood brain barrier disruption plus synaptic degeneration. SVM modeling using 15 protein features, including IGLV2-14, MAMDC2, PRSS35, CD81, BST1, CALB2, and PLPPR4, achieved an AUC of 0.933 (95% CI: 0.778–1.000) and 88.7% accuracy for distinguishing MSA-C from CAUE.

Discussion and Conclusion: CSF proteomics identifies a robust signature distinguishing MSA-C from CAUE, driven by complement activation and synaptic protein loss. High SVM classifier accuracy supports the discriminatory potential of CSF proteomics for these two conditions.

Funding Sources: National Ataxia Foundation and Daniel B. and Florence E. Green Foundation

B64. Integrative Proteomics Analysis of Plasma for Biomarker Discovery in Spinocerebellar Ataxia Type 3 Mouse Model

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Spinocerebellar Ataxia Type 3 (SCA3) is caused by an expansion of a polyglutamine (polyQ) sequence in the ataxin-3 (ATXN3) protein, which triggers neuropathological processes that lead to progressive motor dysfunction. This disease is clinically and biologically heterogeneous, and no disease-modifying therapy is available. Biomarker discovery can help validate disease mechanisms, monitor disease progression, and support improved translation from preclinical to clinical studies. Neurofilament light and phosphorylated neurofilament heavy were the first fluid biomarker candidates, identified in blood, for both human and preclinical treatment trials in SCA3. In this study, we aimed to identify molecular signatures associated with disease pathology and explore potential biomarkers that reflect SCA3-related biological changes, by analyzing SCA3transgenic mouse plasma.

Unbiased proteomic profiling, using High Reaction Monitoring mass spectrometry, was performed in the plasma of wild-type (WT) and SCA3animals (n=5 animals/genotype, both sexes), at 41 weeks of age. Principal component analysis and partial least squares discriminant analysis were used. Exploratory data analysis was conducted, including multivariate analysis and statistical testing, to identify differentially regulated proteins. QIAGEN Ingenuity Pathway Analysis software was used to analyze canonical pathways and upstream regulators.

Among 3465 proteins detected, 27 were differentially expressed comparing WT and SCA3animals. Nine proteins were identified as upregulated, namely: Uchl1and Sod2(used as biomarkers for other neurodegenerative diseases); PSMD4; DCTN; UCHL1; SOD2; SLFN14; ALDH4A1; APBB1IP; SNTB1and SCP2. Ten differentially expressed proteins were downregulated, specifically: Kirrel1, Copg2, Tom1l1, Apoc4, H2-K1, Proz, Dsc3, Col15a1, Pon3, and Reg1. The top upstream regulators identified were related to inflammation and metabolic pathways.

We are currently performing longitudinal assessment of these proteins and testing their response to therapeutic interventions, for validation as useful and biologically meaningful biomarkers with the potential to guide future therapeutic strategies for SCA3.

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B65. Plasma Lipid Profiles in Patients with Friedreich's Ataxia Before and After Treatment with Nomlabofusp

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Background and Objectives: Friedreich's ataxia (FRDA) is an autosomal recessive disorder caused by frataxin (hFXN) deficiency resulting in mitochondrial metabolic dysfunction and characterized by progressive neurologic and cardiac disease. The mitochondrial metabolic dysfunction in FRDA includes reduced fatty acid (FA) β -oxidation and ATP generation, generation of reactive oxygen species and impaired oxidative stress response, and lipid dysregulation.

Nomlabofusp is an investigational protein therapy intended to address the root cause of FRDA by delivering hFXN to patients with FRDA. The objective of this study was to characterize plasma lipid profile alterations and evaluate the effect of nomlabofusp treatment on lipid dysregulation in patients with FRDA.

Methods: Untargeted plasma lipidomic analyses by liquid chromatography tandem mass spectrometry were conducted on plasma samples collected from fasting healthy volunteers (HV) and from subjects with FRDA from 3 nomlabofusp clinical studies. Lipidomic profiles were compared between HV and untreated subjects with FRDA and baseline versus postdose samples of each clinical study. Relationships between changes in plasma lipid profiles and increases in tissue hFXN concentrations with nomlabofusp treatment were also assessed.

Results: Across studies, plasma TG, predominantly with polyunsaturated fatty acid (PUFA) and frequently with odd chain FA, were elevated in FRDA relative to HV. After up to 1 month of nomlabofusp treatment, levels of plasma TGs with these features were reduced. Nomlabofusp dose dependency of lipid changes was observed by Day 28 in a placebo-controlled dose exploration study. In addition, significant inverse correlations were observed between changes in PUFA containing TG and skin hFXN levels.

Discussion and Conclusion: In subjects with FRDA, increased tissue hFXN concentrations associated with nomlabofusp treatment correlated with a decrease of specific plasma TG values towards those observed in HV, suggesting normalization of FRDA-associated lipid dysregulation.

Funding Sources: Larimar Therapeutics Inc.

B66. Dysregulated Gene Expression in Friedreich's Ataxia and Response to Treatment with Nomlabofusp

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Background and Objectives: Friedreich's ataxia (FRDA) is a hereditary neurodegenerative disease caused by guanine adenine adenine repeat expansions in the frataxin (hFXN) gene, resulting in hFXN protein deficiency. As a rare disease affecting clinically inaccessible tissues, FRDA lacks a minimally invasive, repeatable molecular readout directly linked to therapeutic target engagement. Furthermore, distinguishing primary hFXN-driven gene expression changes from secondary disease adaptations remains a key challenge.

Nomlabofusp is a protein therapy intended to address the root cause of FRDA by delivering hFXN. The goal of this study was to determine FRDA-associated buccal gene expression alterations and evaluate the effect of nomlabofusp treatment on these alterations in subjects with FRDA.

Methods: Throughout the nomlabofusp clinical development program spanning 2 clinical studies, buccal epithelial cells from subjects with FRDA and healthy volunteers were collected for gene expression profiling using NanoString nCounter® and RNA sequencing.

Results: The study identified 68 differentially expressed genes, predominantly downregulated, with pathway analyses implicating cellular, immune, and neurological processes consistent with established FRDA biology. Co-expression network analysis revealed a discrete 8-gene interactome (BASP1, BTG2, CD44, EGR1, LRRK2, LY96, SOD2, XPO6) highly coordinated in healthy volunteers but markedly disrupted in FRDA. After treatment with nomlabofusp, directional reversal of abnormal gene expression toward healthy-volunteer levels was observed in 27 differentially expressed genes. Further analysis using orthogonal approaches confirmed 7 genes (SOD2, SLC25A37, EGR1, BTG2, LRRK2, XPO6, MGAM) that trended towards normalized expression with nomlabofusp treatment, with partial restoration of co-expression network connectivity. SOD2 and SLC25A37 emerged as the strongest FRDA-linked transcriptional markers.

Discussion and Conclusion: Consistent with prior reports, gene expression is altered in patients with FRDA. Reversal of these alterations was observed in subjects with FRDA treated with nomlabofusp. Buccal transcriptomics is a scalable, minimally invasive platform for monitoring pharmacodynamic effects of hFXN replacement therapy.

Funding Sources: Larimar Therapeutics, Inc.

B67. Spatially resolved MRI of skeletal muscle OXPHOS activity in spinal muscular atrophy

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Background. Spinal muscular atrophy (SMA) is increasingly recognized to involve skeletal muscle mitochondrial abnormalities that may relate to disease severity. With multiple FDA approved disease modifying therapies now available, there is a critical need for physiological biomarkers that can detect treatment response before changes in motor function become apparent. Noninvasive assessment of skeletal muscle oxidative phosphorylation (OXPHOS) may provide such a biomarker. We developed relaxation compensated OXPHOS chemical exchange saturation transfer MRI (rOXCEST) to map postexercise creatine (Cr) recovery on a clinical 3T scanner as a surrogate of skeletal muscle mitochondrial function.

Methods. Fifteen healthy adults underwent rOXCEST and phosphorus 31 magnetic resonance spectroscopy (31P-MRS) after standardized plantar flexion exercise. The relaxation-compensated Cr recovery time constant (rTCr) was compared with the phosphocreatine (PCr) recovery time constant (TPCr). Repeatability was assessed in five healthy adults. Feasibility of rOXCEST for measuring skeletal muscle OXPHOS capacity in SMA was evaluated in a 22-year-old participant with SMA type 3 and an 11-year-old participant diagnosed prenatally and treated with nusinersen since birth.

Results. rTCr correlated strongly with TPCr ($R^2 = 0.80$, $P < 0.001$), with excellent agreement (ICC = 0.883) and a regression slope of 0.94. Interscan repeatability was excellent in the lateral and medial gastrocnemius (ICC = 0.912 and 0.981). The participant with SMA type 3 had prolonged gastrocnemius rTCr (93 s), exceeding values in approximately 95% of healthy controls. The prenatally diagnosed participant receiving nusinersen since birth had faster recovery (32 s), within the healthy range.

Conclusions. rOXCEST provides a clinically feasible, spatially resolved measure of skeletal muscle mitochondrial recovery and demonstrates feasibility for characterizing OXPHOS capacity in SMA. The difference between the two SMA participants is descriptive and cannot be attributed to treatment. Larger longitudinal studies are needed to determine whether rOXCEST can quantify disease related bioenergetic impairment and therapeutic response.

Funding Sources: Research grant from Friedreich's Ataxia Research Alliance (FARA). SJLIFE U01(U01CA301480). St. Jude Comprehensive Cancer Center Developmental Funds Award

Clinical Studies

C2. An International Survey of Patients with Ataxia: Trends in Patient-Reported Symptoms

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Background and Objectives: Ataxia disorders have complex symptomology and few treatment options. Limited information is available on symptoms and quality of life with ataxia from the perspective of patients and caregivers. We aimed to assess whether trends in symptoms and quality of life varied by ataxia type and disease stage.

Methods: We conducted an anonymous, international survey of 680 National Ataxia Foundation Members, including 587 people with ataxia and 93 caregivers. Data was analyzed for the whole group, by ataxia type, and by Functional Disability Stage (FDS). Respondents self-reported the first symptoms experienced, symptoms currently experienced, and the one symptom that currently has the greatest impact on their life. Nominal data were analyzed via the chi-squared test and Fisher's exact test to detect differences in responses between populations.

Results: Impaired balance and coordination were the most reported first symptoms noticed by respondents across ataxia types. Impaired balance, impaired coordination, and fatigue were the most prevalent symptoms across ataxia types and FDS. Impaired balance was the symptom most reported as having the greatest impact on respondents' day-to-day life. While there were commonalities between FDS and ataxia types, some subtype differences emerged amongst self-reported first symptom, symptom prevalence, and symptoms impacting daily living; particularly for individuals with SCA2, SCA6, SCA8, SCA27B, RFC1Ataxia, and Friedreich Ataxia.

Discussion and Conclusion: This study highlights trends in symptomology across ataxia types and disease stages from the perspective of people with ataxia and caregivers. These findings may help improve diagnosis procedures, align therapeutic development with patient needs, and validate the experiences of people with ataxia.

Funding Sources: This study was fully supported by the National Ataxia Foundation.

C3. From Insight to Impact: Translating Ataxia Patient Feedback into Programmatic Change

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Background and Objectives: Patient- and caregiver-reported data are increasingly recognized as essential for informing healthcare delivery, supportive programs, and research priorities in rare diseases. The National Ataxia Foundation (NAF) conducted a global community survey to better understand experiences, needs, and barriers among individuals affected by ataxia.

Methods: NAF administered a cross-sectional attitudes and usage survey to patients and caregivers affected by ataxia (n = 680). Survey domains included resource utilization, perceived effectiveness of organizational initiatives, unmet needs, barriers to access, and priorities for education, research, and support services. Subgroup analyses compared patient and caregiver responses. Social determinants of health, including transportation access, employment, income, and insurance status, were incorporated into subgroup analysis.

Results: Respondents reported high overall trust in and reliance on NAF resources, with the majority accessing NAF information at least monthly and expressing positive perceptions of the organization. Half of respondents reported barriers to accessing information or services, most commonly related to navigation challenges, lack of awareness, geographic limitations, and relevance to individual circumstances. Support groups, education, and research participation emerged as top priorities across respondent groups. Higher social risk scores were associated with increased difficulty accessing care, information, and local resources.

Discussion and Conclusion: Survey findings were explicitly used to guide organizational decision-making, resulting in targeted programmatic changes at NAF. This includes the redesign of digital resources for accessibility, expansion of research infrastructure and trial readiness, enhancement of support group models with professional social work integration and increased financial support to reduce participation barriers.

This case study demonstrates how structured patient- and caregiver-reported data can inform scalable, equity-oriented program development within a rare disease organization. The approach offers a replicable model for integrating patient-reported insights into strategic planning and service delivery.

Funding Sources: This study was fully supported by the National Ataxia Foundation.

C5. Convergent Validity of EQ-5D-Y Quality of Life Scale with Neurological Impairment and Clinical Severity Measures in Ataxia-Telangiectasia

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Background: Ataxia-telangiectasia (A-T) is a multisystem disorder characterized by neurodegeneration and cerebellar ataxia. Disease progression impairs quality of life (QoL); however, there is a knowledge gap regarding relationships between measures of neurologic impairment, disease severity, and patient-reported QoL. In a large cohort of children with A-T, we assessed convergent validity between patient/proxy-reported total EQ-5D-Y QoL scores and clinician-measured neurologic impairment using International Cooperative Ataxia Rating Scale (ICARS), Rescored modified ICARS (RmICARS), and global disease severity (CGI-S) scale. Secondary analyses examined domain-level QoL associations with ICARS, RmICARS, and CGI-S.

Methods: Data from the randomized, placebo-controlled trial (NEAT) including 105 6-17-year-olds with A-T were analyzed. Construct validity of the 3-point EQ-5D-Y QoL scale was evaluated using Spearman correlation rho (ρ) at baseline and 6-month follow-up. Rho of 0.3-0.8 indicated that the construct validity was met.

Results: Baseline analyses demonstrated associations between EQ-5D-Y QoL total scores and physician-reported measures: ICARS $\rho=0.42$, RmICARS $\rho=0.38$, and CGI-S $\rho=0.41$. Similar associations were observed at month 6 between EQ-5D-Y total score and: ICARS $\rho=0.55$, RmICARS $\rho=0.47$, and CGI-S $\rho=0.48$. Baseline QoL domain analyses showed associations of functional QoL domains and clinician-reported measures. Mobility correlated with ICARS $\rho=0.51$, RmICARS $\rho=0.48$, and CGI-S $\rho=0.53$. Usual activities correlated with ICARS $\rho=0.35$, RmICARS $\rho=0.33$ and CGI-S $\rho=0.37$; while self-care correlated with ICARS $\rho=0.40$ and RmICARS $\rho=0.34$, but not CGI-S $\rho=0.29$. Correlation with the visual analogue scale, pain/discomfort and anxiety/depression scores with the other clinician-reported measures was poor. Similar correlations between QoL domains and physician-reported measures were obtained at month 6.

Discussion: EQ-5D-Y scale demonstrates meaningful relationships with disease severity and ICARS and RmICARS ataxia scales, in particular with respect to the Total score and functional domains of QoL. These findings support the construct validity of EQ-5D-Y in capturing clinically relevant aspects of disease severity and change over time.

C6. Correlation and Validation of Rescored Modified ICARS (RmICARS) with ICARS and Clinical Disease Severity (CGI-S) in Ataxia-Telangiectasia

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Background: Ataxia-telangiectasia (A-T) is a rare, autosomal recessive disorder characterized by neurodegeneration, cerebellar ataxia, telangiectasia, and multisystem involvement. Neurological impairment is assessed using the International Cooperative Ataxia Rating Scale (ICARS), a clinician-rated 100-point scale across four neurologic domains. The Rescored modified ICARS (RmICARS) is a 29-point scale derived from ICARS by excluding the oculomotor domain and collapsing selected scores to focus on axial cerebellar features (posture, gait, speech). Higher scores indicate greater disease severity. Disease status and symptom severity is assessed using the clinician-rated 7-point Clinical Global Impression-Severity (CGI-S) scale (1=normal, not at all ill, 7= the most extremely ill). This study aims to evaluate associations between ICARS and RmICARS ataxia scales to determine whether RmICARS preserves clinical meaning of ICARS, as well as associations between ICARS, RmICARS, and CGI-S to evaluate clinician-rated neurological impairment and global disease severity measures.

Methods: A secondary analysis was conducted of the NEAT randomized, placebo-controlled trial including 105 6-17-year-olds with A-T. Association among ICARS, RmICARS, and CGI-S were conducted using Spearman correlation (ρ) at baseline and 6-month follow-up. Analysis was done for all patients and by treatment groups (patients treated with encapsulated dexamethasone sodium phosphate or placebo).

Results: ICARS and RmICARS scores demonstrated strong correlations at baseline ($\rho=0.938$, 95% CI 0.909–0.958) and Month 6 ($\rho=0.950$, 95% CI 0.926–0.966). Findings were consistent across treatment groups ($\rho=0.927$ –0.957). Both scales demonstrated moderate correlations with CGI-S at baseline and Month 6 (ICARS: $\rho=0.622$, 0.675; RmICARS: $\rho=0.632$, 0.660) for all patients.

Discussion: These findings indicate that RmICARS preserves the clinical information of ICARS and maintains consistent measurement across timepoints and treatment groups. Both scales show moderate associations with CGI-S, with correlation magnitude being similar across treatment arms. These results support the use of RmICARS for assessing A-T neurological impairment, and its use alongside global-clinician rated disease severity.

C7. MJDFhub: A clinical monitoring platform for Indigenous Australians living with MJD/SCA3 and SCA7

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Background and Objectives: Aboriginal Australian communities living with Machado-Joseph Disease (MJD)/spinocerebellar ataxia type 3 (SCA3) and SCA7 face additional barriers to effective clinical monitoring and specialist care. These include infrequent specialist neurology input, and the cultural and language limitations of existing assessment tools, especially those used to assess cognitive and emotional symptoms. We developed an online platform to allow individuals with MJD/SCA3 or SCA7 and their healthcare professionals to assess and track their symptoms over time. **Methods:** Drawing on clinical experience from working with the MJD/SCA3 and SCA7 Aboriginal communities, we developed a culturally- and linguistically-appropriate online self-report questionnaire of MJD/SCA3 and SCA7 symptoms across movement, speech, cognition, and emotions. The “MJDFhub” online platform was created to allow multi-visit, personalised access to the questionnaire. Participants used the platform with the assistance of a support person, predominantly around annual visiting neurology clinics. Participants completed a survey about their experience. Participants’ responses to the questionnaire were provided to the treating neurologist, with neurologists invited to provide feedback on the utility of this information. **Results:** Twenty-eight individuals with MJD/SCA3 and three with SCA7 used MJDFhub for at least one clinical encounter. Areas reported as a notable issue (≥6 on a scale from 1= “no problem” to 9 = “big problem”) in at least 30% of sessions were mobility, vision, emotional control, anxiety, sleep, and memory. Qualitatively, participants generally enjoyed using MJDFhub and found the assessments easy to complete. Neurologist feedback indicated that questionnaire data provided insights into client functioning and influenced client care. **Discussion and Conclusion:** MJDFhub is an accessible tool to enhance serial monitoring of a multiple areas of function for Aboriginal Australians with MJD/SCA3 and SCA7, in a culturally-relevant way. The platform is promising for use with a wide range of ataxias and patient groups, including in under-resourced and culturally-distinct settings.

Funding Sources: auDA Foundation, Australia; Saunders Family Trust

C8. Cerebello-cortical diaschisis in Friedreich ataxia: converging evidence from connectivity and intrinsic activity fMRI

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Background and objectives: Friedreich ataxia (FA) is characterized by cerebellar degeneration and disruption of dentato–thalamo–cortical pathways, leading to motor impairment and cerebellar cognitive affective syndrome (CCAS). While altered functional connectivity has been reported, it remains unclear whether cortical dysfunction reflects true diaschisis. We combined resting-state functional connectivity and intrinsic activity (fALFF) analyses to test whether cerebello-cortical dysfunction in FA reflects a unified diaschisis mechanism underlying cognitive symptoms.

Methods: Twenty-eight individuals with genetically confirmed FA and 28 matched controls underwent resting-state fMRI. Seed-based connectivity (Crus I) was assessed using CONN. Intrinsic activity was quantified using fALFF. Associations with CCAS-S and SARA were examined, and subgroup analyses compared patients with and without definite CCAS.

Results: FA patients showed marked disruption of Crus I–prefrontal connectivity, with progressive loss across cognitive subgroups and complete disconnection in definite CCAS. In parallel, widespread reductions in fALFF were observed in cerebellar and cortical regions, including prefrontal, sensorimotor, and parietal areas. Patients with CCAS exhibited pronounced fALFF reductions within cerebello-prefrontal circuits. Increased connectivity with insular/opercular regions accompanied prefrontal disconnection. fALFF correlated with motor severity, whereas CCAS was associated with network-level disruption rather than linear activity changes.

Discussion and Conclusion: The convergence of connectivity breakdown and reduced intrinsic cortical activity supports a model of functional cortical diaschisis in FA. Cerebellar degeneration leads not only to disrupted communication but also to downstream physiological depression of structurally intact cortical regions, particularly within prefrontal networks. Cognitive impairment appears to emerge when compensatory network mechanisms fail, marking a transition from dysconnectivity to diaschisis. This unified framework links cerebellar pathology to distributed cortical dysfunction and provides a mechanistic basis for cognitive symptoms in FA, with implications for neuromodulation strategies targeting cerebello-cortical circuits.

Funding Sources: FARA

C9. Accelerated Brain Aging and Anatomical Substrates in Spinocerebellar Ataxia Type 3

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Background: Brain age is an important biomarker of neurological health, but its characteristics in spinocerebellar ataxia type 3 (SCA3) remain unclear.

Objective: To determine whether SCA3 is associated with accelerated brain aging and to examine its clinical, genetic, and neuroanatomical correlates.

Methods: We developed an XGBoost-based brain age model using gray matter features from structural MRI. The model was trained in 393 healthy individuals and validated in a local healthy control group (HL, $n = 96$), then applied to 223 patients with SCA3. Brain age and brain age gap (BAG) were compared between groups. Associations of BAG with clinical and genetic variables were assessed, and key brain regions contributing to brain age prediction were further examined.

Results: The model showed good predictive performance in healthy individuals. Compared with HL, patients with SCA3 exhibited significantly increased BAG ($p < 0.05$). BAG was positively correlated with CAG repeat length, disease duration, and SARA score, and negatively correlated with age at onset (all $p < 0.05$). In addition, 19 of 28 key regions showed greater gray matter atrophy in SCA3, and several were significantly associated with clinical and genetic measures.

Conclusions: SCA3 is characterized by accelerated brain aging. BAG may serve as a quantitative biomarker for disease progression and therapeutic evaluation.

C10. Transcranial Alternating Current Stimulation for Treatment of Multiple System Atrophy Cerebellar Type: A Randomized Controlled Trial

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Background: No disease-modifying treatments exist for the cerebellar subtype of multiple system atrophy (MSA-C). Because transcranial alternating current stimulation (tACS) has demonstrated safety and efficacy in spinocerebellar ataxia type 3, a condition marked by cerebellar dysfunction, we tested whether tACS could improve clinical outcomes in MSA-C.

Methods: Here, we designed a randomized, double-blinded, placebo-controlled clinical trial and studied the tACS efficacy and safety in MSA-C patients. Enrolled participants were assigned to receive daily 40-minute sessions of 70 Hz, 2 mA tACS 5 days/week for 2 weeks (active-tACS) or to a sham-tACS group. The primary outcome was the change from baseline to 2 weeks in the Unified Multiple System Atrophy Rating Scale (UMSARS) total score. The Scale for the Assessment and Rating of Ataxia (SARA), Scales for Outcomes in Parkinson's Disease - Autonomic Dysfunction (SCOPA-AUT), Measuring health-related quality of life in MSA (MSA-QoL), gait analysis, and functional magnetic resonance imaging (fMRI) were also performed to evaluate the efficacy of the tACS in MSA-C patients.

Result: The primary endpoint—the change from baseline in UMSARS scores—favored active stimulation: A-tACS -4.72 (3.69) versus S-tACS 0.41 (3.94), with a mean difference of -5.12 (95% CI -6.80 to -3.45), $P < 0.001$. No serious adverse events were reported, indicating a favorable safety profile. Besides, participants in the A-tACS group had better performance in SARA, and gait analysis. However, A-tACS was not superior to S-tACS in improving SCOPA-AUT and MSA-QoL. In addition, seed-based functional connectivity analyses revealed the Left Frontal Control (ContB.PFC_{lv}) and Default Mode (DefaultB.PFC_d) seeds showed the most extensive post-stimulation connectivity increases following A-tACS.

Conclusion: Our results show that tACS produced a statistically significant clinical improvement in MSA-C and was well tolerated. The underlying mechanisms may include enhanced brain network function.

C12. Experiences of Dysphagia Among Persons with Ataxia and Recommendations for Enhanced Service Provision

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Background & Objectives: Swallowing impairment (dysphagia) is increasingly being recognized as a symptom of ataxia. Dysphagia is associated with decreased quality of life (QOL) and higher morbidity and mortality in those with neurological disorders, yet patient experiences with ataxia-associated dysphagia have received limited attention. The objectives of this study were to explore patient experiences of dysphagia, examine practice patterns in dysphagia service provision from the patient perspective, and suggest enhancements to clinical and research practices.

Methods: Four hundred fifty persons with ataxia completed an exploratory, cross-sectional, mixed methods survey about their experiences with dysphagia and related clinical care via Qualtrics. Analysis included a combination of quantitative statistical techniques and qualitative analyses of open-ended responses. Methodological integration occurred across data collection and analysis.

Results: Ataxia progression was associated with increased odds of dysphagia. 75.1% of survey respondents reported clinically significant dysphagia symptoms, yet only 49.4% of these respondents had been referred for diagnostic work-up. Disrupted QOL was frequently reported, but the degree of disruption was reduced when participants received satisfactory clinical care. Qualitative analyses of open-ended questions revealed key themes related to experiences of dysphagia and potential explanations for observed statistical relationships.

Discussion: Findings indicate that dysphagia symptoms are common in ataxia, with negative impact on QOL. Although clinical dysphagia care is associated with improvements in QOL, many individuals are not referred to such specialists. Neurologists can enhance appropriate referral rates by querying patients about specific symptoms and/or incorporating validated screening instruments into clinical protocols. Ataxia progression can also inform referral timing. Researchers should incorporate swallowing status as an outcome variable in natural history and intervention studies.

Conclusion: This study provides insight into the prevalence and characteristics of ataxia-associated dysphagia as well as patient experiences with dysphagia service provision. Findings have practical implications for clinical practice and ataxia research.

C13. Most bothersome symptoms in hereditary ataxia

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Background and objectives: Symptoms in hereditary ataxias affect quality of life to varying degrees and may not correlate with impairment measured by clinical rating scales. We aimed to identify the most bothersome symptom reported by patients and their correlation with clinical scales.

Methods: A standardized questionnaire including 12 items rated how much each symptom bothered patients in daily life (none, mild, moderate, severe), identifying the most bothersome. Scale for Assessment and Rating of Ataxia and Inventory of Non-Ataxia Signs were assessed at each visit. We computed Spearman correlations between symptom burden and clinical scale scores and analyzed the effects of clinical severity, sex, age, and genotype using multivariable ordinal regression models.

Results: A total of 327 participants were included: 16 SCA1, 46 SCA2, 72 SCA3, 37 SCA7, 60 SCA27B, and 96 other SCAs. Walking difficulty was the most bothersome symptom of 58% of the participants, followed by visual disturbances (11%), dysarthria (7%), and fatigue (5%). Gait difficulties were rated mild in 20% of patients, moderate in 38% and severe in 33%. Mild or greater bother from pain and sleep disturbance was reported by 30% and 29% of participants, respectively. SARA scores showed moderate to strong correlations with patient-reported symptom burden, particularly for walking difficulties ($p=0.65$), fine motor impairment ($p=0.54$), dysarthria ($p=0.45$), and swallowing difficulties ($p=0.43$) (all $p<0.0001$). Urinary dysfunction and dysphagia items from INAS strongly correlated with corresponding patient-reported bother (both $p=0.81$, $p<0.0001$), whereas the correlation between patient-reported leg stiffness and lower-limb spasticity was weaker ($p=0.32$, $p<0.0001$). Adjusted models showed the largest genotype effects for diplopia highest in SCA3 and SCA27B, and visual impairment in SCA7.

Discussion and conclusion: Clinical rating scales correlate with the type of symptoms that patients reported as bothersome. However, symptoms not captured by these scales, such as sleep and pain, are frequent and clinically relevant.

C14. Knowledge Gains, Self-Reported Confidence and Competence, and Clinical and Research Interest After Ataxia Clinical Training (ACT)

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Background and Objectives: Given the complexity and rarity of ataxic disorders, clinicians often have limited exposure to ataxia-specific training. Ataxia Clinical Training (ACT) is a foundation-sponsored, 3-day clinical education program designed to improve knowledge, diagnosis, and management of ataxia. This study evaluated changes in knowledge, self-reported confidence and competence, and interest in ataxia-related clinical care and research following the 2026 ACT course.

Methods: Participants completed pre- and post-course knowledge assessments and self-evaluations of confidence, competence, and interest in further work in the ataxia field. Knowledge was assessed using a 24-point multiple-choice test developed by ACT faculty. Confidence and competence across key domains and interest in pursuing ataxia-related career opportunities were measured using Likert-type items. Changes in knowledge were analyzed using paired t-tests, paired Likert-type responses were analyzed using Wilcoxon signed-rank tests with Pratt modification, and course evaluation data were summarized descriptively.

Results: Mean knowledge test scores increased from 55% to 68% ($p < 0.0001$; $n=43$), and 84% of participants improved. Self-reported preparedness to provide care for patients with a variety of ataxic disorders increased from 23% to 91% ($p < 0.0001$; $n=43$), and awareness of current clinical trials increased from 5% to 65% ($p < 0.0001$; $n=43$). Eighty-one percent of clinicians reported greater interest in pursuing ataxia clinical care opportunities, and 65% reported greater interest in participating in ataxia research ($n=41$).

Discussion and Conclusion: Participation in ACT was associated with significant improvements in ataxia-related knowledge and self-reported confidence and competence. Participants also reported increased interest in ataxia clinical care and research. These findings support the value of targeted ataxia-specific training in building clinical capacity and research engagement.

Funding Sources: This study was fully supported by the National Ataxia Foundation.

C15. Clinical features and mobility milestones of dentatorubral-pallidoluysian atrophy: a comparative study of juvenile- and adult-onset patients

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Background and objectives: Dentatorubral-pallidoluysian atrophy (DRPLA) is a rare subtype of spinocerebellar ataxia, characterized by diverse clinical phenotypes depending on age at disease onset. Motor and swallowing functions gradually worsen over the disease course. However, longitudinal data on disease progression remain limited. This study aimed to evaluate clinical features and mobility milestones of DRPLA by comparing juvenile-onset (< 20 years) and adult-onset ($\geq$ 20 years) patients.

Methods: We retrospectively analyzed 23 patients with DRPLA (juvenile-onset: n = 10, adult-onset: n = 13), investigating age at disease onset and clinical features. We also examined age at onset of gait disturbance, wheelchair dependence, and becoming bedridden as mobility milestones, along with age at initiation of enteral nutrition.

Results: The mean ages at disease onset were 12.4 and 36.7 years in the juvenile- and adult-onset groups, respectively. Ataxia and cognitive impairment were observed in all patients. Epilepsy and myoclonus were more frequent in the juvenile-onset than in the adult-onset group, whereas choreoathetosis and psychiatric symptoms showed the opposite trend. In addition, median intervals from the onset of gait disturbance to wheelchair dependence and to bedridden state were 4.5 and 9.0 years in the juvenile-onset group, compared with 7.0 and 12.0 years in the adult-onset group. Enteral nutrition was introduced in 18 patients (78.3%), and its initiation age closely matched the age at becoming bedridden.

Discussion and conclusion: We found that the duration from wheelchair dependence to becoming bedridden was approximately five years in both groups despite differences in clinical phenotypes. These findings provide valuable insights for patients and their families in predicting disease progression and prognosis.

Funding Sources: Grants-in-Aid from the Research Committee of Ataxia, Health Labour Sciences Research Grant, the Ministry of Health, Labour and Welfare, Japan (JPMH20FC1041)

C16. Emotion recognition from facial expressions and prosody is impaired in Friedreich ataxia patients

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Background and objectives: The cerebellum has been largely implicated in social cognition, yet this domain remains underexplored in Friedreich ataxia (FA). One of its key components is emotion recognition, defined as the ability to discern emotions from verbal or nonverbal cues. While facial emotion recognition has been sparsely studied in FA, emotion recognition from prosody has not been systematically investigated. This study aimed to assess emotion recognition from both facial expressions and prosody in FA and to examine its relationship with disease severity.

Methods: We recruited 30 FA patients and 29 age-matched healthy volunteers (HV). Participants completed the Facial Emotion Recognition Test (ERT) which comprised 25 Ekman-based facial expressions and a Prosody ERT consisting of 30 recordings of semantically neutral sentences and non-verbal vocalizations delivered by professional actors expressing specific emotions. Both tasks assessed five basic emotions (anger, disgust, fear, happiness, sadness). Disease severity was assessed using the Scale for the Assessment and Rating of Ataxia (SARA), and the scale of the Activities of Daily Living (ADL).

Results: Patients performed significantly worse than HV on both tasks. Mean correct responses were 19.8 vs. 22.4 ($p < .001$) for Facial ERT and 23.4 vs. 26.2 ($p = .004$) for Prosody ERT. Only prosody recognition showed an association with disease severity, correlating with both SARA ($r = -0.63$, $p < .001$) and ADL ($r = -0.48$, $p = .008$), whereas facial emotion recognition showed no such relationship (SARA $r = -0.243$, $p = .195$, ADL $r = -0.18$, $p = .341$).

Discussion and Conclusion: Emotion recognition is impaired in FA patients, but only prosody recognition appears to be linked to disease severity, suggesting a particular vulnerability of auditory-affective processing. These findings provide the first evidence that prosody-based social cognition is affected in FA and support the role of cerebellar dysfunction in higher-order social processing. Further work is needed to determine its clinical relevance and impact on social interactions and quality of life.

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C17. Symptoms, impact on quality of life and ASO knowledge of people with SCA3 and SCA2 in the US

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Background: Spinocerebellar ataxia types 3 and 2 (SCA3/SCA2) are hereditary, slowly progressive disorders. For first-in-human studies, it is important to understand participants' perspective on disease burden and ASO acceptability to optimize protocol design.

Objectives: To assess symptom burden and impact on Quality of Life (QoL), treatment priorities, ASO knowledge, and willingness to participate in ASO clinical trials among ambulant participants with SCA3 and SCA2 or genetically at risk for these disorders, and caregivers.

Methods: A cross-sectional online survey was disseminated through the National Ataxia Foundation. Eligibility required residence in the United States, ambulatory status without permanent walking aids, or caregiver status for such individuals. Descriptive statistics were generated from 111 eligible respondents.

Results: 54% had SCA3, 38% SCA2, and 8% were caregivers. Mean age at survey for SCA3/SCA2 was 50/50 years; mean ages at symptom onset was 43/39 years, respectively. Across both genotypes, balance or walking impairment was the first symptom to be noticed in 81–83% of patients and the most QoL-limiting symptom in 83–87%. Treatment priority rankings similarly placed balance or walking problems first (SCA3/SCA2: 87/76%). Initial ASO knowledge was low (SCA3/SCA2: 30/ 16%, reporting agree/strongly agree) but improved markedly after an educational introduction to the survey (SCA3/SCA2: 60/57%).

Willingness to join an ASO clinical trial was high across all therapeutic expectations: stabilization (50–52%), symptom reduction (55–57%), prevention of worsening (57–64%), and cure (63–79%). Acceptability of intrathecal dosing was strongly frequency dependent, with highest agreement at 3–6 month intervals (SCA3/SCA2: ~72–73%/~83–86%). Placebo-controlled designs and procedural risks were acceptable to patients.

Conclusion: Survey findings demonstrate a consistent, quantifiable prioritization of gait and balance outcomes and a high readiness to participate in ASO clinical trials, supporting their feasibility and informing patient-centred protocol design in SCA3 and SCA2 participants.

Funding Sources: Servier Bio-Innovation LLC and Institut de Recherches Internationales Servier

C18. Level and variability of self-reported emotional function, psychosocial function, cognition, and fatigue in spinocerebellar types 1, 2, 3, and 6

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Background and Objectives: In spinocerebellar ataxias (SCAs), non-motor symptoms such as cognitive and emotional difficulties can impact daily function and participation, but their breadth and their temporal patterns are not well understood. Given that non-motor symptoms are potentially modifiable, it is important to better understand and track them across the disease course through patient-reported measures, allowing patient-centred interventions to support quality of life and participation. We investigated the level and month-to-month variability of multiple non-motor symptoms in SCAs.

Methods: Individuals with symptomatic SCA types 1, 2, 3, and 6, and unaffected controls (CON), completed self-report questionnaires on depression, emotional dyscontrol, cognition, social participation, and fatigue, within a two-year longitudinal online study. Ataxic motor symptoms were evaluated by self-report on the Patient-Reported Outcome Measure of Ataxia – Physical scale.

Results: At the cross-sectional group level, individuals with SCA (n=90) reported significantly elevated symptoms of depression ($p=.002$) and fatigue ($p>.001$) relative to controls (n=62), and reduced ability to participate in social roles/activities (69 SCA vs. 35 CON; $p<.001$). Across all non-motor measures, poorer outcomes were significantly associated with motor severe motor ataxia symptoms (absolute $r = 0.224-0.557$). Across ≥ 10 monthly questionnaire completions, variability in total scores across individuals and measures ranged from 0 to $>50\%$ of the possible score range. This temporal variability did not significantly differ between SCA (n=62) and CON (n=29) participants; however, higher variability of self-reported cognitive function was strongly associated with more severe baseline ataxic motor symptoms ($r=.52$, $p<.001$).

Discussion and Conclusion: Individuals with SCAs 1/2/3/6 experience significant non-motor symptoms, which are associated with ataxic motor severity. Further work should investigate the role of disease stage/burden on symptom level and variability, and non-motor symptoms in other ataxias. Notable variability in symptoms across months highlights the necessity of serial assessments to characterise non-motor function in clinical and research settings.

Funding Sources: National Ataxia Foundation (Young Investigator Award, Ian Harding)

C19. Experiences and help-seeking for psychological difficulties in ataxia: the consumer voice

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Background and Objectives: Psychological and cognitive-affective symptoms are increasingly recognised as important features of hereditary and acquired ataxias. However, their perceived impact on daily life, their priority for affected individuals and families, and access to appropriate psychological support remain insufficiently characterised. We aimed to explore these issues from the perspective of people living with ataxia and close others/carers.

Methods: We conducted an online survey among adults from Australian- and Czech ataxia patient mailing lists/registries, followed by an optional individual or group interview. Participants had an ataxia diagnosis or were a close other/carer. The surveys and interviews covered psychological symptoms in the person with ataxia, and experiences and views on accessing psychological support.

Results: 144 individuals with various ataxia diagnoses, and 23 close others, completed the survey. The most frequently self-reported symptoms among people with ataxia were low mood, word-finding difficulties, irritability, and difficulty multitasking. Close others most commonly reported irritability, difficulty multitasking, emotional instability, and low mood. Interview data highlighted the impact of these symptoms on occupational function and interpersonal relationships. Most individuals with ataxia (57%) and close others (83%) reported that it was “very important” or “quite important” to discuss and address these issues. About one-third of people with ataxia had sought help for psychological difficulties; most of these individuals had received help. Barriers to seeking help included costs, travel, time, waitlists, and a lack of mental health professionals with knowledge of ataxia. Furthermore, some individuals were unaware that psychological symptoms could be associated with ataxia.

Discussion and Conclusion: Psychological symptoms are commonly reported by people with ataxia and close others, and can impact daily life. There are several barriers to appropriate care. There is an important need for tailored and accessible educational and interventional resources to support psychological wellbeing in ataxias.

C20. Real-world experiences with omaveloxolone in Friedreich ataxia: baseline demographics and patient-reported outcomes from the ARIES study

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Background and objectives: Omaveloxolone was approved in February 2023 for the treatment of Friedreich ataxia (FA) in patients aged ≥16 years in the US. The ARIES (Assessing Real-World Experiences on SKYCLARYS®) study aims to characterize omaveloxolone treatment experience, including patient-reported outcomes (PROs), in the US real-world setting. This initial report describes baseline demographics and PROs in enrolled patients.

Methods: ARIES is an ongoing, observational, ambispective, noninterventional study enrolling voluntarily participating patients aged ≥16 years with healthcare provider–diagnosed, genetically confirmed FA receiving or initiating omaveloxolone in the US. Data sources include medical records and prospective self-reported questionnaires, with all assessments completed directly by patients. The following are summarized for enrolled patients: baseline demographics and survey-based PROs, including quarterly custom survey of ambulation and quality of life; quarterly Modified Fatigue Impact Scale (MFIS); semiannual Friedreich Ataxia Activities of Daily Living (FA-ADL); and annual Patient Health Questionnaire-8 (PHQ-8).

Results: As of April 20, 2026, 146 patients enrolled, reflecting US geographic representation; 79.5% were aged 20-49 years, 54.1% were women, and 89.0% were White. Overall, 64% reported wheelchair use for shorter distances and 75% reported wheelchair or motorized scooter use for longer distances. All participants were able to take part in hobbies/social activities (30% a little, 36% a moderate amount); 69% reported some or quite a bit of difficulty and 17% were unable to perform work-related tasks. Most (95%) reported no emergency department visits, urgent care visits, or hospitalizations in the past 3 months.

Discussion and conclusion: ARIES enables longitudinal real-world follow-up, including in patients underrepresented in clinical trials and in a different age distribution compared with the MOXIe clinical trial. This initial analysis provides early insight into baseline characteristics and PROs among US patients with FA receiving omaveloxolone. Ongoing enrollment and follow-up will further characterize long-term patient experience and outcomes with omaveloxolone.

Funding Sources: Biogen

C21. Online CME Improves Multidisciplinary Recognition and Clinical Competence in Friedreich Ataxia

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Background and objectives: Friedreich Ataxia (FRDA) diagnosis is frequently delayed due to low clinician awareness of clinical red flags and complex differential diagnosis. This educational initiative aimed to address these gaps by increasing knowledge of key symptoms and the evolving management landscape.

Methods: Neurologists and pediatricians participated in a multi-presentation online accredited activity featuring 5 experts and 1 patient/parent perspective (available at [medscape.org/viewarticle/clinicians-primer-friedreich-ataxia-2025a1000zuh](https://www.medscape.org/viewarticle/clinicians-primer-friedreich-ataxia-2025a1000zuh)). A paired pre- and post-test study design assessed changes in knowledge and competence. Statistical significance was determined using paired samples t-tests and McNemar's test, with $P < .05$ considered significant. The activity launched January 12, 2026, and data were collected through March 19, 2026.

Results: Overall significant improvements in correct response rates were observed for neurologists (41% pre-assessment vs 81% post-assessment; $P < .001$, Cohen's $d = 1.34$; $n=135$) and pediatricians (33% pre-assessment vs 80% post-assessment; $P < .001$, Cohen's $d = 1.59$; $n=185$). Specific significant knowledge gains included identifying hypertrophic cardiomyopathy as the most clinically relevant non-neurological manifestation of FRDA, differentiating FRDA from another hereditary ataxia based on EMG findings (sensory neuropathy excluding FRDA), awareness of what genetic test result confirms FRDA (GAA repeat expansion), and awareness of the impact of omaveloxolone on slowing FRDA disease progression. Following the education, 22% of neurologists and 24% of pediatricians reported increased confidence in identifying signs of possible FRDA. Top intended practice changes included considering FRDA in young patients with poor balance/speech (37% neurologists; 32% pediatricians) and in those with hypertrophic cardiomyopathy (24% neurologists; 21% pediatricians).

Discussion and Conclusion: This online CME successfully bridged critical knowledge gaps, particularly for pediatricians who demonstrated the most substantial educational growth. Improving competence in recognizing red flags and genetic markers enhances clinical readiness for earlier diagnosis and appropriate specialist referral, potentially improving patient outcomes in FRDA.

C22. Urinary dysfunction in Friedreich Ataxia

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Background and objectives: Friedreich ataxia (FRDA) is an inherited ataxia with multisystemic involvement. Lower urinary tract symptoms (LUTS) have been reported but poorly characterized. We aim to investigate LUTS prevalence in the FRDA population and to explore possible predictors.

Methods: Retrospective analysis of data from genetically confirmed FRDA patients enrolled in the FACOMS natural history study with a focus on medical history, medication list, and ADL questionnaire. Statistical analysis included a regression model for predictors.

Results: 661 FRDA subjects were included (age at baseline: 21±14, 50.4% female, mFARS: 42±18, GAA1: 644±261, GAA2: 939±234) with an overall LUTS prevalence of 48.5% (263/542) in medical history (physician-reported), ADL questionnaire or both. Cohen's kappa agreement between physician-reported and ADL questionnaire was 0.371 (fair). Multivariate regression showed that age at baseline, male sex and mFARS were significant predictors of LUTS as measured by the ADL questionnaire, OR (95% CI) respectively: 1.05 (1.02-1.09), 0.39 (0.23-0.65), 1.05 (1.02-1.07).

Discussion and conclusion: LUTS are prevalent in the FRDA population but remain an underexplored subject. Further work is needed to standardize a method of detection for more accurate identification and treatment.

Funding Sources: FARA

C24. Understanding progression of mobility during and after loss of ambulation for people with Friedreich ataxia: A qualitative descriptive study.

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Background and Objectives: A significant moment for people with Friedreich ataxia (FRDA) is loss-of-ambulation and the transition to using a wheelchair. Little research has explored factors contributing to transitions in mobility status over time, nor the mobility journey beyond loss-of-ambulation. This study explored the lived experience of individuals with FRDA who are transitioning to non-ambulation or are non-ambulatory. Improved understanding of lived experience will inform clinical practice and the design of sensitive, clinically meaningful mobility outcome measures.

Methods: This qualitative descriptive study used a narrative inquiry approach. People with FRDA with a Friedreich Ataxia Rating Scale Functional Disability Staging score between 4.0-6.0 participated in interviews and focus groups. Journey maps depicting the experience of mobility transitions were created for each participant. Data were analysed using thematic framework analysis with interpretation informed by the journey maps and two lived experience advisors.

Results: Twenty adults with FRDA participated (n=15 interviewees, n=5 focus group participants). Meaningful transitions continue after loss-of-ambulation (e.g., transition from pivot to hoist transfers). Mobility journeys are underpinned by a complex interplay of physical change, psychosocial factors, external barriers/enablers and key events (e.g., injury) dictating transitions. Participant narratives of transitions revealed a cyclical, three-phase pattern: 1) determination to maintain current function despite an increasing physical and cognitive load 2) realisation that transition is needed and 3) transition and re-entering the determination phase. Individual priorities influenced when and why participants made transitions. Some priorities conflicted within (e.g., maintaining independence versus safety), requiring 'trade-offs.' Participants reflected that readiness to transition is not based solely on physical function but also a psychosocial process.

Discussion and Conclusion: Drivers of transitions in the mobility journey are physical and psychosocial. Incorporating these findings into clinical practice will facilitate more nuanced assessment of readiness to transition. Results have informed the development of a new clinical-reported outcome measure.

Funding Sources: This study is funded by the Friedreich Ataxia Research Alliance (FARA), USA.

C25. Introducing the FAB-MI: A new instrumented measure of dynamic balance and mobility for people with Friedreich ataxia

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Background and Objectives: There are no valid outcome measures for examining loss of dynamic balance, nor a sensitive measure of balance decline beyond loss-of-ambulation for people with Friedreich ataxia (FRDA). This study aimed to develop a sensitive and clinically meaningful clinician-reported outcome measure (Clin-RO) of dynamic balance and mobility for individuals with FRDA who are ambulant and non-ambulant.

Methods: The Friedreich Ataxia Balance and Mobility Index (FAB-MI) was developed over three stages. First, twenty people with FRDA participated in a qualitative journey mapping study to identify important indicators of change or decline in mobility status over time. Pictorial journey maps were developed from individual interviews (n=6), refined in a subsequent focus group (n=2) and additional individual interviews (n=9), and verified in a final focus group (n=3). From the journey maps, draft items and response categories were developed for the FAB-MI. Two clinicians and three individuals with FRDA reviewed the items and provided feedback on importance, relevance and clarity. The FAB-MI was field tested with a further 12 people with FRDA, with additional refinements made prior to finalisation.

Results: The FAB-MI is to be administered by a trained healthcare professional via a structured interview with a person with FRDA. It comprises 35 items across nine mobility and balance domains. Lower scores equal better function. Participants in the qualitative study described how the cognitive effort required to perform tasks (e.g., concentrating on body positioning) changes over time. To reflect this, most items include a Visual Analogue Scale to quantify cognitive effort associated with the item.

Discussion and Conclusion: The FAB-MI is a new Clin-RO, informed by people with FRDA, that measures changes in mobility and dynamic balance across the disease spectrum. A Rasch analysis will be conducted to further refine the instrument, potentially reducing items, and to determine the scoring structure.

Funding Sources: This study is funded by the Friedreich Ataxia Research Alliance (FARA), USA.

C26. Evaluating the potential of at-home wearable sensors to produce sensitive and meaningful digital measures in spinocerebellar ataxia types 2 and 3

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Interventional trials for spinocerebellar ataxias (SCA) require sensitive and meaningful motor outcome measures to evaluate therapy efficacy. We aim to validate and optimize genotype-specific motor outcome measures for SCA2 and SCA3 using wearable sensors worn continuously at home. Building on work demonstrating sensitivity to progression across broad ataxia cohorts, we initiated a new study in SCA2 and SCA3 with increased assessment frequency.

Target enrollment is 30 individuals with SCA3, 30 individuals with SCA2 (Scale for the Assessment and Rating of Ataxia (SARA) score 3–15; gait subscore 1–4), and 15 healthy controls. Participants are recruited from U.S. CRC-SCA Natural History Study sites. Participants wear Sysnav Syde wrist and ankle sensors (accelerometer/gyroscope) during waking hours for two weeks every three months over 18 months. Cross-sectional and longitudinal analysis will be performed to evaluate digital measures, including submovements and stride velocity. Sensor data will be correlated with patient-reported outcomes (Patient-Reported Outcome Measure of Ataxia (PROM-Ataxia), Activities-specific Balance Confidence (ABC), Falls Questionnaire, Patient Global Impression of Improvement (PGI-I)) and annual SARA scales.

To date, 26 participants are enrolled (16 SCA3, 3 SCA2, 7 Controls). Preliminary mean $\pm$ SD PROM-Ataxia and ABC scores for SCA participants (n=14) are 73.6 $\pm$ 44.3 and 71.9% $\pm$ 19.4%, respectively. Four SCA3 participants reported feeling “worse” than three months ago via PGI-I; others remain “stable.” Per two-week period, the SCA group (n=15) averaged 166.9 (SD=24, range=117.1–202.9) wrist and 165.9 (SD=24.6, range=122.9–207.0) ankle hours; Controls (n=8) averaged 149.4 (SD=19.6, range=129.1–186.0) wrist and 151.0 (SD=23.5, range=110.3–189.7) ankle hours.

Final longitudinal analysis will determine the sensitivity of wearable-derived measures in capturing disease progression. Evaluating these measures will provide clinical trial teams with the information necessary to further support use in future SCA2 and SCA3 therapeutic trials.

Funding Sources: Servier Pharmaceuticals is funding this work.

C28. Validation of the Scale for Ataxia Severity: A Novel Utilization of Cross-Sectional Data for Longitudinal Severity Score in Hereditary Ataxias

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Background and Objectives: Hereditary ataxias are chronic neurological conditions with predictable progression. Symptom severity is commonly assessed via the Scale for the Assessment and Rating of Ataxia (SARA), but this score gives no insight into disease progression. We aim to validate a Scale for Ataxia Severity (SAS), combining SARA scores with disease duration at assessment, to provide information on both disease progression and current symptom severity with even single-assessment SARA data.

Methods: We analysed SARA scores of 355 patients across three genetic ataxias: ARSACS, SPG7 and Ataxia Telangiectasia. Using non-linear quantile regression, we generated a disease progression model with quantiles representing different severities of progression over the disease course. We validated model robustness using repeated randomised sub-setting to divide the dataset and confirm similar results between subgroups. We assessed SAS score stability, that SAS scores are similarly distributed at each disease duration and individual patients maintain similar SAS scores between assessments, in all patients together and within disease-specific groups of each genetic ataxia. We further analysed the application of severity groupings (based on 5 SARA points).

Results: Derived SAS was robust and distributed similarly across disease durations. At the individual patient level, no significant difference was detected in disease-specific groups and when applying severity groupings, but a modest significant difference was detected when using all patients together.

Discussion and Conclusion: The present study validates the SAS methodology which represents a robust, transferable tool with the potential to increase the value of single-assessment SARA data in understanding disease natural history of hereditary ataxias. However, the sample size was too small to disassociate effects of disease type from individual variation. Including longitudinal data from more patients with different genetic ataxias and validation against other clinical scales would further improve accuracy of the SAS. With these further refinements, the SAS could serve as a valuable clinical prognostic tool.

Funding Sources: This project was supported by several national research agencies (e.g. the Deutsche Forschungsgemeinschaft, DFG, German Research Foundation No 441409627), as part of the PROSPAX consortium under the frame of EJP RD, the European Joint Programme on Rare Diseases, under the EJP RD COFUND-EJP N° 825575.

C29. Cerebello-cerebral connectivity alterations reflect verbal fluency impairment in cerebellar degeneration

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The cerebellum is known as an active hub in language processes, particularly in verbal fluency (VF). Analyzing VF performance in patients with cerebellar ataxia may improve our understanding of how cerebellar dysfunction affects language networks. This study investigated resting-state functional connectivity between cerebellar and cerebral language networks in relation to VF performance in ataxia patients, and whether these relationships differ across fluency subtypes.

22 ataxia patients with predominantly cerebellar degeneration and matched controls underwent 3T resting-state fMRI and completed VF tasks covering phonemic, semantic, and semantic category-switching VF. Dysarthria severity was assessed via diadochokinesis rate as a control measure. Seed-to-voxel analysis used the right-hemispheric cerebellar functional network S1(Nettekoven atlas, 2024) as seed. Connectivity values were entered into linear regression models with VF T-scores as outcomes, corrected for multiple comparisons ($p < .05$). Robustness was tested using a confound model including depression severity, premorbid intelligence, and ataxia severity.

Group comparison revealed anticorrelated functional connectivity between the cerebellar seed and three cerebral clusters: right fronto-temporal opercular cortex, including Area 44, right premotor cortex, and left superior-temporal cortex and insula. Patients showed increased anticorrelated connectivity across all clusters and reduced VF performance compared to controls. VF performance was not significantly associated with dysarthria severity. Semantic VF was significantly associated with all clusters in the primary model, with effects remaining nominally significant after controlling for covariates.

These findings suggest that increased anticorrelated connectivity between the cerebellar language network and right premotor and temporo-frontal opercular areas may be associated with VF deficits in ataxia patients. The absence of associations between dysarthria and category-switching performance indicates language-specific, rather than motor or executive, processes. We hypothesize that impaired cerebellar function drives compensatory reorganization toward right-hemispheric regions, as observed in Friedreich's ataxia or post-stroke aphasia. Whether this reflects true compensation or interhemispheric dysfunction remains to be determined.

Funding Sources: This project is supported by a doctoral scholarship from the Friedrich Ebert Foundation (FES) in Germany. Travel reimbursement for participants was supported by the Deutsche Heredo-Ataxie Gesellschaft e.V. (DHAG).

C30. A Multidisciplinary Care Model for Hereditary Cerebellar Ataxia

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Background/Objectives: Hereditary cerebellar ataxias are a diverse group of progressive neurodegenerative disorders with substantial genetic and phenotypic heterogeneity. Many patients remain without a molecular diagnosis after years of fragmented evaluations. We sought to evaluate the diagnostic yield of a tiered genetic testing strategy in our multidisciplinary ataxia clinic, which integrates genetic evaluation, treatments and rehabilitation.

Methods: From 2021-2025, 149 adults with clinical features concerning for a hereditary ataxia but without an established genetic diagnosis were enrolled into our IRB-approved study. Genetic testing followed a tiered approach, beginning with repeat expansion testing and followed by targeted and/or whole exome sequencing if initial tests were nondiagnostic. Patients were offered specialized neurologic physical therapy focused on gait and balance.

Results: The cohort included 73 men and 76 women with a mean age of 64 years (range 24-90). After genetic testing through our protocol, 86 patients (57.8%) had confirmed genetic diagnoses: 29 with spinocerebellar ataxia 27B (SCA27B), 18 with cerebellar ataxia with neuropathy and vestibular areflexia syndrome (CANVAS), 6 with SCA2, 5 with SCA6, 4 with SCA8, 4 with Friedreich's ataxia, and 20 with other subtypes of ataxia. 57 patients (38.3%) remained without a genetic diagnosis. Six patients (4%) were later determined to have non-genetic causes (3 with MSA-C, 1 with autoimmune ataxia, 2 deemed secondary to vascular insult). Patients were offered medications based on symptoms and/or genetic diagnoses. All patients were encouraged to attend at least one session of neurologic physical therapy at our institution.

Discussion/Conclusion: Recent advances in genetic testing, particularly the identification of genes for SCA27B and CANVAS, have substantially improved diagnostic yield in hereditary ataxias. Our experience highlights the value of a multidisciplinary clinic model integrating genetic testing, customized treatments and rehabilitation. Ongoing work aims to better quantify response to pharmacotherapy and physical therapy across specific genetic subtypes.

Funding Sources: The institution of Dr. Shakkottai has received research support from the National Institutes of Health.

C31. Nationwide Molecular Epidemiology of Spinocerebellar Degeneration in Japan

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Background and Objectives: Spinocerebellar degeneration (SCD) represents clinically and genetically heterogeneous neurodegenerative disorders characterized by progressive ataxia. Although advances in molecular genetics have expanded causative genes, the molecular epidemiology of SCD in Japan remains unclear. We aimed to define nationwide molecular epidemiology using a stepwise genetic diagnostic strategy in the Japan Consortium of Ataxias (J-CAT), a large-scale nationwide registry platform for SCD.

Methods: From December 2016 to February 2025, 2,688 patients were enrolled. First-line screening included PCR fragment analysis and repeat-primed PCR for SCA1, 2, 3, 6, 7, 8, 12, 17, 31, 36, DRPLA, and HD. Second-line screening included SCA27B testing in patients aged ≥30 years and CANVAS screening in those aged ≥30 years without a family history suggesting autosomal dominant inheritance. WES/WGS and phenotype-driven candidate gene analyses were subsequently performed in genetically undiagnosed cases.

Results: First- and second-line screening established molecular diagnoses in 1,290 patients (48%). WES/WGS or targeted candidate gene analyses in 1,084 undiagnosed patients identified 106 (10%) additional diagnoses. Overall, 1,396 of 2,688 patients (52%) received molecular diagnoses. The most frequent diagnosis was SCA31(n=365), followed by SCA6(n=330), MJD/SCA3(n=218), DRPLA (n=82), SCA8(n=57), CANVAS (n=52), SCA2(n=43), SCA1(n=36), SCA36(n=34), EA2(n=29), and SCA27B (n=24). CANVAS and SCA27B testing improved diagnostic yield. Still, nearly half of the patients remained unresolved.

Discussion and Conclusions: This nationwide study updates the molecular epidemiology of SCD in Japan and supports the utility of our stepwise diagnostic strategy. The predominance of SCA31 highlights Japan's unique genetic background, while CANVAS and SCA27B testing improved diagnostic yield. Many unresolved cases indicate opportunities for novel genes or variants discovery. With standardized nationwide clinical and genetic data, J-CAT represents a valuable resource for international collaborative studies of SCD.

Funding Sources: This work was supported by grants from the Ministry of Health, Labour and Welfare of Japan, the Japan Agency for Medical Research and Development (AMED), and Sanofi.

C32. Quality of Life, Unmet Needs and Disease Burden in Friedreich's Ataxia Patients in Greece: A Nationwide Survey

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Background and Objectives: Friedreich's Ataxia (FA) is a rare, progressive neurodegenerative disorder associated with severe disability and reduced quality of life. Data regarding the lived experience and unmet needs of FA patients in Greece remain scarce. This study aimed to evaluate disease burden, psychosocial impact, healthcare access, and quality of life among individuals living with FA in Greece.

Methods: A nationwide cross-sectional survey was conducted by the Hellenic Friedreich's Ataxia Association (HEFAA) between October and November 2025. Anonymous online questionnaires were completed by patients with FA and close family caregivers. The survey assessed demographics, clinical manifestations, mobility, psychosocial well-being, healthcare access, daily functioning, employment impact, and financial burden. Descriptive statistical analyses were performed.

Results: Thirty-nine respondents participated, representing a broad geographic distribution across Greece. Participants ranged from 8.5 to 65 years old (mean age 31.8 years). Symptom onset typically occurred during childhood or adolescence, while delayed diagnosis was commonly reported. Mobility impairment, gait instability, fatigue, speech disorders, and scoliosis were the most frequently reported symptoms. Most participants required mobility aids, predominantly manual wheelchairs.

FA significantly affected social participation, education, employment, and emotional well-being. Twenty-eight of 39 respondents reported that FA often or always limited attendance at work or school, while 33/39 reported major restrictions in social activities. Anxiety about the future, sadness, and feelings of being a burden were highly prevalent. Many participants expressed a need for psychological support and reported ongoing psychotherapy or psychological treatment. Financial burden and limited access to specialized services were additional major concerns.

Discussion and Conclusion: This study demonstrates the substantial multidimensional burden experienced by individuals with FA in Greece. Findings highlight the urgent need for multidisciplinary care pathways, earlier diagnosis, improved rehabilitation and mental health services, and stronger social support policies to improve quality of life and equity of care for FA patients.

Funding Sources: Genesis Pharma

C33. Real-World Experience with Omaveloxolone in Friedreich's Ataxia Patients in Greece: Results from a Nationwide Survey

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Background and Objectives: Omaveloxolone (Skyclarys®) is the first approved disease-modifying therapy for Friedreich's Ataxia (FA). Real-world evidence regarding patient experience, adherence, perceived effectiveness, and quality-of-life impact remains limited. This study aimed to evaluate real-world experiences with omaveloxolone among FA patients in Greece.

Methods: The Hellenic Friedreich's Ataxia Association (HEFAA) conducted a nationwide anonymous survey between October and November 2025 among patients with FA and close family caregivers. The questionnaire assessed treatment uptake, adherence, treatment interruptions, perceived benefits, side effects, and impact on daily life and psychological well-being. Descriptive analyses were performed.

Results: Thirty-eight individuals participated, including 36 patients currently receiving omaveloxolone. Treatment uptake was high, with 95% of respondents receiving therapy. Most participants had been treated for more than 12 months, and adherence was generally excellent, with 81% reporting no missed doses during the previous month. Temporary treatment interruptions occurred mainly because of administrative delays or laboratory monitoring issues rather than treatment dissatisfaction.

Overall, 61% of treated patients reported positive effects, with higher rates among ambulatory patients (77%) compared to non-ambulatory individuals (52%). Frequently reported benefits included reduced fatigue, improved speech articulation, increased energy, improved balance, and better coordination. Several participants also reported improved confidence and greater independence in daily activities. Side effects were generally mild and manageable, rarely leading to discontinuation of therapy. Overall satisfaction with treatment was high, and nearly all participants stated they would recommend omaveloxolone to other individuals with FA.

Discussion and Conclusion: This nationwide real-world survey demonstrates high adherence and meaningful patient-reported benefits associated with omaveloxolone treatment in Greece. The findings support the clinical and psychosocial value of sustained treatment access and highlight the importance of minimizing administrative barriers to ensure continuity of care for FA patients.

Funding Sources: Self funded by the HEFAA.

C34. NAF Ataxia Centers of Excellence Reporting Demonstrates Disease Representation, Care Service Availability, and Opportunities for Network Optimization

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Background and Objectives: The National Ataxia Foundation (NAF) established the Ataxia Centers of Excellence (ACE) program in 2023 to recognize centers that provide multidisciplinary care, participate in community outreach, and advance ataxia research.

To date, 43 centers from 12 countries have been approved to join this collaborative network. In this analysis, data gathered through ACEs were used to evaluate disease representation patterns, differences in care service availability, and opportunities for network optimization across ACE sites.

Methods: Data from ACE applications, annual reporting forms (2023–2025), and a cross-sectional survey were analyzed using descriptive mixed-method analyses. Variables included disease population counts, multidisciplinary services, clinical staffing, and operational challenges.

Results: Across ACE sites, highest reported patient volumes included Friedreich Ataxia (n=560), Multiple System Atrophy-Cerebellar type (MSA-C; n=459), Spinocerebellar Ataxia (SCA) type 3 (n=455), Progressive Supranuclear Palsy (PSP; n=295), and several other dominant SCAs.

Neurologic care and physical therapy were available at 100% of sites (n=43), while genetic counseling (95%), speech-language pathology (95%), occupational therapy (93%), and social work (86%) were also widely available. ACE sites reported offering dedicated ataxia clinics for a mean of 7 days/month. On average, 0.68 FTE was provided by ataxia-specific neurologist/advanced practice provider.

Meaningful variability in care services and infrastructure was observed. Fewer than 50% of sites had access to mental health, palliative care, and nutrition services. Limited protected clinical time of specialty providers, care coordination challenges, variability in clinical trial start-up processes, and administrative burden associated with annual reporting were identified as challenges.

Discussion and Conclusion: Combined data reveal disease patterns and multidisciplinary care capabilities alongside operational variability across ACE sites. These findings highlight opportunities to utilize the ACE network for clinical trial site selection while strengthening the network through improved reporting standardization, collaboration, and targeted investment in clinical and research infrastructure.

Funding Sources: KT, CS, AA, LPC, and LM are full time employees of the National Ataxia Foundation.

C35. Regional Variation beyond GAA1: Onset Features, Neurological and Patient Reported Function in the UNIFAI Natural History study of Friedreich's Ataxia

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Background & Objectives: UNIFAI is a global natural-history study in Friedreich ataxia (FRDA) formed through the merger of FA-COMS (US, Canada, Australia, New Zealand, India) and EFACTS (Europe). The combined registry for the first time permits comparison of genetic, onset and functional features across US/CA, Europe, India and Oceania. We characterized regional patterns in age of onset (AOO), GAA1expansion length, and baseline examiner-rated mFARS and patient-reported FA-ADL scores.

Methods: Cross-sectional data from UNIFAI participants with known GAA repeat length (shorter allele, gaa1; n = 2,098; US/CA n = 1,069, Europe 676, Oceania 209, India 144) was analyzed using linear and segmented regression. We estimated regional offset patterns in AOO (at matched GAA1), in GAA1at matched AOO, and in baseline mFARS and FA-ADL at matched GAA1with adjustment for age at enrollment and sex.

Results: AOO at matched GAA1was not different between US/CA vs Europe (Europe +0.6 yr, p = 0.11), but drifted in Oceania (+1.3 yr, p = 0.03), and India (+2.9 yr, p < 0.001). On the GAA1axis at matched AOO, Oceania carried more repeats than US/CA (+19 pooled, the main difference being in late-onset cases (median GAA1318 vs 200 in onset > 24 yr); Europe carried 44 fewer repeats on average (p < 0.001). At matched GAA1with age and sex adjustment, India scored +5.9 points on mFARS [3.0, 8.8] (p < 0.001), but -1.3 points on FA-ADL [-2.6, -0.1] (p = 0.04).

Discussion & Conclusion: AOO at matched GAA1is comparable across Europe, US/CA and Oceania. Slightly longer repeats in Australia are most apparent in late-onset cases. India is shifted later in reported AOO, higher scores on examiner-rated mFARS, and lower on patient-reported FA-ADL at matched genotype. Cross-continental natural-history pooling and trial endpoint choice should account for these regional differences.

Funding Sources: Friedreich's Ataxia Research Alliance (FARA)

C36. Advancing Global Trial Readiness in Ataxias: New Educational, Biomarker, and Trial Support Activities of the Ataxia Global Initiative

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The Ataxia Global Initiative (AGI) is a worldwide public–private partnership dedicated to accelerating therapy development and improving trial readiness for inherited ataxias through international collaboration, harmonization, and education. In recent years, AGI has substantially expanded its activities across education, formation of regional networks, outcome improvement, and clinical trial support. New annual educational formats include the AGI Ataxia Expert Masterclass, an advanced international training program focused on genetics, disease mechanisms, biomarkers, and trial readiness in spinocerebellar ataxias (SCAs), bringing together clinicians, translational researchers, and industry experts. In parallel, AGI launched a dedicated webinar series on preventive trials in SCAs, addressing pre-ataxic disease stages, early biomarkers, and lessons learned from preventive approaches in other neurodegenerative diseases. To further strengthen global trial readiness infrastructure, AGI continues to expand its service portfolio through the Ataxia Advisory Committee for Therapeutics (Ataxia ACT), which provides independent multidisciplinary guidance for therapeutic development programs, and the AGI Trial Site Registry (AGI-TSR), a global database of specialized ataxia centers supporting study feasibility and site selection for international trials. AGI also started to establish annual regional conferences to foster collaboration and capacity building across different geographic regions and healthcare systems. The first regional conference with a focus on Latin American countries took place online on 7 May, 2026. Scientific progress is driven by AGI Working Groups, which develop i.a. harmonized protocols, multicenter standards, and interoperable datasets for imaging and digital outcome measures in ataxia trials. A further major initiative is the SARA Qualification Program, which aims to develop an improved and standardized SARA user package for regulatory qualification and application in educational, scientific, and commercial settings. Together, these activities position AGI as a central international platform for outcome harmonization and improvement, education, and clinical trial

C37. From Idiopathic to Genetic to Standardized Natural History: Clinical Features and Progression of SCA27B and RFC1-related ataxia in the CRC-SCA Study

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Background & Objectives: SCA27B and RFC1-related ataxia are late-onset cerebellar ataxias caused by recently identified intronic repeat expansions: a dominant GAA expansion in FGF14(SCA27B) and a recessive AAGGG expansion in RFC1. Standardized natural-history comparisons do not yet exist. We compared phenotype, scale severity, non-ataxia signs and 1-year progression in a prospective, multicenter natural-history cohort.

Methods: CRC-SCA Study participants with confirmed SCA27B (n=117) or RFC1-related ataxia (n = 72). **Outcomes:** SARA, the Upright Stability Score (USS), INAS, first reported symptom, 4-aminopyridine (4-AP) exposure. Groups compared by t-test, Wilcoxon and Fisher exact.

Results: Age at presentation was similar (70 vs 68 years). RFC1-related ataxia had earlier onset (50 vs 59 years), longer disease duration (18 vs 11 years) and higher baseline SARA (11 vs 6). The onset gap reflected symptom definition: gait/ataxia onset was similar (~60 years), but the earliest recorded symptom in RFC1-related ataxia was frequently non-ataxic (chronic cough, sensory disturbance). RFC1-related ataxia was multisystem, with Achilles areflexia (58% vs 23%) and saccadic slowing or hypometria (24-28% vs 8-12%); SCA27B was cerebellar-predominant. On the USS, genotypes were similar with eyes open (feet-apart score ≥ 2 : 19% vs 8%) but diverged sharply with eyes closed (62% vs 12%), a Romberg pattern of sensory and vestibular loss. In SCA27B, 4-AP was widely used and its initiation was followed by descriptive SARA improvement. Overall 1-year SARA change was minimal and similar between groups.

Discussion & Conclusion: In this head-to-head comparison, SCA27B and RFC1-related ataxia share a common ataxia-onset timeline but diverge in system involvement: RFC1-related ataxia is multisystem, SCA27B cerebellar-predominant. 4-AP initiation was associated with short-term SARA improvement in SCA27B, supporting its symptomatic benefit. A cerebellar-only presentation should prompt vestibular and sensory phenotyping before RFC1 is excluded. Apparent onset and severity differences are partly symptom-definition and selection effects, relevant to trial design and comparator matching.

Funding Sources: National Ataxia Foundation (NAF)

C38. Cerebellar Degeneration Favors Divergent Thinking: Increased Divergent/Convergent Thinking Ratio in Friedreich Ataxia

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Background: The cerebellum is increasingly recognized as a major contributor to cognition beyond motor control, notably through predictive processing, associative learning, and flexible mental representations. While cerebellar dysfunction is classically associated with executive and cognitive deficits, theoretical models suggest that reduced cerebellar constraint and predictive modulation may also facilitate divergent thinking and unusual associative processes. We investigated whether cerebellar degeneration in Friedreich ataxia (FA) is associated with enhanced divergent thinking relative to convergent semantic processing. Methods: Twenty-eight individuals with genetically confirmed FA (11 females; mean SARA score: 23 ± 10) and healthy controls matched for age and sex were included. Divergent thinking was assessed using Olson's Divergent Association Task (DAT), which quantifies semantic distance between ten generated words. Convergent semantic processing was evaluated using animal semantic fluency. To capture the balance between divergent and convergent cognition, a Divergent/Convergent ratio was calculated by dividing DAT scores by semantic fluency performance. Group comparisons were performed using independent-sample t-tests. Results: Patients with FA demonstrated significantly higher divergent thinking scores than controls (77 ± 6 vs 68 ± 7 ; $p = 1.38 \times 10^{-5}$). In contrast, semantic fluency performance was significantly reduced in FA patients (18 ± 5 vs 21 ± 4 ; $p = 0.030$). Importantly, the Divergent/Convergent ratio was markedly increased in the FA group compared with controls (4.3 ± 1.2 vs 3.2 ± 0.5 ; $p = 1.51 \times 10^{-5}$). Discussion and Conclusion: These findings support the hypothesis that cerebellar degeneration may favor divergent associative processing while impairing convergent semantic retrieval. These findings suggest that cerebellar degeneration may reduce cognitive filtering and favor broader semantic exploration, leading to enhanced divergent thinking despite impaired semantic fluency. This supports a role of the cerebellum in balancing flexible associative thinking and efficient semantic processing.

C40. Long-term natural history of spastic paraplegia type 7: data from a single-center cohort

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Background and objectives: Spastic paraplegia type 7 (SPG7) represents one of the most common forms of autosomal recessive spastic paraplegia. Despite natural history data from large cohorts have already been published, mean reported follow-up duration was approximately five years, while long-term information on clinical evolution from longitudinal observation is lacking.

Methods

Clinical records of patients referring to our ataxia clinic were retrospectively reviewed. Demographic, genetic and disease features at first and last available visit were recorded.

Results: Sixty-nine patients were included. Mean age at onset was 42.1 years, age at diagnosis 53.3 and disease duration at first visit 8.6 years. Duration of follow-up ranged from 1 to 34 years, with a mean of 10 years. The mean number of visits per patient was 6. Onset symptoms were: limb rigidity in 43%, walking instability in 29%, other in 7%. Oculomotor abnormalities were reported in 52.2% of cases at first visit and 82.6% at last one. Dysarthria and dysphagia were observed in 27.9% and 8.8% of patients at first visit and in 50% and 26.4% at last. Limb ataxia was found at first visit in 26.9%(upper limb) and 46%(lower), at last visit in 53.2%(upper) and 66.6%(lower). At first visit, walking aids were needed by 14.5% of patients, while at last visit 68.7% needed one- or-double-sided support and 5.7% were full-time wheelchair-bounded. Time from disease onset to necessity of a walking aid was 14.6 years and to wheelchair use 24.4 years. Five patients(7%) deceased (mean age 82 years), 54 (78%) were alive and 10 (14%) lost.

Discussion and conclusion: Complex phenotypes are frequent in SPG7. Oculomotor abnormalities, limb ataxia or bulbar dysfunction may be found from the very first assessment, with prevalence increasing over time. The impact of the disease on the ability to walk independently is limited, with approximately 5% of patients wheelchair-bounded at last visit.

C42. The Cerebellar Neuropsychiatric Rating Scale Version 2: Development and validation

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Background and Objectives: The affective component of the cerebellar cognitive affective / Schmahmann syndrome (CCAS) includes neuropsychiatric symptoms across five domains: attentional control, emotional control, autism spectrum, psychosis spectrum, and social skill set, each with overshoot (hypermetric) and undershoot (hypometric) manifestations (Schmahmann et al., 2007). We addressed the critical need for a validated instrument to quantify neuropsychiatric symptoms in this cohort with the 35-item informant-reported Cerebellar Neuropsychiatric Rating Scale (CNRS; Daly et al., 2025). Here we develop and validate both a self- and informant-reported neuropsychiatric rating scale for individuals with cerebellar disorders.

Methods: We amended the CNRS items to produce Self- and Informant-Report versions, conducted cognitive debrief to evaluate each item's readability, relevance, and importance, and incorporated patient feedback to generate a 50-item CNRS Version 2 (CNRS-2). We conducted psychometric evaluation via online administration in 43 individuals with cerebellar disorder (CD) and 25 healthy controls (HC) and their respective study-partners at baseline and two weeks later (Selvadurai et al., 2025).

Results: Neuropsychiatric symptom scores were elevated in the CD group (n=43) relative to HC (n=25), for both self-report and informant-report versions ($P < .05$). Internal consistency was adequate. The CD test-retest reliability was moderate-to-good. CNRS-2 scores were unrelated to motor ataxia severity. Receiver operating characteristic (ROC) analysis determined that the groups were distinguished optimally with scores of 18 and 15 for subjects and informants, respectively. There was concordance within subject-informant pairs, however scoring discrepancies were observed for some. CNRS-2 domains correlated highly with other scales, whether conceptually related or not.

Conclusion: The CNRS-2 is a new, comprehensive screening instrument that incorporates the patient perspective and facilitates assessment of the neuropsychiatric component of the CCAS in patients with ataxias and other cerebellar disorders. Further validation using a large normative dataset will facilitate more granular clinical interpretation at the level of the individual patient.

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C43. Longitudinal change in speech outcomes vs clinical and activities of daily living progression in Friedreich ataxia

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Background and objectives: Speech impairment / dysarthria is a defining feature of Friedreich ataxia (FA) and cross sectional evidence suggests it worsens over time. Using the global natural history study UNIFAI, we assessed the longitudinal sensitivity of speech endpoints (Naturalness, Intelligibility, oral Diadochokinesis (DDK)) over 12–24 months, alongside neurological and Activities of Daily Living (ADL) outcomes.

Methods: Follow-up data were available for 151 individuals with ³² visits, and progression was assessed with linear mixed-effects models (random intercept and slope, REML) and through descriptive change-from-baseline plots. Slope modulation by baseline visit value, disease duration, ambulatory status, and Functional Disability Stages (FDS) was tested via slope-by-time interactions. Comparators: modified Friedreich Ataxia Rating Scale (mFARS) with upright-stability (USS) and upper-limb (FARS B) subscores, FA-ADL, and FA-ADL bulbar items (a1speech + a2swallow). Omaveloxolone exposure was excluded.

Results: Across 12 to 24 months, the objective speech outcomes Intelligibility and Naturalness did not change at the population level ($\beta \approx 0$, both $p > .2$), and DDK average rate showed no significant change ($\beta = +0.04$ syl/s / yr, $p = .31$). Significant decline by stage was observed in naturalness in non-ambulatory patients. Neurological outcomes progressed (mFARS +1.68, USS +0.89 / yr; both $p < .001$), with channel-specific decline by stage (upright stability in ambulatory patients, upper-limb in non-ambulatory). ADL outcomes also progressed (FA-ADL +0.93 / yr; $p < .001$, FA-ADL bulbar items +0.16 / yr; $p = .034$).

Discussion and conclusion: The speech endpoints were stable across the whole population and naturalness appeared to decline in non-ambulant individuals. These data provide a useful natural-history reference, showing that scores remained largely stable over this timeframe. Longer follow-up, denser sampling, and refined metrics may optimize short-term sensitivity in the objective metrics. Suggest combining into one sentence: Speech impairment / dysarthria is common in Friedreich Ataxia

Funding Sources: FARA, Redenlab, Biogen

C44. Identifying Disease Subtypes in Friedreich Ataxia Using Machine Learning

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Background and Objectives: Friedreich ataxia (FRDA) is a rare neurodegenerative disorder with heterogeneous progression. Although previous natural history studies have described differential decline across predefined clinical strata, data-driven latent progression subtypes derived directly from disease-duration-aligned longitudinal clinical data remain incompletely characterized. It is also unclear whether such subtypes show shared or divergent patterns across ataxia severity, functional status, and upper-limb dexterity. This study aimed to identify FRDA progression subtypes using machine learning.

Method: We applied mixture of Gaussian processes (MoGP) modelling to longitudinal TRACK-FA assessments from 177 children and adults with genetically confirmed FRDA across six sites. Participants were assessed at baseline, 1-year, and 2-year follow-up, with trajectories aligned to years since disease onset to estimate progression patterns across the first 25 years of disease. Outcomes included the Scale for the Assessment and Rating of Ataxia, modified Friedreich Ataxia Rating Scale, Activities of Daily Living, and dominant- and non-dominant-hand nine-hole peg tests. Univariate MoGP models identified measure-specific subgroups, while a multivariate MoGP model jointly analysed all outcomes to capture shared progression patterns. Biological plausibility was assessed using age at onset and GAA1repeat length.

Results: Univariate modelling identified three to four progression subgroups per outcome, characterized by distinct severity levels, progression rates, and nonlinear trajectories. Subgroups ranged from consistently slower progression to severe rapid deterioration, with some showing early accelerated decline followed by plateau. Several outcome-specific subgroups were significantly associated with GAA1repeat length. The multivariate model identified four progression subtypes, including consistently mild and severe subtypes across all outcomes, as well as intermediate subtypes in which ataxia/function trajectories differed from upper-limb dexterity trajectories. Multivariate clusters also differed significantly in age at onset and GAA1repeat length.

Discussion: These findings identify clinically and biologically meaningful FRDA progression subtypes, supporting precision prognosis, trial enrichment, and future therapeutic targeting.

Funding Sources: TRACK-FA is funded by grants from the Friedreich's Ataxia Research Alliance (FARA, USA) to each of the academic sites and IXICO with financial support from Takeda Pharmaceuticals Company, Novartis Gene Therapies, IXICO, Larimar Therapeutics Inc., and PTC Therapeutics.

C45. Whole-brain Diffusion Tensor Imaging Reveals Widespread White Matter Abnormalities in Adolescents with Friedreich Ataxia

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Background and Objectives: Friedreich ataxia (FRDA) is a progressive neurodegenerative disorder characterised by cerebellar and spinal atrophy. The disease typically manifests in childhood or adolescence. Existing studies investigating brain microstructural changes (i.e., white matter integrity) have largely focused on adults with FRDA, limiting understanding of how white matter pathology emerges during this developmental period. We identified whole-brain microstructural abnormalities in adolescents with FRDA using diffusion tensor imaging analyses.

Methods: This study uses data from the TRACK-FA study, the largest multi-site neuroimaging study in FRDA. Diffusion magnetic resonance imaging (MRI) data from 52 adolescents aged 11–17 with FRDA and 28 healthy controls were analysed. White matter diffusion metrics, including fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AD), were extracted. Group differences were assessed using site-corrected linear regression models, with multiple comparisons controlled.

Results: Significant widespread white matter abnormalities were observed in adolescents with FRDA compared with controls. Specifically, significant reductions in FA and increases in RD and MD were detected across multiple motor and sensory projection fibres such as inferior and superior cerebellar peduncles, corticospinal tract, thalamo-postcentral and striato-postcentral tracts, and superior thalamic radiation, in adolescents with FRDA compared with controls. However, no significant changes were observed in AD between groups.

Discussion and Conclusion: The observed white matter abnormalities align with the known neuropathology of FRDA and previous adult findings, particularly in the cerebellar peduncles and corticospinal tracts, which are critical for motor coordination and sensory processing. The predominant pattern of increased RD and reduced FA, alongside preserved AD, may reflect early myelin abnormalities preceding substantial axonal degeneration. This work addresses a critical gap in adolescent neuroimaging and supports the use of diffusion measures in future clinical and longitudinal studies.

Funding Sources: TRACK-FA is funded by grants from the Friedreich's Ataxia Research Alliance (FARA, USA) to each of the academic sites and IXICO with financial support from Takeda Pharmaceuticals Company, Novartis Gene Therapies, IXICO, Larimar Therapeutics Inc., and PTC Therapeutics.

C46. A Pharmaceutical–Patient Advocacy Partnership Model for Accelerating Rare Disease Research in Ataxia-Telangiectasia

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1. Ataxia Telangiectasia Children's Project, Cambridge, MA, 2. Insmmed Incorporated, Bridgewater, NJ, 3. Aparito – a wholly owned subsidiary of Eli Lilly and Company, Wrexham, Clwyd

Background and objectives: Ataxia-Telangiectasia (A-T) is a rare, autosomal recessive disease associated with progressive gait abnormalities starting from 12–18 months of age. Here we review the experience of partnering with a Patient Advocacy Group (PAG) to develop and perform a pilot study of home-based, non-invasive digital biomarkers using the Atom5™ app and pose estimation software to quantify involuntary movement in children with A-T.

Methods: We describe the experience of partnering with the A-T Children's Project (ATCP) during trial planning, patient recruitment, and trial performance.

Results: Insmmed conceptualized the pilot study design and engaged the ATCP as a key collaborative partner. ATCP was consulted on the clinical meaningfulness of assessment measures, and both partners collaboratively streamlined the study protocol to improve caregiver and patient experience. Leveraging ATCP's clinical study experience, Insmmed utilized the ATCP's commercial Institutional Review Board (IRB) and engaged ATCP as a decentralized study site. This partnership enabled identification of quantitative measures capable of demonstrating meaningful differences between control subjects and children with A-T, optimizing the study protocol and operational processes. Given ATCP's Good Clinical Practice standards, they were designated Principal Investigator and compensated by Insmmed for all activities and supplies as with any clinical site. ATCP played a central role in recruitment through IRB-approved remote informed consent procedures and its caregiver network. Recruitment was efficient, with 10 A-T patients and 5 controls enrolled over 22 days. Parents were willing to discuss and resolve practical issues regarding video recordings with ATCP study staff. Of the 366 video observations received, only 39 (10.6%) failed quality control.

Discussion and Conclusion: Partnering with PAGs can offer significant benefits during trial planning, accelerate patient recruitment, and improve day-to-day study performance.

Funding Sources: This study was funded by Insmmed Incorporated.

C47. Digital Adherence to Remote Monitoring in Friedreich's Ataxia – Results from the PROFA Study

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Background: Friedreich's ataxia (FA) is the most common autosomal recessive hereditary ataxia and is associated with severe motor impairment. Following the approval of omaveloxolone as first disease-modifying therapy, several clinical trials investigating novel therapeutic approaches are ongoing. However, frequent on-site assessments impose a substantial burden on patients. The PROFA study is a prospective multicenter observational study incorporating multidimensional remote assessments of health-status.

Objectives: To evaluate the acceptability, usability, and feasibility of remote collection of patient-reported outcomes (PROs) and digital activity data (SARAhome) in patients with FA.

Methods: Digital adherence was analyzed in 95 patients participating in the PROFA study. Three complementary adherence metrics were assessed: (1) participation frequency, defined as strict adherence (completed PROs relative to questionnaires sent) and overall quantitative adherence (completed PROs relative to the maximum possible number); (2) temporal adherence (response latency following questionnaire delivery); and (3) interaction intensity, (questionnaire completion duration). Influencing factors were examined using multivariate models.

Results: During six months of participation, patients received 4,453 activity-related tasks and 2,365 questionnaires. Median baseline ataxia severity was 16.0 (IQR 13–22) SARA-points and median age was 33 years (IQR 24–44.5). Participation frequency was higher for questionnaires than for activity-measures (75.6% strict; 66.3% overall vs. 68.9% strict; 52.5% overall, $p < .001$) and was greater in centers offering financial incentives. Higher participation frequency was associated with shorter response latency ($\beta = -0.099$, $p = .008$) and shorter completion duration ($\beta = -0.014$, $p < .001$), although effect sizes were small. Older age showed a moderate positive association with adherence, corresponding to an increase of approximately 0.5%/year. Ataxia severity did not affect digital adherence.

Conclusions: Longitudinal multidimensional remote assessments are feasible in patients with FA, independent of disease severity. Temporal adherence analyses may support the integration of digital PRO-based monitoring into future clinical studies in movement disorders.

Funding Sources: European Joint Program for Rare Diseases (EJP-RD) 2021 Transnational Call for Rare Disease Research Projects with local funding from the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie, Federal Ministry of Education and Research, French National Research Agency and and the Canadian Institutes of Health Research (CIHR-IG).

C48. Characterising Dysarthria Heterogeneity in Ataxia

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Background and Objectives: Existing literature has shown that dysarthria does not manifest as a uniform phenotype across ataxias, with heterogeneous speech profiles reported in hereditary ataxias such as Friedreich's ataxia (FRDA) and spinocerebellar ataxias (SCA). It remains unclear whether similar heterogeneity extends to other subtypes of ataxia. This study characterised speech impairment across motor speech subsystems of three relatively unexplored ataxia types: immune-mediated gluten ataxia (GA), spastic paraplegia type-7 (SPG7), and cerebellar ataxia neuropathy vestibular areflexia syndrome (CANVAS), and examined whether these aetiologies showed distinct dysarthria patterns.

Methods: The study cohort consisted of 43 participants: GA (n=11), SPG7(n=12), CANVAS (n=10), and healthy controls (n=10). Participants completed sustained phonation and diadochokinetic (DDK) tasks, passage reading, and a one-minute monologue. Acoustic and perceptual analyses examined functional speech outcomes and subsystem-level features across respiration, phonation, articulation, resonance, and prosody.

Results: Aetiology-related differences were observed in selected speech features. SPG7 showed significantly more frequent articulatory errors and a strained-strangled voice. CANVAS showed the strongest hypernasality profile, reaching significance in monologue and showing a trend for reading. Loudness patterning also suggested aetiology-weighted variation, with uncontrolled loudness variability occurring more frequently in SPG7 and CANVAS. Beyond these differences, the clearest organising pattern was an instability–inflexibility distinction: SPG7 showed a more unstable speech profile, characterised by variable errors and fluctuating prosodic control, whereas GA and CANVAS more often showed an inflexible profile, such as reduced pitch and loudness variability and equalisation of stress.

Discussion and Conclusion: Dysarthria heterogeneity across the ataxia subtypes appeared partly related to aetiology-associated patterns of speech motor control. The stronger hypernasality profile in CANVAS may relate to sensory neuropathy, which may affect speech monitoring and velopharyngeal control. Furthermore, instability–inflexibility profiles may reflect differential disruption of cerebellar feedforward and feedback mechanisms that support speech planning, monitoring, and adaptive control.

Funding Sources: No financial support or external funding was obtained for the conduct of this research. The work was undertaken as part of my PhD research and was carried out through the researcher's own efforts, without specific grant, industry, charity, or commercial funding.

C49. Rethinking Cognitive Heterogeneity in Ataxia

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Background and Objectives: Cognitive impairment is an increasingly recognised non-motor feature of cerebellar ataxia. Existing literature has described heterogeneous cognitive profiles in hereditary ataxias such as Friedreich's ataxia (FRDA) and spinocerebellar ataxias (SCA). However, the cognitive profile of immune-mediated gluten ataxia (GA), spastic paraplegia type-7 (SPG7), and RFC1-related cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS) remains unclear. This study compared cognitive performance across these aetiologies to examine whether they show distinct cognitive profiles.

Methods: Cognitive performance was assessed in 43 participants comprising individuals with GA (n=11), SPG7(n=12), CANVAS (n=10), and healthy controls (n=10). All participants completed the Cerebellar Cognitive Affective Syndrome Scale (CCAS-S) to characterise the cognitive profile.

Results: All ataxia groups showed poorer global CCAS-S performance than healthy controls, but GA, SPG7, and CANVAS did not differ significantly from one another, even after controlling for age, education, disease duration, and age of onset. Descriptively, both raw and pass/fail CCAS-S scores indicated the greatest involvement of fluency, executive function, and verbal recall. Semantic fluency was reduced across all ataxia groups, category-switching was more affected in SPG7 and CANVAS, and verbal recall was lowest and most frequently impaired in CANVAS. However, these patterns did not establish a statistically significant aetiology-specific separation.

Discussion and Conclusion: GA, SPG7, and CANVAS did not show statistically significant differences in cognitive profiles, suggesting that heterogeneous cognitive patterns reported in FRDA and SCA may not generalise across all ataxia types. However, some trends in performance differences were identified, highlighting the potential for larger studies to identify distinct aetiological profiles. It therefore remains important to assess patients' cognitive profiles, aiming to identify individualised strengths and weaknesses, in the overall management of the ataxia.

Funding Sources: No financial support was obtained for the conduct of this research. The work was undertaken as part of my PhD research and was carried out using available institutional resources and the researcher's own efforts, without specific grant, industry, charity, or commercial funding.

C50. UNIFAI: A Global Platform for Advancing Friedreich Ataxia Research and Treatment

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Background: The Friedreich Ataxia Global Clinical Consortium UNIFIED Natural History Study (UNIFAI) is a global, prospective, longitudinal observational study designed to characterize the natural history of Friedreich ataxia (FA). The FA Global Clinical Consortium (FA GCC) is a network of clinician-researchers and patient advocates dedicated to leveraging patient data to advance understanding of FA, accelerate global research and collaboration, and promoting access to clinical care. By integrating two large, well-established cohorts (FACOMS and EFACTS), UNIFAI provides a uniquely large and harmonized dataset across 32 international sites.

Methods: UNIFAI was established through harmonization of existing protocols, standardized procedures, and integration of legacy datasets into a unified data platform. Participants with genetically confirmed FA are followed through annual study visits, capturing core data including demographics, clinical history, and functional assessments. Ancillary assessments across multiple domains are collected at selected sites.

Results: To date, 2,984 participants have been enrolled across 32 active sites worldwide, with a total of 15,408 study visits recorded, including 12,424 follow-up visits. Some participants contributed more than 20 follow-up visits, highlighting substantial longitudinal depth. The cohort is balanced by sex (50.3% male, 49.7% female) and spans a broad age spectrum at baseline (0–7 years [n=84], 8–14 [n=503], 15–24 [n=930], and >24 years [n=1,458]), with a median age at diagnosis of 15 years (IQR 11–22). This extensive longitudinal follow-up enables robust characterization of disease progression over time.

Conclusion: UNIFAI provides one of the largest and most comprehensive longitudinal datasets in FA, combining global scale with substantial follow-up depth. Beyond advancing understanding of natural history, the infrastructure and harmonized data collection enable efficient implementation of clinical studies, from investigator-initiated projects to large multicenter trials. UNIFAI further supports global trial readiness by enabling benchmarking of disease progression and evaluation of treatment effects as standards of care evolve.

Funding Sources: FARA - Friedreich's Ataxia Research Alliance

C51. Longitudinal assessment of oculomotor parameters over one year of Omaveloxolone treatment in Friedreich's ataxia: a pilot study

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Background and Objectives: Friedreich's ataxia (FRDA) is associated with progressive neurological impairment, including oculomotor abnormalities. This study aimed to longitudinally assess quantitative oculomotor parameters in patients with FRDA over one year of Omaveloxolone treatment and to explore potential changes in fixation instability which has been anecdotally reported by some patients.

Methods: Thirteen patients (23.7 ± 7.69 years, mean $\pm$ SD) were enrolled at Bambino Gesù Children's Hospital, IRCCS, Rome, Italy. The Scale for the Assessment and Rating of Ataxia (SARA) and modified Friedreich's Ataxia Rating Scale (mFARS) were used to assess severity. Eye movements were recorded using video-oculography (EyeSeeCam, 220 Hz). Participants performed saccades, smooth pursuit, fixation, and optokinetic nystagmus in a predefined sequence. Data normality was assessed using the Shapiro–Wilk test. Within-group comparisons (baseline vs. one-year follow-up) were performed using paired t-tests or Wilcoxon signed-rank tests, as appropriate ($p < 0.05$). Pearson or Spearman correlations were applied accordingly.

Results: Mean SARA scores increased from 18.3 ± 7.29 to 20.8 ± 7.14 , whereas mFARS scores changed from 50 ± 16.4 to 54.4 ± 14.6 . Mean horizontal saccade peak velocity (20° target amplitude) decreased from 411 ± 136 to $369 \pm 114^\circ/\text{s}$. At both baseline and follow-up, SARA and mFARS scores correlated with horizontal ($p = 0.61\text{--}0.70$; $p = 0.53\text{--}0.45$) and vertical ($p = 0.51\text{--}0.69$; $p = 0.64\text{--}0.44$) saccade latency. No differences were observed in fixation nystagmus.

Discussion and Conclusion: Over one year of Omaveloxolone treatment, selected clinical and oculomotor parameters changed in this FRDA cohort, consistent with disease progression. Fixation instability did not significantly change over time, suggesting no apparent treatment-related effects. Interpretation of these findings is limited by the small sample size, absence of untreated comparators, and relatively short follow-up. Quantitative oculomotor assessment may provide complementary longitudinal measures for monitoring disease progression in FRDA. Larger longitudinal studies with longer follow-up and controlled comparisons are needed to better define oculomotor trajectories during Omaveloxolone treatment.

Funding Sources: Grant funded by AFAF-FMR call 2024

C52. Developing an Interdisciplinary Clinic for Genetic Ataxia: Identifying Gaps in Care and Aligning Treatment with Patient-Perceived Health

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Background and Objectives: Patients with genetic ataxias face substantial rehabilitation and quality-of-life needs, yet access to coordinated disease-specific interdisciplinary care remains limited, particularly within rehabilitation disciplines. We established a monthly interdisciplinary ataxia clinic to improve comprehensive care delivery and evaluate relationships between patient priorities, perceived health, and clinician-rated disease severity.

Methods: Patients underwent a half-day evaluation by a movement disorders specialist, physical therapy, occupational therapy, speech-language pathology, and pelvic floor physical therapy. Prior to the visit patients complete a medical questionnaire, and EQ-5D-3L. The interdisciplinary team developed a unified care plan. A one-year program evaluation utilized chart review, patient-reported outcomes, and interdisciplinary discussion to identify care gaps and assess associations between patient-perceived health and ataxia severity measures.

Results: Eighteen patients (9 women, mean age 62.7 yrs (± 10.9)) representing multiple hereditary ataxia syndromes were evaluated during the clinic's first year, with referrals originating across North Carolina (16 patients) and neighboring states (2 patients). In 12 patients, both patient-reported health perception scores (EQ-5D-3L) and clinician-rated ataxia severity (ICARS and ataxia scale) were available for analysis. Exploratory analysis demonstrated a moderate association between limitations in usual activities and overall health perception (Spearman $r = -0.44$), although statistical significance was not reached in this small cohort. No meaningful associations were observed between perceived health and clinician-rated ataxia severity measures, including ICARS and ataxia scales. Thirteen of 18 patients had never undergone occupational therapy evaluation, despite all patients reporting goals related to improving quality of life and maintaining independence. All patients endorsed pelvic health concerns; however, 16 of 18 had never received pelvic floor physical therapy, and referrals to urology and continuation of pelvic floor therapy remained inconsistent.

Discussion and Conclusion: Findings highlight a disconnect between objective disease severity and patient experience. Patient perception of health may be driven more by functional ability than by measured neurologic impairment. Underutilization of services targeting function and quality of life reflects misalignment between care delivery and patient priorities. Interdisciplinary ataxia clinics may improve access to specialized rehabilitation services while identifying care gaps not captured by traditional neurologic severity measures. Larger cohorts and longitudinal follow-ups are needed.

C53. Home-based video measurement shows promise for quantifying ataxia in children

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Background and objectives: Ataxia-Telangiectasia (A-T) is a rare, autosomal recessive disease associated with progressive gait abnormalities presenting around 12–18 months of age. This pilot study assessed the feasibility and repeatability of a home-based, video-recorded digital biomarker using a smartphone camera and pose estimation to distinguish head movement instability in children with A-T.

Methods: Ten children with A-T (1–7 years, 7 female) and 5 healthy controls (HC) (1–9 years, 3 female) were included. Body movements were recorded from 4 postures/camera angles (sitting/front, sitting/side, standing/front, standing/side), yielding 366 observations; 327 were included and 39 excluded due to off-task movement or lighting issues. Core digital measures included head control and bobbing (head position, head-to-shoulder distance, head vertical angle) and upper/lower body balance (trunk/shoulder sway angle, leg alignment/support base). These yielded 42 digital features, including summary values for head control and upper- and lower-body balance.

Results: HC videos showed tight skeleton overlays with high-confidence landmark detection. A-T patients exhibited broader movement envelopes, greater pose uncertainty, and increased head displacement. Univariate analysis showed head features had the largest effect size between patients and HC (Cliff's δ) and were most statistically significant after false discovery rate correction. Balance features were significant but with smaller effect sizes. Younger children (1–3 years) moved more than older children, though patient–HC separation was visible across all ages. Multivariate analysis identified a minimal model using standing/front head relative velocity and head tilt time as the highest-performing single-setup model via leave-one-subject-out cross-validation (AUC 0.97, $p=0.0017$).

Discussion and Conclusion: A non-invasive, home-based video digital biomarker of head bobbing reliably distinguishes children with A-T from HC, supporting scalable remote assessment and reducing patient and caregiver burden. A larger study is planned to validate these findings.

Funding Sources: This study was funded by Insmmed Incorporated.

C55. Reduced white matter integrity in cerebellar-cortical loops predicts increased prefrontal and motor cortical activation during gait in FXTAS

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Background and Objectives: Fragile X-Associated Tremor/Ataxia Syndrome (FXTAS) is a neurodegenerative disorder characterized by cerebellar ataxia, tremor, and cognitive dysfunction. No studies have examined the neural underpinnings of gait deficits in FXTAS using combined functional near-infrared spectroscopy (fNIRS) to measure cortical activation and MRI metrics, including white matter integrity (WMI) of frontal-cerebellar-cortical pathways. Our aims were to use these techniques during gait tasks of increasing complexity to assess structure-function relationships of gait in FXTAS. **Methods:** 18 participants with FXTAS (70.3 ± 8.42 years) and 20 controls (67.1 ± 8.64 years) underwent gait assessment using an inertial sensor system, with simultaneous fNIRS data collection from the dorsolateral prefrontal cortex (DLPFC), premotor cortex (PMC), and supplementary motor area (SMA). WMI in the cerebellum and superior, middle and inferior cerebellar peduncles (SCP, MCP and ICP) was measured via DTI. **Results:** FXTAS participants exhibited significantly elevated cortical activation in the DLPFC during single task (ST) and obstacle navigation (ON) gait conditions ($p=0.046$), and in the PMC/SMA during ST and fast-paced (FP) gait conditions ($p=0.006$) compared to controls. FXTAS participants spent a significantly greater proportion of task duration recruiting the DLPFC and the combined PMC/SMA above baseline during all four gait conditions, compared to controls ($p=0.0008-0.027$). FXTAS participants had significantly reduced WMI in the SCP ($p=0.015$), and cerebellum ($p=0.026-0.049$) compared to controls. Reduced WMI in the SCP and cerebellum predicted greater cortical activation in the DLPFC and PMC/SMA during FP and ON gait tasks in FXTAS but not control participants ($p=0.045$). **Discussion and Conclusion:** Individuals with FXTAS over-recruit the DLPFC and PMC/SMA during gait, perhaps to compensate for decreased gait automaticity because of reduced WMI in corticocerebellar pathways. These functional and structural alterations in cortico-cerebellar circuits may underlie gait deficits in FXTAS, making them potential targets for therapeutic interventions, such as neuronavigation.

Funding Sources: Department of Defense

C56. Fear of Falling and Balance Confidence in Spinocerebellar Ataxia: A Cross-Sectional Analysis of SCA1, SCA2, SCA3, and SCA6

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Fear of falling and reduced balance confidence may be early, modifiable targets in spinocerebellar ataxia (SCA), potentially relevant before falls occur. We examined prevalence, clinical correlates, and fall-status associations of fear of falling and balance confidence across SCA1, SCA2, SCA3, and SCA6.

This cross-sectional analysis included 493 SCA1, SCA2, SCA3, and SCA6 patients from the Clinical Research Consortium for Cerebellar Ataxias (CRC-SCA), of whom 441 with complete fear and balance confidence data were analyzed. Fallers were defined as reporting at least one fall in the past year; frequent fallers as falling at least once per month. Fear of falling was assessed with a three-level ordinal item (No/Slightly/Very Much) and a 0–100 balance confidence scale. Ataxia severity was measured using SARA. Logistic regression examined associations of fear and confidence with faller and frequent faller status, repeated in non-fallers only, controlling for SARA and SCA subtype with SCA6 as reference.

Fear of falling was present in 275/441 patients (62.4%): 76/155 non-fallers (49.0%), 199/286 fallers (69.6%), and 36/53 frequent fallers (67.9%). Balance confidence was lower in fallers than non-fallers (51.5 vs 72.9, $p < 0.001$) and correlated with SARA ($r = -0.633$, $p < 0.001$). The logistic regression model showed that fear of falling ($OR = 1.79$, $p = 0.010$) and balance confidence ($OR = 0.98/\text{point}$, $p < 0.001$) were each independently associated with faller status. Neither was associated with frequent faller status, where SARA dominated ($OR = 1.10$, $p < 0.001$; $OR = 1.07$, $p = 0.007$;). Among non-fallers, SARA ($OR = 1.09/\text{point}$, $p < 0.001$) was associated with fear of falling. Additionally, SCA2 showed directionally lower fall odds than SCA6 across models ($OR = 0.06–0.58$).

Fear of falling is independently associated with faller status beyond ataxia severity and are present in nearly half of non-fallers, identifying them as potential targets for physical therapy referral. SARA tracks frequent falling, suggesting a potential clinical thresholds for intervention for falls. Longitudinal studies are needed to confirm temporality.

Funding Sources: Clinical Research Consortium for the Study of Cerebellar Ataxia

C57. Progression Characteristics and Data Fitness over 17 years in the CRC-SCA Natural History Study of Spinocerebellar Ataxia

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Background & Objectives Long-term characterization of the natural history of slowly progressive neurodegenerative diseases is fundamental for drug development. Increasingly, these data are not only used for trial design, but sponsors are turning towards comparisons with externally constructed control groups (ECGs), explicitly foreseen by FDA draft guidance from 2023 (FDA-2022-D-2983). This leads to new challenges in the requirements for ataxia natural history studies, often funded through patient-community/non-profit efforts. A recent CRL (NDA210862) highlights the factors FDA scrutinizes when evaluating the use of natural-history data to create ECGs, spanning both cohort-intrinsic- (baseline covariates, retention, outcome scales) and comparison-specific characteristics (site overlap, pre-specification, baseline imbalance). This report investigates the fitness for purpose of the CRC-SCA study data.

Methods Completeness of variables (demographics, genotype, genetic severity), disease progression rates (SARA, random-slope mixed-effects models), 1y/2y retention rates, concomitant medications and comorbidity capture (RxNorm and SNOMED-CT coding) are reported across the cohort, and stratified by study period: a grant-funded period, an intermediate period (with a funding gap) and a concurrent period (from 2021).

Results Over recent study periods, one-year retention rose 44% → 70%, three-year retention 26% → 40%, RxNorm coded medication coverage 59% → 99%, SNOMED-CT coded comorbidity coverage 28% → 72%. E.g., the medical-history CRF captured 3,723 comorbidity entries in 867 subjects, nearly complete among enrollees. SARA progression was consistent across genotypes (SCA1, 1.05; SCA2, 0.92; SCA3, 1.01; SCA6, 0.84 points/year) and varied little across periods despite very different retention times.

Discussion & Conclusion CRC-SCA retention and standardized medication/comorbidity coding improved dramatically, while SARA progression remained stable. These data appear well suited to external-control use; period-stratified reporting offers a useful framework for future NHx-control submissions. Such comparisons demand carefully curated data; done well, they provide important context for early studies. Whether they suffice as pivotal evidence depends on many factors beyond this work.

Funding Sources: National Ataxia Foundation (NAF)

C58. Stage-Dependent Longitudinal Responsiveness of the Upright Stability Score in Spinocerebellar Ataxia vs Friedreich Ataxia

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Background & Objectives: Spinocerebellar ataxias (SCA) lack a validated, change-sensitive outcome measure. Regulatory concerns about SARA drove a shift towards Functional SARA (f-SARA), yet pivotal f-SARA trials failed. The Upright Stability Score (USS), an established Friedreich ataxia (FRDA) outcome, was recently validated cross-sectionally in ambulatory SCA/FRDA. We assessed its longitudinal responsiveness against SARA, f-SARA, SARA-axial and FA-ADL, and its stage dependence.

Methods: Prospectively enrolled individuals in CRC-SCA (n=137; predominantly SCA3, SCA27B, SCA6 and SCA2, fewer SCA1, SCA8 and others) and UNIFAI/FRDA (n=93), each with follow-up data on USS and SARA (≤ 1.5 years) and ambulatory at baseline, were included. Responsiveness was assessed by mixed-effects models and standardized response means (SRM); stage dependence across baseline USS and SARA bands, with genotype leave-out checks.

Results: In ambulatory SCA, responsiveness was strongly stage dependent. In early disease (baseline USS < 8 , $\approx$ SARA 4.5) only USS captured change (SRM 0.38, $p=0.04$); all other scales $p \geq 0.2$. Through early-to-intermediate disease (USS < 16 , $\approx$ SARA 10.5) USS stayed the most responsive scale (SRM 0.40, $p=0.002$; others ≤ 0.26), leading up to $\sim$ USS 15. In late-ambulatory disease (USS 16–20) the axial SARA items improved, while USS lost sensitivity above $\sim$ USS 22 - still well before loss of ambulation. The early-stage advantage held under SARA-based staging and genotype leave-out. Stage stratification was SCA-only, as FRDA presented far more advanced (median USS 22 vs 15; 3% below USS 8). Across the FRDA range, USS was again most responsive (SRM 0.61), ahead of FA-ADL (0.52) and SARA (0.35, all scales were significant).

Discussion & Conclusion: Comparative responsiveness of the tools was stage-dependent, not scale-intrinsic. In early ambulatory SCA - the most trial-relevant stage - USS outperformed every other scale. The scales converge in late-ambulatory disease, where the more subjective axial SARA-components gain sensitivity. Longer follow-up and larger per-genotype samples are required for subtype-specific estimates.

Funding Sources: The National Ataxia Foundation (NAF) and The Friedreich Ataxia Research Alliance (FARA)

C59. Still Four Years: Persistent Diagnostic Delay in Friedreich's Ataxia Across Onset Age, Genotype, and Calendar Time

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Background and Objectives: Friedreich's ataxia (FA) is a rare, progressive, multisystem disorder in which diagnostic delay may limit timely access to specialist care, genetic confirmation, and research participation. Using the Friedreich Ataxia Global Clinical Consortium UNIFIED Natural History Study (UNIFAI), we characterized time to diagnosis (TtD) in individuals with FA by age at symptom onset, geographic region, and genotype.

Methods: We performed a retrospective analysis of 2,988 UNIFAI participants; 2351 had both age at symptom onset and confirmed diagnosis to calculate TtD available. We summarized median TtD and interquartile ranges (IQR) by onset-age group, region and genotype (GAA1), and tested whether TtD changed over calendar time, adjusted for onset group and genotype severity.

Results: Overall median TtD was 4 years (IQR 2-7). TtD was comparable across onset age through age 24 (0–7y, 4y; 8–14y, 3y; 15–24y, 4y) and longest in late-onset FA (LOFA, onset >24y, 6y; IQR 3–10). Patterns were similar across North America, Europe, Oceania, and India. By GAA1 repeat length, TtD was longest in the lowest-repeat group (<500, 6y), consistent with their later onset. TtD did not shorten over time: the median was stable by year of diagnosis, and after adjustment rose slightly (1.07 per decade, $p < 0.001$), with no improvement since the 2023 approval of omaveloxolone.

Discussion and Conclusion: Diagnostic delay was similar across typical onset-age categories, but increased in late-onset FA, suggesting that adult-onset presentations may reduce clinical suspicion and delay genetic confirmation (consistent across regions). Overall, delays have not improved over more than two decades of enrollment, indicating a persistent gap in recognition. UNIFAI provides a valuable global resource for defining diagnostic timelines in FA. Earlier recognition of non-classic and adult-onset presentations may reduce diagnostic delays and improve access to disease-specific care and research opportunities.

C60. Factors associated with exercise performance in children and adults with Friedreich's ataxia (FRDA)

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Background: Friedreich's ataxia (FRDA) is a rare neurodegenerative disorder that affects multiple organ systems, often leading to exercise intolerance and reduced quality of life. The relationship between clinical factors and exercise performance remains unclear, limiting targeted intervention development.

Objectives: This study aimed to determine how clinical factors, such as neurologic function measured by the modified Friedreich's Ataxia Rating Scale (mFARS), relate to cardiopulmonary exercise test (CPET) performance in FRDA.

Methods: This observational study is a re-analysis of a recently reported clinical trial (NCT0419236; PMID: 42009009). Baseline, pre-intervention data from individuals aged 10-44 years with genetically confirmed FRDA (n=66) who completed graded semi-recumbent cycling cardiopulmonary exercise testing (CPET) were used. Univariate and multivariate linear regression analyses were performed to identify factors associated with exercise performance.

Results: Multivariate regression analyses found that neurologic function, as reflected by the mFARS upright stability score, and skeletal muscle mass, as approximated by lower extremity lean mass via dual energy x-ray absorptiometry (DXA), were the most consistent predictors of either peak VO_2 (in L/min) or peak work (in Watts). Our initial analyses showed that, together with biological sex, mFARS upright stability and lower extremity lean mass explained approximately 63% of variance in peak VO_2 and 74% of variance in peak work, respectively. Lower extremity lean mass was a significant independent predictor of peak VO_2 ($\beta = 4.276$, 95% CI: 1.092 to 7.461, $p = 0.009$) and maximum work ($\beta = 0.070$, 95% CI: 0.044 to 0.095, $p < 0.001$).

Conclusions: Increasing lower extremity lean mass may improve exercise performance in FRDA, supporting the need to test interventions such as resistance training. Study designs should account for neurologic function and muscle mass as key factors influencing exercise outcomes. Future work will test these findings in 2 independent cohorts.

Funding Sources: US National Institutes of Health and Friedreich's Ataxia Research Alliance.

C61. A Tale of Two SCA Forties

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Background and Objectives: Genetic testing has become integral to movement disorders evaluations, leading to the identification of rare and recently characterized spinocerebellar ataxias (SCAs). Spinocerebellar ataxia type 40 (SCA40), associated with variants in the CCDC88C gene, is an exceptionally rare autosomal dominant ataxia with limited genotype–phenotype characterization. Interpretation of uncertain genetic findings remains challenging due to the scarcity of reported cases. This study aims to expand the clinical and genetic spectrum of SCA40.

Methods: We conducted a retrospective review of individuals with negative workups outside of CCDC88C variants, plus updated whole genome sequencing analysis in 2026. Clinical data, neuroimaging, and genetic testing results were reviewed and compared with previous reports.

Results: Case 1 presented with adult-onset progressive gait ataxia and cerebellar dysarthria, and cerebellar atrophy on MRI. The most current SARA ataxia scale score is 15.5. Symptoms improved with dalfampridine and rehabilitation therapies. Genetic testing: nonsynonymous heterozygous c.328G>A p.Asp110Asn CCDC88C variant not found in population databases. Case 2 presented with more rapidly progressive adult-onset generalized dystonia which responded well to deep brain stimulation (DBS). The most current SARA ataxia being 13. TWSTRS improved from 28 pre–deep brain stimulation to 7 post-DBS. Pre-DBS genetic workup uncovered a heterozygous c.1985_1987del p.Glu662del CCDC88C variant. In both cases, the CCDC88C variant has data supporting potential pathogenicity, is previously unreported, and formally classified as uncertain significance.

Discussion: These findings support emerging evidence that SCA40 demonstrates broader phenotypic heterogeneity than previously recognized, including presentations dominated by dystonia rather than cerebellar ataxia. Clinical variation may reflect underlying differences in genetic variants, cis- or trans-acting genetic modifiers, or involvement of extracerebellar networks. Such variability can complicate both clinical recognition and genetic diagnosis, emphasizing the importance of expanded workups and clinical descriptions. These cases also highlight ongoing challenges in interpreting rare genetic variants and communicating diagnostic uncertainty in neurogenetic disorders.

Conclusion: This case series further delineates SCA40's potential phenotypic spectrum and underscores the role of detailed case reporting in improving genotype–phenotype correlations and understanding of CCDC88C genetic variants, which ultimately enhances diagnostic accuracy for rare neurogenetic movement disorders.

C62. Definition and validation of SCA27B clinical diagnostic criteria for carriers of FGF14(GAA•TTC)200-299 repeat expansions

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Background and Objectives: SCA27B is a common cause of late-onset cerebellar ataxia caused by a heterozygous (GAA•TTC) repeat expansion in the FGF14 gene. Expansions of ≥ 300 (GAA•TTC) repeats are considered highly penetrant, whereas expansions of 200–299 repeats exhibit incomplete penetrance. Consequently, such expansions may be identified in healthy individuals, patients with unrelated disorders, or patients with ataxia due to other causes, highlighting the need for guidelines to help diagnosing SCA27B in these carriers.

Methods: We developed a set of clinical criteria to assess the likelihood of SCA27B diagnosis. The criteria were established through expert consensus and subsequently validated in two cohorts: 159 patients with expansions of ≥ 300 repeats and 274 patients with 200–299 repeats. The criteria comprised two segregation criteria and seven clinical and radiological criteria (four major and three minor). Based on the number of criteria fulfilled, a numerical score was assigned, allowing classification as clinically definite, probable, possible, or unlikely SCA27B.

Results: In the cohort of patients with ≥ 300 (GAA•TTC) repeats ($n=159$), agreement between the criteria and expert clinical assessment was observed in 97.5% of cases. Among patients with 200–299 repeat expansions ($n=274$), agreement between the criteria and expert assessment reached 94.2%, with a sensitivity of 92.5% and a specificity of 100%. Notably, the criteria score correlated significantly with repeat expansion size (Pearson's correlation coefficient = 0.56, $R^2 = 0.31$, $p < 0.0001$).

Discussion: In the absence of a biological gold standard to support a SCA27B diagnosis, the proposed SCA27B criteria showed very good concordance with expert clinical assessment, supporting their validity in a clinical setting.

Conclusion: We developed clinically based criteria to assess the likelihood of SCA27B in patients carrying incomplete penetrant alleles. These criteria may assist clinicians in routine practice and provide a standardized diagnostic framework for future studies.

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C63. Lower urinary tract symptoms in Friedreich's Ataxia: Prevalence, risk factors, and urodynamic findings

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Background and objectives: Friedreich's Ataxia (FRDA), the most prevalent early onset hereditary ataxia in the Caucasian population, is a neurodegenerative disorder characterized by progressive motor dysfunction and extraneurological, multisystem involvement. Lower urinary tract symptoms (LUTS) are frequently reported and may significantly contribute to disease burden. However, robust prevalence data and correlation with disease milestones remain poorly characterized. This study aimed to determine the prevalence of LUTS in FRDA and to identify disease-related factors associated with their development in the Innsbruck cohort.

Methods: A longitudinal cohort of 64 genetically confirmed FRDA patients (40.6% female) was followed at the Center of Rare Movement Disorders Innsbruck over a median of 7.4 years (range: 0–13.4). Neurological assessments using the SARA and INAS rating scales were conducted at baseline and annually thereafter. Selected patients additionally underwent invasive urodynamic evaluation.

Results: At baseline, 14 patients (21.9%) reported LUTS. These individuals showed significantly higher median age (33.5 vs. 25.0 years, $p=.031$), longer disease duration (18.5 vs. 9.5 years, $p<.001$), and higher SARA scores (24.75 vs. 14.0, $p<.001$). Among the 50 initially asymptomatic patients, 25 (50.0%) developed LUTS during follow-up. Overall, 60.9% of the cohort reported LUTS considering the whole observation period. In correlation analysis, LUTS were most strongly associated with peripheral neuropathy (HR: 3.906, 95% CI [0.402–31.019], $p=.197$), while spasticity showed an inverse correlation with the presence of LUTS (HR: 0.545, 95% CI [0.217–1.367], $p=.196$). Of ten patients undergoing urodynamic studies, overactive bladder was identified in nine (90.0%) at baseline.

Discussion and Conclusion: The present findings underscore the clinical relevance of LUTS in FRDA and point towards a multifactorial etiology. Given the dynamic nature of LUTS in FRDA, systematic longitudinal reassessment and interdisciplinary therapeutic management are essential components of comprehensive care.

C65. BARS-2: A Refined Brief Ataxia Rating Scale with Half-Point Scoring and Video Anchors for Clinical and Research Use

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Background and Objectives: The Brief Ataxia Rating Scale (BARS) was developed and validated as a streamlined clinical tool derived from the modified International Cooperative Ataxia Rating Scale (MICARS; Schmahmann et al., 2009). BARS captures the five cardinal manifestations of the cerebellar motor syndrome: gait impairment, appendicular dysmetria (arms, legs), dysarthria, and oculomotor deficits. It is part of standard neurological examination and take <5 minutes to administer. It correlates closely with MICARS and SARA. Early experience revealed that cerebellar motor performance frequently fell between integer categories, highlighting the need to develop half-point scoring to reflect gradations of severity, and to capture biological variability within a single examination time-point. The revised BARS incorporating half-point scoring (BARS-2) was introduced into clinical practice and multicenter studies in 2015 and has been used in published work. This is its first formal description.

Methods: Systematic refinement of BARS-2 was informed by clinical experience. Half-point increments were defined across all domains, with unambiguous, behaviorally grounded anchor descriptions for each score. Standardized video recordings were obtained, with patient consent, to exemplify each integer and half-point score.

Results: BARS-2 takes no longer to administer than BARS. The expanded descriptors are useful in defining adjacent half-scores clearly. Video exemplars provide visual reference for each score, facilitating consistent application, and education of test administrators.

Discussion and Conclusion: Half-point scoring and explicit descriptions in BARS-2 preserve the five critical core elements of cerebellar motor ataxia while improving granularity. BARS-2 is used in the CRC-SCA natural history study and in multiple publications demonstrating correlations with other clinician-administered and patient-reported outcome measures in genetic and sporadic neurodegenerative diseases, in clinical trials, and in studies validating digital phenotyping across cerebellar motor, vestibular and cognitive-neuropsychiatric syndromes. Supported by video-based anchors, BARS-2 is a fast, accurate, validated instrument for clinical assessment and research in cerebellar ataxia.

Funding Sources: Great Oaks, Once Upon a Time, ARSACS, National Ataxia, and MINDlink Foundations.

C66. Clinical, Genetic, and Imaging characteristics of SCA27B: Insights from a Large Dutch Cohort

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Background and Objectives: Deep intronic GAA repeat expansions in intron 1 of the FGF14 gene were identified in 2023 as cause of late-onset cerebellar ataxia (LOCA). Since then, GAA-FGF14-related ataxia (SCA27B) has emerged as one of the most common genetic causes of LOCA. We aimed to describe the clinical, genetic and imaging features of a large Dutch diagnostic cohort.

Methods: The Radboudumc genetic database was queried for GAA-FGF14 expansions ≥ 200 . Repeat length was assessed using locus-spanning PCR, repeat-primed PCR, and PacBio sequencing. A subset was validated using Oxford Nanopore. Clinical and imaging data were retrospectively reviewed.

Results: 127 individuals with GAA-FGF14 expansions ≥ 200 were identified; clinical data were available from 116 individuals, including 7 asymptomatic/presymptomatic individuals. Fifteen individuals carried GAA200-249 expansions; 60% exhibited at least one core SCA27B feature. In symptomatic individuals, episodic symptoms occurred in 72.5%, and 24% had visited the emergency department or outpatient TIA clinic with episodic symptoms prior to SCA27B diagnosis. Brain MRI frequently showed non-specific white matter abnormalities (>90%) and the superior cerebellar peduncle sign was present in 67.7%. Among those treated, 54.1% reported symptomatic benefit from 4-aminopyridine. Nanopore and PacBio sequencing results showed high correlations. We observed an inverse relationship between age at onset and disease progression suggesting faster progression in those with a later age at onset.

Conclusions and discussion: This study confirms the significance of GAA-FGF14 repeat expansions as a cause of LOCA in the Netherlands. Although the phenotype is highly recognizable, SCA27B can present as a stroke mimic in some cases. MRI features, especially the SCP sign, may aid in diagnosis. We provide additional evidence supporting the pathogenicity of GAA alleles in the GAA200-249 range. The role and specificity of WMAs in SCA27B warrant further study, as does the relationship between age at onset, GAA-FGF14 length and disease progression.

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C67. The Spinocerebellar Ataxia-Health Index: Development and Evaluation of a Patient-Reported Outcome Measure

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Background: There is a need for reliable, sensitive, spinocerebellar ataxia (SCA)-specific patient-reported outcome (PRO) measures capable of detecting clinically-relevant changes in disease burden during therapeutic studies. The U.S. Food and Drug Administration (FDA) emphasizes the importance of properly validated PROs as valuable instruments for supporting drug-labeling claims.

Objective: To meet the need for a disease-specific outcome measures that effectively quantifies disease burden, we have developed and validated the Spinocerebellar Ataxia-Health Index (SCA-HI), a tool specifically designed for use in therapeutic trials involving patients with SCA.

Methods: We conducted semi-structured qualitative interviews and an international cross-sectional study with individuals with SCA to determine the symptoms with the greatest prevalence and impact. We selected symptom items for the SCA-HI based on their relevance, as determined by the cross-sectional study results, and their potential responsiveness to therapeutic intervention. We used factor analysis to generate instrument subscales representing the core areas of symptomatic health in SCA. Subsequently, beta testing evaluated the comprehensibility of the instrument through participant feedback. Test-retest reliability assessments were conducted to optimize the reliability and responsiveness of the SCA-HI.

Results: Twenty individuals with SCA participated in qualitative interviews and 891 participants completed the international cross-sectional study. Fifteen individuals participated in beta-testing of the SCA-HI and 29 individuals completed test-retest reliability. The SCA-HI contains 16 symptomatic health domains, comprehensively assessing the overall SCA disease burden. Both the SCA-HI total score and its independently validated subscales exhibit high internal consistency, strong relevance, and reliability. Known groups testing demonstrated that the SCA-HI is effective in distinguishing between individuals with varying levels of disease severity.

Conclusions: The SCA-HI has a reliable, regulatory-grade, disease-specific outcome measure for therapeutic trials involving patients with SCA. Collectively, the SCA-HI measures 16 distinct domains of SCA disease burden and is designed to support future therapeutic studies and labeling claims.

C68. An Italian Friedreich Ataxia registry (IFAR)

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Background: Friedreich ataxia (FA) is a rare autosomal recessive neurodegenerative disorder caused by a GAA trinucleotide repeat expansion in the FXN gene, leading to frataxin deficiency. This deficiency results in mitochondrial dysfunction, increased oxidative stress, and progressive cellular damage. Suppression of the Nrf2 signaling pathway further contributes to disease progression. Omaveloxolone, a pharmacological activator of Nrf2, became the first therapy for treating patients with FA approved and reimbursed by the Agenzia Italiana del Farmaco (AIFA) in commercial setting from 30 January 2026. This project aims to establish a nationwide Italian registry of patients with FA followed at specialized centers to improve understanding of disease natural history and evaluate the long-term safety and effectiveness of available treatments, including omaveloxolone.

Methods: This is a multicenter, prospective, observational, open-ended study conducted in a real-world setting. Clinical, neurological, neuropsychological, cardiological, biological, and safety data will be collected. Participants will undergo annual evaluations. Patients receiving omaveloxolone will additionally be assessed at months 3, 6, 12, 18, and 24, and annually thereafter whenever feasible. Core outcome measures include mFARS and/or SARA, FDS, echocardiography, FA-ADL, EQ-5D, and CGI/PGI assessments. Optional assessments include the 9-Hole Peg Test, PATA rate test, CCAS, OCT, cardiopulmonary exercise testing, MFIS, PROM-Ataxia, and WPAI questionnaires.

Results: Approximately 30 specialized Italian centers are participating in the registry. Patient enrollment and data collection are ongoing. The registry is expected to generate longitudinal real-world data on disease progression, and contributing to a more comprehensive epidemiological profile of the disease in Italy.

Discussion: This registry will provide long-term quantitative data on FA progression. The project will harmonize clinical assessments across participating centers, support innovative clinical research, optimize outcome measures for future trials, and facilitate patient recruitment for interventional studies. In addition, participants may benefit from longitudinal assessments for personalized clinical management.

Funding Sources: The registry is supported by Biogen

C69. Retrospective Patient-Completed Questionnaire Exploring Stimulant Medication Effects on Cognitive Symptoms and Quality of Life in Cerebellar Disorders

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Background and Objectives: Patients with cerebellar disorders frequently experience fatigue and cerebellar cognitive affective syndrome (CCAS), including executive dysfunction, impaired attention, language difficulties, and emotion dysregulation. These symptoms can substantially impair daily functioning and quality of life (QoL). Despite the significant burden associated with fatigue and CCAS, there are currently no approved pharmacologic treatments targeting these symptoms in cerebellar disease populations. This study tested whether stimulant medications improve fatigue and CCAS-related symptoms in patients with cerebellar disorders.

Methods: Of 59 patients identified in the MGH Ataxia Center database taking stimulant medications, 27 were eligible and invited to participate; 20 completed the study (16 actively taking stimulants at time of completion, 4 responding retrospectively). We administered two questionnaires to 20 patients with cerebellar disorders who had been prescribed stimulant medications as part of clinical care. The first asked them to assess the perceived effects of stimulants across nine domains: QoL, fatigue, alertness, verbal expression, thinking clearly, organizing, planning, and multitasking, emotional state, social interactions, and physical symptoms. The second was the Patient-Reported Outcome Measure of Ataxia, Mental section (PROM-Ataxia MEN), completed retrospectively for symptoms both before and during stimulant treatment.

Results: Patients reported perceived improvements across all assessed domains. The percentage of patients reporting improvement was highest for alertness (95%) and fatigue (90%), followed by thinking clearly (80%), organizing, planning, and multitasking (80%), verbal expression (75%), quality of life (75%), emotional state (70%), physical symptoms (70%), and social interactions (65%). PROM-Ataxia MEN scores improved by 26% during treatment compared to before treatment.

Conclusions: These preliminary findings suggest that stimulant medications may be associated with perceived improvements in fatigue, cognition, and overall functioning in patients with cerebellar disorders. Given the small and heterogeneous sample, and reliance on retrospective patient-report, larger prospective studies are needed to evaluate efficacy, tolerability, and long-term benefit.

Funding Sources: This study was supported by the National Ataxia, Great Oaks, Once Upon A Time, and MINDlink Foundations.

C70. Phase 1b Trial of Safety & Tolerability of SGT-212 Delivered via Intradentate Nucleus & Intravenous Infusion to Participants with Friedreich's Ataxia

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Background: Friedreich's ataxia (FA) is a rare, progressive, debilitating and fatal multi-organ disease caused by variants of intron 1 of the frataxin gene - most commonly an abnormal homozygous GAA repeat expansion - leading to reduced expression of the frataxin protein. Symptoms are neurologic and cardiac in nature. Neuronal damage often results in loss of upper and lower limb function. The average life expectancy is ~38 years, with cardiac dysfunction as the leading cause of death.

Objective: To assess the safety and tolerability of SGT-212 delivered via intradentate nucleus and intravenous infusion to participants with Friedreich's ataxia.

Method: FALCON (NCT07180355) is a Phase 1b multi-center clinical trial to study the safety and tolerability of SGT-212, a novel gene therapy, designed to deliver the frataxin gene to address key neurologic, cardiac and systemic aspects of FA. Through a novel dual route of administration, we are able to provide both a low-dose systemic delivery (IV) and a precise, targeted local delivery to the dentate nucleus, a key brain structure responsible for the neurologic clinical impact of FA, via a minimally invasive, stereotactic surgical procedure.

Summary: Here we present initial safety and tolerability data of SGT-212 in a cohort of non-ambulatory adults with FA. Our findings provide supportive evidence for a dual route of administration strategy that allows for a targeted treatment of both the neurologic as well as the systemic aspect of this devastating disease.

Funding Sources: This study was sponsored by Solid Biosciences Inc. (Charlestown, MA, USA).

C72. Friedreich's Ataxia in Northeastern Brazil: A Long Diagnostic Journey to Wheelchair Dependency

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Background and Objectives: Friedreich's ataxia (FA) is the most common autosomal recessive ataxia worldwide, yet epidemiological data from underserved regions remain scarce. We aimed to characterize the clinical profile, systemic manifestations, and diagnostic journey of genetically confirmed FA patients from Northeastern Brazil. **Methods:** We performed a retrospective observational study at a tertiary Movement Disorders and Neurogenetics Clinic in Northeastern Brazil. Clinical, demographic, and disease-related data from patients with genetically confirmed FA were reviewed, including age at symptom onset, age at diagnosis, age at loss of ambulation, initial manifestations, and associated comorbidities.

Results: Among 69 patients followed for hereditary and degenerative ataxias, 11 (15.9%) had genetically confirmed FA. Mean age at symptom onset was 15.5 years, while mean age at molecular diagnosis was 33.5 years, corresponding to an average diagnostic delay of approximately 18 years. Mean age at loss of ambulation was 30.0 years. The most common initial manifestations were gait abnormalities (63.7%) and imbalance (45.5%). Extraneurological involvement was frequent, including scoliosis (87.5%), cardiomyopathy (72.7%), visual impairment (37.5%), hearing impairment (37.5%), and diabetes mellitus (18.2%). At first evaluation, 81.8% of patients were wheelchair-dependent and no pediatric patients were identified in the cohort. Notably, diagnosis often occurred near or after the expected period of loss of ambulation.

Discussion and Conclusion: Our findings reveal a profound diagnostic gap for FA in Northeastern Brazil. Compared with previously reported Brazilian cohorts, patients experienced substantially longer delays before genetic confirmation and frequently reached tertiary care only after severe disability had developed. Earlier recognition of progressive gait instability associated with scoliosis, cardiomyopathy, diabetes, and sensory deficits may facilitate timely genetic testing, multidisciplinary management, and access to emerging disease-modifying therapies. These data highlight the urgent need to improve awareness, referral pathways, and access to molecular diagnosis in underserved regions.

Funding Sources: Programa de Iniciação Científica Rede HU Brasil

C73. Beyond Ataxia: Preliminary Findings from an Ongoing Multimodal Friedreich's Ataxia Cohort in Northeastern Brazil

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Background and Objectives: Friedreich's ataxia (FA) is a multisystem neurodegenerative disorder characterized by progressive neurological impairment and frequent cardiac involvement. However, the relationships among neurological disability, myocardial abnormalities, arrhythmias, and sleep-disordered breathing remain poorly understood. We aimed to characterize neurological, cardiological, and sleep-related abnormalities in genetically confirmed FA patients and explore potential associations between disease severity and extracerebellar manifestations.

Methods: This ongoing cross-sectional observational study is being conducted at a tertiary referral center in Northeastern Brazil. Participants undergo neurological, cardiological, and sleep assessments. Disease severity is evaluated using the Scale for the Assessment and Rating of Ataxia (SARA) and the modified Friedreich Ataxia Rating Scale (mFARS). Cardiac evaluation includes cardiac magnetic resonance imaging and seven-day Holter monitoring. Sleep-disordered breathing is assessed using type IV polysomnography.

Results: Preliminary data from five genetically confirmed patients demonstrated a mean SARA score of 25.6 and a mean mFARS score of 58.5, indicating moderate-to-severe neurological impairment. Cardiac magnetic resonance imaging identified subepicardial myocardial fibrosis in two patients and mild left ventricular hypertrophy, predominantly involving the interventricular septum, in three. Left ventricular systolic function was preserved or at the lower limit of normal in all cases. Seven-day Holter monitoring, available in two patients, demonstrated predominant sinus rhythm with episodes of non-sustained atrial tachycardia in both individuals. One patient exhibited isolated polymorphic ventricular ectopy, while another showed corrected QT interval prolongation. Sleep assessment revealed a mean oxygen desaturation index of 15 events/hour without significant sustained hypoxemia.

Discussion and Conclusion: These preliminary findings reinforce the multisystem nature of FA and demonstrate that myocardial fibrosis, structural cardiac remodeling, arrhythmias, and sleep-disordered breathing may coexist even without overt ventricular dysfunction. Ongoing recruitment will allow future analyses exploring relationships among neurological severity, cardiac involvement, and sleep abnormalities. Such integrated biomarkers may improve disease characterization, risk stratification, and clinical monitoring in FA.

Funding Sources: Programa de Iniciação Científica Rede EBSEH

C74. Clinicogenetic Characterization of Late Onset Ataxias in Austria

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Objective: To gain insight into the natural history of late-onset ataxia, identify unrecognized genetic underpinnings and improve early differentiation from other causes of gait instability in the elderly, including multiple system atrophy of cerebellar type (MSA-C).

Background: Late onset ataxias are rare neurodegenerative disorders characterized by progressive coordination deficits resulting from damaged cerebellar pathways. Despite the identification of monogenic drivers, specifically RFC1- and GAA-FGF14-expansions, 70% remain genetically unsolved. Frequent age-related comorbidities and phenotypical overlap with MSA-C are among the challenges that hamper the identification of genetic etiologies. Since pathogenic RFC1- and FGF14-expansions have been found in clinically diagnosed MSA-C, precise clinical phenotyping is imperative to maximize the diagnostic yield of genetic testing.

Methods: Patients with late-onset ataxia, including genetically confirmed cases, cases still classified as SAOA, and cases fulfilling the current MSA-C clinical diagnostic criteria will be recruited at the Center for Rare Movement Disorders Innsbruck. Each patient will undergo a baseline visit and a one-year follow-up examination including ataxia rating scales, autonomic questionnaires and candidate patient reported outcomes. We will perform deep-phenotyping covering brain MRI, autonomic function, sleep disturbances, and peripheral nerve involvement. We will integrate available retrospective data to enhance our understanding of longitudinal evolution.

Results: At the time of abstract submission, 53 female and 49 male patients (n=102) fulfilling the inclusion criteria were identified, 36 of which have been prospectively recruited within 4 months. In a preliminary performed statistics in the prospective cohort, the baseline median SARA was 12 points, with a standard deviation of 6.5, and mean age of onset was 59 years.

Discussion: By integrating retrospective and prospective data, we aim to identify early non-motor red flags differentiating monogenic SAOA from MSA-C and other elderly gait disorders. We will pinpoint trial outcome measures and actionable clinical pathways to optimize SAOA diagnosis and patient

C75. Oculomotor abnormalities are frequent in both pure and complex hereditary spastic paraplegia

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Background: Hereditary spastic paraplegias (HSPs) are genetically heterogeneous neurodegenerative disorders characterized primarily by progressive lower-limb spasticity and weakness. Non-motor features, including oculomotor abnormalities, remain understudied.

Objectives: To determine the frequency and types of oculomotor abnormalities in genetically confirmed HSP and to compare their occurrence in pure (pHSP) versus complex (cHSP) forms.

Methods: Thirty patients with genetically confirmed HSP were classified as pHSP (n=14) or cHSP (n=16) and underwent video-oculography (VOG - EyeSeeCam). Examinations assessed spontaneous and gaze-evoked nystagmus, optokinetic nystagmus (OKN), smooth pursuit quality (horizontal and vertical), and horizontal and vertical saccades.

Results: Oculomotor abnormalities were common in both groups. Spontaneous nystagmus was observed in 29% of pHSP and 44% of cHSP patients; gaze-evoked nystagmus in 7% and 19%, respectively. Horizontal smooth pursuit abnormalities occurred in 50% of pHSP and 75% of cHSP patients; vertical smooth pursuit abnormalities were present in 66% of both groups. Preserved OKN responses were seen in 93% of pHSP compared with 54% of cHSP patients, suggesting more frequent OKN impairment in complex phenotypes. Inaccurate horizontal saccades were observed in 7% of pHSP and 25% of cHSP cases, while vertical saccades were inaccurate in 29% of pHSP and 33% of cHSP. Overall prevalence of oculomotor abnormalities did not differ significantly between pHSP and cHSP.

Conclusions: Oculomotor abnormalities are common in genetically confirmed HSP and are not restricted to complex phenotypes. These findings suggest that oculomotor involvement may represent an underrecognized component of the HSP spectrum, including in patients clinically classified as pure HSP. Systematic VOG assessment may refine phenotyping beyond the traditional pure-versus-complex distinction.

Funding Sources: Grant Agency of Charles University n. 216225

C76. Cardiac and Neurological Characterization of Friedreich's Ataxia: An Exploratory Analysis of a Hispanic Cohort

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Background and Objectives: Friedreich's ataxia (FRDA) is a rare autosomal recessive neurodegenerative disorder caused by GAA trinucleotide repeat expansions in the FXN gene, leading to frataxin deficiency, neurological degeneration, and hypertrophic cardiomyopathy. Associations between neurological, cardiac, and biomarker profiles remain poorly characterized in Hispanic populations. This study aimed to describe these associations in an exploratory cohort.

Methods: A cross-sectional study was conducted in 11 genetically confirmed FRDA patients (100% Hispanic/Latino). Assessments included the modified mFARS, FA-ADL, echocardiography, 12-lead ECG, NT-proBNP, and frataxin measurement. Spearman correlation and Mann-Whitney U tests were applied.

Results: Median age was 24.0 years (IQR 21.5–33.5), disease onset at 13.0 years (IQR 11.5–15.5), and disease duration 14.0 years (IQR 10.5–18.0). Median mFARS was 56.5 (IQR 50.5–64.0) and frataxin 239.8 pg/μg (IQR 201.1–366.6). Frataxin correlated inversely with mFARS lower limb subscore ($\rho=-0.832$, $p=0.001$), total mFARS ($\rho=-0.700$, $p=0.016$), and isovolumetric relaxation time ($\rho=-0.730$, $p=0.011$). NT-proBNP (median 53.4 pg/mL; none >125 pg/mL) correlated with lower limb dysfunction ($\rho=0.799$, $p=0.003$) and inversely with frataxin ($\rho=-0.718$, $p=0.013$). LVEF was preserved in 10/11 patients (median 57%). Age at onset correlated with left ventricular mass ($\rho=0.716$, $p=0.013$). ECG abnormalities were present in 8/11 patients (72.7%).

Discussion: Frataxin emerged as a central biomarker linking neurological severity and cardiac dysfunction. Lower frataxin levels associated with greater lower limb impairment, worse mFARS, prolonged IRT, and elevated NT-proBNP, suggesting parallel progression of spinocerebellar and myocardial stress through shared mitochondrial dysfunction. Patients with later onset showed greater ventricular hypertrophy (LVM 199 vs. 140 g) despite similar disease duration, indicating that timing of frataxin deficiency during cardiac development influences hypertrophic trajectory.

Conclusion: Frataxin functions as a cross-domain biomarker of neurological and cardiac dysfunction in FRDA, with NT-proBNP signaling subclinical myocardial stress linked to lower limb deterioration. Prospective multicenter studies in Hispanic FRDA populations are warranted.

Funding Sources: This work was funded by Mantle Therapeutics Inc.

C77. Performance, Response Time, and Oculomotor Characteristics During Multiple Object Tracking in People with Cerebellar Ataxias

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Quantitative behavioral measures of cerebellar ataxias are needed for detecting onset of disease, quantifying progression, and evaluating treatment efficacy. This study sought to characterize patterns in performance, response time, and eye fixations during a computer-based visuospatial cognition task called multiple object tracking (MOT) in cerebellar ataxia populations.

Cross-sectional data from the first time-point of subjects enrolled in the Neurobooth study were analyzed, including controls (n=71) and ataxia subjects (n=177: SCA3; SCA6; SCA27B; CANVAS; FA; other). Participants' eye movements were collected during the MOT task, which involved tracking smoothly moving targets versus distractors while the speed and number of targets increased incrementally. The task was divided into target registration, tracking, and response phases, and eye fixations within each phase were determined, enabling computation of median fixation frequencies, durations, and gaze entropies (future work). The metrics of accuracy (median percent correct) and response time (median trial response duration) were assessed. Patients completed PROM-Ataxia (Patient-Reported Outcome Measures-Ataxia) prior to their session and were administered BARS (Brief Ataxia Rating Scale) on session day. Correlations between severity and task performance measures (accuracy, response time) were assessed.

For individuals with ataxia, MOT accuracy correlated negatively with BARS ($r=-0.32$), PROM-Ataxia total ($r=-0.36$), and cognitive/mental section ($r=-0.29$), but not emotional/mental section. Response time correlated positively with only BARS ($r=0.56$) and PROM-Ataxia total ($r=0.31$). For all correlations, $p<0.05$.

MOT performance aligned weakly to moderately with self-reported cognitive function and clinical measures of motor severity. MOT may capture cognitive components not reflected in these assessments. Future work will analyze saccades and fixations during stimulus and response phases of the task, enabling characterization of eye movement patterns that reflect visual perception and decision making in ataxias.

Funding Sources: NIH (DHHS) - R01NS117826; Broad Institute; Biogen MA Inc.; Massachusetts Life Sciences Center; U.S. Department of Defense; Duke Family Foundation

C78. Monitoring Ataxia Severity in Daily Life using Wearable Movement Sensors

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Background and Objectives: Wearable movement sensors enable objective quantification of Spinocerebellar Ataxia (SCA) severity and motor behavior during brief clinical assessments (structured environments) and continuous monitoring in daily life (unstructured environments). However, it remains unclear which sensor-derived variables best reflect ataxia severity and whether these relationships differ across environments. This study evaluates relationships between wrist-derived sensor variables and ataxia severity during structured and unstructured environments in people with genetically confirmed SCA.

Methods: This ongoing remote cohort study includes 50 people with genetically confirmed SCA (Types 1, 2, 3, 6) and 50 age- and sex-matched healthy controls. Participants wore a sensor on the wrist of the more affected or dominant upper-limb during the Scale for Assessment and Rating of Ataxia at Home (SARAHome), followed by one-week of continuous sensor wear in daily life. Summary movement variables were derived from raw sensor data and correlated with clinical measures using Spearman correlations. Intraclass correlation coefficients (ICC) assessed agreement between sensor variables in structured and unstructured environments.

Results: Preliminary analyses included 25 participants with SCA spanning mild-to-severe ataxia (SARAHome range 3-22). Among a priori candidate variables, jerk, entropy, and magnitude variability demonstrated moderate negative associations with ataxia severity across structured and unstructured environments ($p_{\text{jerk}} = -0.45$ vs. -0.37 ; $p_{\text{entropy}} = -0.20$ vs. -0.36 , $p_{\text{magnitude_sd}} = -0.40$ vs. -0.39). Exploratory analyses identified frequency-domain variables with the strongest overall associations to ataxia severity in unstructured environments ($p_{\text{magnitude}} = -0.62$, $p_{\text{psd_freq_spectrum}} = 0.74$) compared to structured environments ($p_{\text{magnitude}} = -0.56$, $p_{\text{psd_freq_spectrum}} = 0.04$). ICC analyses demonstrated variable agreement across environments (ICC range 0.00–0.68, median = 0.05, Interquartile range₂₅₋₇₅ = 0.00 – 0.19).

Discussion and Conclusion: These findings suggest that across structured and unstructured environments wearable movement sensors capture distinct aspects of ataxia severity and motor behavior. Stronger associations for exploratory frequency-domain variables and low agreement across environments, support the complementary value of continuous wearable monitoring.

Funding Sources: This work was funded in whole or in part by the National Institutes of Health (NIH, R37HD068290).

C79. Vestibular Evoked Myogenic Potentials in Cerebellar Ataxia, Neuropathy and Vestibular Areflexia Syndrome (CANVAS)

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Background and Objectives: Cerebellar Ataxia, Neuropathy and Vestibular Areflexia Syndrome (CANVAS) is caused by biallelic expansions in the RFC1 gene. Histopathological studies demonstrate severe degeneration of Scarpa's (vestibular) ganglia with relative preservation of vestibular end organs. Consistent with this pathology, semicircular canal (SCC) dysfunction is markedly reduced vestibulo-ocular reflex (VOR) gain. However vestibular evoked myogenic potential (VEMP), a measure of otolith system function, have been variably reported. This study aimed to determine whether otolith function is affected to a similar extent as SCC function. **Methods:** A retrospective multicentre study of 41 patients with CANVAS. Participants underwent vestibular assessment including cervical VEMPs (cVEMPs), ocular VEMPs (oVEMPs), and SCC testing using video head impulse testing. Demographic, clinical and genetic data were compared across vestibular end organ function.

Results: 41 participants were included (mean age at symptom onset 54.7 years; mean disease duration 10.4 years). Among 39 participants undergoing cVEMP assessment, normal responses were present in 73% of ears, whereas only 33% of ears demonstrated preserved oVEMP responses. Twenty-four participants underwent both cVEMP and oVEMP testing, with discordant responses observed in 47% of cases. The predominant pattern was preserved cVEMPs with absent oVEMPs (43%). SCC dysfunction was universal. Disease duration correlated negatively with horizontal SCC VOR gain but not with VEMP responses.

Discussion and Conclusion: Despite universal SCC dysfunction, otolith function was relatively preserved in a substantial proportion of patients, particularly within saccular pathways (cVEMPs). These findings suggest that vestibular involvement in CANVAS is not uniform across vestibular end organs. Discordance between VEMP and SCC measures should not dissuade clinicians from the diagnosis of CANVAS and provides new insights into the pathophysiology of RFC1-related disease.

C80. Deep Phenotyping of Aboriginal Australians with Machado–Joseph Disease (MJD)/Spinocerebellar Ataxia Type 3 (SCA3)

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Background and Objectives: Aboriginal Australians living in remote communities in Northern Australia experience the highest prevalence of Machado–Joseph Disease (MJD)/Spinocerebellar Ataxia Type 3 (SCA3) worldwide. Despite this substantial disease burden, comprehensive clinical phenotyping has not previously been undertaken in this population. This study aimed to perform deep phenotyping of Aboriginal Australians with molecularly confirmed MJD/SCA3 to better characterise disease manifestations and identify potential biomarkers relevant to future therapeutic studies.

Methods: Fifty-two Aboriginal Australians with genetically confirmed MJD/SCA3 underwent prospective deep phenotyping. Data collected included demographic characteristics, age at symptom onset, disease duration, CAG repeat expansion size, neurological examination findings, quantitative oculomotor assessments, vestibular function testing, neurophysiological studies, and functional disability measures. Assessments were conducted through specialist ataxia outreach clinics in partnership with local communities and the Machado-Joseph Disease Foundation.

Results: Comprehensive subjective and objective datasets were obtained across a broad spectrum of disease severity. Distinct neurological, vestibular, oculomotor and neurophysiological impairments were identified. Quantitative assessments provided detailed characterisation of disease manifestations and variability within the cohort, enabling objective evaluation of disease burden and functional impairment. The phenotypic data also identified candidate biomarkers with potential utility in future natural history studies and therapeutic trials.

Discussion and Conclusion: This represents the first systematic deep-phenotyping study of Aboriginal Australians with hereditary ataxia and identifies a distinct phenotype. The findings provide important insights into disease expression within a population experiencing an exceptionally high burden of MJD/SCA3 and highlight opportunities for improved clinical management, culturally responsive models of care, and participation in emerging clinical trials. Comprehensive phenotyping establishes a foundation for biomarker selection, trial-readiness initiatives and longitudinal natural history studies.

Funding Sources: National Health and Medical Research Council, Medical Research Future Fund. VESKI FAIR Fellowship, Perpetual's 2024 IMPACT Philanthropy (The Lionel & Yvonne Spencer Trust, Kevin Darrell Clarke Endowment)

C82. Patient-reported experiences during long-term omaveloxolone treatment in Friedreich ataxia: qualitative analysis of quality-of-life domains

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Background and Objectives: Friedreich ataxia (FA) is a progressive neurodegenerative disease that leads to loss of ambulation, increasing disability over time, and poor quality of life (QoL). Natural history studies demonstrate measurable neurological and functional decline. Within this context, stabilization or slower-than-expected progression due to intervention may represent a clinically meaningful outcome. This survey aimed to assess patient-reported quality-of-life experiences among adults receiving omaveloxolone in the United States.

Methods: Adults with FA (N=14) who received omaveloxolone for ≥3 years through participation in the MOXIe trial (NCT02255435), followed by treatment with the commercially available drug, were recruited for one-time, semi-structured interviews conducted between November 2025 and February 2026. Participant experiences were assessed across five QoL domains: fatigue, mobility, work and education, social participation, and communication. Transcripts were analyzed using a structured qualitative framework to categorize outcomes as improvement, stability/slower progression, or deterioration.

Results: Eight of fourteen respondents reported stability or improvement in at least four domains, with five reporting stability and/or improvement across all five domains. All (fourteen) respondents reported stable or improved social participation. Six participants reported stable mobility. Eight respondents reported ongoing disease progression (deterioration) in mobility; all eight also reported stable or improved social participation, six reported stable or improved work, and six reported stable or improved communication abilities.

Discussion and Conclusion: This patient-reported survey provides qualitative insights into the experiences of adults with FA, highlighting the impact of long-term treatment with omaveloxolone across multiple QoL domains. Interpretation is limited by the small sample size and potential clinical trial, recall and selection bias.

Funding Sources: Biogen

C83. Demographic, clinical and genetic characteristics of the DRPLA Natural History and Biomarkers Study (DRPLANHBS)

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Background and objectives: Dentatorubral-pallidoluysian atrophy (DRPLA) is an ultrarare genetic type of ataxia caused by a CAG repeat expansion in the ATN1 gene. Currently, there are not treatments that can halt the progression of the disease, although therapies with antisense oligonucleotides have been used in n-of-1 trials, and might enter phase I clinical trials in the near future. For those studies, valid outcome measures and biomarkers will be required. We aim to describe the demographic, clinical and genetic characteristics of the cohort of participants enrolled in the DRPLA Natural History and Biomarkers Study (DRPLANHBS).

Methods: We recruited 55 DRPLA carriers and 32 healthy controls in 9 participating centres, from 2022 to 2026. Participants were assessed on an annual basis following a standardised protocol. Information on demographic variables, previous clinical and family history, age of onset for different symptoms, and activities of daily living (FARS- ADL questionnaire) was collected. Data on epilepsy was collected using three different instruments (simplified score of seizure frequency, Liverpool seizure severity scale, VA seizure type and frequency rating). Neurological examination was recorded using SARA, INAS, the Unified HD Rating Scale (UHDRS), and the Unified Myoclonus Rating Scale (UMRS). Functional assessments included SCAFI. We assessed symptoms of anxiety and depression with the PHQ-9 questionnaire, and fatigue with the MFIS. Cognition was assessed with the WAIS-IV. Statistical analysis will include multilevel modelling to account for intrasubject variability.

Results: We collected longitudinal data in the participants, with a maximum of 4 timepoints for some of them. Statistical analysis of the different variables will follow.

Discussion and Conclusion: We have conducted a natural history study in an ultrarare type of genetic ataxia. Results from this study will inform on the evolution of the disease, and will contribute to the selection of outcome measures for future clinical trials.

Funding Sources: Ataxia UK and CureDRPLA grants.

C86. Let's Move: A virtual adaptive exercise and wellness program for individuals living with ataxia and movement disorders

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Background/Objective: : Individuals living with chronic conditions benefit from self-management to improve their health behaviors, outcomes, and quality of life over the long term. Let's Move is a new virtual community resource serving individuals with ataxia, promoting self-management, exercise, and safe mobility. Group exercises and wellness classes have been led by neuro specialized physical therapists via Zoom/virtual format. This poster aims to provide a summary of the community program since inception.

Methods: Virtual sessions have been offered 1 time per week including 40-minutes of exercise followed by 20-minute wellness presentation. Educational topics include self-efficacy, safe mobility strategies, fall prevention, evidenced based benefits of exercise, etc. Participants have completed pre and post surveys and questionnaires related to self-efficacy, balance confidence, and quality of life.

Results: 70+ individuals with ataxia and movement disorders participate each week from across the US and globe. The pre-survey results indicated 52% of participants did not previously have a home program.

Post-survey results revealed 87% reported they were compliant with exercising in between the class sessions. 87% reported they felt the exercises taught in the class were relevant to their condition and 83% reported they plan to continue the exercising beyond the group class.

53% reported experiencing a positive effect on their mood as a result of the class and over half the class (57%) reported that they felt less socially isolated and lonely.

Participant feedback: "Great presentations, resources and expert led exercise."

Discussion/Conclusion: Telerehabilitation is a resource that improves patient access and remains widely underutilized with little knowledge of how effective it may be for individuals with movement disorders. Individuals with movement disorders have received access to evidenced based group exercise and wellness education via a group telerehabilitation format, at no cost. As a group, attendees reported improvement in mood, social connectedness, and exercise compliance.

Funding Sources: Pacing for Parkinson's community grant via the Johns Hopkins Department of Neurology (Funding, Zoom session, marketing support). National Ataxia Foundation (Funding, Zoom session, marketing support)

C87. Characterizing Clinical Milestone Progression Across Key Domains in Friedreich's Ataxia Using Real-World EHR Data

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Background and Objectives: Friedreich's ataxia (FRDA) is a multisystem disease with substantial heterogeneity in its clinical progression. This study leverages real-world electronic health record (EHR) data to quantify the occurrence of clinically meaningful milestones across key disease domains, with the goal of supporting the development of milestone-based outcome measures.

Methods: We conducted a retrospective longitudinal study using the TriNetX EHR research platform. FRDA patients were identified from 1/1/2010 to 4/20/2026 using FRDA-specific diagnosis codes, requiring at least two recorded instances. The index event was the first recorded ataxia or FRDA diagnosis. Milestone ascertainment began 6 months post-index to exclude prevalent milestone conditions. Five clinical domains were evaluated: cardiovascular, diabetes, mental health, mobility/balance, and sensory deficits (vision, hearing). Domains were defined by pre-specified International Classification of Disease-coded events. Patients with a domain-specific milestone prior to the observation window were excluded from the incident analysis and instead summarized as prevalent-at-diagnosis. Kaplan-Meier survival analysis was used to estimate cumulative incidence for each milestone at 3, 4, and 5 years and over all available follow-up, with 5-year milestone-free survival probabilities also reported for each domain.

Results: We identified approximately 1500 FRDA patients with a median follow-up of 5.4 years. Prevalent-at-diagnosis and cumulative incidence from 3 years to maximum follow-up were: 35.1% and 15.6%-31.8% for cardiovascular, 11.3% and 3.5%-10.0% for diabetes, 19.5% and 12.1%-24.6% for mental health, 36.8% and 20.2%-36.5% for mobility/balance, and 11.1% and 7.4%-16.8% for sensory deficits. Five-year survival probabilities were 70.4% for cardiovascular symptoms, 92.0% for diabetes, 77.4% for mental health symptoms, 61.7% for mobility/balance symptoms, and 85.0% for sensory deficits.

Discussion and Conclusion: These findings provide real-world estimates of milestone incidence across multiple clinical domains in FRDA. Substantial variation across domains was observed, and these results help to establish a data-driven foundation for clinical milestone-based endpoint development for FRDA.

Funding Sources: This work is supported by the Friedreich's Ataxia Research Alliance (FARA).

C85. A telephone interview-based prospective natural history study using FARS-ADL revealed disease-specific progression trajectories of SCA3,6 and 31

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Patient registries play an essential role in the development of therapeutic measures, particularly in establishing disease-specific natural history. The Japan Consortium of Ataxias, J-CAT, was launched in 2016 as a nationwide patient registry on ataxias. Previous studies on natural history of ataxias have adopted the SARA score as a standard clinical indicator. However, the SARA score requires face-to-face examination and does not directly reflect the activities of daily living. Recently, the Friedreich's Ataxia Rating Scale – Activities of Daily Living (FARS-ADL) has become widely used as a clinical indicator to address these issues.

To establish a disease-specific prospective natural history using FARS-ADL in ataxia patients registered in J-CAT.

As of May 2026, 3846 ataxia patients have participated in J-CAT using a web-based registry system. The enrollment criteria of this study were as follows: 1) patients registered in J-CAT from April 2019 to March 2025, 2) a confirmed genetic diagnosis and 3) baseline SARA scores of below 16. Structured telephone interviews using FARS-ADL have been conducted semi-annually for up to 3.5 years.

Overall, 303 patients have participated in this study and 226 are currently continuing the semi-annual follow-ups. Detailed analysis of the 2.5-year follow-up in 128 patients consisting of 48 with SCA31, 33 with SCA6, 31 with MJD/SCA3 and 16 with other disease types revealed that the overall FARS-ADL scores at baseline and at 2.5-year timepoint were 10.9 ± 5.9 and 15.1 ± 4.5 . The average annual changes in SCA31, SCA6 and MJD/SCA3 were 1.36, 1.4 and 2.2, respectively. Linear mixed model analyses revealed distinct disease-specific progression trajectories, demonstrating that the progression rate of MJD/SCA3 was higher than that of SCA31 or SCA6.

This study demonstrated that the structured telephone interview using FARS-ADL within the J-CAT framework may be a feasible method to establish a disease-specific prospective natural history of ataxias.

Funding Sources: This study was supported by a Health and Labor Sciences Research Grant from the Ministry of Health, Labor, and Welfare, Japan (grants JPMH20FC1041 and JPMH 23FC1010) and the Japan Agency for Medical Research and Development (grants JP21ek0109532 and 24ek0109734).

C88. A Large Multicentre Spanish Cohort Identifies SCA27B as the Most Common Genetic Cause of Cerebellar Ataxia and Defines a Distinct Clinical Phenotype

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Introduction: GAA–TTC expansions in FGF14(SCA27B) have emerged as a frequent cause of late-onset cerebellar ataxia (LOCA), although their pathogenic threshold and epidemiological impact in Spain remain incompletely defined.

Methods: We assessed the prevalence of SCA27B in a multicentre Spanish cohort of 430 patients with idiopathic late-onset cerebellar ataxia (ILOCA) from 21 hospitals without a previous genetic diagnosis. An additional 90 external SCA27B cases were included. Clinical, neurophysiological, radiological, and progression features were compared between SCA27B and ILOCA.

Results: Among 430 patients, 111 (25.8%) carried >250 GAA–TTC expansions, identifying SCA27B as one of the most frequent causes of ataxia in Spain. 16 patients had 200–249 repeats. 217 patients with >200 GAA–TTC expansions were compared with 283 ILOCA patients. SCA27B patients showed a later age at onset (63 vs. 55 years; $p<0.001$), a higher frequency of episodic manifestations (45.2% vs. 12.7%; $p<0.001$), and more frequent downbeat nystagmus (67.4% vs. 32.9%; $p<0.001$). SCA27B patients also exhibited less extracerebellar involvement, including spasticity and peripheral neuropathy ($p<0.01$), and less cerebellar and brainstem atrophy ($p<0.003$). The response to 4-aminopyridine was also a differential feature between SCA27B and ILOCA (72.5% vs 36%, $p=0.002$). Clinical progression did not differ between groups (SARA score/diseaseduration; $p=0.67$). Expansion size was not associated with age at onset, disease severity, or most clinical, radiological, and neurophysiological variables.

Conclusions: This study reports the largest comparison published between SCA27B ($n=217$) and ILOCA ($n=283$). SCA27B may represent the most frequent genetic cause of cerebellar ataxia in Spain and is associated with a distinctive phenotype characterized by episodic manifestations, downbeat nystagmus, and limited extracerebellar involvement, despite a rate of progression comparable to ILOCA.

Funding Sources: This project is supported by the Spanish Ministry of Science and Innovation - Instituto de Salud Carlos III PI23/01090

C89. Direct Comparison of the Brief Ataxia Rating Scale version 2 and the Scale for the Assessment and Rating of Ataxia in the Spinocerebellar Ataxias

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Background and Objectives: The five core features of cerebellar motor ataxia are gait ataxia, arm and leg dysmetria, dysarthria, and oculomotor abnormalities. The Brief Ataxia Rating Scale version 2 (BARS-2) and Scale for the Assessment and Rating of Ataxia (SARA) variably quantify these features, measuring disease severity and progression. We compared the psychometric properties and clinical utility of BARS-2 and SARA in the spinocerebellar ataxias (SCA).

Methods: We identified 638 patients in the CRC-SCA natural history study who completed both BARS-2 and SARA at the same annual visit > once. We compared floor and ceiling effects, internal consistency, and dimensionality; assessed agreement between BARS-2 and SARA and evaluated their correspondence to Friedreich's Ataxia Rating Scale functional staging (FARS-Func). We tested convergent validity with the Patient-Reported Outcome Measure of Ataxia physical (PROM-PHYS) and activities of daily living (PROM-ADL) and FARS-ADL subscales, and the Inventory of Non-Ataxia Signs (INAS) oculomotor scores. We assessed longitudinal responsiveness of the scales and their ability to predict disease progression.

Results: The psychometric properties of BARS-2 and SARA were comparable. There were no ceiling effects. Several SARA items exhibited strong floor effects (> 20% of scores at minimum). Both scales had high internal consistency ($\alpha \geq 0.90$, $\omega_t \geq 0.94$), although exploratory factor analysis revealed redundancies in SARA. Both scales corresponded to FARS-Func and correlated with PROM-PHYS, PROM-ADL, and FARS-ADL. BARS-2 oculomotor correlated strongly with a composite INAS oculomotor score. Both scales predicted longitudinal progression and captured clinically meaningful change ($\text{MSDR} \geq 0.40$).

Discussion and Conclusion: BARS-2 and SARA are valid measures of the cerebellar motor syndrome, reflecting disease severity, patient meaningfulness, and symptom progression. BARS-2 is faster, simpler to administer, captures all five core features of cerebellar motor ataxia, and is well-positioned as the standard for clinical evaluation, longitudinal monitoring, and outcome measurement in cerebellar ataxia.

Funding Sources: MINDlink Foundation, National Ataxia Foundation, Greak Oaks Foundation, Once Upon a Time Foundation

C90. Cognition and Patient-Reported Outcomes in RFC1-Disease: Cross-Sectional Results Across Disability Stages

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BACKGROUND: RFC1-disease is a progressive, late-onset neurological disorder classically defined by cerebellar ataxia, sensory neuropathy, and vestibular areflexia (CANVAS). Cognitive and affective dysregulation consistent with Cerebellar Cognitive Affective Syndrome (CCAS) is increasingly recognised, but variation across the disease course remains unclear. We investigated cognitive and neuropsychiatric features of RFC1-disease across disability stages.

METHODS: Fifty-four people with RFC1-disease and 57 age-, sex-, and education-matched healthy controls completed the CCAS scale, PROM-Ataxia scale, NeuroQoL health-related quality-of-life scales, and digital finger tapping and reaction-time tasks. A subset of 31 RFC1 participants and 29 controls also completed an in-person neuropsychological battery. RFC1-control differences and Spearman correlations with disability stage (FARS staging) were assessed. Outcomes were residualised for age, sex, and education using normative regression. Inference was Bonferroni-corrected.

RESULTS: CCAS-S fail scores were higher in RFC1-disease than controls ($d=0.91$) and increased with disability severity ($r=0.54$). Definite CCAS prevalence rose from 33% in minimal disability to 60% in mild-moderate disability and 80% in moderate-severe disability. Compared with controls, individuals with RFC1 showed domain-specific deficits in motor speed and pacing, verbal fluency, cognitive flexibility, visuospatial processing, and processing speed/attention. Only finger tapping speed and processing speed/attention, measured by the Symbol Digit Modalities Test, correlated significantly with disability stage. Individuals with RFC1 reported more depressed mood, fatigue, and social-life dissatisfaction; only social-life dissatisfaction increased with disability. PROM-Ataxia physical, ADLs, and cognitive subscales, but not the neuropsychiatric subscale, correlated with disability stage.

DISCUSSION: Cognitive and affective deficits in RFC1-disease are consistent with CCAS, with cognitive deficits becoming more prevalent as disability advances. The composite CCAS scale appears more sensitive to disease-stage variability than domain-specific cognitive tests, possibly reflecting aggregate subscale effects or heterogeneous progression pathways across individuals. Affective symptoms did not clearly worsen with disability. Self-reported PROM-Ataxia physical and ADL subscales are highly convergent with disability staging. Funding Sources: Australian National Health and Medical Research Council

C91. Factor analysis validates internal structure of the Cerebellar Neuropsychiatric Rating Scale Version 2 and five domains of cerebellar neuropsychiatry

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Background and objective: The Cerebellar Neuropsychiatric Rating Scale Version 2 (CNRS-2) measures affective symptoms across the domains of attentional control, emotional control, autism spectrum, psychosis spectrum, and social skill set, each with overshoot and undershoot subdomains. Using a data-driven approach we explored the latent structure of the CNRS-2 to test our a priori five-domain and overshoot-undershoot model. **Methods:** CNRS-2 was administered to 279 cerebellar subjects. Data suitability for factor analysis was assessed. Eigenvalues and parallel analysis scree plots were evaluated for factor extraction. Exploratory factor analysis (EFA) was performed to identify an optimal factor solution. The EFA-derived solution and our a priori model were examined via confirmatory factor analysis (CFA). Fit indices, item loadings, and conceptual interpretability were compared. Bifactor analysis and CFA were performed on each of the five domains of the a priori model to evaluate whether variance in domain scoring was attributable to the construct measured by the domain versus two subfactors, and to test validity of the overshoot-undershoot item groupings.

Results: We extracted 5, 6, and 7 factors. EFA indicated that a six-factor solution best balanced statistical fit and theoretical interpretability. CFA on this EFA-derived solution and our a priori model showed both have acceptable statistical fit and internal consistency, but the a priori model demonstrated stronger conceptual coherence. Bifactor analysis revealed variation in interpretability of two subfactors within each domain, while CFA supported the overshoot-undershoot grouping of symptoms.

Discussion and Conclusion: Factor analysis of the CNRS-2 provided empirical support for the clinically derived conceptual framework that defines five neuropsychiatric domains. Sub-analyses of individual domains supported the overshoot-undershoot dichotomy. Convergence between our a priori domain model and EFA-derived solutions revealed stable and interpretable symptom clusters. These findings underscore the presence of coherent, multidimensional interrelated neuropsychiatric constructs in cerebellar disease that can be identified and measured by CNRS-2.

Funding Sources: MINDlink Foundation, National Ataxia Foundation, Greak Oaks Foundation, Once Upon a Time Foundation.

C92. International Survey on Epidemiology and Prescription Practices in Genetic Ataxias

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Background and Objective: Clinical care of genetic ataxia patients is limited by available technologies and a lack of understanding of epidemiology and prescription practices. This study aimed to provide a current epidemiological landscape for several common genetic ataxias and how those patients are cared for by international providers. The results will help establish best clinical practices and define potential treatment populations for future clinical trials.

Methods: An international survey was distributed through the National Ataxia Foundation, the Ataxia Global Initiative, and the Movement Disorders Society Ataxia Study Group. Responses were collected anonymously between June 2024 and December 2025. Survey participants were asked to identify country of clinical practice, clinical specialty, number of ataxia patients by specific genotype, and prescription practices for various symptoms. Ataxia genotypes included SCAs 1, 2, 3, 6, 7, 8, 10, 12, 27B, RFC1/CANVAS, Friedreich's Ataxia (FA), Fragile X Tremor Ataxia Syndrome, Ataxia-Telangiectasia, and Episodic Ataxia 1/2.

Results: 193 survey responses were obtained from 49 countries, mainly from the United States, Argentina, Brazil, Thailand, and the United Kingdom. The largest patient cohorts were reported from Brazil, followed by the United States, and the Netherlands. The most commonly seen ataxia was FA, followed by SCA3. Aminopyridine and riluzole derivatives were felt to be most helpful for ataxia along with anti-spasmodics, while GABA-ergic medications were cited as additionally helpful for nystagmus. Non-traditional therapies were infrequently used.

Discussion and Conclusion: Genetic ataxia patients are seen across the globe, with focused clusters that are often genotype-specific. Despite no formally approved medications, there was general consensus amongst providers with regards to the most effective medications for select clinical symptoms. While incomplete as a true global database, these results provide a template for the epidemiological landscape of genetic ataxias and offer insights into clinical practices.

C93. Predicted time to events related to loss of ambulation in omaveloxolone-treated patients with Friedreich ataxia

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Background and objectives: Friedreich ataxia (FA) is a progressive neurodegenerative disease characterized by impaired balance, gait dysfunction, and eventual loss of ambulation (LoA). Omaveloxolone, an Nrf2 activator evaluated in the MOXIe trial and its open-label extension (NCT02255435, EudraCT2015-002762-23), showed benefit in neurological function versus untreated patients in natural history up to 5 years. We examined the potential impact of omaveloxolone treatment (up to 5 years) on time to disability milestones leading up to and including LoA.

Methods: In ambulatory patients randomized to omaveloxolone in MOXIe Part 2, LoA and loss of specific functions related to stance and gait measured by the Friedreich Ataxia Rating Scale (FARS) were predicted using time-to-event methods from the time of FA onset. Loss of a specific function related to stance or gait was defined as first attainment of the maximum score for a particular item in the FARS functional disease staging, with LoA defined as attainment of a score ≥ 5 on item E7(gait).

Results: In omaveloxolone-treated patients included in this analysis (n=40), median (IQR) age at onset was 15.0 (12.0-18.5) years, including patients from 2 different age-at-onset groups (<15 and 15-24 years), and median disease duration was ≈ 7.5 years at MOXIe Part 2 baseline. The median (95% CI) time to LoA was 17.2 (13.3-19.0) years. The median (95% CI) times to loss of function of key stance items leading to LoA were 9.0 (7.0-11.0) years for feet apart, eyes closed; 9.5 (8.0-11.0) years for feet together, eyes open; and 14.7 (11.0-18.0) years for feet apart, eyes open.

Discussion and conclusion: The estimated median time to LoA in the selected omaveloxolone-treated cohort was 17.2 years from age at onset. These data help inform the potential impact of omaveloxolone on time to loss of clinically relevant functions, such as the ability to stand, leading to LoA.

Funding Sources: Biogen

C94. Natural history of RFC1 CANVAS highlights the need for integrated outcome measures of cerebellar and neuropathic progression

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Background and Objectives: Cerebellar ataxia, neuropathy, vestibular areflexia syndrome (CANVAS) is a late-onset neurodegenerative disorder involving sensory neurons, the vestibular system, and the cerebellum. Multi-domain, disease-specific scales are currently unavailable, limiting natural history studies and future clinical trial design.

Methods: We prospectively collected clinical data from 119 genetically confirmed CANVAS patients recruited from neuropathy and ataxia clinics in the UK and US. The Scale for the Assessment and Rating of Ataxia (SARA) was collected across all centres. Additionally, modified Friedreich's Ataxia Rating Scale section E (mFARS-E) and the Timed 25-Foot Walk (T25-FW) were used in ataxia clinics, while the Charcot-Marie-Tooth Examination Score (CMTES) and Inflammatory Neuropathy Cause and Treatment (INCAT) Sensory Sum score (ISS) were used in neuropathy clinics. One-year longitudinal follow-up was available in 34 patients.

Results: Mean age at onset and enrolment were lower in the neuropathy cohort than in the ataxia cohort (51.2±9 vs 55.7±11 years, $p=0.02$). SARA scores were significantly higher in patients recruited through ataxia clinics (11.6±5.4 vs 6.1±5.2, $p<0.0001$). SARA and CMTES correlated with age at examination ($r^2=0.16$, $p<0.0001$ and $r^2=0.25$, $p=0.0008$, respectively), while ISS and CMTES correlated with disease duration ($r^2=0.22$, $p=0.008$; $r^2=0.13$, $p=0.02$, respectively). Longitudinally, T25-FW significantly worsened over 12 months (8.5 vs 10.3 seconds, $p=0.04$), while SARA and CMTES showed no significant change. Most scale items demonstrated floor or ceiling effects; among the remaining items, only gait and stance (SARA), upper motor symptoms (CMTES), and upper limb vibration sense (ISS) correlated with age at examination and disease duration.

Discussion and Conclusion: Our findings suggest that currently available clinical scales incompletely capture disease severity and progression in CANVAS. Future disease-specific outcome measures should better capture the clinical trajectory of CANVAS, from early sensory dysfunction to later progression of imbalance, gait impairment, and hand dexterity decline of mixed peripheral and central origin.

Funding Sources: National Ataxia Foundation (NAF). Guarantors of Brain Postdoctoral Fellowship

C95. Proactive Approaches to Health (PATH) for Asymptomatic Adults at Risk for Neurodegenerative Conditions

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Introduction: As genetic testing advances, more asymptomatic adults at risk for hereditary adult-onset neurodegenerative diseases pursue predictive genetic testing. However, there is little guidance surrounding what support and follow-up these asymptomatic individuals have access to after they receive a positive genetic test result. Further, there is little known about what resources this population is currently accessing.

Methods: This was a quantitative, survey study that was available to participants between May and October 2025. Eligible participants were recruited through support group and national advocacy organization listservs and website postings. Statistics were calculated to describe this population's characteristics and resource utilization patterns, with chi-squared analyses used to measure the significance of any differences.

Results: One hundred ninety-two asymptomatic at-risk adults participated; 37 with ataxia (ATX) in their family, 42 with Huntington disease (HD), and 113 with ALS-FTD. Neurologists and genetic counselors were the most frequently used resources. People at risk for hereditary ataxia utilized genetic counselors, mental health providers and social workers significantly less than the other two disease groups and were less likely to participate in support groups ($p=0.003$). Research was the most common activity across all 3 disease groups with the ALS-FTD group participating significantly more than the other two disease groups ($p<0.001$). Participants identified furthering science, participating in research, and receiving additional education about the condition in their family as important needs that were met through the resources and activities they utilized.

Conclusions: At-risk ataxia subjects engage information resources differently than in HD or FTD-ALS and overall appear to be less engaged with information resources than for the other two diseases. Understanding these differences provides possible avenues for improved education on the issues they face. Further nuance into what leads to satisfaction and what needs remain unfulfilled could be explored through additional qualitative studies.

C97. Findings from a longitudinal follow-up study: NAD⁺ precursor supplementation and exercise training to increase aerobic capacity in Friedreich's Ataxia

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Background: There is a knowledge gap regarding the best ways to increase and manage exercise tolerance in Friedreich's Ataxia (FRDA). Our investigative team conducted a 3-month mechanistic clinical trial, ("NAD⁺ and Exercise in FRDA Study" (ExRx, NCT04192136, PMID: 42009009), testing an in-home, combination (aerobic + strength), exercise regimen. We demonstrated excellent adherence to supervised exercise and corresponding fitness benefits in our participants. Previous studies (e.g., PMID: 40946705) have found that continued adherence predicts persistence of gains from supervised exercise in individuals with ataxia.

Objective: The primary objective of this study was to measure the proportion of individuals who continued to participate in intentional exercise (aerobic, resistance, or combination) at each time point (every three months) for the 12 months post completion of the main trial (ExRx).

Methods: Participants who completed the final study visit for the main trial (ExRx) (individuals aged 10-40 years with genetically confirmed FRDA, who were able to safely exercise) were eligible to enroll in the extension study (n=62). Check-in calls were conducted every three months (at 3, 6, 9 and 12 months) for 1 year following completion of the main trial. Participants provided responses regarding their current exercise routine, and completed questionnaires related to physical activity levels, fatigue and quality of life.

Results: A preliminary review of data shows that most participants (80%) reported exercising (aerobic and/or resistance) during the 12M check-in. 24% (aerobic) and 14% (strength) of 12M check-in calls indicated the exercises were those prescribed during the main trial. 73% and 65% of check-ins across all time points reported aerobic and strength exercise, respectively.

Discussion: Developing and sustaining an exercise regimen is an important goal for individuals with FA. Ongoing analyses for this ancillary study will compare reported exercise, and look at questionnaire responses and their relationships with the amount of exercise reported.

Funding Sources: The Friedreich's Ataxia Research Alliance (FARA) provided funding for this study. The funding source had no involvement in study design, or interpretation of results.

C98. Experiences and perceptions of patients with Friedreich ataxia treated with omaveloxolone (Skyclarys) in France

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Background and Objectives: Omaveloxolone (Skyclarys®) is available in France for FA patients under an Early Access Program (EAP) granted by the French National Health Authority since November 2023.

The French Friedreich Ataxia Patients Association (AFAF) collected real-life experiences and perceptions from French patients treated with omaveloxolone (omav) for more than 1 year to support the application for the renewal of the EAP.

Methods: FA patients anonymously agreed to answer a questionnaire about their experiences and perceptions regarding balance, coordination, speech, fatigue and their daily life. Responses were collected from June to July 2025.

Results: From a total of 142 FA patients who answered the survey, 119 were on treatment, representing 27% of patients receiving omav in France. Both ambulatory (n=50) and non-ambulatory (n=67) patients noticed an improvement in speech (44% and 67% respectively), balance (29% and 32%), coordination (33% and 36%) and other motor aspects. 78% of those who experienced an improved speech and 82% of those who reported an improved balance, noticed the improvement within the first 6 months of treatment. All declared a decrease in fatigability (35% and 28% respectively). Moreover, considering all aspects, 42%-75% of ambulatory and 23%-74% of non-ambulatory patients reported neither improvement nor degradation. Side effects (headache, digestive problems, nausea, fatigue, etc.) were mostly reported during the first 3 months of treatment. Overall, 56% of patients said that the treatment improves their quality of life, and 30% expressed satisfaction with Skyclarys.

Discussion and Conclusion: These data revealed expected neurological benefits in terms of coordination and balance, but also improvements in major symptoms affecting quality of life, such as reduced fatigue and improved speech. After more than a year of treatment, perceptions described as "neither improvement nor degradation" of the disease, could be interpreted as a sense of stability, with the disease remaining unchanged.

Funding Sources: This work was funded by AFAF

C100. Detection and quantification of downbeat nystagmus across naturalistic and traditional oculomotor tasks

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Assessment of eye movements in ataxias typically relies on in-person clinical examinations with limited granularity to detect subtle oculomotor abnormalities, grade severity, or treatment-related changes. Here, we evaluate whether quantitative analysis of vertical and horizontal saccades enables detection of downbeat nystagmus (DBN) in ataxias and hypothesize that DBN could be quantified across a range of oculomotor tasks through saccade count direction ratios. Data were collected from participants enrolled in the Neurobooth study. Participants carried diagnoses of SCA27B (max n sessions=47), SCA3(67), SCA6(36), CANVAS (25), FA (25), or control (186). Eye movements were recorded binocularly at 1000Hz using an EyeLink Portable Duo. Artifacts due to blinks, poor calibration, and other noise sources were removed.

Data from the less noisy eye were analyzed. Participants completed gaze-holding, horizontal pursuit, horizontal saccade, and passage-reading tasks. Vertical saccades were detected and labeled (upward/downward). Log-transformed ratios of saccade counts were computed: vertical:horizontal (vh_ratio), down:up (du_ratio), and down:horizontal (dh_ratio).

Comparing DBN-associated ataxias (SCA27B, SCA6, CANVAS) to non-DBN ataxias (FA, SCA3) revealed significant differences across 26/28 task-measurement relationships. Large effect sizes were observed across all tasks (largest in passage-reading, $g=1.69$ and pursuit, $g=1.77$). Log-transformed ratios provided the strongest discrimination between DBN-associated groups (dh_ratio best in gaze-holding, vh_ratio in passage-reading, du_ratio in pursuit and horizontal saccade tasks). In the subset of individuals with detailed clinical oculomotor evaluation, individuals with rest and gaze-evoked DBN (max n=15) had significant differences across all ratio-measured tasks compared to controls.

Saccade count direction ratios enable quantification of DBN across both naturalistic and traditional tasks. Next steps in analysis include correlating DBN metrics with the patient-reported Visual Activities Questionnaire and evaluating changes in DBN severity over time. Quantitative detection and grading of DBN severity across different tasks offers potential to more sensitively detect and assess DBN, a key symptom common in inherited ataxias.

Funding Sources: Massachusetts Life Sciences Center, U.S. Department of Defense, NIH R01NS117826, Biogen, Broad Institute, Duke Family Foundation

C101. Diagnostic Delay in Genetic Ataxia: A Single-Center Retrospective Cohort Study

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Background and Objectives: Adult onset genetic ataxias are a group of heterogeneous disorders that present with variable clinical manifestations, ages of onset, and progression. Limited data exists about diagnostic timelines and presenting clinical features that could result in delayed diagnosis. Here we describe our cohort with a long-term aim of assessing diagnostic delays and presenting clinical characteristics in patients with genetic ataxia.

Methods: We performed a retrospective cohort study of a pilot group of patients with a diagnosis of ataxia enrolled in the Virginia Commonwealth University (VCU) Parkinson's Disease and Movement Disorders clinical registry. Variables collected included age at symptom onset, age at genetic diagnosis, family history, presenting symptoms, neurologic examination findings, and number of providers evaluated prior to diagnosis. Descriptive statistics were used to characterize the clinical features of this pilot cohort.

Results: 40 adults with a diagnosis of ataxia are enrolled in clinical registry. Of these, 26 underwent genetic testing with 12 receiving a definitive genetic diagnosis on testing. Time to diagnosis was calculated in 12 patients with complete clinical data and ranged from 0.2 to 28.4 years. The most common presenting symptom was imbalance/gait difficulty. Family history was absent in 50% of genetically confirmed cases, all of which were autosomal dominant disorders. More than 50% saw multiple providers, including other neurologists, prior to referral to VCU or receipt of a definitive diagnosis.

Discussion and Conclusion: Patients with genetic ataxia may experience substantial variability in time to diagnosis. Clinical heterogeneity and absence of family history contribute to delayed recognition of hereditary disease. Larger studies are needed to better characterize nuanced factors associated with diagnostic delay in adult-onset genetic ataxias.

C102. Access to symptomatic treatments in ataxia: a patient-reported comparison across the UK, Germany and Italy

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Background and objectives: Symptomatic management of non-motor and motor features—including spasticity, tremor, cramps, dystonia and bladder dysfunction—is a key determinant of clinical outcomes and quality of life in ataxia. Previous analyses from our European survey have highlighted variability in care pathways and access to specialist ataxia centres (SACs). This study aimed to assess patient-reported outcomes related to symptom burden and management, and access to symptomatic treatments, in the UK, Germany and Italy.

Methods: A cross-sectional survey collected patient-reported data on symptom burden, treatments received, referral pathways and satisfaction with care across three countries (UK n=273; Germany n=101; Italy n=174). Analyses were descriptive and comparative.

Results: Across all countries, patients reported a high burden of clinically relevant symptoms: stiffness, cramps, tremor, dystonia and bladder dysfunction. Management strategies were broadly similar between countries and predominantly reported as beneficial, and included physiotherapy, exercise and pharmacological treatments. Despite this, a notable proportion of patients reported suboptimal management: in the UK, 25–35% did not receive treatment for specific symptoms, with comparable gaps seen in Germany and Italy. Variability in access to treatment was observed, with some patients not accessing specialist services and differences in availability of multidisciplinary interventions such as occupational therapy and speech and language therapy.

Discussion and Conclusion: These findings highlight variability in patient-reported clinical outcomes related to symptom control in ataxia across three European settings. Despite similar treatment approaches, gaps in access and delivery of symptomatic therapies persist. Improving access to multidisciplinary interventions and supporting more consistent implementation of symptom-focused management strategies may be important to achieve better clinical outcomes for people living with ataxia. This study was part of the Value of the Treatment project, with the aim to converge data evidence to policy recommendations on how to improve treatment and care for people with rare neurological diseases across Europe.

Funding Sources: This study was funded by the European Brain Council (grant code: 551578), Takeda Pharmaceutical Company Ltd and Reata Pharmaceuticals (grant code: NA), then Biogen (grant code: 585006).

C103. Presymptomatic Diagnoses of Friedreich Ataxia from Whole Genome Sequencing (WGS)

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Background and objectives: Diagnosis of FRDA is made typically symptomatically via molecular testing of the FXN gene, while whole genome sequencing can detect the rarer point mutations in remaining cases. Here we review 4 individuals diagnosed incidentally with Friedreich ataxia by WGS.

Methods: This is a retrospective case series of 4 patients with genetically confirmed FRDA by WGS presenting with non-ataxic neurologic symptoms.

Results. Case 1 involves a male subject diagnosed incidentally with FRDA at age 2.5 years following WGS for developmental delay (nonverbal at age 3). This subject had consanguineous parents. Resulting GAA repeats were 180 and 110 indicating a likely symptom onset well into adulthood. Concurrent diagnoses include a homozygous DOCK3 mutation (accounting for all symptomatology) and a heterozygous multi-gene deletion in the 22q11.22q11.23 region. The patients entered the dose-finding study of omaveloxolone in children.

Case 2 was the younger full sibling of the subject described in Case 1 above. He had no symptoms and GAA repeats of 89/>100. He did not have a DOCK3 mutation or CH 22 deletion.

Case 3 describes a highly autistic man who underwent WGS at age 19. No cause of autism was found. GAA repeats were 89/<100, the consistent with mid to late adult onset of FRDA. Although of age to receive omaveloxolone, extreme anxiety with phlebotomy prevents its consideration.

Case 4 involves a 17-month-old male with mild, transient developmental delays. Physical exam was unremarkable, and nerve conduction studies showed normal SNAP and CMAP. WGS identified repeats of 90/596, consistent with likely age of symptom onset of FRDA in later adulthood.

Discussion: In these cases, WGS identified individuals long before presentation, creating the possibility of early therapy. However, in these individuals, age or other symptomatology prevented use of omaveloxolone, showing that treatment of presymptomatic individuals is complicated.

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C104. US medical claims analysis of patients with Friedreich ataxia prescribed omaveloxolone: incidence of new-onset clinical progression events

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Background and objectives: Friedreich ataxia (FA) is a rare, genetic, multisystem, neurodegenerative disorder marked by ataxia and systemic manifestations with variable onset. Real-world data on clinical event onset and progression are limited. Omaveloxolone, an Nrf2activator, is approved for patients aged ≥ 16 years with FA following the MOXIe study and open-label extension (OLE) (NCT02255435, EudraCT2015-002762-23). This study quantified the rate of disease progression using US claims data for omaveloxolone-treated vs untreated patients, based on incidence of new-onset FA progression events.

Methods: This retrospective analysis used multipayer, deidentified claims from Komodo Health (October 2015 to April 2026). Eligible patients had ≥ 2 FA claims ≥ 30 days apart, ≥ 1 inpatient FA claim, or ≥ 1 outpatient FA claim and ≥ 1 early-onset cerebellar ataxia claim ≥ 30 days apart; and ≥ 1 year history with continuous enrollment prior to first FA-related claim. FA progression was measured by cumulative first-occurrence events across bulbar or breathing, mobility, and cardiometabolic domains. Incidence of new-onset FA progression events was estimated using negative binomial regression with inverse probability of treatment weighting to balance baseline differences between cohorts and evaluate the average treatment effect, adjusting for covariates.

Results: A total of 537 omaveloxolone-treated and 1904 untreated patients were included. Cohorts were well matched after weighting. Overall, omaveloxolone-treated patients had an incidence of 0.947 events/patient-year vs 1.354 in untreated patients (30.0% reduction [95% CI, 26.7%-33.1%]). Domain-specific reductions were: bulbar/breathing (50.8% [48.0%-53.4%]), cardiometabolic (37.3% [33.8%-40.6%]), and mobility (25.2% [21.7%-28.7%]). All were statistically significant.

Discussion and conclusion: Claims data may not fully capture all clinical events or follow-up due to differences in coding practices and insurance coverage. Despite limitations, omaveloxolone was associated with significant reductions in clinically relevant new-onset FA progression events vs no treatment in real-world US medical claims data. Building on MOXIe study/OLE efficacy findings, this postmarketing experience supports the real-world effectiveness of omaveloxolone.

Funding Sources: Biogen, Inc.

C105. Diagnosis of Genetic Ataxias: a 3-Year Experience from a New Outpatient Neurogenetics Clinic

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Background & Objective: As genetic testing continues to rapidly evolve, establishing an accurate genetic diagnosis in ataxia has become increasingly available, effective, and important, yet complex. Genetic testing panels may not cover the full range of known genetic etiologies. Next generation genetic testing requires specific consent processes and possible coordination of family member sample collection. Any testing could yield uncertain results requiring further investigation. At the same time, requests for predictive testing from non-symptomatic individuals are growing. This study characterizes the demographic and clinical profiles of individuals referred for evaluation of a possible genetic etiology of ataxia.

Methods: Study cohort included referrals during the 3-year period from July 2023 to June 2026 for pre-test and post-test genetic counseling for ataxia. Visits for predictive testing for at-risk individuals and result interpretation from previous genetic testing were also included.

Results: Preliminary review of cases found seventy individuals with a clinical diagnosis or family history of ataxia. Those seeking a genetic etiology for a clinical diagnosis of ataxia represented approximately 70% of the study cohort. Spinocerebellar ataxias were the most common genetic diagnoses in this cohort. Approximately 61% of those with positive genetic test results reported a family history of ataxia symptoms. Of the 38 positive genetic tests, there were six cases of SCA27B with at least one case being diagnosed from updated genetic testing.

Discussion and Conclusion: Similar to previously published single-clinic experiences, this review of cases from a newly established outpatient neurogenetics clinic found that interdisciplinary evaluation and genetic testing allowed for diagnosis of genetic ataxias in the majority of patients. Reported family history and established clinical diagnosis of ataxia increased diagnostic yield; however, many patients with autosomal dominant etiologies presented without family history. Diagnosis required comprehensive panel testing, including single nucleotide variants and repeat expansion analysis, or whole genome sequencing.

C106. The cup that shatters balance: caffeine-induced paroxysmal neurological signs in patients affected by spinocerebellar ataxia type 27B (SCA27B)

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Background and Objectives. Spinocerebellar ataxia type 27B (SCA27B) is caused by an intronic GAA repeat expansion in FGF14, with cerebellar ataxia and paroxysmal neurological episodes. Although alcohol is a well-recognized trigger, the contribution of other environmental factors remains incompletely characterized. We investigated the role of caffeine as a potential precipitating factor and report a severe phenotype associated with biallelic FGF14/GAA repeat expansions.

Methods. We report a woman carrying biallelic FGF14/GAA repeat expansions who developed marked caffeine sensitivity. We assessed aggravating factors in SCA27B patients enrolled in the SPATAX/BIOMOV cohort at the Paris Brain Institute, France. GAA repeat expansion sizes were compared between patients with and without caffeine-related symptoms worsening (Mann–Whitney U test).

Results. The patient carried two FGF14/GAA repeat expansions (473/510) and developed daily paroxysmal episodes (dysarthria and oromandibular dystonia) at age 27, triggered by alcohol, sleep deprivation, and physical exertion. Caffeine subsequently emerged as a potent trigger of severe attacks. Progressive ataxia resulted in wheelchair dependence by age 40. Her brother, carrying biallelic FGF14/GAA expansions (534/483), developed an ataxic–spastic syndrome at age 28, whereas both heterozygous parents exhibited a milder phenotype with later disease onset. Among 82 patients in the SPATAX/BIOMOV cohort, caffeine-induced symptoms worsening was reported by 34 (41.5%), making caffeine the second most common trigger after alcohol (54/82, 65.9%). Caffeine sensitivity was not associated with GAA repeat expansion size ($p=0.21$).

Discussion and Conclusions. Our findings expand the phenotypic spectrum of SCA27B and highlight caffeine as a frequent trigger of paroxysmal symptoms. The severe manifestations observed in individuals with biallelic FGF14/GAA expansions further support a gene-dosage effect. Impaired sodium channel regulation resulting from FGF14 deficiency may increase susceptibility to caffeine-triggered episodes, possibly mediated by enhanced glutamatergic and NMDA receptor activity. Routine assessment of caffeine intake may identify a modifiable trigger whose avoidance could reduce symptom burden.

C107. AtaxiCare: a novel digital platform for objective assessment of upper-limb motor symptoms in ataxia

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Background & Objectives. Ataxia rating scales such as the Scale for the Assessment and Rating of Ataxia (SARA) are ordinal and rater-dependent. We aim to develop portable infrastructure for quantifying upper-limb ataxia using continuous metrics that overcome the limitations of discrete rating scales.

Methods. We developed AtaxiCare, a digital ataxia assessment platform that uses a touchscreen tablet and wristworn inertial measurement units (IMUs) to capture upper-limb movement. We analyzed 17 upper limbs from 8 individuals, including 8 limbs with SARA upper-limb subscore of 0 and 9 limbs with subscore ≥ 1 . Participants completed two upper-limb motor tasks translated into touchscreen-based modules: "Automated Finger Chase" (AFC) and "Static Tapping" (ST), with concurrent IMU recording. In AFC, participants tracked a moving on-screen target with the index finger; we computed Mean Dimensionless Jerk (MDJ) and Maximum Deviation from Path (MDP) from the finger trajectory. In ST, participants repeatedly tapped a static target; we quantified speed and regularity using mean Inter-Tap Interval (ITI) and its standard deviation (ITI-SD).

Results. AFC-derived MDJ and MDP distinguished upper limbs with SARA subscore of 0 vs ≥ 1 (MDJ: $t=-2.55$, $p=0.026$; MDP: $t=-2.37$, $p=0.033$). ST-derived mean ITI and ITI-SD similarly separated zero vs non-zero scorers (ITI: $t=-3.11$, $p=0.010$; ITI-SD: $t=-2.32$, $p=0.049$). Mean ITI correlated with MDJ ($r=0.70$, $p=0.0066$) and MDP ($r=0.64$, $p=0.017$), and ITI-SD correlated with MDP ($r=0.59$, $p=0.031$), supporting between-task validity.

Discussion. These findings demonstrate the feasibility of quantifying upper-limb ataxia using tablet-based assessments and continuous performance metrics. The observed relationships between AFC and ST measures suggest that AtaxiCare captures shared features of upper-limb motor dysfunction across distinct task paradigms.

Conclusions. AtaxiCare-derived metrics distinguish between upper limbs with normal and abnormal SARA scores and may complement conventional clinical rating scales. Future work will evaluate sensitivity across a broader range of ataxia severity and responsiveness to longitudinal at-home symptom fluctuations.

Funding Sources: Raynor Cerebellum Project, Next Gen Researchers Award

C108. Acquired Strabismus in Adult-Onset Cerebellar Ataxia: Prevalence, Patterns, and Diagnostic Implications

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Background and Objectives: Strabismus is common in patients with cerebellar disease but remains incompletely characterized in adult-onset cerebellar ataxia (AOCA). We aimed to determine the prevalence and patterns of strabismus across AOCA subtypes and evaluate whether combinations of ocular motor findings provide clinically useful clues to specific etiologies, particularly spinocerebellar ataxia type 27B (SCA27B).

Methods: We performed a retrospective multicenter cross-sectional study of 211 adults with AOCA. Participants included 87 patients with idiopathic late-onset cerebellar ataxia (ILOCA), 75 with genetically confirmed SCA27B, and 49 with other genetically confirmed SCAs.

Results: Strabismus was present in 67.8% (143/211) of patients and occurred with similar frequency across ILOCA, SCA27B, and other SCAs (64.4%, 73.3%, and 65.3%, respectively; $p=0.436$). Diplopia was strongly associated with strabismus ($OR=18.56$, 95% CI 8.80–39.13; $p<0.001$). Skew deviation was the most common strabismus subtype (38.3%), followed by divergence insufficiency (29.2%) and convergence insufficiency (20.6%). Downbeat nystagmus (DBN) occurred in approximately 70% of both ILOCA and SCA27B patients. The combination of DBN and skew deviation was significantly more frequent in SCA27B than ILOCA ($OR=2.22$, $p=0.022$). The triad of DBN, skew deviation, and divergence insufficiency further enriched for SCA27B ($OR=3.24$, $p=0.038$).

Discussion: In the largest AOCA strabismus cohort to date, nearly 70% of patients exhibited ocular misalignment, highlighting strabismus as a common but underrecognized manifestation of cerebellar degeneration across both genetic and idiopathic ataxias. While no individual ocular motor sign distinguished SCA27B from ILOCA, composite vestibulocerebellar profiles improved phenotypic recognition and may assist diagnostic stratification in clinical practice.

Conclusion: Strabismus is a highly prevalent and underrecognized feature of AOCA. A composite profile characterized by DBN, skew deviation, and divergence insufficiency is enriched in SCA27B and may provide a practical bedside clue to FGF14-related disease.

C109. Symptomatic effects in SCA27B as a paradigm to evaluate the responsiveness of digital motor markers

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Background: There is a need for applicable digital performance markers to monitor disease course in ataxias. Sensitivity to change of novel measures has been difficult to establish due to generally slow symptom progression. We here exemplify the setting of symptomatic treatment in SCA27B to comparatively evaluate the responsiveness of digital motor markers.

Methods: Motor features of gait and balance were captured by two established set-ups 1) wearable sensors (MobilityLab) and 2) RGB-Depth camera (PASS) according to published protocols. Both were applied in the same sessions in an individual with SCA27B on and off fampridine (>24h withdrawal). Selected parameters of both methods (7 MobilityLab and 9 PASS) were transformed into Z-scores based on published normative datasets to compare drug effects between parameters and methods.

Results: With fampridine, the patient experienced improvement with reductions in SARA (8 to 2), PROM-Ataxia total (-54) and 8m walk (-3.4 sec). For Mobility Lab, absolute Z-scores were lower with than without Fampridine (0.14-5.51 and 1.5-7.8 SD of norm, respectively) and highest for lateral step variability. Changes in absolute Z scores were 0.53-2.45 with changes >2SD of norm for gait measures ElevMidswing, LatStepVar and Circumduction. For PASS, absolute Z-scores ranged from 0.26-4.51 on fampridine and 0.27-17.36 without and were highest for sway speed in stance, step width in gait and sit-to-stance time. Changes ON to OFF expressed in SD of norm were 0.45-12.85 with >2SD seen for sit-to-stance time, trunk roll in comfortable gait, and most pronounced for sway speed in closed stance.

Conclusion: The symptomatic effect of fampridine in SCA27B provides an opportunity to comparatively evaluate the sensitivity to change in features of ataxia with novel digital performance outcomes within applicable timeframes. This helps to effectively establish a set of meaningful digital motor markers for ataxia symptoms and margins of important change for different technologies.

C110. Continuous at-home wearables and machine learning sensitively track progression in pediatric and adult Friedreich's Ataxia

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Background: Friedreich's Ataxia (FA) is the most common hereditary ataxia, characterized by progressive cerebellar and sensory ataxia affecting patients from early childhood through adulthood. Current clinical outcome measures are limited by rater subjectivity, coarse scoring, infrequent in-person assessments, and inability to assess behavior continuously in naturalistic settings. We test whether submovement-based measures derived from wrist and ankle accelerometers worn continuously at home can capture motor impairment and disease progression across a wide age and severity spectrum.

Methods: 95 participants (57 FA, 38 age-matched controls; age 2-75) wore GENEActiv wrist and ankle sensors for one week per visit; 60 completed two or more visits over up to 2.2 years. 85 movement features were extracted including submovement kinematics and activity intensity. The FA cohort included ambulatory and non-ambulatory participants spanning early, mid, and late stages of disease. Five machine-learned composite models were evaluated per sensor location, including FA-specific models, and models previously trained on datasets from other neurologic populations including ataxia-telangiectasia and ALS. Sensor measures were evaluated for reliability, relationship with clinician and patient-reported measures, and sensitivity to capture progression.

Results: Ankle sensor measures demonstrated strong cross-sectional correlations with FARS functional staging ($|r| = 0.66-0.87$), mFARS total ($|r| = 0.53-0.73$), FARS-ADL ($|r| = 0.58-0.74$), and FA Health Index (FA-HI) total ($|r| = 0.43-0.57$), with high within-week reliability ($ICC = 0.89-0.96$) and large differences between FA and control groups ($|e.s.| = 2.0-2.6$). FA-specific composite models showed significant longitudinal sensitivity to disease progression ($|MSDR| = 0.67-0.89$), and outperformed models trained on other disease populations.

Discussion: At-home ankle accelerometer data yields reliable, ecologically valid measures reflecting clinician-rated and patient-reported outcomes, and captures longitudinal disease progression in FA. These results support remote wearables as scalable, sensitive digital outcome measures for FA clinical trials and naturalistic disease monitoring.

Funding Sources: Sponsor / Funding, Ataxia Telangiectasia Children's Project (A-T Children's Project) Broad Institute, inc., Children's Hospital Corporation, Boston Children's Hospital, Cure DRPLA, NIH-National Institutes of Health, Trustees of the University of Pennsylvania.

C111. What is Ataxia? Using Digital Phenotyping to Educate and Illuminate How Cerebellar Disease Affects Behavior

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Background: Ataxia is characterized by progressive decline in coordination, balance, speech, and eye movements resulting from cerebellar dysfunction. Despite its large impact on an individual's daily life, ataxia remains a misunderstood condition to those around them, often leading others to make incorrect assumptions. The phenotypic changes that define the disease experience, including saccadic pursuit, dysarthria, and impaired coordination are challenging to convey through text alone. Improved disease understanding is central to patient engagement and shared decision making, yet accessible educational resources for rare conditions like ataxia remain limited. Digital phenotyping technologies now capture ataxia related behaviors with unprecedented precision, yet this data has not been broadly shared with the communities who would benefit most.

Methods: 'What is Ataxia?' (WIA) is a long term, iterative, public facing educational initiative structured as a virtual interactive museum. Using patient phenotyping data from the Neurobooth at Massachusetts General Hospital, WIA intends to make abstract neurological signs visible and interpretable. Each exhibit focuses on a specific behavior affected by ataxia and follows a consistent narrative: establishing real world context, demonstrating digital data capture, illustrating disruption by ataxia, and conveying the impact on daily life. Target audiences include patients and families, advocacy organizations, clinicians, drug developers and regulators, and the general public.

Results: Our first exhibit highlights smooth pursuit eye movements—a behavior universally experienced in everyday life and commonly disrupted in ataxias. Viewers are guided from the evolutionary and biological foundations of smooth pursuit to high resolution data visualizations from Neurobooth comparing healthy controls to individuals with ataxia, culminating in a reflection of how impaired pursuit affects daily experience, from tracking a moving ball to safely crossing a busy street.

Discussion: Future exhibits will emphasize speech, motor movements, and other ocular behaviors shaped by feedback from clinicians, researchers, and patient communities.

Funding Sources: Massachusetts Life Sciences Center, U.S. Department of Defense, NIH R01NS117826, Biogen, Broad Institute, Duke Family Foundation

C112. Multi-System Milestones and Care Gaps in Friedreich's Ataxia: Insights from a Routine Care-Based Real-World FA Disease Repository

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Background and Objectives: Friedreich's ataxia (FA) is a progressive multisystem disorder with substantial neurological, cardiac, and metabolic morbidity. This study uses a longitudinal, routine-care FA patient-data-repository to characterize cross-system clinical milestones, phenotypic subgroups, comorbidity burden, and care gaps beyond what exists in current registries and trials.

Methods: This retrospective database study used deidentified, longitudinal data from genetically-confirmed patients receiving routine care, curated from the Clinakos real-world FA repository. Data were derived from existing medical records, ~14.5 years of follow-up per patient, and abstracted into a structured dataset capturing ages at milestones across six domains (neurological, cardiovascular, musculoskeletal, metabolic, sensory, psychiatric), diagnostic timing, rehabilitation initiation, and healthcare utilization. Patients were assigned to phenotypic clusters (A–D) based on age at onset, time to loss of ambulation, cardiac involvement, and atypical genotype/clinical criteria. This analysis targets 20 deeply phenotyped patients (14 currently analyzed).

Results: The cohort (93% female; symptom onset 6-51 years) was distributed across all phenotypic clusters (A: 21%, B: 36%, C: 21%, D: 21%), reflecting the routine-care clinical spectrum. Diabetes mellitus was documented in 71% of patients, exceeding the 10-40% published prevalence. Vision impairment was documented in 71%, and depression/anxiety in 57%. Diagnostic delay averaged ~11 years in late-onset versus ~5 years in childhood/adolescent-onset disease. Mean delay to first PT/OT was 7.9 years from symptom onset. Multispecialty care was documented for 10 of 14 patients. Omaveloxolone was used by 13 of 14 patients, indicating high real-world uptake in routine care.

Discussion and Conclusion: This routine-care FA repository reveals high metabolic and psychiatric comorbidity burden, substantial diagnostic delays in late-onset disease, persistent rehabilitation underuse, and high real-world omaveloxolone uptake, features not fully characterized in existing FA registries and trials. The dataset's granular, multi-domain longitudinal structure provides a foundation for future natural history characterization, burden-of-illness modeling, and external comparator analyses in routine-care populations.

Funding Sources: This work is funded by Clinakos Inc.

C113. WEAR-FA: A Remote Wearable Sensor Study for Digital Motor Outcome Validation in Friedreich's Ataxia

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Background: Sensitive and scalable outcome measures are needed to support clinical trials in Friedreich's Ataxia (FA), a progressive neurodegenerative disease. In a related study presented at this conference, we demonstrate that at-home wrist and ankle accelerometer data captures motor impairment and disease progression in FA. These findings motivate extension of this assessment approach in a larger, multi-site natural history study that can more fully capture the breadth of the diverse FA population and enable additional comparisons between digital measures and in-person clinical assessments.

Methods: WEAR-FA is a prospective and longitudinal sub-study of FA Global Clinical Consortium's (GCC) UNIFAI natural history study. The study will enroll up to 120 participants across 3 distinct cohorts: non ambulatory adults (FDS $\geq$ 4.5, ages 18-40; n=40), pediatric (FDS $\geq$ 1, ages 8-17; n=50), and late onset (FDS $\geq$ 1, onset $\geq$ 25, ages 25-60; n=30). Wrist and ankle Syde sensor data will be collected during two-to three-week wear periods every three months over 24 months. Sensor-derived metrics implicating lower and upper limb function will be correlated with clinical measures (e.g., mFARS) and patient reported outcomes, compared between ataxia and control populations, and assessed for sensitivity to capture longitudinal change.

This remote study will anchor wearable sensor data to annual visits from the UNIFAI natural history study, with analysis conducted by teams at Massachusetts General Hospital and Sysnav.

Results: Preliminary data from an initial cohort of participants will be presented.

Discussion: WEAR-FA aims to provide analytically validated digital outcome measures across FA subpopulations that were under-represented in prior work, generating natural history data for future interventional trials.

Funding Sources: Friedreich's Ataxia Research Alliance (FARA), Biogen

C114. Speech in Children and Adolescents with Friedreich's Ataxia

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Background and objectives: In adults with Friedreich's ataxia (FA), speech intelligibility and naturalness are diminished, causing communication difficulties. However, little is known about how speech is affected in children and adolescents with FA. To address this gap, we analyzed speech samples of children and adolescents with FA to characterize symptom severity and relate speech to overall disease progression.

Methods: Participants included 43 FA patients and 28 controls from the TRACK-FA study who were stratified into two age bands: ages 5-12 years (n = 16 FA patients, n = 14 controls) and 13-19 years (n = 27 FA; n = 14 controls). Recordings of speech tasks were rated for intelligibility, naturalness, and overall voice quality by two speech-language pathologists. Acoustic measurements such as speech rate and pause percentage were calculated. Welch's t-tests assessed group differences within age bands. Multiple linear regressions were conducted for all FA patients to assess predictors of speech intelligibility and naturalness.

Results: Patients aged 13-19 had significantly reduced speech intelligibility ($t=-3.64$, $p<.001$), reduced naturalness ($t=-5.15$, $p<.001$), slower speech rates ($t=-4.72$, $p<.001$), and higher pause percentages ($t=2.29$, $p=.03$) during monologue than controls. No group differences were found for ages 5-12. Intelligibility was predicted by overall voice quality but not speech rate or mFARS, $F(3,39)=7.34$, $p<.001$. Speech naturalness was predicted by overall voice quality and speech rate, but not mFARS, $F(3,39) = 13.5$, $p<.001$.

Discussion and Conclusions: For adolescents with FA, speech intelligibility and naturalness were significantly affected. For children 12 and under, any changes in their speech may be subtler than what the current measures reveal. Across all patients, reduced vocal quality and slow speech rate predicted intelligibility and/or naturalness whereas mFARS scores were not predictive. Thus, for these patients changes in speech may have a different trajectory than changes in gross and fine motor function.

Funding Sources: TRACK-FA is funded by grants from the Friedreich's Ataxia Research Alliance (FARA, USA) to each of the academic sites and IXICO with financial support from Takeda Pharmaceuticals Company, Novartis Gene Therapies, IXICO, Larimar Therapeutics Inc., and PTC Therapeutics.

C115. Amplifying the Patient Voice: Impact of a Friedreich Ataxia Cardiac Advisory Council in Its First Year

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Background and Objectives: Friedreich ataxia (FA) is a rare, hereditary disease affecting approximately 15,000 people worldwide. While historically recognized as a neuromuscular disorder, FA is a multisystem disease with cardiac manifestations being the most common cause of premature death.

In 2025, Lexeo Therapeutics established an FA Cardiac Advisory Council comprising individuals living with FA cardiomyopathy (FA-CM) and caregivers. The Council was created to incorporate lived experience into the gene therapy development process and to serve as a long-term strategic partnership informing program direction. The Council aims to provide insights into unmet needs, improve understanding of patient and caregiver priorities, and foster collaboration to address gaps in FA cardiac care, including advancing multidisciplinary management and elevating the role of cardiology in disease management.

This analysis aimed to evaluate the Council's first-year impact, including the ongoing need for increased discussion of FA-CM in the community, barriers to FA-related cardiac care, and perceived contributions to Lexeo's FA program.

Methods: The FA Cardiac Advisory Council included individuals reflecting diverse disease stages and experiences. Over its first year, the Council participated in two virtual meetings, ad hoc consultations, and one in-person meeting. Members completed multiple surveys throughout the year, including a comprehensive post-meeting survey assessing their experiences over the preceding year.

Results: Respondents reported that participation in the Advisory Council was highly beneficial. Participation enhanced understanding of the FA drug development process and was reported to be impactful, with members perceiving meaningful contributions to Lexeo's FA program.

During the in-person meeting, Council members emphasized that cardiac involvement in FA remains underrecognized and identified future Council priorities including improved education, transparent communication, and continued engagement in research.

Conclusions: The Council demonstrated meaningful value and impact in its first year, supporting the role of patient advisory engagement in advancing patient-centered FA drug development.

Funding Sources: Funding for this work was provided by Lexeo Therapeutics

C116. Clinical Management of People with Hereditary Ataxia: A Single Center Review

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BACKGROUND AND OBJECTIVES: The most common hereditary ataxias are Friedreich's Ataxia (FA), spinocerebellar ataxia (SCA) 3, SCA2, and SCA6. Management approach includes physical (PT), occupational (OT), and speech-language therapies (SLP), and medications for symptom control. In the U.S., the only FDA-approved medication for ataxia is omaveloxolone for FA. Dalfampridine is used off-label for gait and nystagmus in SCA27b. Recently, trilorizole, a prodrug of riluzole, showed promise for SCA, but is not FDA-approved. We aim to evaluate management approach at our ataxia center.

METHODS: We conducted a retrospective chart review of patients with hereditary ataxia seen at our ataxia center between January 2024 and March 2026. We reviewed demographics, disease types, Scale for the Assessment and Rating of Ataxia (SARA) scores, medications, and use of PT, OT, and SLP.

RESULTS: Of 106 patients seen, 43.4% (n=43) had genetically confirmed ataxia. Mean age was 56 +/- 20 years, 44.2% (n=19) were female, and 88.4% were White (n=38). Most common diagnoses were SCA6(20.9%, n=9), SCA27b (20.9%, n=9), and FA (18.6%, n=8). Most participated in at least PT (n=40). Common medications used were: riluzole (22.2% SCA6), dalfampridine (88.9% SCA27b; 11.1% SCA6), omaveloxolone (62.5% FA). Median SARA score after medication initiation changed from 16.5 (IQR 16-24) to 15.5 (IQR 14.5-23.5) over 3-12 months on riluzole, 12.5 (IQR 8.4-13.9) to 10.3 (IQR 8.5-11.3) over 6-18 months on dalfampridine, and 31 (IQR 19-32) to 22.5 (IQR 17.5-32) over 6-8 months on omaveloxolone.

DISCUSSION AND CONCLUSION: The most common hereditary ataxias seen at our center are SCA6, SCA27b and FA, mirroring global prevalence. Most patients participated in PT, OT or SLP. People with SCA27b and FA were most commonly on medications for ataxia and demonstrated clinical improvement. Further multicenter studies to understand treatment approach and response in people with ataxia will inform providers and help standardize care.

C117. Expanded ATXN10 Alleles in Neurodegenerative Disorders: A Case Series and Review of the Literature

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Background and objectives. Spinocerebellar ataxia type 10 (SCA10) is typically attributed to large intronic ATTCT repeat expansions in ATXN10, yet interpretation is complicated by reduced penetrance, repeat interruptions, and assay limitations. We describe three patients referred for SCA10 evaluation in whom ATXN10 repeat-primed PCR (RP-PCR) reported “>32 repeats, probable pathogenic. Methods. Targeted long-read sequencing (LRS) was performed to size the ATXN10 expansion and analyze internal structure. We compared these cases with other reports in literature, describing clinical features, disease evolution, and diagnostic methods applied for genetic confirmation using traditional repeat expansion analysis, targeted long-read sequencing (LRS) and whole-genome sequencing (WGS). Results. Targeted long-read sequencing (LRS) identified two individuals with intermediate, mixed repeat tracts (~100–150 repeats) and one individual with a very large, highly pure ATTCT expansion (~1,127 repeats). Each phenotype was explained by an alternative disorder: spastic ataxia syndrome caused by biallelic pathogenic FA2H variants, full-penetrance ATXN3CAG expansion consistent with MJD/SCA3, and clinically established MSA-C. Literature Review: We identified three studies reporting four SCA10 cases coexisting with Huntington’s disease (one case) and SCA2 (three cases). Discussion and conclusion. Our three cases suggest that intermediate ATXN10 alleles in the 100–150 range may not be pathogenic and that even very large, pure expansions may be non-pathogenic. Comprehensive genetic testing beyond RP-PCR is necessary for accurate diagnosis.

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C118. CANVAS Deep Phenotyping Reveals Young Onset in This Highly Heterogeneous Condition

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Background and Objectives: Cerebellar Ataxia with Neuropathy and Bilateral Vestibular Areflexia Syndrome (CANVAS) is a progressive neurodegenerative disorder caused by biallelic RFC1AAGGG repeat expansions and characterised by marked phenotypic heterogeneity. This study aimed to characterise the clinical and genetic features of RFC1-spectrum disorder in a large specialist ataxia cohort.

Methods: We retrospectively analysed 136 individuals referred to specialist ataxia clinics in London and Girona between 2019 and 2025. Fifty-five patients carried biallelic pathogenic RFC1expansions and were compared with 81 RFC1-negative individuals with suspected CANVAS. Assessments included neurological examination, SARA and INAS scoring, vestibular testing, neurophysiology, autonomic assessment, neuroimaging, and neuropsychological evaluation. Genetic testing used flanking and repeat-primed PCR, with Southern blot confirmation when available. Longitudinal SARA progression was analysed using linear mixed-effects models.

Results: Of the CANVAS positives, 54.5% had a positive family history and 14% showed pseudodominant inheritance. Alternative genetic diagnoses were identified in 14 RFC1-negative individuals, most commonly FGF14repeat expansions. In addition to the classical CANVAS triad (present in 74%), a broader multisystem phenotype including chronic cough, downbeat nystagmus, dysautonomia, and neuropathic pain was significantly associated with RFC1positivity compared with RFC1-negative individuals. Objective vestibular impairment was present in 81.5%, although only 18.2% reported dizziness. Neurophysiology consistently showed sensory neuronopathy or neuropathy with frequent small fibre involvement. Longitudinal analysis of 38 patients demonstrated a mean SARA progression of 1.38 points/year with marked variability. Earlier onset correlated with faster progression and larger repeat expansions of the smaller allele ($r=-0.39$, $p=0.031$). Two siblings with exceptionally large expansions developed childhood-onset cough, cognitive decline and premature death.

Discussion and Conclusion: RFC1-spectrum disorder shows broader multisystem involvement than previously recognised, with autonomic, small fibre and cognitive features contributing substantially to disease burden. These findings expand the phenotypic spectrum of CANVAS and provide data relevant for future therapeutic trials.

C119. A Novel Clinical Rating Scale for Late-Onset Tay-Sachs and Sandhoff Diseases: Development and Baseline Findings

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Late-onset GM2gangliosidosis (LOGG), comprising late-onset Tay-Sachs (LOTS) and Sandhoff disease (LOSD), is an ultra-rare lysosomal disorder caused by pathogenic variants in HEXA and HEXB. Symptoms result from central and peripheral nervous system involvement, with frequent cerebellar ataxia presenting from childhood through adulthood.

We sought to develop a disease-specific LOGG clinical rating scale (GM2-CRS), to measure symptom severity and progression.

Insights gathered during a 2015-2019 natural history study, qualitative interviews, and expert input, as well as assessments of related disease rating scales and FDA guidance documents, informed initial scale development. The GM2-CRS includes standardized neurological assessments and performance-based outcome measures. From 2023-20224, the scale was administered and refined, incorporating feedback from disease experts and patients. Since 2024, the revised scale has been used in a longitudinal, multicenter, observational LOGG natural history study in the US.

Twelve LOTS and six LOSD patients have completed a baseline visit. Baseline scores demonstrated substantial heterogeneity, and performance-based measures reflected a range of functional impairment.

Lower extremity weakness was more consistently and severely affected than upper extremity weakness. Ataxia was prevalent. Sensory abnormalities were more common in LOSD than in LOTS.

Strong correlations were found between the total GM2-CRS score, the scale domains, and performance-based measures. The total scale score was strongly correlated with the Timed Up-and-Go ($r \approx 0.89$), supporting the relationship between clinical impairment and real-world mobility limitations. Subgroup analysis revealed disease-specific relationships.

Baseline findings highlight shared disease features and specific differences. Functional performance measures correlated with domain-based neurological examination findings.

This baseline analysis demonstrates that the GM2-CRS captures key features of LOGG, while accommodating clinical heterogeneity. Including examination-based and performance-based components strengthens the scale's ability to reflect clinically meaningful impairment. The ongoing natural history study will enable longitudinal assessment of disease progression and sensitivity to change, facilitating further

Funding Sources: a { text-decoration: none; color: #464feb; } tr th, tr td { border: 1px solid #e6e6e6; } tr th { background-color: #f5f5f5; }. This research is supported by a grant from the National Tay-Sachs & Allied Diseases Association (NTSAD).

C120. Improvements in vestibulo-ocular reflex gain, dynamic balance, and gait speed following vestibular rehabilitation in an individual with CANVAS

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Background and Objectives: Evidence in the literature has shown that individuals with neurodegenerative cerebellar ataxias have potential to improve balance and coordination and aerobic capacity with rehabilitation. Yet there is scant literature reporting positive efficacy of vestibular physical therapy (VPT) in ataxia and even more so among individuals living with Cerebellar Ataxia with Neuropathy and Vestibular Areflexia Syndrome (CANVAS).

Presented here is a unique case of an individual genetically diagnosed with CANVAS (biallelic RFC-1 positive) with bilateral vestibulopathy, who performed comprehensive VPT interventions in the clinic and home settings, and whose vestibular physiology (i.e., vestibular ocular reflex (VOR) gains), gait speed, balance, and clinical neurological symptoms improved or remained stable over a 4-year period.

Intervention: Gaze and postural stability tasks (2-3 sets, 1.5 minutes each task), gait training, aerobic exercise, core strengthening at a perceived level of challenge, "somewhat difficult", for 4-5 days per week minimum.

Results: Improvements or stable measures were observed comparing baseline versus 4-year including: vestibulo-ocular reflex gains (VOR) via passive video head impulse testing: Left yaw mean/(0.23 +/- 0.06 versus 0.45 +/- 0.1; Right mean/sd 0.27 +/- 0.07 vs 0.19 +/- 0.8), gait velocity (0.87 m/s vs 1.1m/s), dynamic balance via the Functional Gait Assessment, 11/30 vs 17/30 (higher is better for each outcome), and the clinical neurological measure (Scale for Assessment and Rating of Ataxia, 8/40 vs 7/40, lower is better). The patient's severity of symptoms and vestibular physiology impairment did not correlate.

Discussion/Conclusion: This unique case of an individual diagnosed with CANVAS demonstrates that vestibular physiological, functional, and clinical improvements are possible with the help of intensive VPT and intensive cardiovascular exercise.

C121. Postgraduate Curriculum for Ataxia/HSP: Development and First Implementation

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Background and objectives: Specialised expertise in cerebellar ataxias and hereditary spastic paraplegias is rare and unevenly distributed, while advances in neurogenetics, diagnostics, multidisciplinary care and emerging therapies require structured clinical postgraduate training. The European Reference Network for Rare Neurological Diseases (ERN-RND) has developed the first ERN-based postgraduate curriculum approved and adopted under the European Union of Medical Specialists (UEMS, board certificate level) framework. The underlying European Training Requirement for the Competency Rare Neurological Diseases represents the first formal European Training Requirement for a group of rare diseases. We report the development methodology and first implementation of the Ataxia/HSP module. **Methods:** A competency-based curriculum-development methodology was applied. The Body of Knowledge was used as the central framework to define domains, competencies and learning objectives. Existing educational material was mapped to these objectives; gaps informed development of additional learning content. The module was structured around online learning, e-learning patient cases, recommended readings, supervised clinical training, logbook documentation and assessment towards the European Certificate in Rare Neurological Diseases. **Results:** The Ataxia/HSP module was launched on 28 June 2026 as the first ERN-RND curriculum module. Application deadline for the first class is 31 October 2026. It covers five domains, 21 competencies and 96 learning objectives. The online learning path is implemented on EANCampus (European Academy of Neurology) and includes educational webinars, interactive patient cases, readings and knowledge assessment. This is complemented by a supervised 3–6-month on-site training period at a recognized clinical expertise centre. Competence development is documented in a structured logbook. The module concludes with an oral exit examination. **Discussion and Conclusion:** The ERN-RND Ataxia/HSP module establishes a new European model for rare-disease postgraduate clinical education by combining network-based expertise, UEMS-aligned competency standards, flexible online learning and supervised clinical practice. Early implementation has started; prospective evaluation is planned.

Funding Sources: European Reference Network for Rare Neurological Diseases, Funding by Eu4Health program

C122. Allogeneic Adipose-derived MSCs delay disease progression in moderately severe SCA

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Background and Objectives: Spinocerebellar ataxia (SCA) is a progressive neurodegenerative disorder without an effective therapy. Stemchymal®, an allogeneic adipose-derived mesenchymal stem cell product, has previously shown its potential in the treatment of SCA. We evaluated the extended safety and potential efficacy of Stemchymal®, in patients with SCA2 and SCA3 and explored the therapeutic effects in SCA3 subgroups.

Methods: This Phase II randomized, double-blind, placebo-controlled trial enrolled 56 patients with SCA2 or SCA3. Participants received three monthly intravenous infusions of Stemchymal® (70 million cells per infusion) or placebo. Efficacy was assessed using Scale for Assessment and Rating of Ataxia (SARA) through Week 60, including subgroup analyses of patients with baseline SARA ≥ 9 .

Results: Although Stemchymal® did not significantly delay disease progression in the overall SCA3 cohort, it was associated with delayed SCA progression in participants with baseline SARA scores ≥ 9 (moderately severe to severe SCA). Treatment benefits were observed in appendicular function at earlier time points and in the preservation of axial function at later time points. Stemchymal® was well tolerated throughout the study.

Discussion and Conclusion: Three intravenous Stemchymal® infusion were safe and well tolerated and demonstrated clinically meaningful reductions in SARA score in SCA3 participants with baseline SARA ≥ 9 for over one year. These findings suggest that Stemchymal® can potentially be a safe, effective and feasible treatment for SCA3.

Funding Sources: Steminent Biotherapeutics, Inc.

C123. PROVE-FA: Evaluation of Long-Term Efficacy of Vatiquinone for the Treatment of Friedreich's Ataxia

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Objective: PROVE-FA (NCT07681713) is a global phase 3, open label, single arm study to confirm the treatment effects of vatiquinone in young ambulatory patients with Friedreich's Ataxia (FA) on the key measures of disease progression demonstrated in MOVE-FA (NCT05515536).

Background: Vatiquinone (PTC743) is an investigational small molecule treatment that inhibits 15 lipoxygenase (15-LO), an oxidoreductase enzyme that is essential to inflammation, oxidation, and cell death (ferroptosis), which have been shown to be associated with FA pathogenesis. Previous vatiquinone studies have demonstrated favorable safety and tolerability in children and adults as well as evidence of short- and long-term effects on slowing disease progression. This study is designed to confirm these observations in an open-label study with a natural history external control group.

Design/Methods: This study was designed in consultation with the FDA to enable the use of an external control cohort to provide data to support regulatory approval application submission. The study will enroll approximately 120 ambulatory patients with FA aged 7-21 years with a diagnosis of homozygous GAA repeat expansion FA, and a total modified Friedreich's Ataxia Rating Scale (mFARS) score ≥ 20 to ≤ 70 . The study comprises a 4-week screening period followed by a 24-month treatment period. Eligible participants will receive up to 400 mg vatiquinone three times a day. The primary study objective is to confirm the benefit of vatiquinone on disease progression assessed by the change in mFARS after 24-months relative to a propensity matched natural history cohort. Secondary endpoints include the subscales of mFARS, FARS-activity of daily living (ADL), timed 25-foot walk (T25FW), safety and tolerability. PROVE-FA is currently enrolling participants at selected FA clinical care centers worldwide.

Funding Sources: This work is funded by PTC Therapeutics

C124. A single-center experience with Omaveloxolone in Friedreich Ataxia

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Introduction: Omaveloxolone is the first approved treatment for Friedreich Ataxia (FA), an autosomal recessive neurodegenerative disorder caused by GAA trinucleotide expansions in the FXN gene, resulting in reduced frataxin levels. This leads to suppression of the Nrf2 pathway, oxidative stress, mitochondrial dysfunction, and cellular damage. Omaveloxolone is a synthetic oleanane triterpenoid that activates Nrf2 and enhances antioxidant responses. In a phase II trial, omaveloxolone (150 mg/day) significantly improved neurological function compared with placebo. This retrospective study evaluated its efficacy and safety in patients treated in clinical practice. **Methods:** Patients underwent baseline and follow-up assessments at 3, 6, and 12 months. Neurological outcomes included the modified Friedreich Ataxia Rating Scale (mFARS) and Friedreich Ataxia Activities of Daily Living (FA-ADL). Liver enzymes and lipid profiles were assessed at baseline and after 1, 2, 3, 6, and 12 months. Changes over time and interactions with baseline disease severity were analyzed using linear mixed models for repeated measures. **Results:** Sixty-two patients (32 females, 30 males; mean age 40.2 ± 13.6 years; mean disease duration 20.0 ± 10.7 years) were enrolled. All completed 6 months of follow-up, while 45 reached the 12-month assessment. mFARS scores significantly decreased over time ($p=0.028$), from 56.9 ± 18.2 at baseline to 54.7 ± 16.5 at 12 months. Patients with higher baseline mFARS showed a trend toward greater improvement, although the interaction was not significant ($p=0.488$). FA-ADL scores showed a modest but significant worsening (16.2 ± 7.4 to 16.7 ± 7.4 ; $p<0.001$), with greater baseline disability associated with a trend toward functional stability. AST and ALT increased until month 3 and subsequently decreased ($p<0.001$). Eleven patients temporarily discontinued treatment because of adverse events or patient choice and subsequently restarted with slow titration. Three permanently discontinued treatment due to gastrointestinal side effects or patient choice. **Conclusion:** Omaveloxolone was generally safe and well tolerated. mFARS significantly improved over time, with a greater benefit suggested in patients with more advanced disease, while FA-ADL showed only mild functional decline.

C125. A Randomized, Double-Blind, Placebo-Controlled Phase 3 Trial of SLX-100 (Immediate-Release 4-Aminopyridine) in SCA27B: Study Design and Population

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Background & Objectives: SCA27B (GAA-FGF14ataxia) presents late, with a milder and more variable phenotype than other dominant ataxias: gait and balance impairment is near-universal from onset, while speech, oculomotor and appendicular involvement are variably penetrant. 4-Aminopyridine is known to produce a symptomatic benefit in patients, but no approved, controlled formulations exist for ataxias. SLX-100 is an immediate-release formulation, dosed three times daily to cover waking hours.

Methods: Randomized, double-blind, placebo-controlled, parallel-group Phase 3 trial: 1:1 to SLX-100 5 mg TID or placebo for 12 weeks; approximately 88 individuals at 10 sites across the US, Canada, Germany, and the UK for at least 70 completers (90% power for a 3-point difference in Upright Stability). Inclusion criteria: age 40–80; ≥250 GAA repeats in FGF14, confirmed before randomization; ataxia not exclusively episodic; Upright Stability Score (USS) ≥10; able to walk 10 m unaided. Participants must be 4-aminopyridine-naïve; experienced individuals are studied separately. Exclusions include seizures, acetazolamide or OCT2inhibitors, creatinine clearance ≤50 mL/min, uncontrolled hypertension, a second neurological disease with functional impact, and psychogenic gait disturbance.

Results: The primary endpoint is change from baseline to Week 12 in the USS (mFARS Section E, 0–36). Secondary endpoints, tested hierarchically, are Patient Global Impression, harmonic ratio from wearable sensors, m-SARA, FA-ADL, and the ABC scale. Speech assessments and further digital gait metrics are exploratory. Enrollment is planned to begin in late 2026.

Discussion & Conclusion: A primary endpoint confined to axial function concentrates measurement where SCA27B is expressed in nearly every individual, and avoids domains in which age-related variability rivals the disease effect. The USS correlates with m-SARA and SARA ($r \approx 0.85$) yet retains range where both floor: in CRC-SCA, 90% of individuals with SCA27B scored ≤4 on m-SARA. A baseline USS ≥10 leaves headroom of about three times the minimal detectable change.

Funding Sources: Solaxa PBC, supported by a grant from the U.S. Department of War and a development partnership with Alvogen.

C126. How the real-world evidence differed from the results of the randomised clinical trials in omaveloxolone in Friedreich ataxia

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Background / introduction: A Cochrane review into Pharmacological treatments for Friedreich ataxia (FRDA) including epoetin alpha, CoQ10 and vitamin E, idebenone, leriglitazone, omaveloxolone, and RT001 reviewed the data from randomised clinical trials (RCT) of greater than 1 year and found that pharmacological treatment probably makes little or no difference in primary outcomes compared with placebo after 12 months treatment in individuals with FA1. This important information was derived using Cochrane guidelines and Grading of Recommendations Assessment, Development and Evaluation (GRADE). A further recent systematic review indicated omaveloxolone offered minimal clinical efficacy based on performance on clinical rating scales². In contrast, a recent update to the Clinical Management Guidelines for Friedreich Ataxia recommended the use of omaveloxolone over no omaveloxolone for providing neurological benefit for adults with FA³. All studies used performance on clinical rating scales as primary endpoints.

Method: We reviewed the data from two recent systematic reviews and additional real-world data specifically related to omaveloxolone from individuals with FRDA.

Results : Clinically meaningful outcomes (such as quality of life, speech or fatigue measurements) did not constitute primary endpoints in RCTs examining efficacy of pharmacological intervention in FRDA therefore were not reported in the systematic reviews. Most studies however did include clinically meaningful outcomes as secondary measures.

Discussion and Conclusion: Anecdotal evidence from real-world data reports improvement in domains such as speech and fatigue however this continues not to be captured in efficacy trials. Countries seeking reimbursement from regulatory agencies face challenges in justifying efficacy based on clinical rating scales rather than real life experience. There is an urgent need to capture real-world evidence that supports the ongoing use of expensive medication in a rare disease

Disease Mechanisms (Dominant Ataxias)

DD1. The NF- κ B inducing kinase (NIK) is a selective enhancer of mutant ataxin-3 levels in SCA3 brain cells

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Background and Objectives: Spinocerebellar ataxia type 3 (SCA3) is caused by expanded-polyglutamine ATXN3, whose accumulation and aggregation drive neurodegeneration. Thus, reducing mutant ATXN3 levels is a compelling therapeutic strategy, yet mechanisms regulating mutant ATXN3 proteostasis remain poorly understood. Through a genetic screen, we identified the NF- κ B-inducing kinase (NIK/MAP3K14), a central activator of the non-canonical NF- κ B pathway, as an enhancer of mutant ATXN3 abundance. While NIK regulates cell survival and stress responses, its role in neurodegeneration remains largely unexplored. This work aimed to elucidate how NIK regulates mutant ATXN3 stability and contributes to SCA3 pathogenesis.

Methods: NIK involvement in SCA3 was examined using patient brain tissue, patient-derived neural progenitor cells (NPCs), and SCA3 transgenic mice. Genetic and pharmacological perturbation of NIK, combined with protein stability/degradation assays, were used to define mechanisms regulating mutant ATXN3 abundance.

Results: NIK accumulated in SCA3 patient brain areas showing neuronal loss. In NPCs, NIK positively regulated mutant ATXN3 abundance in a kinase activity-dependent manner, while NIK inhibition selectively reduced mutant, but not wild-type, ATXN3 without altering transcript levels. Proteasome inhibition reversed the ATXN3-lowering effect of NIK inhibitors, whereas lysosomal inhibition did not, supporting that NIK prevents mutant ATXN3 proteasomal degradation. Furthermore, NIK inhibition accelerated mutant ATXN3 turnover in cycloheximide chase assays. Importantly, genetic ablation of Nik in SCA3 mice reduced human mutant ATXN3 levels, including its nuclear accumulation.

Discussion and Conclusion: These findings indicate that NIK regulates mutant ATXN3 proteostasis and support a model in which NIK inhibition promotes clearance of pathogenic ATXN3, highlighting NIK-mediated regulation of ATXN3 abundance as a potential selective therapeutic target for SCA3.

Funding Sources: National Ataxia Foundation - SCA3 Translational Research Grant (2019-2020) to MCC, Cayman Biomedical Research Institute - Research Grant (2021-2022) to MCC, Michigan State University to MCC, NINDS (R01NS038712) to HLP.

DD2. Domain-dependent Decoupling of Co-chaperone and E3 Ligase Functions in CHIP (STUB1) Drives Biochemical and Clinical Heterogeneity in SCA48

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Background and Objectives: The carboxyl terminus of Hsp70-interacting protein (CHIP, encoded by STUB1) combines co-chaperone and E3ubiquitin ligase activities to regulate protein quality control. Heterozygous mutations in STUB1 cause spinocerebellar ataxia type 48 (SCA48), a progressive cerebellar ataxia with varying extrapyramidal and cognitive features.

Methods: To understand the molecular basis of this variability, we systematically analyzed 13 SCA48-related variants in the TPR and U-box domains using recombinant protein biochemistry and cellular models.

Results: TPR variants maintained their intrinsic ligase activity but showed significantly reduced HSC70 binding, impaired substrate ubiquitination, and decreased stability. Conversely, U-box variants abolished ligase function, promoted the formation of high-molecular-weight oligomers, and often increased CHIP levels while only partially impairing co-chaperone activity. Many mutants displayed temperature-sensitive defects and defective stress-induced nuclear translocation. Principal component analysis revealed distinct biochemical clustering specific to each domain. RNA-seq after STUB1 knockdown confirmed that HSF1-dependent transactivation was preserved, but CHIP's role in amplifying ubiquitination, chaperone function, and stress-related transcription was lost. Meta-analysis of 87 SCA48 patients showed correlations between these biochemical profiles and clinical features, with TPR-like signatures linked to upper motor neuron signs and U-box profiles associated with prominent dysarthria.

Discussion and Conclusion: Overall, SCA48 results from domain-specific decoupling of CHIP's dual functions, leading to dominant-negative or gain-of-toxic effects that drive phenotypic diversity. These findings offer a mechanistic understanding of SCA48 pathogenesis and suggest opportunities for targeted therapies that leverage residual CHIP activity.

Funding Sources: This work was supported by the National Institutes of Health R35GM128855(N.G.B.), R01AG066710(J.C.S.), R01AG061188(J.C.S.), the American Heart Association 23IPA1048749(N.G.B.), and the National Ataxia Foundation.

DD5. PUMILIO1 and the post-transcriptional regulation of alpha-synuclein

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What is it that causes alpha-synuclein, the protein encoded by SNCA, to accumulate in Parkinson's disease (PD)? One possibility is that the normal regulation of alpha-synuclein protein levels is somehow disrupted through alterations in the 3'UTR of SNCA transcripts. There are shorter and longer isoforms of SNCA mRNA, and several studies have shown that the longer isoform predominates in Parkinson's Disease (PD) post-mortem brain tissue. So far, only the microRNA miR-7 has been shown to post-transcriptionally suppress alpha-synuclein levels.

In this study, we found that the RNA-binding protein Pumilio1(PUM1) directly interacts with the long 3'UTR of SNCA mRNA to modulate alpha-synuclein protein levels in a brain region-specific manner in mice. RNAscope in mouse brain and single-nucleus RNA sequencing (snRNA-Seq) of post-mortem Parkinson's disease (PD) brain tissue revealed an inverse correlation between PUM1 and SNCA mRNA expression in dopaminergic neurons of the substantia nigra pars compacta. Similarly, in patient-derived induced neurons (iNs) carrying a triplication of the SNCA locus, PUM1 mRNA levels were significantly lower than wild-type, but restoring PUM1 expression normalized both SNCA mRNA and alpha-synuclein protein levels. Using microfluidic chamber assays, we found that PUM1 knockdown in the neuronal soma led to increased SNCA mRNA in axons and decreased levels in the soma, suggesting that PUM1 also directs subcellular localization of SNCA mRNA. We also show that miR-7 requires PUM1 to regulate SNCA expression, and a search through a PD clinical database has identified several individuals with variants in PUM1. These findings highlight a novel post-transcriptional mechanism that suppresses alpha-synuclein accumulation and may offer new therapeutic strategies for PD.

Funding Sources: R01NS109858, NIH/NINDS, National Ataxia Foundation Research Seed Money Total Award

DD6. RNA splicing dysregulation across SCA3 vulnerable regions and cell types reveals disease mechanisms

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Background and Objectives: Dysregulated RNA splicing has been implicated in neurodegenerative disorders, including polyglutamine spinocerebellar ataxias (SCAs). While RNA splicing is prevalent in SCAs, affected cell types and functional implications remain unclear. Our work seeks to define cell populations exhibiting RNA splicing changes in SCA3, one of the most common dominantly inherited ataxias, and define mechanisms connecting polyglutamine repeat expansion, alternative splicing, and ataxic motor impairments.

Methods: We performed alternative splicing analysis on published sequencing data from cerebellar and brainstem regions from YACQ84transgenic SCA3mice, including mice treated with an anti-ATXN3ASO. We sequenced RNA from the cerebellum of post-mortem SCA3patients and a conditional-off knock-in (cKI) SCA3mouse model crossed with Baf53b-Cre, and Sox10-Cre for genetic silencing of the mutant allele in neurons or neural crest derived cells and oligodendrocyte lineage, respectively.

Results: Our data revealed widespread RNA splicing changes conserved across SCA3-vulnerable regions in patients and mouse models. The most conserved genes with significant skipped exon events implicate neurons and oligodendrocytes as affected cell types across models. CNS delivered ASO treatment, previously shown to rescue pathology and motor impairments in YACQ84mice, also rescued RNA splicing across affected brain regions. Alternatively, cell type-selective genetic silencing of mutant ATXN3in cKI mice provided a modest rescue of splicing changes. Comparison of enriched RNA binding protein motifs surrounding alternatively spliced exons between ASO and cKI mice identified candidate factors driving splicing changes in specific cell types in SCA3.

Discussion and Conclusion: Together, these studies highlight the importance of RNA splicing biology in SCA3. We provide evidence of RNA splicing dysregulation in the cerebellum of patients with SCA3. Additionally, our ASO and cKI rescue data suggests connections between functionally relevant RNA splicing changes and disease associated motor impairments.

Funding Sources: JE F31NS145373, HSM R01NS122751, NS140063

DD7. Generation of patient-derived iPSC lines and iNeurons for SCA27B and SCA27A: a disease-relevant cell model for mechanistic studies

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Background and Objectives: SCA27B and SCA27A are autosomal dominant cerebellar ataxias caused by an intronic GAA•TTC repeat expansion and by heterozygous loss-of-function variants in FGF14, respectively. Because FGF14 is predominantly expressed in neurons, mechanistic studies require neuronal models derived from patient cells. We sought to generate, characterize, and differentiate iPSC lines from SCA27B and SCA27A patients into neurons to enable downstream investigation of disease mechanisms.

Methods: Dermal fibroblasts from three SCA27B patients (monoallelic expansions of 583 and 391 repeats; biallelic expansions of 315/290 repeats) and two SCA27A patients (one carrying a heterozygous and the other a homozygous NM_175929.3:c.75del; p.(Leu26Serfs*51) variant) were reprogrammed into iPSCs. All five lines underwent comprehensive characterization. iPSCs were induced into neuronal progenitor cells (NPCs) by dual SMAD inhibition. NPCs were next differentiated and matured for up to 21 days into FOXG1-positive forebrain cortical neurons. FGF14 expression was tracked across neuronal maturation. SCA27B iNeurons are currently being processed for Fiber-seq, R-loop profiling using the anti-DNA:RNA hybrid antibody clone S9.6, and long-read RNA sequencing (PacBio).

Results: iPSC lines were successfully generated from patient fibroblasts. iPSC colonies were isolated based on morphology and characterized using karyotyping, pluripotency markers, and in vitro directed trilineage differentiation. Successful differentiation into forebrain cortical neurons was achieved across all lines. Longitudinal characterization demonstrated progressive FGF14 upregulation during neuronal maturation, with high levels of expression reached after 21 days. Results from further genomic studies are currently being generated and analyzed.

Discussion and Conclusion: These iPSC lines and their iNeuron derivatives establish a disease-relevant cell model for mechanistic studies of SCA27B and SCA27A. With the SCA27B lines, ongoing Fiber-seq, R-loop profiling, and long-read RNAseq analyses aim to define chromatin features and R-loop dynamics at the expanded locus, and to identify novel and aberrant FGF14 splice transcripts.

Funding Sources: Suna and İnan Kırarç Foundation, Canadian Institutes of Health Research, Peripheral nerve society, Ataxia UK, National Ataxia Foundation, NINDS

DD8. Karyopherin alpha 3 (KPNA3) is a genetic and core mechanistic modifier of Spinocerebellar ataxia type 3 (SCA3)

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Background and Objectives: Spinocerebellar ataxia type 3 (SCA3) or Machado-Joseph disease (MJD) is caused by the expansion of a polyglutamine repeat in the ataxin-3 protein. A hallmark of both SCA3 and other polyglutamine diseases is the formation of protein aggregates. Interestingly, while ataxin-3 is normally a cytoplasmic protein, these protein aggregates form in the nucleus of neurons. We already demonstrated that this nucleocytoplasmic translocation of ataxin-3 is critical for the pathophysiology of SCA3, that ataxin-3 contains both two nuclear export and one nuclear localization signal and that Karyopherin alpha 3 (KPNA3) is the nuclear transport protein which shuttles ataxin-3 from the cytoplasm to the nucleus. Knowing this critical impact of KPNA3, we examined whether genetic variations within KPNA3 modify the disease.

Methods: We characterized the single nucleotide polymorphism (SNP) rs11618521, located in the KPNA3 promoter, as genetic modifier of the age at onset in SCA3 and a regulator of the KPNA3 promoter activity. We therefore combined genotyping of hundreds of SCA3 patients with functional promoter and transcription factor studies.

Results: We identified the SNP rs11618521 as a regulator of the KPNA3 promoter activity and as a genetic modifier of SCA3. Moreover, we identified the transcription factor Nanog Homeobox (NANOG) as a key regulator that exerts allele-specific modulatory effects on the KPNA3 promoter activity. This modulates expression and contributes to SCA3-associated pathogenic mechanisms. Pharmacological inhibition of NANOG reduced KPNA3 levels and ameliorated aggregate formation.

Discussion and Conclusion: The mechanistic insights we provide may guide future therapeutic strategies targeting the intracellular localization and toxicity of ataxin-3. Our results highlight NANOG as a regulator of neurodegeneration-relevant mechanisms through modulation of KPNA3, representing a potential therapeutic target for SCA3 and other conditions involving this nuclear transport pathway.

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DD10. CAG repeat expansion in THAP11 impairs autophagy and causes neurodegeneration in a Drosophila model

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Background: Spinocerebellar ataxia (SCA) type 51 is a neurodegenerative disease caused by CAG repeat expansions in exon 1 of the THAP11 gene. These repeats are translated into a glutamine-rich protein, THAP11-polyQ, which forms protein aggregates and exhibits toxicity in cell models; however, the underlying mechanism remains unclear.

Methods: In this study, we generate transgenic Drosophila models expressing varying lengths of THAP11-polyQ using the UAS-GAL4 system and assess neurodegeneration through pathological and behavioral analyses.

Results: Our results demonstrate that expression of THAP11-polyQ in transgenic flies leads to progressive neuronal cell loss, locomotor deficiency, and reduced survival. RNA sequencing of patient-derived skin fibroblasts reveals significant enrichment of the PI3K-Akt-mTOR pathway, and electron microscopy of transgenic flies shows an increase in multilamellar bodies, suggesting involvement of autophagy in SCA51. We treat the fly model with rapamycin, an mTOR inhibitor known to enhance autophagy. This treatment reduces toxic THAP11-polyQ protein aggregates, significantly alleviates neuronal degeneration, and improves locomotor function, consistent with the rescue effects observed upon overexpression of Atg8a.

Conclusions: Overall, these findings suggest that the Drosophila model, which recapitulates the neurodegenerative features of SCA51, can be used to investigate pathogenic mechanisms and that rapamycin holds promising potential as a therapeutic approach for this disease.

Funding Sources: National Natural Science Foundations of China (Grants 82402177, 82171846, 82422025); The Beijing High-Level Innovation and Entrepreneurship Talent Support Program-Healthcare Platform young backbone talent projects (G202531202)

DD11. Ataxin-3 regulates the ubiquitination and proteostasis of RNA-binding proteins in SCA3

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Background and objectives: Ataxin-3, the pathogenic protein in spinocerebellar ataxia type 3 (SCA3), is a deubiquitinase with an important role in proteostasis. Emerging evidence suggests that polyglutamine expansion within the ataxin-3 protein may alter its deubiquitinase function. However, whether this results in a gain or loss of ataxin-3's deubiquitinase function, and the extent to which this contributes to SCA3 pathogenesis, remains to be established.

Methods: To identify proteins that may be differentially K48-ubiquitinated by polyglutamine-expanded ataxin-3, we have immunoprecipitated K48-ubiquitinated proteins in Neuro-2A cells expressing wild-type or polyglutamine-expanded human ataxin-3, and performed LC-MS/MS analysis. We have subsequently used a combination of immunoblotting, co-immunoprecipitation, ubiquitination assays, RT-qPCRs, and immunofluorescent staining, in cellular and mouse models of SCA3 to evaluate ataxin-3's regulation of RNA-binding proteins.

Results: Our proteomic analysis revealed many RNA-binding proteins that may be differentially K48-ubiquitinated in cells expressing polyglutamine-expanded ataxin-3, possibly due to changes in ataxin-3's deubiquitinase function. This includes several RNA-binding proteins implicated in amyotrophic lateral sclerosis (ALS), including matrin-3 and TDP-43. We subsequently discovered an increase in matrin-3 protein in Neuro-2A cells expressing wild-type ataxin-3 compared to deubiquitinase inactive ataxin-3. Matrin-3 protein is also increased in the brain of SCA3(CMVMJD135) mice at end-stage of disease, with a corresponding decrease in the amount of K63-ubiquitinated matrin-3. The matrin-3 and ataxin-3 proteins were also found to co-immunoprecipitate, and the stability of the matrin-3 protein is increased in cells expressing polyglutamine-expanded ataxin-3, which appears to be due to decreased clearance of matrin-3 by autophagy within these cells.

Discussion and conclusion: Our findings highlight a role for ataxin-3's deubiquitinase function in regulating the ubiquitination and proteostasis of matrin-3, which may be important to the pathogenesis of SCA3 and ALS. We are continuing to investigate how targeting the deubiquitinase function of ataxin-3 may lead to new treatment avenues for SCA3.

Funding Sources: The MJD Foundation Australia, Macquarie University Research Excellence Scholarship (MQRES), Skipper Postgraduate and Early Career Researcher Travel Award, MND Australia PhD Top-Up Scholarship.

DD12. A Pain in the Tail: Characterizing patient-relevant peripheral neuropathy features in a preclinical mouse model of SCA3

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Background and Objectives. Spinocerebellar ataxia type 3 (SCA3) is the most common dominantly inherited ataxia, caused by a gain of function CAG expansion in ATXN3. No effective preventative or disease-modifying treatments currently exist for this progressive, multisystem disorder. Although SCA3 is classically associated with central nervous system degeneration and demyelination, more than half of individuals with SCA3 also exhibit peripheral neuropathy symptoms, including reduced limb sensation and burning pain, which significantly impact quality of life and mobility. However, the cell types and disease mechanisms underlying SCA3-associated peripheral nervous system abnormalities remain underexplored. As such, characterizing a patient-relevant model is essential to link peripheral pathology with functional deficits and guide therapeutic development.

Methods. We used the homozygous YACQ84 mouse model, which carries four copies of full-length human mutant ATXN3, to define the progression of sensorimotor behaviors, nerve conduction abnormalities, ultrastructural pathology, and transcriptomic dysregulation in the dorsal root ganglia (DRG) and sciatic nerve.

Results. YACQ84 mice display prominent sensorimotor nerve conduction velocity reductions in sciatic and sural nerves, along with blunted sural nerve signal amplitudes, suggestive of axon loss and demyelination. Behaviorally, YACQ84 mice have abnormal responses to thermal and mechanical stimuli. Histological analysis of their peripheral nerves highlights both neuronal and glial involvement with reduced myelinated and unmyelinated fiber densities, decreased axon diameters, demyelination of large fibers, and loss of small epidermal nociceptive fibers. Transcriptomic analyses identified dysregulation of diverse somatosensory pathways and a significant increase in alternative splicing events.

Discussion and Conclusion. Our results newly characterize significant peripheral neuropathy in a preclinical SCA3 mouse model, providing a strong foundation for future mechanistic and therapeutic studies. Moving forward, targeting DRG neurons and peripheral nerves in YACQ84 mice with therapies that reduce mutant ATXN3 is of particular interest.

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DD13. Repeat sequence of CAG repeats in spinocerebellar ataxias

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Background and Objectives: Expansions of repeats account for 60% of the autosomal dominant cerebellar ataxias (SCAs) known causes. The threshold size of these repeats defines their pathogenicity, while the presence of interruptions in the repeat sequence can modify the clinical presentation.

Methods: We analyzed, by short-read amplicon sequencing, the repeat sequence of the main forms of SCAs caused by CAG coding expansion in the ATXN1, ATXN2, ATXN3 and ATXN7 genes, in 993 individuals carrying a CAG expansion.

Results: CAA interruptions within the CAG expansion were identified in ATXN2 in 5 out of 214 patients (2.3%). The CAG expansions in ATXN1 (n=208), ATXN3 (n=461), and ATXN7 (n=110) were pure, without interruptions. Depending on the size of the CAG expansion, CAA interruptions in ATXN2 modify the SCA2 phenotype: for small expansions, CAA interruptions are associated with a parkinsonian syndrome, and for large expansions, with a later age of onset of ataxia (of 30 years later in the case studied). The usual somatic instability of the expansions was absent in the interrupted expansions. Short-read sequencing allowed us to obtain both the size and sequence of the CAG expansion, within the current read size limits of 600 bases.

Discussion and Conclusion: Short-read sequencing allows for the characterization of the size and composition of typical CAG expansions. This approach highlights the role of CAA interruptions in ATXN2, which modify the clinical phenotype of SCA2, shifting it from ataxia to a parkinsonian syndrome. This finding enables improved genetic and clinical stratification of SCA2. The variability in the age of symptom onset, beyond the size of the CAG expansion, suggests the existence of genetic modifiers yet to be identified.

Funding Sources: Paris Brain Institute, SPATAX and BIOMOV Studies, Inserm, APHP.

DD15. Investigating non-ion flux-related pathogenesis for neurodegeneration in CACNA1A disorders

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Background/Objectives: The CACNA1A gene encodes the voltage-gated calcium channel, Cav2.1. Mutations in CACNA1A produce a spectrum of disorders including childhood-onset disease and late-onset ataxia. The diverse clinical phenotype remains unexplained in CACNA1A-related disorders. We recently identified a mechanism of disease in a murine model of SCA6 that arises from Cav2.1 misfolding and causing endoplasmic reticulum (ER) stress-related membrane hyperexcitability. Our identification of a misfolding-based mechanism in SCA6 suggests that this gain-of-function mechanism may apply more broadly to CACNA1A-related disorders. **Methods:** To study the role for ER stress in CACNA1A mediated disease, we identified families with pathogenic CACNA1A variants, p.R1661H and p.R1667P, that respectively exhibit adult onset and childhood onset of ataxia. We have developed patient and mutation-corrected induced pluripotent stem cells (iPSCs) lines. These cell lines are being differentiated into cortical neurons. Using patch-clamp electrophysiology, transcriptome analysis, immunoblots for UPR-related pathways, and assessment of cell viability in response to ER stressors, we will examine whether Cav2.1 channel currents are normal whereas ER stress pathways are engaged.

Results: We anticipate finding no alterations in Cav2.1 current density in iPSC neurons derived from individuals with p.R1667H and p.R1667P Cav2.1 mutations. Using transcriptome analysis from p.R1661H Cav2.1 and p.R1667P Cav2.1 iPSC derived cortical neurons, we expect to identify a shared gain-of-function pathway related to ER stress from mutant Cav2.1. We expect to identify cortical neuron hyperexcitability in p.R1667P Cav2.1 but not in p.R1661H Cav2.1 iPSC derived neurons. In late onset CACNA1A disease, we predict the ER stress responses to be adequately compensated without an increase in cortical neuron excitability.

Discussion/Conclusion: We expect to identify a mechanism, independent of impaired calcium flux in CACNA1A-related disorders, that explains variable age of onset. Examining a role for misfolded Cav2.1 in neurodegeneration will provide insight into a novel role for channelopathies to behave as proteinopathies.

Funding Sources: Raynor Cerebellum Project

DD16. Spinocerebellar ataxia–associated mGluR1 hyperactivity disrupts postnatal Purkinje cell maturation and cerebellar circuit development

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Developmental abnormalities in cerebellar circuitry are increasingly recognised in spinocerebellar ataxias (SCAs), yet the underlying mechanisms remain poorly understood. Metabotropic glutamate receptor 1 (mGluR1) is highly expressed in Purkinje cells (PCs), where it plays a critical role in cerebellar development and motor learning. Notably, enhanced mGluR1 signalling has been implicated across multiple SCA subtypes. We previously demonstrated that mice carrying a SCA44-associated gain-of-function mGluR1 mutation recapitulate key features of SCA, establishing enhanced mGluR1 activity as a direct pathogenic driver. Here, we investigated whether increased mGluR1 function disrupts cerebellar development, with a particular focus on Purkinje cell structure and function.

Wildtype and mGluR1-mutant PCs were filled with biocytin for morphological reconstruction, and dendritic complexity was quantified using Sholl analysis. Dendritic development was further examined in organotypic cerebellar slice cultures. Bulk RNA sequencing was performed at postnatal day 21 to identify key altered molecular pathways. PC spontaneous firing was characterised using whole-cell patch-clamp recordings. Molecular layer height and climbing fibre (CF) extension along PC dendrites were assessed by immunohistochemistry during the critical postnatal developmental period.

We found that mGluR1 gain-of-function results in aberrant PC development and reduced dendritic complexity. Using transcriptomic analysis, we identified dysregulated genes and pathways such as neurotrophin signalling in the mutant cerebellum. PC dendritic deficits were rescued by pharmacological and genetic modulation of key downstream signalling molecules. Functionally, mutant PCs exhibited significantly higher spontaneous firing rates than wildtype PCs while maintaining normal firing precision. Moreover, CF territory was reduced in the mutant cerebellum with lobule- and genotype-specific differences.

Enhanced mGluR1 activity disrupts multiple aspects of early postnatal cerebellar development, including Purkinje cell dendritic maturation, spontaneous activity, and climbing fibre innervation. Together, our findings identify a pathogenic mechanism that may contribute to neurodevelopmental deficits across multiple SCA subtypes and provides a potential target for therapeutic intervention.

Funding Sources: This work has been supported by the UK Research and Innovation - Medical Research Council. Yin Chun Cheng has been the recipient of a 2024 Graduate Research Fellowship from the National Ataxia Foundation.

DD18. Developmental and Early Degenerative Purkinje Cell Changes in SCA2

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Spinocerebellar ataxias (SCAs) are a family of dominantly inherited neurodegenerative diseases predominantly affecting the cerebellum. To date, more than 40 distinct genetic subtypes have been identified in SCA patients. Late-onset clinical symptoms range from poor balance to slurred speech and eye movement abnormalities. However, there is emerging evidence that late-onset ataxias are not only degenerative in nature, with molecular and electrophysiological deficits preceding the onset of motor impairments in mouse models.

This study aimed to characterize early-disease changes in SCA2, one of the most common SCAs. SCA2 is caused by an expansion of a polyQ (CAG) repeat in exon 1 of the ATXN2 gene. We used a transgenic mouse model of SCA2 (Pcp2-tgATXN2) to assess Purkinje cell (PC) dendritic morphology and climbing fiber (CF) innervation during development and early-disease stages. Immunohistochemistry-based analysis of biocytin-filled SCA2PCs revealed reduced dendritic arborization as early as four weeks of age, with progressive degeneration after six weeks. Similarly, SCA2PCs in organotypic slice cultures from P9 pups exhibited reductions in dendritic tree and soma size after 12 days in vitro. Analysis of molecular layer height and CF innervation showed molecular layer thinning after 12 weeks of age, with no significant deficits in CF innervation of PCs. Ongoing experiments are investigating the molecular mechanisms underlying these developmental and early-disease changes and probing the therapeutic potential of their genetic or pharmacological targeting. Together, our findings suggest that, in addition to the previously described neurodegeneration, developmental deficits might contribute to SCA2 pathophysiology, emphasizing the importance of early intervention in late-onset ataxias.

Funding Sources: National Ataxia Foundation (NAF). The Margaret Thatcher Scholarship Trust (MTST)

DD19. Targeting circuit dysfunction in a mouse model of SCA1

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Background and Objectives: Spinocerebellar ataxias (SCAs) are neurological disorders causing disabling ataxia symptoms like imbalance, frequent falls, and loss of movement control. Despite diverse causes, our lab's data shows a common cerebellar rhythm alteration due to climbing fiber (CF) degeneration compared to controls. This study aims to determine if direct cerebellar stimulation with a pattern mimicking CFs input restores circuit dysfunction and ameliorates ataxia in a mouse model of SCA1.

Methods: ATXN1mice and wild-type littermate older than 8 months underwent local field potential (LFP) recordings in the cerebellar cortex and downstream cerebellar targets (primary and accessory motor cortex) coupled with motor tests (accelerated rotarod, balance Beam test, gait analysis). Various bipolar electrical stimulation protocols were tested in the bilateral cerebellar cortex (Lobe IV/V), comparing "CF-like" stimulation (300 Hz bursts delivered at 1 Hz) with tonic stimulation (10 Hz, 50 Hz, 200 Hz). Network connectivity was analyzed using coherence, phase amplitude coupling and Granger causality.

Results: During motor tasks, SCA1mice exhibited a motor deficit associated with a shift in the peak frequency in the high-gamma band (80-100 Hz compared to 100-120 Hz in WT mice) and impaired connectivity between cerebellum and motor cortex. Only CF-like stimulation significantly reduced the number of slips. At the neurophysiological level, the CF-like protocol reduced pathological oscillation and attenuated the altered connectivity between the cerebellum and the motor cortex.

Discussion and Conclusion: The high-gamma oscillatory shift in SCA1mice is closely linked to motor function and correlates with the degree of clinical impairment. CF-like electrical stimulation effectively ameliorates cerebellum-motor cortex communication and improves motor coordination, suggesting that targeting microcircuit dysfunction may represent a promising adaptive neuromodulation strategy for spinocerebellar ataxias.

Funding Sources: National Institutes of Health (NIH), National Ataxia Foundation Postdoctoral Fellowship, Parkinson's Foundation, International Essential Tremor Foundation, Brain Research Foundation

DD20. Anaphase-Promoting Complex post-translationally regulates Pumilio1 expression

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In actively dividing cells, the E3ubiquitin ligase Anaphase-Promoting Complex/Cyclosome (APC/C) and its co-activator CDH1/FZR1 are essential for successful cell-cycle progression. Interestingly, APC/C–CDH1 activity is retained in post-mitotic neurons, where it contributes to neuronal arborization and health. However, the identification of neuronal proteins targeted for degradation by APC/C requires further investigation.

Recently, in mouse brain, we identified an interaction between APC/C and Pumilio1 (PUM1), an RNA-binding protein associated with neurodevelopmental disorders and ataxia, known as SCA47. Here, we aim to characterize this interaction and determine whether PUM1 is targeted for degradation by the APC/C complex.

Experiments were performed in diverse model systems, including mammalian cell lines, patient-derived cell lines, and mice. To identify PUM1 protein interactors, we performed immunoprecipitation followed by mass spectrometry. To investigate the role of the APC/C co-activator CDH1/FZR1 in PUM1 degradation, we performed siRNA knockdown and overexpression studies. Finally, levels of PUM1 mRNA targets were quantified by qRT-PCR.

We found that PUM1 interacts with the complete APC/C complex and its co-activator CDH1 in an RNA-independent manner across different cell lines and in murine brain. We also found that PUM1 levels and ubiquitylation depend on APC/C and CDH1 activity. Together, our results suggest that PUM1 is a potential substrate of the APC/C complex.

Future studies will aim to identify the degron in PUM1 required for APC/C-mediated degradation, resolve the structure of the APC/C–PUM1 interaction, and examine the effects of APC/C–CDH1-dependent modulation on PUM1 mRNA targets. Ultimately, this work will uncover the post-translational regulation of PUM1 expression and the functional consequences of this regulation in neurons.

Funding Sources: R01NS109858, NIH-NINDS. ICAR

DD21. Investigating the mechanism underlying Purkinje neuron firing abnormalities in SCA6 mice

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Background: Spinocerebellar ataxia type 6 (SCA6), an autosomal dominant late-onset ataxia, is due to a hyperexpanded CAG repeat on the CACNA1A gene, which encodes voltage-gated calcium channel Cav2.1. In a heterozygous model of SCA6 with hyperexpanded CACNA1A, we recently identified a disease mechanism independent of Cav2.1 ion-flux; Cav2.1 misfolding causes endoplasmic reticulum (ER) stress-related membrane hyperexcitability in cerebellar Purkinje cells (PC) concurrent with symptom onset at 19 months. In homozygous SCA6 mice, firing abnormality and symptom onset is at 7 months. The mechanism of disease in the homozygous mice has not been determined.

Methods: Changes in PC firing were analyzed using patch-clamp electrophysiology in acute cerebellar slices from 7 to 12-month homozygous SCA6 mice. Pharmacological studies in slices were used to determine whether ER-stress related calcium currents are activated. To determine whether ER stress is occurring, we used Western Blot and Tissue immunofluorescence to detect changes in UPR-related proteins.

Results: Homozygous SCA6 mice PCs show a significant increase in firing irregularity and no changes in firing frequency. A CRAC inhibitor did not improve firing regularity, but significantly increased firing frequency. Whole-cell patch clamp recordings show a significant reduction in the amplitude of Cav2.1-dependent afterhyperpolarization potential. Western blotting of cerebellar tissue showed no significant changes in UPR-related proteins. Nevertheless, tissue immunohistochemistry showed a modest but significant decrease in the UPR-related protein ATF6 in PCs.

Discussion: ER stress-related activation of calcium currents does not fully explain the firing abnormalities in the homozygous SCA6 model. The reduction in the Cav2.1-dependent AHP and the reduction in ATF6 in PCs concurrent with symptom onset is most consistent with an ER-stress response that targets Cav2.1 for degradation. The advancement of symptoms in the homozygous SCA6 mice is likely due to ER-stress related loss of Cav2.1 current and activation of store operated calcium currents.

Funding Sources: NIH. Raynor Cerebellum Project

DD22. Phenotypic characterization of BAC transgenic mice carrying SCA31 pentanucleotide repeats

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Background and objectives: Spinocerebellar ataxia type 31 (SCA31) is one of the most common autosomal-dominant cerebellar ataxias in Japan. SCA31 is caused by a complex pentanucleotide repeat containing a long stretch of TGGAA repeat within an intronic region shared by BEAN1 (brain expressed associated with NEDD4-1) and TK2 (thymidine kinase 2) genes. To characterize pathological and motor features of SCA31, we analyzed a bacterial artificial chromosome (BAC) transgenic mouse model carrying the human SCA31 repeat region.

Methods: We generated BAC transgenic mouse lines carrying the entire human BEAN1 locus derived from a homozygous SCA31 patient, thereby preserving the pathogenic repeat within its native human genomic context. Motor function was evaluated using rotarod and treadmill gait analyses at 68 weeks of age, together with histopathological analyses.

Results: BAC transgenic mice exhibited RNA foci containing UGGAA repeats co-localized with TDP-43 in Purkinje cell nuclei, consistent with pathological findings in patients with SCA31. In the rotarod test, BAC transgenic mice tended to show shorter retention times compared with wild-type (WT) mice (Tg, n = 9; WT, n = 13). In treadmill gait analysis, transgenic mice showed significantly increased maximal toe height ($P = 0.027$) and larger ankle angles at foot contact ($P = 0.040$) compared with WT mice. These gait abnormalities were consistent with previously reported mouse models of cerebellar dysfunction.

Discussion and conclusion: SCA31 BAC transgenic mice exhibited pathological and motor features consistent with cerebellar dysfunction. These findings indicate that this model recapitulates key features of SCA31 and may provide a useful platform for investigating repeat-mediated pathogenesis and therapeutic strategies in SCA31.

Funding Sources: The AMED "Practical Application Research Project for Intractable Diseases, Step 1 (Project Title: Oligonucleotide Therapeutic Development for Spinocerebellar Ataxia Type 31)

DD23. Identification of RNA molecules driving SCA1 progression in patient-derived neurons

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Background and objectives: Spinocerebellar ataxia type 1 (SCA1) is a progressive, autosomal dominant neurodegenerative disease caused by CAG repeat expansions in the ATXN1 gene. This expansion results in an abnormally elongated polyglutamine (polyQ) tract in the mutant ataxin-1 (ATXN1) protein, leading to the formation of toxic polyQ inclusion in the nucleus of affected neurons. Although polyQ aggregation is considered a major pathological hallmark of SCA1, the mechanisms driving disease progression remain incompletely understood. There is evidence that toxic aggregates in the nucleus sequester RNA molecules, potentially causing transcriptional dysregulation. Therefore, there is a critical need for cellular models that closely recapitulate the human neuronal environment to investigate the molecular mechanisms underlying SCA1.

Methods/Results: Here, we present the generation of a human-derived cellular model of SCA1. Peripheral blood mononuclear cells (PBMCs) from SCA1 patients and age/sex matched healthy controls were directly trans-differentiated into induced neural stem cells (iNSCs), which were spontaneously differentiated into neurons. In order to identify RNA molecules that bind to pathological ATXN1 and potentially contribute to disease progression, iNSC-derived neurons from SCA1 patients and controls were subjected to cross-linked RNA immunoprecipitation followed by high-throughput sequencing (RIP-Seq).

Discussion/Conclusions: In this study, we successfully generated human SCA1 neurons that represent a valuable platform for studying SCA1 pathophysiology. Cross-linked RIP-Seq enabled the identification of RNA molecules that are selectively associated with pathological ATXN1, which may play a critical role in SCA1 progression. The RNAs identified by RIP-Seq as binding partners of ATXN1 may contribute to a better understanding of disease mechanisms and represent promising targets for therapeutic development.

Funding Sources: Panhellenic Federation of Alzheimer's Disease and Related Disorders

DD24. Integrated transcriptomic and metabolomic profiling of SCA36 cerebellum reveals molecular pathways associated with neurodegeneration

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Background and objectives: Spinocerebellar ataxia type 36 (SCA36) is a late-onset dominant ataxia caused by a GGCCTG hexanucleotide repeat expansion in NOP56 gene. Current research in SCA36 is very limited, so the underlying mechanisms and molecular pathways involved are not already known. We performed the first transcriptomic and metabolic analysis in human SCA36 cerebellum in order to identify pathways involved in disease pathogenesis and potential biomarkers. In parallel, we developed zebrafish models of SCA36 to investigate pathogenic mechanisms in vivo and facilitate future therapeutic studies.

Methods: Post-mortem cerebellar tissue from SCA36 patients and healthy controls was analyzed using whole-transcriptome sequencing including mRNA, lncRNAs and small RNAs and metabolomics. Multi-omics integration was performed to identify altered molecular networks associated with neurodegeneration. Findings were compared with molecular signatures obtained from zebrafish models of SCA36 to identify conserved disease-associated pathways across species.

Results: Integrated multi-omics analyses revealed significant molecular alterations affecting multiple biological processes in SCA36 cerebellum. Whole transcriptomic analyses provided a comprehensive characterization of coding and non-coding RNA alterations affecting multiple biological pathways while metabolomic profiling identified dysregulated pathways related to cellular metabolism and stress responses. Comparative analyses with zebrafish models identified shared molecular signatures, highlighting conserved pathways involved in disease pathogenesis. These findings indicate the utility of zebrafish as a relevant experimental model for investigating SCA36 mechanisms and therapeutic targets.

Discussion and Conclusion: This study represents the first integrated transcriptomic and metabolomic analyses of human SCA36 cerebellum. The combination of human brain tissue studies and zebrafish models provides a unique opportunity to identify molecular mechanisms underlying SCA36 and to uncover candidate biomarkers and therapeutic targets. These findings will contribute to a more comprehensive understanding of disease biology and may reveal pathways shared with other ataxias and neurodegenerative diseases.

Funding Sources: This work has been supported by FRC (Fédération pour la Recherche sur le Cerveau) project: Translation of repeat expansion mutations into toxic proteins as a common pathogenic mechanism in microsatellite neurodegenerative diseases and a postdoctoral fellowship from the Xunta de Galicia (2025) to the presenting author.

DD25. Transcriptomic analysis of SCA4 patient-derived neurons reveals disrupted synaptic, metabolic, and cell-death pathways

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Background and Objectives: Spinocerebellar ataxia type 4 (SCA4) is a rare neurodegenerative disorder characterized by progressive cerebellar ataxia, sensory neuropathy, and degeneration of the cerebellum and sensory ganglia. SCA4 is caused by a pathogenic GGC-repeat expansion in ZFH3, which encodes an expanded polyglycine tract. ZFH3 loss-of-function variants have been linked to neurodevelopmental disorders and childhood epilepsy, while altered ZFH3 function has also been implicated in atrial fibrillation and associated metabolic and calcium-handling defects. To identify disease-associated pathways downstream of expanded ZFH3, we performed transcriptomic profiling of SCA4 patient-derived neurons.

Methods: We generated iPSC lines from three SCA4 patients and two unaffected controls, engineered them for inducible NGN2 expression, and differentiated them into neurons. After three weeks in culture, triplicate samples from each subject were collected for RNA sequencing.

Results: SCA4 neurons expressed neuronal and glutamatergic markers, supporting successful neuronal differentiation. Differential gene expression analysis identified 23 upregulated and 14 downregulated genes in SCA4 neurons compared with controls (adjusted p-value <0.05). Gene Set Enrichment Analysis revealed negative enrichment of pathways involved in neuronal identity and function, including axon and dendrite growth, synaptic organization, neurotransmitter transport, vesicle exocytosis, ion channel activity, and glutamatergic synapse function. SCA4 neurons showed positive enrichment of oxidative phosphorylation, electron transport chain activity, ATP synthesis, protein synthesis, DNA repair, p53-associated programs, apoptosis, and cell-death signaling.

Discussion and Conclusion: These findings indicate that SCA4 patient-derived neurons exhibit impaired neuronal and synaptic programs, altered mitochondrial and metabolic activity, increased protein synthesis, DNA damage responses, and activation of cell-death pathways. This transcriptomic profile suggests that both loss- and gain-of-function mechanisms involving ZFH3 may contribute to SCA4 pathogenesis.

Funding Sources: NINDS R01NS137233, R33NS124965, R35NS127253, R21NS128630.

DD26. Identity-by-Descent Analysis Refines the Ancestral History of the ZFH3GGC Repeat Expansion Underlying Spinocerebellar Ataxia Type 4

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Background and Objectives: Spinocerebellar ataxia type 4 (SCA4) is a rare, autosomal dominant neurodegenerative disorder caused by a pathogenic GGC repeat expansion in ZFH3. While initial identity-by-state (IBS) mapping suggested the mutation arose from an ancient founder event approximately 88.5 generations ago (~2,200 years), its low prevalence and geographic clustering in the United States challenge this timeline. This study aimed to utilize high-resolution pairwise identity-by-descent (IBD) analysis to resolve the fine-scale genetic relationships between American families and recalculate the mutation's age.

Methods: We analyzed single nucleotide polymorphism (SNP) microarray data from 84 individuals representing three distinct US families (Utah, Georgia, and Los Angeles). Quality-filtered genotypes (117,893 SNPs) underwent pairwise IBD analysis using XIBD against a European reference panel. Haplotypes spanning chromosome 16 were phased using Beagle 5.5. The time to the most recent common ancestor (MRCA) was estimated using a likelihood-based model that assumes a correlated genealogy.

Results: IBD network analysis revealed two primary carrier clusters (Utah vs. Georgia/Los Angeles) bridged by only two distant relative pairs sharing minimal segments (<1 cM). Mapping these shared regions defined a minimum overlapping interval of 690 Kb centered on ZFH3. Within this region, a core 67-SNP founder haplotype was conserved across all 30 expansion carriers. Likelihood modeling placed the MRCA at 12.4 generations (95% CI: 3.9–43.5), translating to approximately 310 years ago.

Discussion and Conclusion: Our findings revise the origin of the US ZFH3 expansion from an ancient event to a recent founder effect occurring ~310 years ago. This younger timeline explains the mutation's rarity compared to ancient expansions like C9orf72. Defining this localized founder haplotype provides the essential genetic architecture needed to develop targeted, variant-specific therapies for SCA4.

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DD27. Integrated metabolomic and transcriptomic profiling reveals broad molecular remodeling in SCA36

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Background and objectives: Spinocerebellar ataxia type 36 (SCA36) is an adult-onset neurodegenerative ataxia caused by a (GGCCTG)_n repeat expansion in intron 1 of NOP56. The broader molecular alterations associated with disease status remain insufficiently characterized. This study aimed to provide a general multi-omics overview of disease-associated metabolic and transcriptomic signatures in SCA36.

Methods: Metabolomic profiling using UHPLC-QTOF-MS was performed in SCA36 and non-SCA36 samples and analyzed using hierarchical clustering, differential metabolite analysis, and variable importance in projection ranking. Transcriptomic profiling was performed by paired-end short-read bulk RNA sequencing. Transcriptomic landscape of SCA36 was characterized by analyzing differential gene expression and gene set enrichment analysis. Results were visualized using principal component analysis, heatmaps, volcano plots, and Gene Ontology pathway enrichment summaries to identify global disease-associated patterns.

Results: Metabolomic analysis revealed a distinct separation between SCA36 and non-SCA36 samples, indicating a disease-associated metabolic signature. Differential metabolites involved multiple biochemical categories, including amino acid, nucleotide-related, redox-associated, energy-related, lipid-related, and neurotransmitter-related metabolites. Several amino acid and amino acid-related metabolites ranked among the most discriminating features.

Transcriptomic analysis similarly distinguished SCA36 from non-SCA36 samples, with differentially expressed genes forming clear disease-associated expression clusters. Pathway-level analysis showed increased enrichment of nucleotide metabolism, RNA metabolism, and DNA/RNA stress signatures in SCA36. In contrast, transcriptomic analysis revealed relative suppression of several cellular homeostasis-related pathways, including lipid membrane, oxidative stress, autophagy-lysosome, apoptosis/cell death, neurodegeneration-related, mitochondrial energy, amino acid metabolism, and ubiquitin-proteasome pathways.

Discussion and Conclusion: Together, metabolomic and transcriptomic profiling consistently indicate that SCA36 is associated with broad molecular remodeling rather than alteration of a single pathway. The convergent findings highlight coordinated changes in amino acid and nucleotide metabolism, RNA-related stress responses, and multiple cellular homeostasis pathways. This integrated multi-omics overview provides a framework for future studies exploring disease mechanisms, biomarkers, and therapeutic targets in SCA36.

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DD28. Calpain-mediated TBP cleavage disturbs neuronal AMOT-YAP1 signalling in spinocerebellar ataxia type 17

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Background and Objectives: Spinocerebellar ataxia type 17 (SCA17) is a neurodegenerative disorder caused by expansion of a polyglutamine (polyQ) tract in the transcription factor TATA-box-binding protein (TBP), which is essential for neuronal function. While disease mechanisms remain incompletely understood, we previously identified calcium-dependent calpains as key modulators, showing their overactivation and enhanced TBP cleavage in SCA17 models. Here, we aimed to define calpain-dependent cleavage sites and elucidate the role of resulting TBP fragments in disease pathogenesis.

Methods: We combined in silico, in vitro, and cell-based approaches with mass spectrometry to identify TBP cleavage sites. TBP fragment constructs were expressed in cellular models to assess aggregation, localization, and effects on calpain activation and cell viability. Multi-omics analyses were performed in SCA17 cell systems and complemented by studies in patient-derived fibroblasts, induced neurons, and animal models.

Results: We identified a major calpain cleavage site after threonine 106 (T106), generating polyQ-containing N-terminal and C-terminal TBP fragments. N-terminal fragments mislocalized to the cytoplasm and showed strong aggregation, whereas C-terminal fragments localized mainly to the nucleus and displayed rapid turnover. Both fragment types contributed to calpain overactivation. Multi-omics analyses revealed disruption of pathways linked to neuronal function and neurodegeneration and identified angiomin (AMOT) as a key downstream factor. Follow-up studies in cellular and in vivo models (rat and zebrafish) confirmed a pivotal neuronal role of AMOT, regulated by TBP and mediated via YAP1-dependent signaling.

Discussion and Conclusion: These findings highlight calpains as central drivers of SCA17 pathogenesis and demonstrate that TBP fragments actively contribute to cellular toxicity. Identification of the TBP-AMOT-YAP1 axis provides new insight into TBP function and suggests broader relevance for neurodegenerative and neurodevelopmental disorders, offering potential therapeutic targets.

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DD29. Ngn2-derived induced neurons as a model for drug screening in Spinocerebellar ataxia type 3

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Background and Objectives: Spinocerebellar ataxia type 3 (SCA3) is the most common autosomal dominant ataxia. Clinically, SCA3 is characterized by progressive cerebellar degeneration. Molecularly, it results from a CAG repeat expansion within the ATXN3 gene, leading to an expanded polyglutamine tract in the ataxin-3 protein. The mutant protein misfolds and aggregates, undergoes excessive proteolytic cleavage, disrupts protein quality-control pathways, impairs mitochondrial function, and ultimately causes selective neurodegeneration. Developing neuronal cell models for SCA3 is therefore essential for dissecting disease mechanisms and facilitating drug discovery for this currently incurable disorder. To this end, we focused on establishing a neuronal model to investigate the molecular pathogenesis of SCA3 and evaluate therapeutic strategies, including potentially repurposable drugs currently used to treat other diseases.

Methods: We present a neurogenin 2 (Ngn2)-induced neuronal model (iNeurons) of SCA3 generated using a rapid differentiation protocol that yields mature early neurons within just a few days and without the need for supporting cells. Using protein biochemical and microscopy-based analyses, we validated the iNeurons as a model for studying the molecular pathogenesis of SCA3. This approach provides a practical alternative to classical iPSC-derived neuronal differentiation, avoiding the lengthy and labor-intensive procedures associated with conventional protocols.

Results: We demonstrate that SCA3Ngn2iNeurons recapitulate key molecular phenotypes of SCA3, including altered protease activity and impaired mitochondrial function, underscoring the model's suitability for reproducible mechanistic studies and potential drug screening. We further validated the model using compounds targeting pathways known to be disrupted in SCA3, showing that SCA3Ngn2iNeurons are functional and responsive to pharmacological intervention.

Discussion and Conclusion: We establish Ngn2-derived iNeurons as a practical and scalable human model of SCA3 and a valuable alternative to traditional iPSC-derived neuronal systems, enabling more rapid translational progress toward targeted interventions for SCA3 and potentially other neurodegenerative disorders.

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DD30. Wild Type ATXN3 reduces Neuroinflammation in SCA3

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Background and Objectives: Spinocerebellar ataxia type 3 (SCA³) is a dominant disorder caused by a CAG repeat expansion in the ATXN3 gene, encoding expanded polyglutamine ATXN3 that accumulates and aggregates in the brain. The contribution of the normal Atxn3 allele to disease modulation remains poorly understood. We investigated whether loss of the wild-type Atxn3 allele exacerbates transcriptional dysregulation and neurodegenerative pathways in a SCA3 mouse model.

Methods: SCA3 knock-in mice carrying an expanded CAG repeat allele (~230 repeats) were crossed with Atxn3 knockout mice to generate five genotypes: Atxn3Q6/Q6, Atxn3Q6/-, Atxn3-/-, Atxn3Q230/Q6, and Atxn3Q230/-. Genotypes were confirmed by PCR analysis of tail DNA. Bulk-RNA sequencing was performed on brainstem tissue from ~ 40 weeks old mice (n = 3/genotype), when mice harboring the Atxn3Q230 allele exhibited pronounced motor impairment. Differential expression analysis was conducted using DESeq2, followed by pathway and gene ontology enrichment analyses using iPathwayGuide.

Results: Atxn3Q230/- mice (expressing a sole mutant Atxn3 allele (Q230)) exhibited greater transcriptional dysregulation than Atxn3Q230/Q6 mice (expressing one mutant (Q230) and one wildtype (Q6) Atxn3) with 1,517 and 1,319 differentially expressed genes, respectively, relative to Atxn3Q6/Q6 controls identified in the brainstem. Both genotypes showed increased innate immune responses, regulation of interleukin-6 production and complement-mediated pruning compared to controls. However, the brainstem of Atxn3Q230/- mice showed further unique activation of interleukin-1 β production and integrin-mediated processes, accompanied by upregulation of inflammasome-associated genes including Casp1 and Casp4, suggesting that loss of the wild-type Atxn3 allele enhances innate immune activation characterized by inflammasome signaling and IL-1 β -associated inflammatory pathways.

Discussion and Conclusion: These findings indicate that loss of the wild-type Atxn3 allele amplifies inflammasome associated neuroinflammation in the brainstem of SCA3. Together, these results support a modulatory role for normal ATXN3 in neuroimmune responses.

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DD31. The effect of lysosomal, transmembrane protein SIDT2 on the reduction of mutant ataxin-3 in spinocerebellar ataxia type 3

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Background and Objectives: Despite ongoing therapeutic efforts, effective strategies for treating the progressive and fatal neurodegenerative disorder spinocerebellar ataxia type 3 (SCA3) remain limited. The removal of pathogenic forms of the disease-associated polyglutamine (polyQ)-expanded ataxin-3 (Atx3) protein may represent a promising strategy to delay disease progression and improve the quality of life for individuals with SCA3. SIDT2, a heavily glycosylated, lysosomal transmembrane protein, has been reported to mediate the degradation of CAG-rich mRNAs via RNA autophagy. Notably, SIDT2 has been shown to reduce levels of both soluble and aggregated polyQ-expanded huntingtin exon 1 through this mechanism, highlighting its potential as a therapeutic modulator of polyQ-related toxicity in Huntington's disease. Building on these findings, we aim to investigate the role of SIDT2 in regulating polyQ-expanded Atx3 in SCA3.

Methods: We employed protein biochemistry techniques (Western blot, filter-retardation assays, co-immunoprecipitation), RNA-based analysis (qPCR), and omics-based approaches (proteomics, interactomics, transcriptomics) to analyse the mechanisms underlying SIDT2-dependent lowering of Atx3 levels in cellular SCA3 models.

Results: We demonstrate a SIDT2-dependent reduction of soluble, oligomeric, and aggregated polyQ-expanded Atx3 protein in SCA3 cellular models. Our qPCR-based and transcriptomic analyses suggest that this reduction is mediated by RNA autophagy. In parallel, proteomics and interactome data reveal a complex interplay of post-translational modifications and protein-protein interactions that are essential for SIDT2's cellular function.

Discussion and Conclusion: This study advances our understanding on the molecular mechanisms underlying SIDT2-mediated lowering of polyglutamine-expanded Atx3. Our findings introduce a novel perspective for therapeutic intervention. If validated in vivo, this project may lay the groundwork for the development of SIDT2-based therapeutic strategies, with broader relevance for SCA3 and other polyglutamine-associated spinocerebellar ataxias.

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DD32. CACNA1A-related disorders: genotype-phenotype analysis in a cohort of 463 patients

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Background and objectives: Pathogenic single-nucleotide variants and small indels in CACNA1A are associated with a broad phenotypic spectrum characterized by marked inter- and intrafamilial variability, which remains poorly understood. We investigated genotype–phenotype correlations in a large CACNA1A cohort.

Methods: Clinical and genetic data were collected through the international SCANOP network. We included patients carrying a pathogenic CACNA1A variant, excluding CAG expansions and hemiplegic migraine phenotype.

Results: We included 463 patients carrying 188 pathogenic CACNA1A variants: 62% patients had missense variants (36% voltage sensor S4, 31% P-loop, and 11% pore forming S5-S6domains), 26% stop variants, 6.5% splicing variants, and 4% intragenic CNV. Three recurrent variants (>10 index cases) were identified, p.Arg583Gln, p.Thr666Met, p.Arg1346Gln, not associated with congenital or neurodevelopmental disorders.

The median age at onset was 17.4 years [0-76], without association with variant type ($p=0.8$). Position of stop variants did not explain variability of age at onset (12.0 years [0-63], $p=0.15$). Missense variants located in the S4domain were associated with a later age at onset (12 to 22 years later, $p<0.01$).

Episodic ataxia occurred in 48% (155/320) of patients and was less frequently associated with missense variants ($p<0.001$) than with other types of variants. Chronic ataxia occurred in 64% (197/310), neurodevelopmental disorders in 43% (143/333), and epilepsy in 18% (48/267) of patients without correlations with types of variants. Ongoing structural analysis of 93 missense variants based on the 8X90crystal structure is assessing their impact on protein stability, with preliminary results predicting 44 destabilizing, 43 neutral, and 6 stabilizing variants.

Discussion and conclusion: Phenotypic variability in CACNA1A-related disorders is only partially explained by variant location and is not predicted by variant type. These findings support the contribution of additional factors, including genetic modifiers, to disease expression, and highlight the need for integrative approaches to improve genotype-based prognostic stratification.

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DD33. Spinocerebellar ataxia type 27B predominantly manifests as an astrogliaopathy

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Background and objectives: Spinocerebellar ataxia type 27B (SCA27B), caused by an intronic GAA expansion in FGF14, is the most common late-onset cerebellar ataxia. We performed neuropathological and plasma biomarker analyses in patients with SCA27B compared with other ataxia subtypes, astrocyte-associated diseases, and controls.

Methods: Plasma samples were collected from 30 patients with SCA27B and 145 patients with polyglutamine SCAs. GFAP and NfL levels were quantified using the Simoa® HD-1/Human Neurology 2-Plex assay. Neuropathological examination was performed on 22 brains (eight SCA27B, one SCA1, two SCA3, two SCA7, one Friedreich ataxia, two fragile X-associated tremor/ataxia syndrome, one aging-related tau astrogliaopathy, and five controls).

Results: Plasma biomarker analyses demonstrated higher GFAP levels in SCA27B patients than in polyglutamine SCAs (215 pg/ml [138;290] vs 104 pg/ml [69; 140], $p < 0.01$), and remained increased after age-adjustment ($+ 45 \pm 18$ pg/mL, $p = 0.016$). Compared with other cases, post-mortem examination of SCA27B patients revealed a reduced astrocytic fibrillar network and focal Purkinje cell loss, predominantly affecting the anterior vermis. Aquaporin-4 (AQP-4) showed perineuronal positivity in the Bergman glia resulting in an aberrant “pompom-like” accumulation around Purkinje cells. Significant regional differences were observed overall, with the inferior vermis (lobule VIII) showing the highest density of AQP4-positive pompom lesions ($p < 0.01$), which was positively correlated with disease duration (Spearman's $\rho = 1.0$, $p < 0.01$). In contrast, in the anterior vermis (lobule I-IV), the density of AQP-4-positive perineuronal lesions was significantly negatively correlated with GAA repeat size (Spearman's $\rho = -0.90$, $p < 0.05$). FGF14 expression in the adult human cerebellum was similarly high in neurons and astrocytes, namely in Bergman glia, that is not the case in the mouse brain, suggesting an evolutionary gain of function.

Discussion and conclusion: Increased plasma GFAP levels and the presence of marked astrogliaopathy support a key role for astrocytic dysfunction in the pathogenesis of SCA27B.

Funding Sources: iCRIN NEUROPAGE funding (Alexandra Durr & Danielle Seilhean)

DD34. TUDCA treatment reshapes brainstem lipidome in a mouse model of SCA3

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Background and objectives: Spinocerebellar ataxia type 3 / Machado-Joseph disease is an expanded polyglutamine-associated neurodegenerative disease characterized by motor dysfunction and neuronal loss. Although several aspects of the pathogenic mechanism underlying this disease have been mapped, lipid metabolism alterations remain underexplored. This study aimed to characterize the brainstem lipidomic profile in a validated mouse model of SCA3 and to investigate the impact of tauroursodeoxycholic acid (TUDCA) treatment on this lipidomic profile.

Methods: Brainstem samples from 24-weeks old WT (n=8), CMVMJD135 (n=7) and CMVMJD135+TUDCA (n=7) mice were analyzed by LC-MS/MS-based lipidomics. Mice were treated for 19 weeks, daily, with TUDCA 50 mg/kg or with vehicle, by intraperitoneal injection. Lipid classes and individual species were compared between groups to identify disease- and treatment-associated changes.

Results: Mid-stage SCA3 animals (24-weeks old) showed a clear brainstem lipidomic shift. At the lipid class level, transgenic mice displayed reduction in several structural and signalling-related lipids, including phosphatidic acid, phosphatidylcholine, ether-linked phosphatidylcholine, sphingomyelin, sulfatide and acylcarnitine. In contrast, various lysophospholipid classes, phosphatidylserine and N-acyl-serine were increased. At the species level, among the multiple observed changes, SCA3 mice showed more marked increased levels of N-acyl-serine, phosphatidylserine and phosphatidylglycerol species. TUDCA selectively reshaped specific lipid classes and species, particularly lysophospholipids, acylcarnitines, triacylglycerols and N-acyl-phosphatidylserines.

Discussion and conclusion: The brainstem lipidomic signature observed in CMVMJD135 mice suggests that lipid dysregulation may be more than a downstream consequence of neurodegeneration. The disruption of the brainstem lipidome points to altered membrane homeostasis, lipid signalling and cellular stress responses as convergent features of disease. These findings identify lipid metabolism as a disease-relevant and potentially as a targetable axis in SCA3.

Funding Sources: National Ataxia Foundation (NAF). Sara Duarte Silva (CEECIND/00685/2020)

DD35. Serum proteomics identifies Brorin as a novel disease-associated protein in SCA3: from human cohorts to gene therapy in a preclinical model

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Background/objectives: Spinocerebellar Ataxia Type 3 (SCA3), one of the most common dominantly inherited ataxias worldwide, is a progressive neurodegenerative disease caused by a CAG repeat expansion in the ATXN3 gene. The molecular mechanisms driving disease progression remain incompletely understood, limiting the identification of therapeutic targets and robust biomarkers. We aimed to identify novel disease-associated proteins in SCA3 through a proteomic approach and investigate their biomarker and therapeutic relevance.

Methods: Serum proteomic profiling of 1,000 proteins was performed in 12 controls (CTRL), 10 pre-ataxic (PA) mutation carriers, and 10 ataxic (A) SCA3 patients using a commercial array. Differentially abundant proteins were identified and used to select 15 candidates for validation in a larger cohort (CTRL, n=49; PA, n=36; A, n=54). One lead candidate was further characterized in a transgenic SCA3 mouse model treated with an ATXN3-silencing gene therapy.

Results: Proteomic screening identified 36, 66, and 82 differentially abundant proteins between CTRL and PA, CTRL and A, and PA and A individuals, respectively. Among these, brorin, a bone morphogenetic protein predominantly expressed in the brain, emerged as a novel biomarker and therapeutic target candidate. Serum brorin levels were significantly decreased in SCA3 patients compared with controls. In a transgenic SCA3 mouse model, brorin mRNA and protein levels were increased in the cerebellum, and a negative correlation between cerebellar and serum brorin levels was observed. Following administration of ATXN3-silencing gene therapy, cerebellar brorin mRNA levels decreased in a dose-dependent manner. Importantly, reduced cerebellar brorin expression correlated with improved motor performance, linking brorin levels to therapeutic response.

Discussion/conclusion: Our findings identify brorin as a novel SCA3-associated protein detectable in serum. Its dysregulation in the cerebellum, responsiveness to ATXN3-silencing therapy, and correlation with motor outcomes implicate brorin in disease-relevant pathways and support its further investigation as a potential therapeutic target and pharmacodynamic biomarker in SCA3.

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DD36. Transgenic SCA10 mouse models show a phenotype that closely recapitulate human SCA10 depending on the different internal repeat unit composition.

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Background & Objective: Spinocerebellar ataxia type 10 (SCA10) is an autosomal dominant neurodegenerative disorder found in the Americas and East Asia, characterized by progressive cerebellar ataxia variably accompanied by epilepsy. Although SCA10 is attributed to an expanded intronic ATXN10(ATTCT)_n repeat, recent studies suggest that heterogeneous 3' repeat interruptions, including (ATTCC)_n or (ATCCT)_n(ATCCC)_n, influence phenotypic severity. We aimed to develop transgenic mouse models to investigate pathogenic mechanisms driven by these 3' heterogeneous repeats and to support preclinical therapeutic studies.

Methods: We generated transgenic mice expressing three SCA10 repeat expansion structures: Type A, consisting of (ATTCT)_n with scattered heptanucleotide ATATTCT, TTATTCT, and CTATTCT units; Type B, consisting of 5'-(ATTCT)_n followed by 3'-(ATTCC)_n; and Type C, consisting of 5'-(ATTCT)_n followed by 3'-(ATCCT)_n(ATCCC)_n. Each expanded repeat, derived from different SCA10 patients and cloned in somatic cell hybrid lines, was inserted into an artificial intron within an enhanced GFP reporter gene. Transgene expression was driven by either the neuron-specific enolase promoter or the Purkinje cell-specific PCP2 promoter.

Results: Type C transgene expression produced eGFP, indicating expected splicing of the artificial intron. Type C mice developed progressive ataxia, convulsive seizures, Purkinje cell degeneration, and shortened lifespan, recapitulating key features of human SCA10. Both neuron-wide and Purkinje cell-specific Type C models showed similar phenotypes, supporting a Purkinje cell-autonomous pathogenic mechanism, although the neuron-wide model was more severe. In contrast, Type A and Type B mice showed no detectable clinical phenotype. Histopathology confirmed selective Purkinje cell loss and abnormal dendritic arborization. FISH demonstrated widespread AUUCU RNA foci in neuron-wide Type C animals.

Discussion & Conclusions: These SCA10 mouse models provide useful tools for pathogenic studies and preclinical therapeutic development, highlighting the importance of repeat structure in disease severity and long-read sequencing for resolving complex repeat expansions.

Funding Sources: NIH (NS083564, NS1150024)

DD38. Mutation- and cell-type-dependent neuronal network dysfunction in DRPLA

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Background and objectives: Dentatorubral-pallidoluysian atrophy (DRPLA) is a rare, progressive neurodegenerative disorder caused by a CAG expansion within the ATN1 gene. DRPLA is characterized by ataxia, epilepsy, cognitive decline, and astrogliosis; however, the disease mechanisms underlying these manifestations remain poorly understood. Here, we developed a clinically relevant human induced pluripotent stem cell (iPSC)-based platform to model DRPLA-associated epileptiform phenotypes and dissect the cellular mechanisms contributing to disease pathology. Antisense oligonucleotide (ASO)-induced ATN1 knockdown and CAG repeat expansion correction were used as experimental tools to further interrogate the contributions of ATN1 mutation to disease-associated phenotypes.

Methods: We generated multicellular neuro-glial co-cultures from juvenile-onset DRPLA patient-derived iPSCs (70/23Q) and gene-edited isogenic controls (15/23Q), consisting of excitatory and inhibitory neurons and astrocytes. To investigate non-cell-autonomous mechanisms underlying neuronal network dysfunction, we defined co-cultures combining excitatory and inhibitory cortical neurons and astrocytes with mixed ATN1 CAG expansion status. Network activity was assessed using live-cell GCaMP6 calcium imaging and microelectrode array (MEA) electrophysiology. ATN1 expression and CAG expansion were additionally manipulated using (i) ASOs targeting and (ii) CRISPR-Cas13d-mediated RNA targeting of expanded CAG repeats.

Results: Preliminary analyses revealed that DRPLA excitatory-inhibitory-astrocytic tri-cultures exhibit impaired neuronal function characterized by reduced neuronal firing rates and shortened network burst length compared to gene-corrected controls. Notably, replacing DRPLA astrocytes with gene-corrected astrocytes was sufficient to normalize these neuronal activity features, despite the continued presence of the DRPLA neurons. These findings suggest a prominent non-cell-autonomous astrocytic contribution to DRPLA pathophysiology. Our ongoing studies examine ATN1 knockdown and CAG expansion in these complex excitatory-inhibitory astrocytic co-cultures.

Discussion and Conclusion: Together, these cellular models provide a platform to investigate the non-cell-autonomous mechanisms underlying DRPLA-associated neuronal network dysfunction. Our initial findings support the contribution of astrocytes to the disease pathophysiology and establish a framework for future studies dissecting the cell-type-specific mechanisms in DRPLA.

Funding Sources: National Ataxia Foundation, Silva Ataxia Foundation

DD41. NOVEL GENETIC MODIFIERS OF SCA3/MJD: AN EMS SCREENING IN A C. ELEGANS MODEL OF THE DISEASE.

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In Spinocerebellar ataxia type 3 (SCA3)/Machado-Joseph disease (MJD), an abnormal CAG repeat expansion in ATXN3 gene causes cerebellar ataxia of late-onset. Considering that the CAG repeat size explains about half of the disease heterogeneity, additional modifier loci must predict the remaining phenotypic variability verified in SCA3/MJD, namely at the age at onset (AO), clinical presentation and disease severity.

The identification of novel modifier genes, potentially amenable to be targeted pharmacologically, 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 feature of the disease.

The model was treated with EMS to introduce random mutations into its genome. We then isolated mutants from the F2 progeny that displayed an improved motor phenotype. Various motor behavioral assays were conducted to assess the impact of the EMS-induced mutations on motor function and mutant ATXN3 aggregation. Whole genome sequencing of mutants revealed potential gene modifiers with neuronal expression. Among these candidates, *clh-2*, which encodes a chloride channel in C. elegans, emerged as a particularly relevant genetic modifier of the disease phenotype. Importantly, *clh-2* is orthologous to the mammalian *CLCN2* gene, which encodes the voltage-gated chloride channel *ClC-2*. The identification of *clh-2/CLCN2* highlights a conserved molecular pathway that may influence neurodegeneration and disease progression in SCA3/MJD. Given the known roles of *ClC-2* channels in neuronal excitability, ion homeostasis, and brain function, *CLCN2* represents a strong candidate for further validation in patients.

Identifying genetic modifiers of SCA3/MJD offers significant promise in uncovering new genetic factors that influence the disease. Most importantly, these genetic pathways can serve as potential targets for novel drug development, opening up new therapeutic possibilities for patients affected by this debilitating disorder.

Funding Sources: National Ataxia Foundation

DD42. A gene-edited isogenic iPSC panel recapitulates neuronal dysfunction in a novel human model of spinocerebellar ataxia type 3

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Background and objectives: Spinocerebellar ataxia type 3 (SCA3) is a dominantly inherited neurodegenerative disorder caused by an abnormal CAG expansion in the ATXN3 gene. SCA3 patient-derived iPSC models are valuable but limited by inter-individual phenotypic variability and inefficient, heterogeneous neuronal differentiation. To address these hurdles, we developed a precise genome editing approach and a rapid direct neuronal conversion method to generate isogenic SCA3 neurons with robust disease-associated phenotypes.

Methods: We developed a novel, selection-marker-free approach to precisely edit the ATXN3-CAG expansion in SCA3 patient iPSC lines by co-delivery of CRISPR/Cas9 ribonucleoprotein and AAV donors for targeted knock-in of CAG tracts into ATXN3 exon 10. Editing was assessed by Sanger sequencing and isogenic lines validated by PCR and western blot. Isogenic iPSCs were directly converted into glutamatergic induced neurons (iNeurons) via Neurogenin-2 overexpression for functional characterization.

Results: Our editing method achieved 25–30% ATXN3-CAG editing in the bulk population. To better correlate ATXN3-CAG genotype-phenotype, we performed clonal selection to attain a complete SCA3 isogenic panel comprising a monoallelic CAG-expanded patient line, a biallelic CAG-corrected control, and a biallelic CAG-expanded line to accelerate disease phenotype. Homogeneous iNeurons expressed mature neuronal and synaptic markers and remained viable >90 days. CAG-expanded iNeurons exhibited reduced dendritic complexity and synaptic density versus patient and corrected lines. Multi-electrode array recordings revealed reduced firing rate but increased network burst frequency in CAG-expanded iNeurons, suggesting dissociation between intrinsic excitability and network synchronization.

Discussion and conclusion: We developed an effective, selection-marker-free method to precisely edit ATXN3-CAG expansions, with potential for autologous cell therapy across polyglutamine disorders. Our SCA3 isogenic iNeuron model recapitulates early hallmarks—synaptic loss, dendritic impairment, and distinct electrophysiological dysfunction—under basal conditions. Ongoing work investigates the contribution of astrocytes in driving neuronal dysfunction. Our platform enables precise genotype-phenotype correlation and offers a scalable tool for therapeutic discovery.

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DD43. CHIP Mislocalization and Dysregulated Nuclear Shuttling in Spinocerebellar Ataxia Type 48

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Spinocerebellar ataxias (SCAs) are progressive neurodegenerative disorders linked to disruptions in protein quality control (PQC). The C-terminus of Hsc70Interacting Protein (CHIP), encoded by STUB1, is a critical E3ubiquitin ligase and co-chaperone essential for degrading aberrant proteins and maintaining proteostasis. STUB1mutations cause SCA type 48 (SCA48), and loss of CHIP function is associated with accelerated neurodegeneration. This study aimed to characterize the spatiotemporal consequences of SCA48-associated CHIP mutations on nucleocytoplasmic shuttling and stress-response signaling. Wild-type (WT) CHIP and three SCA48mutants (G33S, P228S, L275Dfs16) were overexpressed in cell models. Heat shock responses were assessed using a dual-luciferase reporter assay driven by heat shock elements. Subcellular localization of CHIP and HSF1was evaluated by fractionated immunoblotting and immunofluorescence under basal (37°C) and heat shock conditions. WT CHIP overexpression enhanced HSF1transcriptional activity and facilitated nuclear co-translocation of both CHIP and HSF1during heat shock. All SCA48mutants preserved or augmented heat shock transcriptional responses and HSF1nuclear translocation comparable to WT. However, heat shock-induced nuclear translocation of CHIP itself was abolished across all mutants. Strikingly, under basal conditions (37°C), all mutants exhibited elevated nuclear CHIP accumulation relative to WT, suggesting misregulated nucleocytoplasmic retention. U-box mutants (P228S, L275Dfs16) maintained or increased HSF1nuclear levels despite reduced CHIP nuclear entry during heat shock. These findings demonstrate that SCA48mutations disrupt CHIP's dynamic nucleocytoplasmic shuttling, causing aberrant basal nuclear retention while impairing heat shock-induced nuclear import. The dissociation between CHIP and HSF1nuclear translocation suggests CHIP-independent mechanisms can sustain the acute heat shock response; however, heightened HSF1activity in U-box mutants may reflect a dysregulated or gain-of-toxic-function stress response rather than simple loss of function. Collectively, these data implicate impaired CHIP nuclear trafficking as a mechanistic feature of SCA48and identify CHIP's noncanonical nuclear functions as a potential therapeutic target in SCA-related neurodegeneration.

Funding Sources: NAF Postdoctoral Fellowship - 1379501

DD44. Characterizing Regional Microglial Heterogeneity Across Disease Progression in Spinocerebellar Ataxia Type 1 (SCA1)

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Spinocerebellar Ataxia Type 1 (SCA1) is a fatal, inherited neurodegenerative disease caused by CAG repeat expansion in the ATXN1 gene. The resulting polyglutamine-expanded ATXN1 protein drives progressive cerebellar Purkinje cell dysfunction and loss, leading to motor impairment. Despite three decades since the genetic cause was identified, no disease-modifying therapies exist. Although SCA1 pathology prominently affects the cerebellum, it also involves cortical regions and non-neuronal cell types. The contribution of microglia, the brain's resident immune cells, remains poorly defined. In other neurodegenerative diseases, microglia adopt diverse activation states that are neuroprotective or neurotoxic depending on local environment and disease stage. Recent findings that microglial depletion yields modest motor improvement in SCA1 mice implicate microglial involvement, yet it remains unclear whether activation is primarily reactive or causally contributes to neurodegeneration, and whether responses differ across brain regions. We hypothesize that microglia develop distinct, region-specific activation programs across SCA1 disease stages, differentially influencing neurodegeneration in the cerebellum and cortex. Using the Atxn1^{154Q/2Q} knock-in mouse model, we will isolate microglia from cerebellum and cortex at 12 and 24 weeks by FACS, followed by bulk RNA-sequencing and gene ontology analyses to identify region- and stage-specific transcriptional signatures. We will validate transcriptional changes using quantitative morphological profiling with the MicrogliaMorphologyR pipeline and targeted immunofluorescence. To test functional contributions, we will perform stage-dependent microglial depletion and repopulation using the CSF1R inhibitor PLX5622, followed by motor behavioral testing and neuropathological assessment. Preliminary morphological data show a significant genotype-by-cluster interaction in cerebellar microglia and a cortical shift toward amoeboid morphology in SCA1 mice not observed in the cerebellum. Transcriptional profiling and longitudinal morphology analyses are ongoing. This study will provide the first comprehensive, region-resolved characterization of microglial heterogeneity in SCA1, defining stage-specific neuroinflammatory programs and informing immunomodulatory therapeutic strategies relevant to SCA1 and other polyglutamine ataxias.

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DD45. Suppressing somatic CAG repeat instability rescues motor deficits and extends lifespan in SCA1 mice

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Spinocerebellar Ataxia Type 1 (SCA1) is a neurodegenerative disorder caused by an expanded CAG repeat in the ATXN1 gene. While inherited repeat length correlates inversely with age at onset, whether progressive somatic CAG expansion contributes to SCA1 pathogenesis remains unclear. Based on recent discoveries in Huntington's disease (HD), we hypothesized that somatic CAG expansions also contribute to SCA1 disease onset and/or progression.

To test this hypothesis, we used the f-ATXN1146Q/2Q mouse model, which recapitulates multiple SCA1-like motor and molecular phenotypes, including age-dependent and tissue-specific somatic CAG instability. To identify modifiers of instability, we first performed an in vivo CRISPR/Cas9 screen and found that the DNA mismatch repair pathway is the primary mechanism driving somatic ATXN1 CAG expansions. Notably, we identified Msh3, Mlh3, and Pms1 as the most promising therapeutic targets, based on their critical roles in promoting somatic expansions and reduced oncogenic risk.

To determine whether somatic instability contributes to SCA1 phenotypes, we crossed the f-ATXN1146Q/2Q mouse model with a constitutive Msh3 knockout, which completely suppresses somatic CAG expansions. Preliminary results indicate that suppression of somatic expansions has no significant effect on kyphosis, as assessed by CT scan analysis. Because kyphosis in this model has been explicitly attributed to mutant ATXN1 expression in muscle cells, and because somatic instability is comparatively limited in muscle of this model, these results suggest that this phenotype is largely independent of somatic CAG repeat instability. In contrast, we observed significant improvement of gait abnormalities and motor performance. Remarkably, suppression of somatic CAG expansions also significantly extended average lifespan.

These findings demonstrate that somatic CAG instability directly contributes to SCA1 phenotypes, justifying the repurposing of MMR-targeting therapeutics already in development for HD, thereby providing a clear, translational path to treatment.

Funding Sources: National Ataxia Foundation

DD48. Investigating the developmental alterations in the SCA1 using 3D cerebellar organoids

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Background and Objectives: Spinocerebellar Ataxia type 1 (SCA1) is an adult-onset neurodegenerative disease with progressive loss of motor coordination and cognitive impairment, characterized by severe Purkinje neuron degeneration. Previous study identified alterations in the cerebellar development in SCA1 mice, but whether this occurs in human cerebellums remains unclear. We investigated whether cerebellar development is altered in SCA1 cerebellar organoids.

Methods:

1. Generation of cerebellar organoids: We utilized SCA1 patient and control iPSC lines to generate 3D cerebellar organoids using Atamian et al., 2024.
2. Quantification of cerebellar neural progenitors and cells: Cerebellar organoids were dissociated at days 30, 60, 90 and 120 and flow cytometry was used to quantify the percentage of neural progenitors and differentiated cells. Cryosectioning and immunofluorescence were used to confirm flow-cytometry results.
3. Basal and glutamate evoked (25 μ M) activity of cerebellar neurons were assessed using MEA. Electrophysiology recordings and calcium imaging. For MEA, mesh type microelectrode arrays were used to measure the electrical activity in the organoids. For calcium imaging we used viral vectors expressing calcium indicator (AAV9-hsyn-Gcamp6s).

Results and discussion: SCA1 cerebellar organoids show early imbalance in the ventricular zone derived Purkinje progenitor (SKOR2) population. The calcium imaging and MEA data identified altered neuronal firing patterns in SCA1 organoids compared to healthy controls.

Conclusion:

We identified altered cerebellar development and neuronal activity in the human SCA1 organoids suggesting that cerebellar changes in SCA1 occur from the developmental stage. Our work highlights the need for further examination of altered cerebellar function and development of early-stage therapeutic strategies for SCA1.

Funding Sources: R21

DD49. Purkinje Nuclear Isolation and Granule Neuron Depletion for Single-Cell Investigations of Spinocerebellar Ataxia Type 7

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Background: Cell type selective vulnerability is a hallmark feature of neurodegenerative diseases, manifesting in the dysfunction and eventual death of specific neuronal populations depending on a combination of genetic and environmental factors. In the polyglutamine spinocerebellar ataxias, Purkinje cells (PCs) of the cerebellum are the primary targets of disease, yet technical barriers have prevented their isolation from in-vivo models for proper investigation of disease mechanisms. We sought to develop a method for purification of Purkinje nuclei, and depletion of granule neuron nuclei for single-cell profiling in a mouse model of spinocerebellar ataxia type 7 (SCA7).

Methods: Using a commercially available Alexa Fluor-conjugated antibody against the nuclear pore subunit Ranbp2, nuclear extracts from mouse cerebellar tissues were stained for fluorescence assisted nuclear sorting (FANS). Nuclei from granule neurons and Purkinje cells (PCs) are detected by differences in nuclear membrane fluorescence, and sorting gates are drawn for populations of interest. Real-time PCR for canonical PC markers validates their successful purification, and customizable enrichment schemes are applied for snRNA-seq and snATAC-seq investigation of SCA7 mouse cerebellar tissues.

Results: Purified PC nuclei with Rbp2 labeling and FANS show significant enrichment in Calb1, Pcp2, and other canonical PC markers. Purkinje-enriched snRNA-seq between WT and SCA7-266Q mouse cerebellum finds hundreds of differentially expressed genes in symptomatic animals, highlighting a breakdown of Purkinje zebrin subtypes in SCA7. Granule neuron depleted snATAC-seq in SCA7-266Q mice yields a high-resolution map of cerebellar cell types, highlighting significant chromatin remodeling in oligodendrocytes during the SCA7 disease process.

Disease Mechanisms (Recessive and Non-Hereditary Ataxias)

DR1. Frataxin-Linked Impairment of DNA Damage Signaling in Friedreich's Ataxia and Its Pharmacological Modulation

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Background and Objectives: Friedreich's Ataxia (FA) is a neurodegenerative disorder caused by reduced frataxin expression, leading to mitochondrial dysfunction and oxidative stress. Emerging evidence suggests that genome maintenance pathways may also be affected. This study aimed to investigate whether frataxin deficiency alters DNA double-strand break (DSB) signaling and repair following ionizing radiation (IR), and to evaluate whether pharmacological agents can modulate these responses.

Methods: Three FA and two control human skin fibroblast lines received a single irradiation of 2 Gy, with or without 24 h pretreatment using zoledronic acid, pravastatin, or their combination. DNA damage response (DDR) dynamics were assessed by immunofluorescence analysis of pATM, γ H2AX, 53BP1, MRE11 and DNA-PKcs foci at multiple time points. Clonogenic assay was used to determine cellular survival following irradiation.

Results: FA fibroblasts exhibited significantly fewer pATM foci at 10 min and 1 h post-IR and increased residual γ H2AX foci at 24 h post-IR compared with controls ($p < 0.001$), indicating compromised DSB detection and repair. Persistent accumulation of 53BP1 and MRE11 foci further supported defective DDR signaling. While no significant effects were observed in control cells, treatment with zoledronic acid alone or in combination enhanced early DDR activation in FA cells, as reflected by increased pATM and γ H2AX foci. Combination treatment also decreased residual DNA damage and improved post-irradiation clonogenic survival ($p < 0.05$).

Discussion and Conclusion: Frataxin deficiency is associated with impaired DSB signaling and delayed repair kinetics, contributing to genomic instability in FA. Pharmacological intervention partially restores DDR efficiency and improves cellular survival. These findings highlight DNA repair dysregulation as a relevant pathogenic mechanism in FA and support further exploration of targeted therapeutic strategies.

Funding Sources: This research project is funded by the Medical Practice Plan (MPP) Fund, an internal funding mechanism at the American University of Beirut.

DR2. Cerebellar Modulation of Cortical Hierarchies: Evidence for a Domain-General Transform Across Cognitive Networks in Friedreich Ataxia

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Background and objectives: The cerebellum is increasingly recognized as a key contributor to higher cognitive functions through its involvement in large-scale brain networks. Converging theories propose that it implements a domain-general “universal transform”, applying similar computational operations across motor and cognitive domains to regulate cortical processing. However, how this putative transform shapes hierarchical organization across cognitive networks remains unclear. We aimed to characterize cerebello-cortical hierarchy using effective connectivity (EC) and to determine how this organization is altered in Friedreich ataxia (FA). To probe the cerebellum’s role in higher cognition, we examined three networks supporting social cognition (mentalizing), self-referential processing (default mode network, DMN), and executive control. **Methods:** Twenty-eight individuals with FA and 28 age- and sex-matched healthy controls participated. Patients had a mean age of 32 ± 14 years, mean GAA1expansion size of 672 ± 254 repeats, mean disease duration of 14 ± 7 years, and mean SARA score of 23 ± 9 . Resting-state fMRI data were analyzed using dynamic causal modelling and parametric empirical Bayes to estimate directed EC. Only connections with posterior probability >0.95 were retained. Hierarchical levels were defined from the distribution and directionality of EC, distinguishing top-level cortical drivers, intermediate integrative nodes, and bottom-level cerebellar modulators. **Results:** In controls, all networks exhibited a consistent three-level hierarchy. Cortical regions (precuneus, mPFC/PCC, fronto-parietal cortex) acted as top-level organizers with broad outgoing connectivity, while intermediate cortical nodes (TPJ, IPL, PFC) integrated cortical and cerebellar inputs. Cerebellar regions (Crus I/II) occupied a bottom level, showing selective and predominantly negative EC toward cortex (-0.17 to -0.37 Hz), a pattern conserved across networks. In FA, the global architecture was preserved but cerebellar contributions were reduced and redistributed. The mentalizing network showed marked sparsification, whereas DMN and executive networks retained cortical forganization but exhibited attenuated cerebellar outputs. Significant group differences involved both cortical and

Funding Sources: FARA

DR3. PCDHGA10 Drives Cardiomyocyte Injury and Mitochondrial Oxidative Stress in a Human iPSC Model of Friedreich Ataxia

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Introduction: Friedreich ataxia (FRDA) is a progressive neuromuscular disorder caused by pathogenic GAA repeat expansions in FXN, leading to frataxin deficiency and cardiomyopathy, the primary cause of mortality. Complementary therapeutic targets acting downstream of frataxin deficiency remain an important priority alongside frataxin replacement strategies.

Methods: Three FRDA patient-derived iPSC lines and their CRISPR/Cas9-corrected isogenic controls, in which frataxin expression was restored to healthy donor levels, were differentiated into cardiomyocytes (iPSC-CMs) for genetically matched disease comparisons. Contractile function, calcium handling, mitochondrial ROS, mitochondrial morphology, and cell viability were assessed across 2D iPSC-CMs and multicellular 3D cardiac microtissues incorporating endothelial cells, smooth muscle cells, autonomic neurons, and cardiac fibroblasts. Bulk RNA sequencing defined the transcriptional signature of frataxin deficiency, and candidate genes were functionally interrogated by SMARTpool siRNA knockdown (all reported comparisons $n \geq 3$ independent experiments, $P < 0.05$).

Results: FRDA iPSC-CMs exhibited prolonged contraction and relaxation times, increased cell size, irregular Ca^{2+} transients, elevated mitochondrial ROS, increased mitochondrial fission, and increased cell death, exacerbated by pathological iron exposure consistent with myocardial iron dysregulation in FRDA. In 3D microtissues, FRDA models additionally displayed increased beat rate variability, consistent with arrhythmic susceptibility. Transcriptomic profiling of iPSC-CMs identified PCDHGA10, a gamma-protocadherin implicated in cardiac myofibrillar organisation and contractile impairment, as consistently upregulated across all three FRDA lines. siRNA-mediated silencing of PCDHGA10 in FRDA iPSC-CMs rescued cardiomyocyte death, normalised elevated mitochondrial ROS, and improved cardiomyocyte contractile function, restoring relaxation time and beating rate toward isogenic control levels, without affecting FXN expression.

Conclusion: This isogenic iPSC platform captures early, clinically relevant features of FRDA cardiomyopathy and establishes PCDHGA10 as a functionally active contributor to cardiomyocyte injury. Its downstream role in redox stress, cell death, and contractile dysfunction, independent of frataxin expression, nominates PCDHGA10 as a priority target for mechanistic and therapeutic investigation in FRDA cardiomyopathy.

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DR4. Mechanistic insights into microglia-mediated neuronal loss in Friedreich's Ataxia

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Friedreich's ataxia (FA) is a severe inherited multisystem neurodegenerative disorder caused by a homozygous GAA trinucleotide repeat expansion in intron 1 of the FXN gene, resulting in frataxin (FXN) deficiency, mitochondrial dysfunction, and progressive neuronal loss. Although mitochondrial impairment is a hallmark of FA, the cell-type-specific mechanisms driving disease pathogenesis remain incompletely understood. To address this, we generated induced pluripotent stem cell (iPSC)-derived neurons and microglia from multiple FA patient lines, alongside isogenic controls generated via CRISPR/Cas9-mediated excision of the pathogenic repeat expansion using dual sgRNAs (UP4/DN4). Focusing on microglia, we identified a previously underappreciated cell-autonomous hyperactivated state in FXN-deficient cells, characterized by mitochondrial and lysosomal dysfunction, impaired mitophagy, iron dysregulation, lipid peroxidation, oxidative stress, and a pronounced pro-inflammatory signature. Mechanistically, we identify disruption of mitochondrial-endoplasmic reticulum contacts (MERCs) as a central pathological feature linking FXN deficiency to microglial activation. FXN-deficient microglia exhibit impaired mito-ER coupling, a key signaling hub regulating Ca²⁺ dynamics, metabolism, organelle quality control, and immune responses. Pharmacological modulation of MERCs bidirectionally regulates mitochondrial reactive oxygen species, Ca²⁺ signaling, lysosomal acidification, and microglial activation, supporting a causal role for organelle contact dysfunction. Functionally, FA microglia were sufficient to induce degeneration of otherwise healthy neurons in vitro and to drive Purkinje cell loss and cerebellar neurodegeneration following xenotransplantation in vivo, demonstrating that microglia act as active drivers of FA pathology. In vivo, xenotransplantation of FRDA microglial progenitors into a humanized mouse model disrupts cerebellar circuit integrity, including imbalanced excitatory and inhibitory inputs onto Purkinje cells, leading to ataxia-like phenotypes, motor deficits, and reduced survival. Importantly, genetic correction of the FXN mutation restored microglial homeostasis and prevented microglia-mediated neurotoxicity. Collectively, these findings establish microglial organelle dysfunction—centered on impaired MERC-dependent Ca²⁺ homeostasis—as a key driver of neurodegeneration in FA and highlight restoration of inter-organelle communication as a promising therapeutic strategy.

Funding Sources: FARA postdoctoral fellowship (started in April 2024, PI Carla Pernaci, presenting author)
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DR5. Impaired Glur2 Palmitoylation in Cerebellar Purkinje Cells of a Friedreich Ataxia Mouse Model

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Friedreich's ataxia (FRDA) is a neurodegenerative disorder caused by frataxin deficiency, characterized by progressive cerebellar dysfunction and neuronal loss. Although synaptic abnormalities are an early feature of FRDA, the molecular mechanisms linking frataxin deficiency to synaptic dysfunction remain incompletely defined. In this study, we investigated post-translational regulation of the AMPA receptor subunit GluR2 in the cerebellum of the frataxin knockdown (FRDA^{kd}) mouse model. GluR2 protein levels are reduced early following frataxin knockdown, despite unchanged mRNA expression and preserved Purkinje cell number. Phosphorylation of GluR2 at regulatory sites (Ser880 and Tyr876) are unchanged relative to total protein, indicating that altered phosphorylation does not account for reduced GluR2 levels. In contrast, acyl-biotin exchange assays and proximity ligation analysis reveal a significant reduction in GluR2 palmitoylation, localized primarily to Purkinje cell somata. This deficit is selective, as palmitoylation of other synaptic proteins is variably affected. Mechanistically, reduced GluR2 palmitoylation associates with decreased expression and palmitoylation of the palmitoyl acyltransferase DHHC3, while levels of depalmitoylating enzymes remain unchanged. In vitro, DHHC3 enhances GluR2 palmitoylation, supporting a direct enzymatic relationship. Notably, partial restoration of frataxin expression partially rescues GluR2 and DHHC3 protein levels and palmitoylation. These findings identify impaired GluR2 palmitoylation as an early, selective synaptic alteration in FRDA and implicate dysregulated lipid-dependent post-translational modification as a mechanism linking frataxin deficiency to cerebellar synaptic vulnerability.

Funding Sources: FARA Graduate Student Research Grant. CHOP Friedreich ataxia Center of Excellence

DR6. Beyond Frataxin Loss: Mitochondrial Dysfunction and Lysosomal Loss Drives Cellular Dysfunction in Friedreich's Ataxia

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Background: Friedreich's Ataxia (FRA) is the most common autosomal recessive ataxia, caused by a GAA-trinucleotide expansion in the frataxin gene resulting in frataxin deficiency. This causes mitochondrial iron dysregulation, respiratory chain dysfunction, oxidative stress, impaired cellular energy metabolism, driving neurodegeneration and cardiomyopathy. We aimed to characterise mitochondrial and lysosomal dysfunction in FRA patient-derived fibroblasts using high-content phenotypic profiling to identify therapeutic targets.

Methods: Fibroblasts from seven FRA patients (aged 21–59 years, variable disease onset and repeat length) were analysed alongside age- and sex-matched controls. High-content imaging assessed mitochondrial and lysosomal morphology, mitophagy flux and frataxin levels. ATP production was quantified using luminescent assays, and oxygen consumption rate (OCR) assessed by longitudinal time-lapse respirometry over seven days. Results: FRA fibroblasts demonstrated significantly reduced mitochondrial and lysosomal numbers and smaller cell size compared with controls (all $p < 0.0001$). Despite fewer lysosomes, mitochondrial-lysosomal colocalisation was preserved, suggesting maintained mitophagy. Frataxin levels were reduced ($p = 0.0078$) and uncoupled from mitochondrial content.

Mitochondrial membrane potential (MMP), mitophagy, morphology, and total ATP levels were preserved despite a reduced OCR over seven days ($p = 0.0039$), indicating compensatory adaptations despite impaired oxidative phosphorylation. OCR decreased with age but showed no association with repeat length or onset. Longer disease duration correlated with fewer lysosomes ($r = -0.72$), whereas later onset and shorter repeat length correlated with more functional mitochondria ($r = 0.70$, $r = -0.68$ respectively), MMP ($r = 0.48$, $r = -0.43$), and increased mitophagy ($r = 0.48$, $r = -0.43$). Frataxin content correlated with OCR in controls ($r = 0.86$) but not FRA cells ($r = 0.29$). Mitophagy correlated with frataxin only in FRA cells ($r = 0.82$), suggesting disease-specific mitochondrial adaptation.

Conclusion: FRA fibroblasts display fewer mitochondria compensated by preserved MMP and increased mitophagy but impaired oxidative capacity. Disease onset relates more to mitochondrial integrity than frataxin levels. These findings implicate mitochondrial-lysosomal dysfunction as a central to FRA and support further characterisation of lysosomal function for targeted therapeutic exploration.

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DR7. Haploinsufficiency of Pumilio2 is associated with developmental delay and ataxia in mice and humans.

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RNA-binding proteins (RBPs) regulate dozens of mRNAs and are essential for neurodevelopment and neuronal function, yet their roles in brain disease remain poorly defined. We previously showed that PUM1, a member of the PUMILIO/FBF (PUF) family, is critical for neurological function: Pum1-Het mice develop ataxia and Purkinje cell degeneration, while Pum1-KO mice display seizures, growth defects, and severe cerebellar degeneration. PUM1 mutations in humans cause spinocerebellar ataxia type 47 (SCA47), with phenotypic severity tracking protein dosage, ranging from mild adult-onset ataxia (~25% reduction, known as PRCA) to early-onset neurodevelopmental disease with seizures and cognitive delay (40–60% reduction, known as PADDAS).

While mapping PUM1 interactors in the mouse brain, we identified its closest paralog, PUM2. Pum2 levels increase proportionally to Pum1 loss across brain regions, consistent with compensation. Unexpectedly, PUM2 is destabilized in mouse brains and patient-derived cells carrying the severe early-onset PUM1-R1147W mutation, suggesting PUM2 loss contributes to disease. This prompted us to search for patients with PUM2 variants. We identified 34 individuals with de novo missense PUM2 mutations presenting with intellectual disability, ataxia, cerebellar atrophy, and seizures, phenotypes overlapping SCA47, alongside distinct features including headaches, strabismus, and cardiomyopathy, defining a previously unrecognized PUM2-associated neurodevelopmental and ataxic disorder. We obtained Pum2-Het and -KO mice to conduct behavioral and neuropathological experiments to detect behavioral defects and neuronal abnormalities to represent this disease and submitted brain tissues for omics assays. The mutant mice recapitulate patient phenotypes: ataxia, hyperactivity, and muscle weakness at 12 weeks; reduced dendritic arborization at 10 weeks; and adult-onset Purkinje cell degeneration. Integrated RNA-seq, quantitative proteomics, and eCLIP from the Pum2 mouse brain reveal both shared and unique targets with Pum1, defining convergent and divergent pathways that explain overlapping and distinct clinical features. Together, these findings establish PUM2 as the cause of a novel human neurological syndrome.

Funding Sources: This project has been funded by the National Ataxia Predoctoral Fellowship and the TL1TRANSFORM Predoctoral Grant for about two years.

DR8. Investigating the Role of Microglial RNF216 in Motor Function and Neuroimmune Profiles in a Mouse Model of Gordon Holmes Syndrome

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Background: Gordon Holmes Syndrome (GHS) is a neurodegenerative disorder marked by cerebellar ataxia and hypogonadotropic hypogonadism, and is caused by mutations in the E3ubiquitin ligase RNF216. While global Rnf216knockout mouse models demonstrate motor changes and cognitive deficits, the cell-specific contribution of microglial RNF216to motor dysfunction remains unclear. Motor impairment is a defining feature of ataxia, and microglia are increasingly recognized as key regulators of neuronal function and circuit integrity, including those involved in motor control. Coupled with prior evidence of microglial alterations in global Rnf216knockout mice across multiple brain regions, this study tested whether microglia-specific deletion of Rnf216contributes to motor changes. **Methods:** Microglia-specific Rnf216conditional knockout (cKO) mice and littermate controls were generated and assessed at middle age (P365). Male and female mice underwent a battery of motor function tests including rotarod, pole climb, beam walk and limb clasping assays, to evaluate coordination, balance, and motor function. Behavioral outcomes were analyzed for genotype and sex and compared to global Rnf216KO motor behaviors. **Results:** Preliminary results suggest subtle differences in motor performance between cKO mice and controls, with potential sex-dependent trends emerging across assays. Notably, increased incidence of abnormal limb clasping was observed in male cKO mice, suggesting microglial RNF216may influence motor circuit integrity during aging. **Discussion and Conclusion:** Preliminary results suggest a potential role for microglial RNF216in maintaining motor function, with its loss contributing to early features of ataxia-like phenotypes. Ongoing and future studies will examine whether microglial alterations are present, define their temporal relationship to pathology, and assess motor behavior at 18 months (P545) to determine whether impairments worsen with age and more closely recapitulate disease progression. Cumulatively, this work highlights microglia as a potential contributor to ataxia pathophysiology and as a potential cellular target for GHS therapeutic strategies.

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DR9. Identification of Neuronal E3 ubiquitin ligase substrates for RNF216 using Orthogonal Ubiquitin Transfer (OUT)

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Background and objectives: Protein ubiquitination (UB) is a posttranslational modification that is mediated by E1-E2-E3 enzymatic cascades. Ubiquitination regulates key pathways of neuronal cell biology that include signal transduction, protein degradation, and neural development. E3UB ligases can specify the targets, timing, and subcellular location of protein ubiquitination reactions. Mutations in E3UB ligases disrupt key regulatory circuits in the cell and are implicated in many neurodevelopmental and neurodegenerative disorders. Mutations in the E3UB ligase RNF216 have been identified in Gordon Holmes syndrome (GHS), an autosomal recessive cerebellar ataxia that presents with cognitive impairment, ataxia and chorea, reproductive dysfunction, and neurodegeneration. Although GHS causative mutations have been shown to decrease ubiquitination of RNF216 targets, a knowledge gap remains on the molecular mechanisms that drive the development of GHS due to limitation in the ability to identify E3 ligase substrates in neurons.

Methods: We adopted an orthogonal approach in combination with mass spectrometry-based proteomics to identify RNF216 substrates in neuronal cells.

Results: ATXN2, ATXN10 and FMRP were found in our substrate list indicating that RNF216-mediated ubiquitination of these proteins may be involved in GHS pathogenesis. Bioinformatics assessment showed that RNF216 dominantly regulated substrates that were involved in protein synthesis across different cell systems that altered global protein synthesis rates.

Conclusions: Taken together, our findings provide support that the dominant function RNF216 is to balance rates of protein synthesis and degradation in living systems. Our findings also highlight that GHS is not only a disorder resulting in disrupted ubiquitination but also of protein synthesis.

Funding Sources: NIH:R01NS136464

DR10. Analyzing the possible contribution of non-neuronal components in ARSACS

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Background and Objectives: Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is caused by mutations in the SACS gene, resulting in loss-of-function of the protein saccin. While most ARSACS studies have focused on neuronal cells, we observed high levels of saccin in astroglia, microglia, endothelial cells and pericytes. Our aim is to analyze the effects of saccin loss of function on non-neuronal cells.

Methods: Saccin expression was blocked by means of CRISPR/Cas9 approaches in a series of rodent and human cell lines from astroglial, microglial, endothelial and pericyte lineages.

Results: Saccin knockout (KO) in rat astroglial-like C6 cells or HMC3 human microglial cells leads to accumulation of intermediate filaments (IFs) in the juxtanuclear area and disruption of mitochondrial networks, just as in neurons. This is accompanied by functional alterations, such as disruption of response to inflammatory cytokines, cell proliferation and motility, phagocytosis, and secretion of alarmins involved in neuroinflammation such as S100B. Upregulation of the S100B protein can also have protective effects, as it interferes with the formation of toxic protein aggregates in the context of other neurodegenerative disorders. siRNA- or pentamidine-induced inhibition of S100B led to increased mitochondrial fragmentation and IF aggregation. Higher concentrations of exogenous S100B reduced juxtanuclear accumulation of IFs.

Discussion and Conclusion: Our results indicate that glial cells can also be functionally altered by saccin loss. We are currently developing similar cellular models to study alterations in blood-brain-barrier cells and oligodendrocytes. Our results may provide relevant information about the contribution of non-neuronal cells to ARSACS pathology, and the rational design of glial-targeted therapeutics.

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DR11. Avatar mouse model of a compound heterozygous patient with Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS)

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Background: ARSACS is a rare neurodegenerative disorder characterized by progressive cerebellar ataxia, spasticity, and peripheral neuropathy. In 2023, the first case was diagnosed in a 3-year-old girl in Uruguay. Exome sequencing revealed a compound heterozygous variant in exon 10 of the SACS gene: a previously described frameshift mutation (p.His1915Argfs*19) and a novel missense mutation (p.Pro2369Leu) predicted as pathogenic (Raggio et al., 2025). Few regional cases have been reported, highlighting the need for deeper etiological characterization.

Objective: To generate a patient-specific "avatar" mouse model and characterize its phenotype at the genetic, behavioral, protein, and neuropathological levels.

Methods and Current Status: Given the high sequence conservation between human and murine SACS, CRISPR/Cas9 reagents were delivered via electroporation into C57BL/6J mouse zygotes to independently recreate both parental mutations. These lines are maintained as heterozygous in our facility. Crossing these parental lines generates, among other genotypes, the compound heterozygous avatar ARSACS model. These animals are born in a Mendelian ratio with no sex bias. We are currently conducting rotarod tests, and preliminary results show motor discoordination in both male and female avatar ARSACS mice compared to wild-type controls. Additionally, neurobehavioral tests evaluating locomotor activity, balance, and gait dynamics will be performed shortly to identify early functional deficits associated with cerebellar and corticospinal tract impairments.

Conclusions: This avatar model provides a vital translational platform to better understand the pathophysiology of ARSACS and evaluate future targeted neuroprotective therapies.

Funding Sources: Ataxia Charlevoix-Saguenay Foundation. PEDECIBA, Basic Sciences development program, University of the Republic, Uruguay. Laboratory Animal Biotechnology Unit, Institut Pasteur de Montevideo, Uruguay

DR12. Anterior vermis as a hotspot of early neuroinflammatory pathology in Shaker rat cerebellum, a model of Christianson Syndrome

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Background and objectives: Christianson Syndrome (CS) is caused by SLC9A6 mutation coding for NHE6 resulting in a rare X-linked neurodevelopmental disorder with intellectual disability, autism-like behaviors, epilepsy, and ataxia. The Shaker rat carries a spontaneous X-linked LoF mutation in Slc9a6 model of tremor/ataxia with progressive Purkinje-cell degeneration. As part of our efforts to positionally clone shaker including transcriptomics, we noticed RNA profiles very different from SCA mouse models with abundant neuroinflammatory changes. Here, we aimed to define the time course, cellular and region-specific changes relating inflammatory and glial activation signatures to neurodegeneration in the Shaker rat cerebellum.

Methods: Wild-type and affected Shaker rat cerebellum (6 weeks) were micro-dissected into anterior-hemisphere, posterior-hemisphere, anterior and posterior vermis regions (n=3/genotype/region). Bulk RNA-seq was analyzed using DESeq2 (FDR<0.05) and enrichment pathways across Hallmark, KEGG, Reactome, Gene-Ontology, and cell-marker datasets. Protein-protein interaction (PPI) networks were constructed using the STRING database. Key findings were validated by qPCR and immunohistochemistry (IHC).

Results: Shaker cerebellum showed a strong regional transcriptomic response dominated by inflammatory genes. The anterior vermis had the greatest transcriptional perturbation (659 DEGs), followed by the posterior vermis (405 DEGs), posterior hemisphere (188 DEGs), and anterior hemisphere (73 DEGs). Upregulated genes across regions were enriched for microglial activation, phagocytosis, interferon signaling, TNF/NF- κ B, and IL-6/JAK/STAT3 pathways. Downregulated genes were enriched for synaptic organization and axon guidance, most prominently in the anterior vermis. IHC confirmed increased immunoreactivity for Iba1, CD68, and GFAP, indicating microglial activation and reactive astrogliosis. qPCR supported the RNA-seq pattern by validating immune-associated and Purkinje-associated marker genes.

Discussion and Conclusion: 6-week-old Shaker rats exhibit early, spatially patterned cerebellar neuroinflammation, with the anterior vermis leading transcriptional changes. Glial-neuroimmune pathways represent candidate therapeutic targets in CS and potentially in other cerebellar ataxias. These findings highlight the role of glial and microglial activation in neurodevelopmental and neurodegenerative disorders.

Funding Sources: R35NS127253, R37NS033123, R21NS104799

DR14. Mitochondrial Protein Disruption in Frataxin Knock-down Friedreich Ataxia Mouse Model

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Background and Objectives: Friedreich ataxia (FRDA) is an autosomal recessive, neurodegenerative disorder caused by insufficient expression of the mitochondrial protein frataxin, which is important in iron-sulfur cluster assembly and mitochondrial energy metabolism. The disruption of mitochondrial homeostasis includes iron accumulation, impaired ATP synthesis, oxidative stress, and progressive neuronal and cardiac degeneration. To expand on how these mitochondrial pathways are affected, we utilized cerebellum tissue from the doxycycline-inducible frataxin knock-down (FRDAkd) mouse model and examined levels of the mitochondrial dynamics proteins dynamin-related protein 1 (Drp1), mitofusin-2 (Mfn2), and optic atrophy 1 (Opa1), and oxidative phosphorylation complexes nicotinamide adenine dinucleotide + hydrogen:ubiquinone oxidoreductase subunit B8(NDUFB8), succinate dehydrogenase complex iron-sulfur subunit B (SDHB), ubiquinol-cytochrome C reductase core protein 2 (UQCRC2), mitochondrially encoded cytochrome C oxidase I (MTCO1), and adenosine triphosphate synthase F1subunit alpha (ATP5A).

Methods: At 8 weeks post-doxycycline induction, wild-type and FRDAkd mice were sacrificed, and tissues were harvested. Protein expression analysis was performed via Western blot.

Results: Western blot analysis demonstrated lower levels of Mfn2, increased levels of Drp1, and unchanged levels of Opa1, NDUFB8, SDHB, UQCRC2, MTCO1, and ATP5A in the 8 week-induced FRDAkd mice.

Discussion and Conclusions: These findings emphasize how proteins that govern mitochondrial dynamics are affected in the FRDAkd mouse model. Future studies should include immunohistochemistry and Western blot protein expression analysis at different doxycycline-induction timepoints, different tissue types like heart and liver, and in other FRDA models, such as the frataxin knock-in knockout mouse model. By further understanding how mitochondrial proteins affect disease mechanisms in FRDA, there is therapeutic potential of effectively targeting mitochondrial pathways.

Funding Sources: GRT-00000770, FRIEDREICH'S_ATAXIA_CENTER_OF_EXCELLENCE_DAVID_LYNCH

DR15. Isogenic ARSACS iPSC models enable human disease modeling and therapeutic screening

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Background and objectives: Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a severe neurodegenerative disorder caused by mutations in the SACS gene, leading to progressive motor dysfunction. However, its underlying disease mechanisms remain poorly understood, limiting therapeutic development. We aim to generate isogenic induced pluripotent stem cell (iPSC) models and establish a human cellular platform for mechanistic studies and drug screening.

Methods: We used a panel of 10 ARSACS patient-derived iPSC lines carrying distinct SACS mutations, including five homozygous c.8844delT lines, one line carrying c.8844delT/c.7504C>T, one line carrying c.4205A>T/c.5836T>C, one homozygous c.7504C>T, one homozygous c.9284dupC, and one homozygous c.9497-9498delTT. CRISPR/Cas9 genome editing was applied to correct these mutations and generate isogenic controls. Editing efficiency was evaluated using droplet digital PCR and Sanger sequencing. All iPSC lines were characterized for pluripotency, genomic integrity, and differentiation potential using standard assays.

Results: We successfully generated and validated a panel of ARSACS iPSC lines and derived isogenic controls through CRISPR/Cas9-mediated gene correction. Correction of target mutations was achieved, and edited clones exhibited restoration of the wild-type sequence while maintaining pluripotency and genomic stability. These findings demonstrate the efficiency and reproducibility of our gene-editing approach and support the establishment of a robust isogenic iPSC resource for ARSACS research.

Discussion and Conclusion: This study establishes a reliable and scalable human iPSC-based resource to investigate ARSACS while minimizing confounding effects of inter-individual genetic variability. The availability of matched patient-derived and isogenic control lines provides a powerful platform to dissect mutation-specific mechanisms, and represents a critical component of iPSC disease modeling nowadays. Future work will focus on differentiating these iPSCs into disease-relevant cell types, including motor neurons and Purkinje cells, to model ARSACS pathology and enable therapeutic screening. Open dissemination of these resources will foster collaboration and accelerate translational research in ataxia.

Funding Sources: National Ataxia Foundation. Fondation de l'Ataxie Charlevoix-Saguenay

DR16. Classifying autosomal recessive ataxias by clinical phenotype converges on molecular pathways and specific cell types

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Background and objectives: Genetic ataxias are a group of neurologic disorders characterized by gait imbalance, slurred speech, abnormal eye movements, and lack of coordination. Autosomal recessive genetic ataxias encompass more than 200 disorders and often result in diverse neurologic symptoms, leading to a large amount of clinical heterogeneity. The objective of this study is to investigate if shared clinical phenotypes among recessive ataxias have common molecular pathways. We chose to focus on autosomal recessive ataxias because the mechanism is presumably loss of function and therefore better represents a gene's true function and participation in molecular networks.

Methods: In this study, we mined clinical phenotype databases (HPO and OMIM) to identify a comprehensive and unbiased list of autosomal recessive ataxias along with their secondary clinical phenotypes. This yielded three distinct gene lists for different ataxia plus dystonia, ataxia plus neuropathy, and ataxia plus seizure syndromes. These three gene lists were used for downstream analysis of biological processes using gene ontology, protein-protein interactions using STRING, functional expression networks using WGCNA, cell type enrichment and brain region enrichment using EWCE.

Results: We found that ataxia plus dystonia, ataxia plus neuropathy, and ataxia plus seizure genes were indeed enriched for distinct biological processes. The most notable result was ataxia plus neuropathy genes converged on DNA repair pathways, which was also reflected in specific DNA repair protein-protein interactions. Ataxia plus dystonia genes were found to be significantly enriched in oligodendrocytes as well as a myelin/oligodendrocyte gene expression module. We did not find any neuro-anatomical enrichment of ataxia plus genes, suggesting ataxia plus syndromes to be a diffuse, multi-network disease across the brain.

Discussion and Conclusion

Overall we demonstrate a novel approach towards dissecting the relationship between clinical phenotype and gene function, which can be extended and applied to other complex and heterogeneous genetic disorders.

Funding Sources: Denning Ataxia Center

DR17. Metabolic rewiring through ketosis rescues frataxin deficiency by promoting mitochondrial biogenesis

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Background and objectives: Frataxin deficiency reduces OXCT1 protein levels and compromises ketone body metabolism. This study aims to investigate the mechanism by which frataxin regulates OXCT1 protein levels and to determine how OXCT1 deficiency contributes to FRDA pathogenesis.

Methods: Plasmids encoding cyto-HA-ubiquitin, mito-HA-ubiquitin, and frataxin truncation mutants were transfected into HEK293 cells. Immunoprecipitation, immunocytochemistry, and Western blotting were performed to examine OXCT1 ubiquitination and the effect of frataxin on this process. To investigate the impact of OXCT1 deficiency on FRDA pathogenesis, we further reduced OXCT1 expression in KIKO mice by introducing a heterozygous OXCT1 deletion, generating KIKO/OXCT1^{+/-} mice. Lipidomic profiling, Western blot and rotarod analyses were subsequently performed to assess the metabolic, mitochondrial and neurobehavioral effects of OXCT1 deficiency.

Results: We demonstrate that frataxin regulates OXCT1 protein turnover by inhibiting its ubiquitination and subsequent proteasomal degradation. This effect is mediated by the N-terminal 40 amino acids of frataxin, as overexpression of this region alone blocks ubiquitin-proteasome system-dependent OXCT1 degradation. The impact of OXCT1 deficiency was studied in KIKO/OXCT1^{+/-} mice. Further OXCT1 reduction in KIKO mice induces ketosis, promotes mitochondrial biogenesis, increases frataxin levels, normalizes fatty acid metabolism, and improves neurobehavioral performance. The increase in frataxin does not reflect elevated FXN gene transcription but rather enhanced mitochondrial biogenesis, as evidenced by increased mitochondrial biogenesis markers, elevated mitochondrial DNA copy number, enhanced mitochondrial gene transcription, and improved mitochondrial morphology. These effects were further supported by fasting-induced ketosis and the direct effects of β -hydroxybutyrate in vivo in KIKO mice and in vitro in cardiomyocytes derived from induced pluripotent stem cells from FRDA patients.

Discussion and Conclusion: Our findings demonstrate that ketosis partially restores frataxin levels and ameliorates FRDA-related phenotypes. These results support ketosis-based interventions as a potential therapeutic strategy for FRDA and related mitochondrial disorders by enhancing mitochondrial biogenesis and metabolic function.

Funding Sources: Department of Defense CDMRP. Friedreich's Ataxia Research Alliance

DR18. Targeting peripheral neurodegeneration in Friedreich's Ataxia: a novel mechanism and therapeutic strategy

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Introduction Friedreich's ataxia (FA) is a neurodegenerative disorder caused by frataxin deficiency, leading to progressive sensorimotor dysfunction. DRG neurons are early affected, but the basis of their vulnerability is unclear. Given that FA onset occurs during periods of active neuronal growth, we examined whether frataxin regulates axonal development and cytoskeletal organization.

Methods To investigate frataxin's role in neuronal development, we generated an inducible conditional knockout mouse model with tamoxifen-induced FXN deletion at different postnatal stages. We performed behavioral, histological, and molecular analyses, complemented by studies in human FA iPSC-derived DRG organoids. In parallel, we tested pharmacological modulation of microtubule dynamics to explore disease mechanisms and therapeutic strategies.

Results Our data show that frataxin depletion before P25 caused severe ataxia and premature lethality, whereas deletion after P35 was non-lethal and did not induce overt motor defects. These findings identify a critical developmental window in which frataxin is essential, while later loss is better tolerated in adulthood. Based on these findings, P15 was selected for mechanistic studies. At this stage, frataxin loss induced motor deficits, impaired weight gain, and degeneration of large DRG neurons, while later deletion caused only mild phenotypes. DRG neurons from cKO mice and FA iPSC-derived patient DRG organoids exhibited impaired neurite elongation and progressive degeneration. Mechanistically, frataxin loss impacts on the neuronal cytoskeleton inducing a significant microtubule hyperstabilization. To target these alterations, we tested microtubule-modulating drugs and identified a compound that normalized tubulin modifications, improved survival, and reduced neurological deficits.

Discussion Our findings identify a post-developmental window particularly sensitive to frataxin loss, supporting the neurodevelopmental component of FA. This vulnerability likely reflects the role of frataxin in axonal growth and cytoskeletal regulation. Altered tubulin modifications may contribute to impaired neuronal plasticity and neurodegeneration. Importantly, the beneficial effects of a microtubule-targeting compound highlight this pathway as a promising therapeutic

Funding Sources: GOFAR (Friedreich's ataxia research) and FARA (Postdoctoral research award)

DR19. Cerebellar pathology in a humanized Friedreich's ataxia mouse is corrected by excision of the expanded GAA repeats

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Background and Objectives: Friedreich's ataxia (FA) is caused by expanded GAA repeats within intron 1 of the frataxin (FXN) gene. A variety of FA mouse models have been used to study disease mechanisms. The YG800mouse model closely resembles FA genetically, harboring a human FXN transgene containing >800 GAA repeats. These mice display neurobehavioral, cardiac, and musculoskeletal deficits. Recently, using CRISPR-Cas9mediated excision of the expanded GAAs, we developed an isogenic control strain to the YG800, termed YG-del.800. Removal of the GAAs corrected numerous neurobehavioral, motor, and metabolic deficits observed in YG800mice. Our objective was to further characterize the anatomical and physiological differences between cerebella of YG800and YG-del.800 mice in the context of somatic instability of the expanded GAAs.

Methods: Whole brains from 3, 6, 9, and 12 month-old YG800, YG-del.800, and WT animals were perfused, sectioned, and stained with thionin acetate to reveal brain/cerebellar structure. A set of cerebellar-specific anatomical measurements were taken and Purkinje cell (PC) autonomous spiking was analyzed using patch-clamp recordings from acute cerebellar slices. Genomic DNA was isolated from 10 tissues and used in small-pool PCR to assess somatic instability.

Results: YG-del.800 mice displayed similar cerebellar anatomy to WT mice. At a single cell level, there was no difference in the rate of regularity of spontaneous spiking of PCs among all three strains. Brain, cerebellum, and spinal cord tissue exhibited the highest somatic instability, with expansions frequently exceeding 1,000 GAAs.

Discussion and Conclusion: These results emphasize the efficacy of GAA excision in the YG800mouse model and underscore the utility of an isogenic control mouse for FA research. Somatic instability appears to be profound and highly variable among different tissues, demonstrating the practicality of the YG800mouse model in testing the link between somatic expansion and pathology.

Funding Sources: This work was supported by grants from Friedreich's Ataxia Research Alliance and the Congressionally Directed Medical Research Programs (CDMRP) under Award Number HT9425-23-1-0337.

DR20. Astrocytic frataxin deficiency impairs the neuronal phenotypes in models of Friedreich's ataxia

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Background and objectives: Friedreich's ataxia (FRDA) is a rare multisystem disorder which primarily affects the nervous system, heart, and musculoskeletal system. The majority of FRDA patients carry biallelic GAA repeat expansions in intron 1 of the frataxin (FXN) gene, whereas a small proportion is compound heterozygous, carrying a GAA expansion on one allele and a point mutation on the other. These mutations lead to deficiency of frataxin mRNA and protein. Limited access to cell types and tissues primarily affected in FRDA remain as major bottlenecks for the field. Moreover, most induced pluripotent stem cell (iPSC)-based studies focus on autonomous effects of frataxin deficiency on neurons of different subtypes while non-cell-autonomous effects remain to be discovered. In the current study, we utilize iPSC-derived neural cells to study the autonomous and non-autonomous effects of frataxin deficiency on neuronal phenotypes.

Methods: Neurons and astrocytes were differentiated from FRDA, isogenic (in which the biallelic GAA repeat expansions were removed), and non-FRDA control iPSCs. Neurons in mono-culture or co-cultured with iPSC-derived astrocytes (iAS) were immunostained for MAP2, imaged and analyzed for neurite outgrowth and cell body size.

Results: There is no significant difference in the neurite outgrowth and soma size between GAA (biallelic GAA expansion), G130V (GAA/G130V compound heterozygotes), isogenic, and non-FRDA control neurons in mono-culture. However, when neurons were co-cultured with GAA or isogenic iPSC-derived astrocytes (iAS), significant differences in neuronal phenotypes were observed. Specifically, neurons co-cultured with GAA iAS have significantly smaller cell body and fewer neurites as compared to mono-cultured neurons. Interestingly, these negative effects were mitigated when the neurons were co-cultured with isogenic iAS.

Discussion and conclusion: Together, our results indicate that FRDA iAS exert pathological effects on neurons in co-culture, and that restoring FXN expression levels by removing the GAA repeat tracts diminishes these effects.

Funding Sources: This work was supported by grants from Friedreich's Ataxia Research Alliance and National Institutes of Health (NIH), National Institute of Neurological Disorders and Stroke (NINDS).

DR21. Frataxin deficiency uncovers glial-driven regional degeneration in CNS and DRG

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Background and objectives: Friedreich ataxia (FA) has traditionally been attributed to neuronal dysfunction; however, increasing -though still limited- evidence suggests that glial cells play a critical role in disease progression. Moreover, it remains unclear why the PNS is affected earlier than CNS. In FA, damage spreads from the sensory neurons in the DRG upward into the spinal cord and cerebellum, leading to ataxia.

Our main objective was to investigate region- and cell-type-specific pathophysiological mechanisms in vivo and to identify potential therapeutic targets.

Methods: Neurons and glial cells from the DRG, cerebellum, and cerebrum of the FXN151F mice model were isolated by immunomagnetic separation and analyzed for mitochondrial function, iron metabolism, oxidative stress, and ferroptosis. Analyses were performed at two ages: 21 weeks, prior to the onset of neurological symptoms, and 39 weeks, when neurological deficits were evident. The FXN151F mouse model harbors the pathogenic human point mutation I154F in the FXN gene and exhibits low frataxin protein levels across tissues, resembling FA patients.

Results and Discussion: The DRG were identified as the most severely affected region, exhibiting early and pronounced mitochondrial respiratory deficits, increased ROS, mitochondrial iron accumulation, lipid peroxidation, and reduced levels of GPX4 and NRF2 in both neuronal and non-neuronal cells. In contrast, in the cerebrum and cerebellum, astrocytes displayed earlier and more severe alterations than neurons, including impaired respiratory chain efficiency, disrupted supercomplex interactions, elevated ROS, and hallmarks of ferroptosis. Neuronal abnormalities emerged later, suggesting that glial dysfunction precedes -or potentially drives- neuronal pathology within the CNS.

Conclusion: This study provides the first systematic, cell-type-specific comparison of neurons and glial cells across CNS and PNS regions in FA. It supports a paradigm shift in FA pathology by identifying glial cells as the earliest and most profoundly affected compartment and positions them as a central target for disease-modifying interventions.

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DR22. Merscope-based spatial transcriptomic profiling of a mouse model of ARSACS

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Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a childhood-onset cerebellar ataxia caused by mutations in SACS, which encodes the protein saccin. ARSACS-associated cellular phenotypes include mitochondrial dysfunction, intermediate filament disorganisation, disrupted synaptic adhesion and loss of Purkinje neurons. We have previously used multi-omic profiling of CRISPR/Cas9-generated saccin-knockout cell lines and an ARSACS mouse model to investigate mechanisms linking these cellular phenotypes. We now extend this approach to Merscope-based spatial transcriptomics profiling of brains from an ARSACS mouse model.

High-resolution spatial transcriptomics data were generated from juvenile and adult mouse brains at multiple ages, using a custom 960-plex Merfish 2.0 assay, informed by previous multi-omics datasets. Sagittal whole-brain and cerebellar sections from Sacs^{+/+} and Sacs^{-/-} mice were imaged at single-cell resolution. Initial regions of interest (ROI)-based analysis of the whole cerebellum, as well as the isolated Purkinje cell layer, was performed. We then implemented a bespoke pipeline combining image- and transcript-based cell segmentation and established marker genes to validate the assay and detect differential expression in distinct cell populations. We then extended this modelling with post-segmentation analysis to identify distance-based metrics that associated neuronal and glial phenotypes with neighbourhood-based cell status.

The initial analysis of the Purkinje cell layer identified robust differential transcriptional changes between control and saccin knockout cerebellum, including for genes associated with synaptic vesicle trafficking and other pathways consistent with our previous multi-omic analysis. Differential expression analyses identified cell-type-specific transcriptional shifts across neuronal and glial populations consistent with activation of neuroinflammatory pathways and a disease-associated microglial-like phenotype. Neighbourhood-driven analysis provided detailed insight, including localised patterns of these processes.

This first spatial transcriptomics analysis of ARSACS brains reveals cerebellar cell-type-specific alterations. These include changes in Purkinje neurons, alongside glial responses, supporting combined neuronal and non-cell-autonomous mechanisms, as well as evidence for neuroimmune activation.

Funding Sources: The Ataxia Charlevoix-Saguenay Foundation

DR23. Mitochondrial Calcium Uniporter Hypoactivity Drives Cerebellar Circuit Dysfunction in Friedreich Ataxia

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Background and Objectives: Friedreich ataxia (FRDA) is an autosomal recessive neurodegenerative disorder caused by GAA repeat expansion mutations in the mitochondrial protein frataxin (FXN), leading to progressive ataxia and neurological dysfunction. Although mitochondrial abnormalities are a hallmark of FRDA, increasing evidence suggests that neuronal dysfunction precedes overt neurodegeneration. We previously demonstrated concordant impairments in cerebellar synaptic transmission and plasticity in the shRNA-frataxin knockdown (FRDAkd) and FXN knock in-knockout (KIKO) FRDA mouse models. Here, we investigated whether impaired intrinsic neuronal function and dysregulated mitochondrial calcium handling through reduced mitochondrial calcium uniporter (MCU) activity contribute to these concordant cerebellar abnormalities.

Methods: Advanced-stage FRDAkd and KIKO mice were used for electrophysiological and biochemical analyses. Whole-cell patch clamp recordings were performed in cerebellar Purkinje cells, granule cells, and dentate nucleus neurons to assess intrinsic membrane properties and synaptic function. Cerebellar network activity and synaptic plasticity were evaluated using field potential recordings combined with pharmacological modulation of MCU activity. Western blotting was used to assess MCU protein expression.

Results: Both FRDA mouse models exhibited marked reductions in intrinsic membrane excitability across cerebellar neuronal populations, including decreased action potential firing, impaired membrane responsiveness, and altered excitability thresholds. These physiological abnormalities were associated with reduced MCU channel activity. Notably, western blot analysis demonstrated decreased MCU protein expression in the FRDAkd model, supporting a molecular basis for impaired mitochondrial calcium handling. Pharmacological enhancement of mitochondrial calcium uptake restored synaptic responses, supporting a causal role for MCU hypoactivity in cerebellar dysfunction.

Discussion and Conclusions: These findings identify mitochondrial calcium dysregulation as a key mechanism underlying cerebellar circuit dysfunction in FRDA. Rather than widespread neurodegeneration, FRDA appears to involve profound impairments in neuronal physiological stability driven by defective mitochondrial calcium handling. Importantly, restoration of mitochondrial calcium signaling rescued synaptic dysfunction, highlighting MCU signaling pathways as promising therapeutic targets for FRDA.

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DR24. Evidence of Disease-Associated Microglia (DAM) in ARSACS: a mouse-human transcriptomic study

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Background and Objectives: Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS) is driven by loss-of-function mutations in the SACS gene encoding saccin. Purkinje neurons (PNs)—which show the highest SACS expression—are particularly vulnerable, both in human and in the well-established *Sacs*^{-/-} mouse model. We recently uncovered prominent neuroinflammation in the *Sacs*^{-/-} mouse cerebellum, with bulk transcriptomics revealing a clear Disease-Associated Microglia (DAM) signature. Microglia adapt continuously to their microenvironment, shifting from a homeostatic state to DAM in various neurodegenerative diseases. However, the role of DAM in cerebellar ataxia is unexplored, and its role in ARSACS is still completely unmapped. This work represents the first effort to investigate and profile DAM in both mouse and human ARSACS cerebellar tissue.

Methods: In the mouse, cerebellar microglia from 5-month-old *Sacs*^{-/-} and *Sacs*^{+/+} mice were enriched via FACS-sorting and profiled using single-cell RNAseq (scRNAseq). For the human validation, single-nucleus RNAseq (snRNAseq) was conducted on frozen post-mortem specimens (anterior cortex, posterior cortex, and arbor vitae) from one ARSACS patient and an age/sex-matched control. Microglial subclusters and their respective differentially expressed genes (DEGs) were mapped, and DAM spatial localization was confirmed via immunofluorescence on cerebellar slices.

Results: We annotated seven microglial subclusters in mice. Notably, two specific subclusters were heavily enriched in *Sacs*^{-/-} mice, with their DEG profiles capturing a striking transition from a resting/homeostatic state to a full DAM phenotype. Similarly, we identified nine microglial subclusters in the human cerebellum. DEG analysis confirmed a matching DAM-related transcriptome unique to ARSACS compared to the healthy control. Immunofluorescence mapped these DAM predominantly within the molecular layer and in proximity to degenerating PNs.

Discussion and Conclusion: We report the first description of DAM in ARSACS. Establishing whether this microglial transition accelerates or modifies the trajectory of cerebellar degeneration will expose new, potentially druggable neuroinflammatory mechanisms to alleviate patient symptoms.

Funding Sources: Ataxia Charlevoix-Saguenay Foundation

DR26. AMAFAC, Opening gaps and promoting research in neurogenetics for Friedreich's ataxia in Mexico.

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Background: Friedreich's ataxia is a rare, progressive, multisystem neurodegenerative disease caused by the expansion of GAA trinucleotides in the FTX gene, which leads to frataxin deficiency, mitochondrial dysfunction, and clinical disability. The Mexican Association of Friedreich's Ataxia, Civil Association (AMAFAC), was established in 2016 due to the scarce documented information available in Mexico and Latin America, both regarding cases and research lines and management for Friedreich's ataxia.

Objective: Promote the dissemination and research of Friedreich's Ataxia in Mexico.

Methods: A narrative description was carried out of the activities developed by the association since its creation, including the preparation and updating of the census of Mexican patients with Friedreich's Ataxia, implementation of specific actions to achieve timely and accurate diagnosis of suspected cases, as well as timely multidisciplinary assistance, medical guidance, dissemination of scientific information, emotional support, educational activities, and connection with specialists, in addition to developing protocols in clinical research.

Results: The association has positioned itself as a reference in Friedreich's Ataxia in Mexico, through the dissemination of scientific information about Friedreich's Ataxia and other rare and disabling diseases, the establishment of timely diagnostic protocols, the follow-up of patients and families through support networks, data collection, and the development of diagnostic and research protocols, promoting greater visibility of the disease within the community.

Conclusions: The Mexican Association of Friedreich's Ataxia, A.C., represents a fundamental institution to strengthen comprehensive support in rare diseases such as Friedreich's ataxia, promoting education, community support, visibility, and research, while enhancing the human rights of patients.

DR27. Reduction of intestinal iron absorption is neuroprotective in a Drosophila model of Friedreich's ataxia

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Background and Objectives: Friedreich ataxia (FRDA) is a neurodegenerative and cardiac disease caused by GAA repeat expansions within the first intron of the FXN gene, resulting in reduced frataxin expression. Frataxin is required for iron–sulfur cluster biosynthesis, and its deficiency causes multiple cellular dysfunctions, including mitochondrial iron overload. Although altered iron homeostasis has been reported in several frataxin-deficient models and in FRDA patients, its contribution to the progression of the disease remains debated. This study investigated the effects of reducing intestinal iron absorption in a Drosophila model of FRDA with frataxin deficiency.

Methods: Iron accumulation and cellular alterations were analyzed in a GAA expansion-based Drosophila model. Intestinal iron uptake was reduced either pharmacologically using the extracellular iron chelator bathophenanthroline disulfonic acid (BPS) or genetically through gut-specific silencing of the iron transporter Malvolio. Survival, sensitivity to dietary iron, locomotor activity, brain and ventral nerve cord alterations were assessed.

Results: Iron accumulation was tissue-specific and predominantly affected the central nervous system. In the ventral nerve cord, glial cells were affected more severely than neurons, suggesting increased glial vulnerability to frataxin deficiency. Reducing intestinal iron uptake, either by BPS treatment or Malvolio silencing, nearly doubled fly survival. BPS treatment also improved sensitivity to dietary iron and locomotor performance, restored normal brain size, and prevented glial alterations.

Discussion and Conclusion: These findings demonstrate that modulation of dietary iron availability alleviates major pathological phenotypes in a Drosophila model of FRDA and identify glial cells as early and preferential targets of frataxin deficiency in an iron-dependent manner. Altogether, this study supports the therapeutic potential of targeting intestinal iron absorption and dietary iron availability in FRDA.

Funding Sources: This study was supported by the French Association of Friedreich Ataxia (AFAF call 2023).

DR28. TLR4-mediated neuroimmune signalling drives proprioceptive neuron degeneration in Friedreich ataxia

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Background: Friedreich ataxia (FA) is one of the most common inherited neurodegenerative disorders and is caused by frataxin deficiency resulting from a GAA repeat expansion in the FXN gene. Degeneration of proprioceptive sensory neurons (pSNs) in the dorsal root ganglia (DRG) is an early and major contributor to sensory ataxia. Although mitochondrial dysfunction is a hallmark of FA, how neuron-intrinsic metabolic stress leads to progressive neuronal degeneration remains poorly understood.

Methods: We combined single-cell RNA sequencing, molecular and histological analyses, and in vivo studies in mouse models of frataxin deficiency. The functional contribution of Toll-like receptor 4 (TLR4) signaling was assessed using pharmacological inhibition.

Results: We identify a previously unrecognized neuroimmune response triggered by metabolic stress in frataxin-deficient sensory neurons, revealing a non-cell-autonomous component of FA pathogenesis. TLR4 signaling emerged as a key mediator linking neuronal dysfunction to inflammatory activation within the DRG microenvironment. Importantly, pharmacological inhibition of TLR4 reduced neuronal stress, improved neuronal integrity, and delayed disease progression in vivo.

Conclusions: These findings uncover a neuroimmune axis driving disease progression in FA and provide the first proof-of-concept that targeting an innate immune receptor can delay neurodegeneration in this disease. Our work identifies TLR4 signaling as a potential therapeutic target and highlights neuroimmune mechanisms as an important component of FA pathology.

Funding Sources: This work was supported by BMIC (to D.M.C.), the Association Française pour l'Ataxie de Friedreich (AFAF; to H.P. and M.P.), the Fondation pour la Recherche Médicale (FRM; grant number FDT202304016821 to D.M.C.), and the Friedreich's Ataxia Research Alliance (FARA; to D.M.C.).

DR29. Shared glial dysfunction across three cerebellar ataxias reveals convergent mechanisms of Purkinje cells vulnerability

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Background and Objectives: Cerebellar ataxias is a wide group of rare and diverse genetic neurodegenerative diseases characterized by cerebellar dysfunction and prominent Purkinje cell (PC) degeneration, resulting in loss of coordination and ataxia symptoms. To date, the mechanisms underlying the selective vulnerability of PCs remain in many cases incompletely understood and may involve non-cell autonomous processes.

Methods: To identify convergent pathogenic pathways, we performed single-cell transcriptomic analyses of three mouse models of cerebellar ataxia, Spinocerebellar ataxia type-1 (SCA1), Spinocerebellar ataxia type-7 (SCA7), and Friedreich's ataxia (FA), at two stages of disease progression.

Results: This approach revealed shared dysregulated pathways across multiple cerebellar cell populations. Among the most promising altered pathways, we found Bergmann glia genes encoding for synapse-modifying signaling proteins to be significantly dysregulated in the three models. In line with these *in silico* data, we observed structural alterations of Bergmann glia radial processes, and a reduction in the levels of several Bergmann glia-secreted synaptogenic cues in the cerebellum of each model. Given a peculiar alteration of Climbing fibers-Purkinje cells (CF-PC) synapses in the cerebellum of many cerebellar ataxia mouse models, and the key role of astroglia in synapse formation, maintenance, elimination and function, we are dissecting and experimentally modulating this dysregulated pathway to determine its contribution to the loss of the glutamatergic synaptic contacts onto PCs.

Discussion and Conclusions: Together our data suggest an early Bergmann glia pathology that likely contributes to the characteristic PC degeneration observed in most cerebellar ataxias.

Funding Sources: MSCA Postdoctoral fellowship (European Commission). FRM - Fondation pour la Recherche Médicale (France)

DR30. Calcium dysregulation exacerbates TGF β -induced fibrotic responses in Friedreich's ataxia human fibroblasts

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Background and Objectives: The leading cause of death in Friedreich's ataxia (FA) is cardiomyopathy, characterized by extensive fibrosis. Yet, the multicellular programs driving FA cardiac fibrosis remain largely unknown. While TGF β -driven fibroblast-to-myofibroblast transition is central to the fibrotic process, whether FA fibroblasts exhibit disease-specific alterations in this transition remains to be elucidated. Here, using multi-omics and biochemical approaches, we investigated if FA human fibroblasts are more susceptible to undergoing pro-fibrotic reprogramming than healthy controls.

Methods: We investigated 12 FA and 12 control skin fibroblast lines from the UT Southwestern repository. Fibroblast-to-myofibroblast transition was induced with TGF β 1 (10 ng/ml). Multi-omic profiling, including bulk RNAseq, proteomics, and phosphoproteomics, was performed on both fibroblasts and myofibroblasts. Cytosolic Calcium (Ca) dynamics were measured using GCaMP6f. The plasma membrane Na/Ca exchanger NCX1 was inhibited pharmacologically with SEA0400 (1 μ M) or overexpressed via lentiviral transduction.

Results: FA fibroblasts displayed pro-fibrotic transcriptional priming at baseline, including upregulation of TGF β receptors and SMAD2/3, alongside elevated expression of genes controlled by NFAT, a Ca-responsive transcription factor. Upon TGF β 1 stimulation, compared to controls FA myofibroblasts showed amplified induction of pro-fibrotic genes (CCN2, NOX4) and suppression of anti-fibrotic CCN3, correlating inversely with residual frataxin mRNA levels. FA fibroblasts exhibited elevated cytosolic Ca. RNAseq and proteomics identified NCX1 as the most downregulated Ca-handling protein in FA. Interestingly, NCX1 expression levels correlated directly with frataxin levels. NCX1 inhibition in control fibroblasts recapitulated FA Ca phenotype, whereas NCX1 overexpression in FA fibroblasts normalized Ca dynamics and blunted CCN2 induction in the fibroblasts-to-myofibroblast transition.

Conclusions: These findings highlight NCX1 as a modulator of the fibrotic reprogramming and identify Ca dyshomeostasis as an intrinsic fibrosis-susceptibility mechanism in FA that could be targeted therapeutically.

Funding Sources: FARA-MDA Molecular and cellular mechanisms of cardiac fibrosis in Friedreich's Ataxia (Payne & Manfredi). FARA Calcium communication among intracellular compartments in FA patient-derived cells (Stepanova)

DR32. Patient-derived cerebellar organoids recapitulate key pathological features of Friedreich's Ataxia

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Background and Objectives: Friedreich's ataxia (FRDA) is a debilitating inherited neurodegenerative disorder caused by a GAA repeat expansion mutation in the FXN gene, resulting in reduced expression of the mitochondrial protein Frataxin. The cerebellum is among the most affected tissues in FRDA and undergoes progressive atrophy. However, the mechanisms underlying this selective vulnerability remain unclear. Human induced pluripotent stem cells (iPSCs) provide a powerful platform for disease modelling. Here, we generated cerebellar organoids from FRDA patient-derived iPSCs and isogenic controls to investigate disease-associated molecular and functional phenotypes.

Methods: FRDA iPSC lines and isogenic controls from the Friedreich's Ataxia Cell Line Repository (FACLR) were differentiated into cerebellar organoids using a reproducible cerebellar differentiation protocol. Cerebellar identity and expression of key markers including Frataxin were assessed by qPCR, immunostaining and Western blotting. Bulk RNA sequencing was performed at multiple timepoints during differentiation to identify altered molecular pathways. Mitochondrial function and reactive oxygen species (ROS) production were analysed by flow cytometry.

Results: Control cerebellar organoids showed robust Frataxin expression throughout cerebellar differentiation, with predominant mitochondrial localisation. FRDA organoids exhibited markedly reduced Frataxin expression while retaining mitochondrial localisation. Patient-derived cerebellar organoids recapitulated key features of FRDA, including mitochondrial dysfunction and increased oxidative stress. Transcriptomic analyses identified dysregulated pathways including iron homeostasis and mitochondrial metabolism, and upregulation of recently established FRDA-associated biomarkers. Ongoing studies are assessing the response of FRDA organoids to candidate therapeutic compounds.

Discussion and Conclusion: These findings demonstrate that human cerebellar organoids faithfully model key molecular and cellular features of FRDA. This model provides a valuable tool for investigating disease mechanisms underlying cerebellar vulnerability and offers a scalable system for therapeutic screening and development.

Funding Sources: This work was funded by the Friedreich's Ataxia Research Alliance (FARA).

DR33. DRG neurogenic defects during embryogenesis in the G127V mouse model

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Friedreich ataxia (FA) is a progressive multisystemic disorder characterized by degeneration of spinocerebellar tracts, cerebellar dysfunction, and peripheral sensory neuropathy. The most vulnerable sensory cells are proprioceptive neurons located in the dorsal root ganglia (DRG). DRG originate from neural crest cells that migrate along the neural tube and differentiate into sensory lineages during early embryogenesis, while neuronal maturation continues postnatally.

In this study, we investigated whether frataxin (FXN) deficiency disrupts DRG neurogenesis. Because complete knockout of FXN is embryonic lethal, we used the G127V knock-in mouse model, which displays low FXN levels throughout development. To assess the impact of reduced FXN during DRG formation, we performed immunostaining and molecular analyses on posterior embryos at key embryonic stages, corresponding to the major steps of DRG neurogenesis.

Our data revealed neurogenic defects at E12.5, a critical stage of early neuronal specification. At this stage, the expression of key neurogenic markers of sensory neuronal progenitors were altered, and apoptosis was increased within the developing DRG. Although these early defects appeared partially compensated by E14.5, abnormalities persisted at E16.5, when altered neurofilament staining revealed defects in neuronal maturation and organization.

This study provides, to our knowledge, the first developmental characterization of DRG formation in a FA mouse model with chronically reduced FXN levels. Defining the temporal sequence of developmental abnormalities caused by FXN deficiency offers new insight into FA pathogenesis.

Funding Sources: FARA Graduate Research Fellowship (3rd year funding) 2023.- Equipe FRM 2024. Fondation Neurodis 2023

DR34. The FXN c.165+5G>C point mutation confers unique clinical and molecular phenotypes

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Background: The FXN c.165+5G>C point mutation identified in Friedreich's ataxia (FA) compound heterozygotes disrupts canonical FXN splicing, resulting in production of a truncated, apparently nonfunctional frataxin protein. Compared with patients carrying biallelic GAA expansions, FA patients harboring the FXN c.165+5G>C mutation exhibit selective early motor neuropathy/spasticity of the lower extremities with relative sparing of upper extremities, speech and cardiac function. In contrast, vision loss and diabetes develop early in the disease course. Importantly, FXN levels measured in patient fibroblasts are comparable between individuals with biallelic GAA expansions and those carrying the FXN c.165+5G>C mutation. The objective of this project is to define the transcriptomic signatures of FXN c.165+5G>C and biallelic GAA expansion cells to help elucidate the molecular basis underlying their distinct clinical presentations.

Methods: The transcriptomes of primary fibroblasts from individuals with the FXN c.165+5G>C mutation, biallelic GAA expansions, and unaffected controls were defined in two independent RNA sequencing experiments. Induced pluripotent stem cells (iPSCs) were reprogrammed from FXN c.165+5G>C FA fibroblasts and characterized for subsequent studies in disease-relevant cell types and organoids.

Results: Comparative transcriptomic analyses identified a distinct FXN c.165+5G>C signature compared with biallelic GAA expansion samples. More than 500 transcripts were upregulated and nearly 700 were downregulated in FXN c.165+5G>C versus biallelic GAA expansion samples (FDR < 0.05). Genes involved in cardiac contractility, axon guidance, and G protein-coupled receptor (GPCR) signaling were among the top-ranked categories in ontology and pathway analyses. We successfully generated and characterized FXN c.165+5G>C iPSCs.

Discussion/Conclusion: Frataxin levels alone cannot fully explain the differences in disease manifestation between the two groups. Transcriptomic analysis of FXN c.165+5G>C and biallelic GAA expansion samples revealed profound molecular differences between these distinct FXN mutations. Current studies are focused on generating brain and retinal organoids from FXN c.165+5G>C, biallelic GAA expansion and control iPSCs.

Funding Sources: This work was supported by grants from Friedreich's Ataxia Research Alliance and the Congressionally Directed Medical Research Programs (CDMRP) under Award Number HT9425-23-1-0337.

DR35. First Radiopathologic Correlation of SWI Lesions with Postmortem Neurovascular Pathology in Ataxia-Telangiectasia

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Background: Susceptibility-weighted imaging (SWI) hypointense foci and cystic white matter lesions have been reported on MRI in ataxia-telangiectasia (A-T), but their pathologic substrate has never been established. Historical autopsy studies documented vascular abnormalities in A-T brain tissue, yet none included antemortem SWI for direct radiopathologic correlation. We aimed to provide the first such correlation and characterize the neurovascular pathology of A-T.

Methods: Comprehensive general and neuropathologic autopsy was performed 38 hours postmortem in a 31-year-old man with clinically diagnosed A-T. Histopathology included H&E, special stains (LFB-PAS, Masson trichrome, Prussian blue), CD31 immunohistochemistry, and neurodegeneration panels. Radiopathologic correlation was performed by mapping the anatomic locations of SWI hypointense foci and cystic lesions on antemortem MRI to corresponding gross coronal brain sections, with targeted histopathologic sampling of regions showing imaging abnormalities.

Results: Classic A-T pathology was confirmed: severe cerebellar cortical degeneration with Purkinje cell loss, posterior column and spinocerebellar tract demyelination, and motor neuron loss. Beyond these findings, widespread vascular abnormalities were identified. Multifocal gliovascular nodules contained tortuous vessels with endothelial damage, thrombosis, fibrin deposition, and recanalization. Large cavitory "lakes" lacked vascular walls and hemosiderin on CD31 and Prussian blue staining, suggesting chronic plasma extravasation rather than hemorrhagic infarction. Similar vascular lesions were present in the spinal cord. SWI hypointense foci corresponded to gliovascular nodules rather than simple hemosiderin deposits. A mixed perivascular inflammatory infiltrate surrounded the lesions without systemic vasculitis.

Discussion and Conclusion: This case provides the first direct correlation between antemortem SWI abnormalities and postmortem vascular neuropathology in A-T, establishing that SWI hypointense foci represent complex gliovascular lesions with chronic endothelial injury. These findings demonstrate that A-T harbors a vascular phenotype extending along the neuraxis, superimposed on classical neurodegeneration. This has implications for MRI surveillance, vascular biomarker development, and therapeutic strategies — including gene therapy approaches that may

Funding Sources: Ataxia Telangiectasia Children's Project (ATCP)

DR37. Molecular mechanisms by which intronic RFC1 AAGGG repeat expansions cause cerebellar ataxia with neuropathy and vestibular areflexia syndrome (CANVAS)

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Background and Objectives: Cerebellar ataxia with neuropathy and vestibular areflexia syndrome (CANVAS) is a debilitating, late-onset neurodegenerative disorder with patients displaying both progressive cerebellar atrophy and peripheral sensory neuronopathy. CANVAS results from biallelic, pentanucleotide CCCTT(AAGGG) repeat expansions in the second intron of replication factor C subunit 1 (RFC1). There are currently no effective treatments. The objectives of this study are to examine the molecular mechanisms by which repeat expansions elicit CANVAS.

Methods: To investigate molecular mechanisms underlying this disorder, we generated CANVAS patient-derived induced pluripotent stem cells (iPSCs) differentiated into neural progenitor cells (NPCs), cortical forebrain-like neurons (iNeurons), and nociceptive sensory neurons. The cells are characterized through functional neuronal assays, proliferation assays, and survival analyses.

Results: We do not observe changes in RFC1 expression or splicing in patient samples or iNeurons. CANVAS iNeurons display downregulation of synaptic genes as well as reduced neuronal connectivity, which are not recapitulated by RFC1 knockdown but which do correct with CRISPR mediated deletion of a single expanded CCCTT(AAGGG) repeat. CANVAS NPCs exhibit a proliferation deficit compared to control cells that is normalized with re-provision of RFC1 protein. Overexpression of 61 AAGGG repeats did not affect survival of either rodent neurons or iNeurons. Experiments examining the synergistic roles of RFC1 loss and pathogenic AAGGG repeat expression in patient-derived iNeurons and nociceptive sensory neurons are ongoing.

Discussion and Conclusion: In patient-derived iNeurons and neural progenitor cells, we observe data supporting both gain-of-function and loss-of-function mechanisms by which AAGGG repeats cause disease. Despite these advances, the underlying molecular mechanisms that drive neurodegeneration in CANVAS remain enigmatic.

Funding Sources: NIH T32GM141840

DR38. Unravelling cell type sensitivity to frataxin depletion versus partial frataxin dysfunction using zebrafish models

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Friedreich's ataxia (FA) is a progressive neurodegenerative disease most commonly caused by a homozygous (GAA)_n repeat expansion within the first intron of the frataxin gene (FXN), which encodes a mitochondrial protein essential for iron-sulfur cluster biogenesis. As an essential gene, constitutive loss of function in the mouse (and many other organisms) is embryonic lethal at atmospheric oxygen levels. While homozygous point mutations in FXN were long assumed to be absent in patients, this assumption has recently been challenged with the recent identification of a Turkish family carrying a homozygous R165C missense mutation. Unlike the (GAA)_n repeat expansion mutation, this mutation does not reduce frataxin mRNA or protein levels, but leads to partially functional frataxin protein. Interestingly, the R165C mutation leads to an atypical clinical phenotype marked by relatively mild ataxia, absence of cardiac disease, and the presence of both sensory and motor neuropathies, features rarely seen in classical FA. To investigate the pathophysiological consequence of partial versus complete loss of frataxin function, we are developing a novel zebrafish knock-in model harbouring the R128C-mutation equivalent to the human R165C-mutation. This model will be systematically compared to a frataxin knockout (Fxn-KO) through behavioural and morphological analysis focused on sensorimotor development and neuronal circuitry. By integrating these phenotypic assessments with metabolic and mitochondrial profiling, we aim to understand how frataxin deficiency versus dysfunction affects mitochondrial physiology and function. This work will provide new insights into cell type vulnerability in FA and may reveal alternative functions of frataxin beyond Fe-S biogenesis. Finally, this work may uncover novel therapeutic targets tailored to specific mutation types in FA.

Funding Sources: This work is supported by Friedreich's Ataxia Research Alliance (FARA), Fondation Maladies Rares (FMR) and Association Française Ataxie de Friedreich (AFAF).

DR39. Neurodevelopmental Origins and Cell Type Dependent Mechanisms of Neurodegeneration in ARSACS Revealed by Longitudinal snRNA-seq

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Background and Objectives: ARSACS is characterized by progressive loss of cerebellar Purkinje neurons, particularly the Aldoc- subpopulation. The mechanisms driving selective vulnerability are unknown. Moreover, it is unclear the extent to which the neurodegenerative cascade originates in the adult brain or has earlier developmental roots, and how cell types besides Purkinje neurons are affected. Here we characterize the temporal trajectory of ARSACS progression using single-nucleus RNA sequencing (snRNA-seq).

Methods: Neonatal behavioral assessments were conducted from P2 through P14 using developmental milestone batteries. snRNA-seq was performed on ARSACS mice spanning early cerebellar development through advanced motor dysfunction (n=48 male mice; age p5,30,60,120; 225k nuclei). Gene expression was quantified using an ensemble DGE approach, alongside orthogonal methods to characterize cell-type-specific transcriptional dynamics, and compositional shifts. Candidate cellular and synaptic phenotypes were validated by immunofluorescence.

Results: ARSACS mice exhibited transient delays on neonatal behavioral tests, which coincide with significant transcriptional changes in immature Purkinje neurons. In adult mice, multiple neuron types showed early, temporally dynamic changes of synaptic transmission genes, while non-neuronal cells showed a temporally delayed response that coincided with the onset of Purkinje loss. Aldoc- Purkinje neurons showed progressive loss of canonical subtype identity markers, while Aldoc+ Purkinje neurons selectively upregulated multiple signatures hypothesized to impart resilience to the loss of Sacs1. Histological validation revealed lobule and cell type specific changes in synaptic structure that mirror the pattern of selective vulnerability.

Discussion and Conclusion: These findings reveal that ARSACS has clear and distinct changes during cerebellar development, manifesting both in developmental delay and transcriptional changes. Analysis of resilient/susceptible Purkinje subtypes supports a model in which intrinsic differences in transcriptional response distinguish susceptibility from resilience. Future studies aim to activate these resilience signatures in vulnerable neurons. In all, this longitudinal atlas provides a platform for understanding the neurodegenerative cascade in ARSACS.

Background and Objectives: ARSACS is characterized by progressive loss of

Funding Sources: ARSACS Foundation Research Grant. National Ataxia Foundation New Investigator Award

DR42. Transcriptome-wide aberrant splicing as a pathogenic mechanism in Friedreich's ataxia

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Background and objectives: Friedreich's ataxia (FA) is caused by biallelic intronic GAA-repeat expansions in FXN leading to frataxin deficiency and mitochondrial dysfunction. Beyond FXN downregulation, the transcriptomic landscape in FA brain remains poorly characterised. We sought to define the extent and mechanistic basis of RNA biology in FA brain tissue. RNA splicing dysregulation is a crucial contributor and potential therapeutic target across neurodegenerative disorders such as amyotrophic lateral sclerosis. We were particularly interested in how alternative splicing, a critical regulatory process, may also be affected in FA.

Methods: We performed differential splicing analysis on high-depth bulk and single-nucleus RNA-sequencing data generated from cerebellar and frontal cortex post-mortem brain tissue from people with FA and neurologically unaffected controls. Differential splicing was identified using Leafcutter, an annotation agnostic tool. Downstream analysis included functional enrichment for biological pathways and Human MitoCarta3.0 mitochondrial gene enrichment. RNA-binding protein (RBP) activity was inferred using publicly available experimental datasets inferring RBP function.

Results: We identified widespread differential splicing affecting approximately 15,000 intron clusters across approximately 2,000 genes in FA brain. The differentially spliced gene set was entirely distinct from differentially expressed genes, indicating a mechanistically distinct pathological process. Compared to other repeat expansion ataxias, mis-splicing events markedly exceeded differential expression events, suggesting mis-splicing as a predominant mechanism in FA brain specifically. Differentially spliced genes were enriched for RBP-encoding genes in the cerebellum. Critically, 8% of differentially spliced genes were implicated in mitochondrial function, enriched in iron-sulphur cluster biogenesis and oxidative phosphorylation pathways (OR=1.44, FDR $p=3.6 \times 10^{-5}$).

Discussion and Conclusion: These data reveal transcriptome-wide aberrant splicing as an unanticipated and potentially dominant layer of dysregulation in FA brain, mechanistically linked to both mitochondrial dysfunction and RBP perturbation, with previously unrecognised commonalities with other neurodegenerative diseases. This establishes aberrant splicing as a novel and potentially targetable pathogenic mechanism in FA.

Funding Sources: Leonard Wolfson Foundation, Horne Family Foundation.

DR43. Defining the Gene Expression Phenotype of Friedreich's Ataxia Cardiomyocytes Using Bulk and Single-Cell RNA Sequencing

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Background: Friedreich's ataxia (FRDA) is a rare neurodegenerative disease caused by GAA trinucleotide repeat expansion within the first intron of the FXN gene. The major manifestations of FRDA include progressive neuromuscular degeneration and cardiomyopathy. Friedreich's ataxia cardiomyopathy (FA-CM) is the leading cause of mortality in FRDA, accounting for approximately 60% of patient deaths. Despite its clinical significance, the molecular mechanisms underlying FA-CM remain incompletely understood. Objective: To better define the molecular pathology of FA-CM and identify candidate therapeutic targets, we sought to characterize a FRDA-associated gene expression phenotype (GEP) in induced pluripotent stem cell-derived ventricular cardiomyocytes (iPSC-CMs) using both bulk and single-cell RNA sequencing. Methods: Nine iPSC lines representing three genotypes (FRDA, isogenic control, and unaffected control; n=3 per genotype) were propagated and differentiated into ventricular cardiomyocytes. Following cardiomyocyte enrichment, samples underwent both bulk RNA sequencing and single-cell RNA sequencing. Differential gene expression analyses were performed between FRDA and matched isogenic control cardiomyocytes, and concordance between platforms was assessed. Results: The iPSC lines demonstrated an average differentiation efficiency of 70%, and flow cytometry indicated approximately 95% cardiomyocyte enrichment following purification. Differential expression analysis identified numerous genes dysregulated in FRDA cardiomyocytes relative to matched isogenic controls in both bulk and single-cell datasets. Comparison of the two platforms demonstrated moderate concordance ($p \approx 0.6$), with most shared differentially expressed genes exhibiting consistent directionality of expression changes. Single-cell transcriptomic annotation using Azimuth classified approximately 90% of cells as cardiomyocytes while also identifying additional non-cardiomyocyte populations. Discussion and Conclusion: Bulk and single-cell RNA sequencing identified a reproducible FRDA-associated transcriptional signature in iPSC-derived cardiomyocytes. While bulk and single-cell analyses showed substantial agreement, single-cell profiling provided additional resolution of cellular heterogeneity not captured by conventional purity assessments. These findings establish a framework for defining the FRDA cardiomyocyte gene expression phenotype and provide insight into the molecular

Funding Sources: National Institute of Environmental Health (NIEHS), National Ataxia Foundation (NAF)

DR44. Purkinje Neuron-Specific ATM Deletion Drives Cerebellar Dysfunction in a Mouse Model of Ataxia Telangiectasia

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Ataxia-telangiectasia (A-T) is a rare inherited neurodegenerative disorder characterized by progressive cerebellar ataxia, immunodeficiency, cancer susceptibility, and premature death. A-T results from loss-of-function mutations in ATM, a critical regulator of the DNA damage response. Although ATM is broadly expressed, the cerebellum is selectively vulnerable to its loss. In A-T patients, neuropathology is dominated by cerebellar cortical atrophy with prominent loss and degeneration of Purkinje neurons (PNs), the sole output neurons of the cerebellar cortex, suggesting that these neurons are especially vulnerable to ATM loss.

To test whether PN-specific ATM deficiency causally contributes to cerebellar pathology and ataxia, we generated mice with L7-Cre-mediated *Atm* deletion restricted to PNs on the published *Aptx*-null sensitized A-T model background. We compared PN-*Atm* cKO mice with *Aptx*^{-/-} controls retaining *Atm* and *Atm*R35X/R35X; *Aptx*^{-/-} mice with constitutive *Atm* loss. Using an in-house composite ataxia score optimized to detect early-stage disease, PN-*Atm* cKO mice were indistinguishable from controls at P60 and P120 but developed significant motor coordination deficits by P200 that persisted at P280. At P200, scores were 0.78 ± 0.12 in controls, 1.32 ± 0.11 in PN-*Atm* cKO mice, and 2.62 ± 0.34 in constitutive *Atm*R35X/R35X; *Aptx*^{-/-} mice, indicating that PN-restricted *Atm* loss produces a milder but significant ataxic phenotype compared with controls (Welch's t-test, $p=0.0041$). Consistent with this phenotype, western blot analysis of P100 PN-*Atm* cKO cerebellum revealed reduced ITPR1, a Purkinje neuron calcium channel implicated in spinocerebellar ataxia.

Ongoing studies will further characterize disease progression at functional, cellular, structural, and molecular levels using multi-electrode array electrophysiology, immunohistochemistry, and transcriptomics. Together, this work defines the PN-intrinsic contribution of ATM loss to cerebellar vulnerability in A-T and may inform targeted neurotherapeutic strategies.

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DR45. A New Rat Model of Friedreich's Ataxia Through AAV-Mediated Frataxin Silencing

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Background: Friedreich's ataxia (FA) is a neurodegenerative disorder characterized by loss of coordination, muscle weakness, and sensory deficits, with a neuropathology that progresses from the dorsal root ganglia (DRG) to the posterior columns and spinocerebellar tracts. The primary cause of FA is the reduced expression of frataxin, a mitochondrial protein encoded by the gene Fxn. However, the mechanisms linking frataxin deficiency to the neuropathological defects observed in FA are yet to be completely elucidated. To provide an additional resource for further investigating FA disease mechanisms, we are developing a new rat model of targeted frataxin silencing.

Methods and Results: We generated an adeno-associated viral vector expressing an shRNA targeting rat frataxin. This AAV-Fxn-shRNA induced a significant reduction of Fxn mRNA and frataxin protein in rat neural stem cells in vitro, and following intracerebral injection to the rat cerebellum in vivo. To target the rat sensorimotor system, AAV-Fxn-shRNA was administered intrathecally, resulting in widespread viral transduction throughout the spinal cord, cerebellum and DRG. Preliminary findings indicate motor behavior deficits in rats receiving Fxn-shRNA compared to scrambled-shRNA controls.

Discussion and Conclusion: Our results demonstrate that AAV-mediated delivery of Fxn-shRNA is efficient at silencing rat frataxin. We hypothesize that sustained frataxin knockdown in the rat sensorimotor pathways will successfully reproduce key features of FA neuropathology, providing a new experimental platform for studying FA pathogenesis and evaluating novel therapeutic strategies.

Funding Sources: This work was supported by the Houston Methodist NeuralCODR Fellowship program.

DR46. Comparative proteomic analysis of tissue-specific responses to frataxin ablation in striated muscles

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Background: Friedreich's ataxia is caused by reduced expression of frataxin (FXN), a mitochondrial protein required for iron-sulfur cluster (ISC) biogenesis. Despite sharing this genetic deficiency, cardiac tissue shows higher mortality than skeletal muscle. Objectives: We aimed to identify the key protein groups underlying this differential tissue vulnerability.

Methods: Using high-throughput LC-MS/MS with TMT, we profiled the proteome of heart (HT) and skeletal muscle (SM) in male control (CON; #000664, Jackson Laboratory) and cardiac/skeletal muscle-specific FXN knockout (KO; #029720, Jackson Laboratory) mice (8-10 weeks; n=6 per group). Results: We quantified 5,465 protein groups. In KO mice, frataxin deficiency induced severe, asymmetric remodeling. Skeletal muscle showed mild adaptation, while the heart exhibited profound downregulation of mitochondrial respiratory components, particularly ISC-containing complex I (NDUFS1, NDUFS8) and II (SDHB) subunits. In the heart, heme synthesis proteins (FECH) decreased, while chronic mitochondrial stress markers (MTHFD2, GRPEL2, CHCHD4, BDH1) and the iron uptake protein TFR1 were significantly elevated. Concurrently, the mitochondrial iron balance protein C15orf65 was depleted. Conversely, skeletal muscle mitochondrial pathways remained relatively preserved. Discussion and Conclusion: These findings identify impaired mitochondrial iron utilization as a central pathogenic mechanism in the heart. Coordinated depletion of ISC and heme synthesis drives upstream respiratory chain dysfunction. Ultimately, profound cardiotoxicity stems from the heart's strong reliance on continuous oxidative phosphorylation for contractility, rendering it uniquely vulnerable compared to skeletal muscle.

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DR48. Modeling Friedreich's Ataxia-Associated Retinal Pathology Using Patient-Derived Retinal Organoids

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Background and Objectives: Friedreich's ataxia (FRDA) is a rare autosomal recessive multisystem disorder caused primarily by biallelic GAA repeat expansions in intron 1 of the FXN gene, leading to reduced frataxin expression. Approximately 20% of FRDA patients experience progressive vision loss, significantly impacting quality of life. The retina, with its high metabolic demand and mitochondrial density, is particularly vulnerable to FXN deficiency. Despite clinical evidence linking FRDA to retinal hypoplasia, retinal nerve fiber degeneration, and optic nerve dysfunction, the molecular mechanisms underlying vision impairment remain poorly understood due to the lack of disease models and focused research. This study aimed to determine the effects of frataxin deficiency and GAA repeat expansions on retinal pathology and retinal DNA integrity using FRDA induced pluripotent stem cell (iPSC)-derived retinal organoids (ROs) compared with isogenic and healthy control iPSC-derived ROs.

Methods: iPSC-derived ROs were generated from FRDA patients, their matched isogenic controls, and healthy controls. GAA repeat instability, FXN mRNA and protein expression, mitochondrial DNA damage, and retinal organoid differentiation and maturation were assessed over time.

Results: FRDA ROs exhibited progressive GAA repeat expansion during retinal differentiation, accompanied by significantly reduced FXN mRNA and protein levels compared with controls. In addition, FRDA ROs demonstrated increased mitochondrial DNA damage compared with isogenic and healthy controls. These abnormalities were associated with impaired photoreceptor differentiation and maturation relative to control ROs.

Discussion: The findings suggest that frataxin deficiency and progressive repeat expansion may contribute to defective retinal cell development through mechanisms involving mitochondrial DNA damage. The high metabolic demand of retinal tissue may render it particularly susceptible to FXN deficiency.

Conclusion: FRDA iPSC-derived retinal organoids showed delayed retinal differentiation and maturation and provide a relevant human model for investigating mechanisms of visual dysfunction and evaluating potential therapeutic strategies.

Funding Sources: FARA and O'Donnell Brain Institute Sprouts Program

DR49. Computational Design and Experimental Screening of Anti-FDX2 Nanobodies for Friedreich's Ataxia Research

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Background and objectives: Friedreich's ataxia (FRDA) is an inherited neurodegenerative disorder caused by reduced frataxin expression, leading to altered mitochondrial iron–sulfur (Fe–S) cluster metabolism, iron homeostasis, and redox balance. Ferredoxin 2 (FDX2), a mitochondrial electron-transfer protein involved in Fe–S cluster biogenesis, is a potential target for investigating FRDA-relevant mitochondrial processes. We developed an integrated computational workflow to design and prioritize FDX2-binding nanobody (NB) candidates, followed by initial experimental assessment.

Methods: Mature human FDX2 was used as the design target, with putatively accessible residues selected in the reported structural context of the mitochondrial Fe–S cluster assembly machinery. NeuroBind Advance was run independently five times to generate FDX2–NB complex models. Complexes underwent 5,000 steps of AMBER energy minimization and relaxation. Candidates were ranked using predicted interface features, PRODIGY binding estimates, MM/GBSA calculations, hydrogen-bond networks, structural-confidence metrics, molecular-dynamics-derived fluctuations, and predicted developability properties. Selected complexes were independently modeled with Chai-1, Boltz-2, and AlphaFold3. Recombinant holo-FDX2 and His-tagged NB candidates were produced in *Escherichia coli*, purified, and assessed by exploratory biolayer interferometry (BLI).

Results: Five NB candidates were generated and ranked computationally; three were expressed and purified under the tested conditions. Exploratory BLI indicated detectable FDX2-dependent binding for ADV1, whereas the remaining tested candidates produced no interpretable interaction signal under the same conditions. ADV1 was selected for interface-focused ProteinMPNN redesign. Three variants—ADV1_s20, ADV1_s20_cdr3, and ADV1_s20_a60tfwS1—retained favorable predicted conformational and developability profiles and showed improved computational interaction scores relative to the parental design. These predictions have not yet been experimentally tested.

Discussion and conclusion: We established a computationally driven framework that incorporates early experimental feasibility and interaction evidence to prioritize anti-FDX2NBs. ADV1 is a lead candidate for continued optimization rather than a validated high-affinity binder. Future work will quantify binding kinetics and affinity, assess specificity and epitope engagement, and determine suitability for biochemical applications.

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DR50. Transcriptomic Profiling Reveals Altered Ferroptosis Regulation in Friedreich's Ataxia Fibroblasts

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Background & Objectives: Friedreich's ataxia (FRDA) is a rare, life-limiting, frataxin-deficiency disorder resulting in iron mislocalization, increased oxidative burden, altered lipid metabolism and neurodegeneration. Cellular sequelae common to FRDA, including accumulation of lipid peroxides, are proposed drivers of ferroptosis—a type of iron-mediated cell death that remains underexplored. To better characterize the molecular underpinnings that precede ferroptosis in FRDA and lay groundwork for identifying a coordinated gene expression profile to guide future biomarker development, we describe a GEO2R-based transcriptomic analysis of ferroptosis regulator gene expression in FRDA patient-derived fibroblasts relative to controls.

Methods: Publicly available expression data from FRDA (n=18) and control (n=17) fibroblasts (Gene Expression Omnibus GSE104288) were analyzed using GEO2R's limma workflow to yield normalized expression counts for an initial panel of ferroptosis suppressor genes (AIFM2, AKR1C1/2/3, CDKN1A, GPX4, SLC7A11, TP53)—and FXN—which were selected based on literature-supported roles in regulating ferroptosis. Statistical comparisons between the groups were conducted in Excel using a two-tailed, unequal-variance t-test.

Results: Of the genes selected for this analysis, AIFM2(p=0.002), AKR1C1(p=0.005) & AKR1C3(p=0.007) were significantly upregulated in FRDA fibroblasts. Differential expression of AKR1C2 was also observed, approaching significance (p=0.06). FXN expression was significantly reduced (p≤0.001) across FRDA samples when compared against controls, while no difference in CDKN1A, GPX4, SLC7A11, or TP53 was observed.

Discussion and Conclusion: As expected for a frataxin-deficiency disorder, FXN expression was significantly reduced in FRDA fibroblasts, supporting the validity of our dataset and our analysis. Simultaneous upregulation of multiple ferroptosis suppressors, including AIFM2, AKR1C1, and AKR1C3, suggests a coordinated response to increased ferroptotic stress in FRDA fibroblasts. Together, these findings provide transcriptomic evidence of altered ferroptosis regulation in FRDA and identify a potential molecular signature warranting further investigation for future biomarker development.

DR51. Temporal Dyscoordination of Cerebellar Firing in Ataxia

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Ataxia-telangiectasia (A-T) is an autosomal-recessive disease caused by A-T mutated (ATM) protein deficiency. Mice harboring a clinically relevant ATM nonsense mutation develop a severe cerebellar-ataxia without an overt cell-loss, indicating that circuit-level dysfunction alone can produce motor deficits. Cerebellar neural-circuits critical for coordinated movement are progressively altered in A-T, but the circuit-level impairments driving this dysfunction remain unknown. We hypothesize that A-T pathophysiology reflects a breakdown in temporal-coordination of neural firing, leading to abnormal population-dynamics. To test this, we performed the first dense silicon-probe recordings from ~1,300-cerebellar cortex (CC)-units, ~1200-cerebellar nuclei (CN)-units, and 145-putative Purkinje neurons (PNs) from late-stage ataxic (EXP, n=9) and healthy control (HC, n=9) mice during head-fixed voluntary locomotion on a running-wheel.

We identified cell-types based on peak firing-rate (FR) modulation aligned to movement-onset and offset, and observed higher-FR concentrated in movement-activated units ($p=0.025$), with trending irregularity (higher CV2, shorter Inter-Spike Interval (ISI); both $p<0.01$). PNs showed comparable movement-FR ($p=1.00$), but significantly irregular spiking (CV2: $p=0.014$) and elevated complex-spike rates ($p=0.044$), most pronounced in movement-activated PNs ($p=0.005$). Downstream, CN-units in EXP mice showed elevated tonic-FR alongside blunted locomotion driven FR-modulation ($p=0.021$) and a trend towards shorter ISI ($p=0.052$). Movement-suppressed CN-units failed to differentiate locomotion from rest (FR ratio $\approx$ 1.0). Together, these findings point to poorly regulated CN output, consistent with irregular, insufficiently patterned PN-inhibition upstream. This breakdown extended beyond FR to the temporal structure of network activity: although FR-locomotion:speed coupling was similarly weak in both groups ($p\approx 0.10$; $p=0.662$), the temporal-lag between the two showed greater variance in EXP, local pairwise synchrony was elevated across both CC and CN, spatial decay of CN-synchrony was shorter in EXP ($\lambda\approx 106\mu\text{m}$ vs $>393\mu\text{m}$), and the coherent locomotion-state modulation of CC population-synchrony present in HC was absent in EXP.

Our observations suggest ATM deficiency degrades temporal-precision of PN-output and downstream movement-preparatory signals across the cerebellar-thalamic-cortical pathway.

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DR52. Repeat Expansion–Induced Gain of Function and RFC1 Loss of Function mechanisms in Drosophila Models of CANVAS

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Background and Objectives: Cerebellar ataxia with neuropathy and vestibular areflexia syndrome (CANVAS) is a late onset, progressive, recessive disorder characterized by diffuse Purkinje cell loss and peripheral sensory neuropathy. CANVAS is caused by a non-reference Alu-element associated, biallelic short tandem repeat (STR) expansion (CCCTT/AAGGG) in the second intron of replication factor C subunit 1 (RFC1). How RFC1STR expansion elicits neurodegeneration is unknown. Here, we developed a series of Drosophila models of CANVAS to investigate potential pathogenic mechanisms involving gain of function of RFC1STR expansion and loss of RFC1 function.

Methods: A set of transgenic flies expressing the RFC1STR with flanking human sequences or with an upstream ATG start codon 5' to the repeat were generated to determine whether the repeat RNA elicits toxicity in Drosophila neurons. Multiple RNAi transgenes were used to knock down gnf1, the Drosophila homolog of RFC1. RFC1STR and gnf1RNAi constructs were expressed in motor neurons, sensory neurons, or pan-neuronally during development or in adulthood.

Results: In flies, RFC1AAGGG repeat expression supported repeat associated non-AUG (RAN) translation to produce KGREG repeat peptides similar to those observed in patient brains. Expression of the RFC1STR in motor neurons caused an age-dependent decline in climbing ability. In parallel, knockdown of gnf1 in distinct neuronal populations also resulted in neuronal dysfunction: knockdown in larval sensory neurons impaired heat-avoidance responses, whereas adult-specific pan-neuronal knockdown shortened lifespan and reduced climbing ability.

Discussion and Conclusion: Neuronal expression of the RFC1STR and knockdown of gnf1 both impair neuronal function, suggesting that CANVAS may arise through a combination of repeat-induced gain-of-function and RFC1 loss-of-function mechanisms that combinatorially drive neurodegeneration.

Funding Sources: NIH [R01NS086810, R01NS099280, and P50HD104463]. Department of Veteran's Affairs [I01BX004842-04].

Emerging and Existing Therapeutic (Clinical Research)

EC1. Relative bioavailability of omaveloxolone when capsules contents are sprinkled over low-fat, non-Greek yogurt versus administration as intact capsules

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Background and objectives: Omaveloxolone, an oral Nrf2activator, is approved for Friedreich ataxia in patients aged ≥ 16 years at 150 mg daily, administered on an empty stomach as intact capsules or sprinkled over applesauce. This study (NCT07019064) assessed the relative bioavailability, safety, tolerability, and palatability of omaveloxolone administered as capsule contents sprinkled over low-fat, non-Greek yogurt versus intact capsules in healthy adults. This sprinkled-over-yogurt option expands administration vehicles for omaveloxolone and could benefit patients with dysphagia.

Methods: In this phase 1, open-label study, healthy adult participants were randomized to 1 of 2 treatment sequences to receive a single 150-mg dose of omaveloxolone: 3 intact 50-mg capsules in Period 1 followed by capsule contents sprinkled over and mixed in 1 tablespoon (15 mL) of low-fat, non-Greek yogurt in Period 2, or the reverse sequence, with a 14-day washout between periods. The primary endpoints were C_{max}, AUC_{0-last}, and AUC_{0-∞}. Secondary endpoints included safety and tolerability. Exploratory endpoints included acceptability and palatability of the sprinkled-over-yogurt administration using a 5-point hedonic scale.

Results: Of 52 participants, 50 completed the study; 1 participant per randomized group discontinued after Period 1. Sprinkled-over-yogurt administration showed similar exposure to intact capsules; geometric mean ratios (90% CIs) were 1.03 (0.953-1.119) for C_{max}, 0.93 (0.873-0.997) for AUC_{0-last}, and 0.93 (0.871-0.995) for AUC_{0-∞}, all within bioequivalence bounds (0.80-1.25). Adverse events (AEs) were reported in 44.2% of participants (31.4% during intact-capsule administration and 29.4% during sprinkled-over-yogurt administration); all were non-serious and most were mild. One participant withdrew due to AEs. Most participants rated sprinkled-over-yogurt administration as “good” or “okay” for taste, “no odor” or “neutral” for smell, “easy to swallow” for swallowability, and “smooth” for texture.

Discussion and conclusion: Sprinkling omaveloxolone over low-fat, non-Greek yogurt is a feasible alternative to intact capsules, achieving bioequivalence and being generally safe, well tolerated, and palatable.

Funding Sources: Biogen

EC2. Ten Days of Cerebellar ctDCS in Friedreich Ataxia: A Double-Blind Sham-Controlled Crossover Study

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Background and objectives: Cerebellar transcranial direct current stimulation (ctDCS) improves motor and cognitive symptoms in Friedreich ataxia (FA). A previous sham-controlled crossover trial demonstrated significant benefit after 5 days of anodal ctDCS. Because longer stimulation regimens have been proposed to enhance clinical effects, we aimed to determine whether extending cerebellar ctDCS to 10 sessions increases benefit in FA.

Methods: In a randomized, double-blind, sham-controlled crossover study, 22 patients with FA (age 31.2 ± 14.3 years; age at symptom onset 14.3 ± 9.9 years; disease duration 16.9 ± 9.1 years; GAA1repeats 689 ± 175) received 10 sessions of anodal or sham cerebellar ctDCS delivered over 2 consecutive 5-day blocks. The two stimulation conditions were separated by a 12-week washout period. Motor and cognitive performance were assessed before and after each condition using the Scale for the Assessment and Rating of Ataxia (SARA) and the Cerebellar Cognitive Affective Syndrome scale (CCAS). Outcomes were analyzed using linear mixed-effects models.

Results: Anodal ctDCS significantly improved SARA compared with sham (condition $\times$ time interaction: $\beta = -1.50$, $p = 0.033$; $d_z = 0.88$), corresponding to a mean reduction of 1.39 points (-6.3% from baseline). CCAS also improved significantly after anodal ctDCS compared with sham ($\beta = 11.11$, $p = 0.00005$; $d_z = 0.93$), corresponding to a mean increase of 8.95 points ($+9.7\%$ from baseline). However, these effects were not greater than those previously observed after a 5-day protocol (-6.5% for SARA and $+11\%$ for CCAS).

Discussion and Conclusion: Ten sessions of anodal cerebellar ctDCS significantly improve motor and cognitive symptoms in FA, but do not confer greater benefit than a 5-day protocol. These findings confirm the efficacy of cerebellar ctDCS while suggesting that extending stimulation duration from 5 to 10 days may not provide additional short-term clinical gain.

Funding Sources: FARA

EC3. Pharmacological Interventions for Cardiac Outcomes in Friedreich's Ataxia: A Systematic Review and Meta-analysis of RCTs

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Background: Cardiac complications particularly, hypertrophic cardiomyopathy accounts for approximately 60% of mortality in Friedreich's Ataxia (FRDA). Despite the severity of cardiac involvement, no pharmacological agent has established cardiac benefit. Multiple drugs have been introduced and tested but these individual studies lack statistical power to establish meaningful clinical impact.

Objective: To synthesize and pool the outcomes of existing studies and assess the efficacy of these pharmacological agents on cardiomyopathy.

Methods: We searched PubMed, MEDLINE, Cochrane, EMBASE, Google Scholar and ClinicalTrials.gov including the reference list of prior meta-analysis to identify additional studies. We included all published randomized controlled trials on FRDA comparing any pharmacological agent with placebo or standard of care and reporting at least one cardiac outcome. We excluded non-randomized studies, animal studies and trials reporting only non-cardiac endpoints. The review was registered on PROSPERO (CRD420261325132) and standard PRISMA guidelines were followed. We assessed the risk of bias using Cochrane Risk of Bias 2 (RoB 2) tool and pooled standardized mean difference using random-effects model.

Results: Seven RCTs involving 364 participants, evaluated deferiprone, idebenone, L-carnitine plus creatine, etravirine and epoetin alfa over 4-12 months of follow-up. Pooled analysis did not show statistically significant improvement with any intervention. LVEF: SMD 2.70 (95% CI -1.18 to 6.58), $p = 0.17$, $I^2 = 98\%$. LVM: SMD -0.27 (95% CI -0.79 to 0.25), $p = 0.31$, $I^2 = 0\%$. LVMI: SMD -0.45 (95% CI -1.81 to 0.90), $p = 0.51$, $I^2 = 85\%$. IVS thickness SMD -0.48 (95% CI -1.01 to 0.05), $p = 0.07$, $I^2 = 0\%$.

Conclusion: Current evidence from randomized controlled trials does not show any statistically significant cardiac benefit of pharmacological therapy. The observed trend towards reduction in LVM and IVS thickness is only hypothesis-generating and has not reached statistical significance. Larger, adequately powered RCTs using standardized cardiac endpoints are needed.

EC4. Efficacy and safety of omaveloxolone in Friedreich ataxia : 18-month real-world outcomes from a French early access program

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Background and objectives: Since January 2024, omaveloxolone is available in France for patients with Friedreich ataxia aged ≥ 16 years, through an early access program. This study describes real-world efficacy and safety over 18 months of prescription.

Methods: Scale for the Assessment and Rating of Ataxia (SARA) and national pharmacovigilance data were collected. In addition, laboratory safety outcomes from patients treated at Pitié-Salpêtrière Hospital, Paris, are described.

Results: 448 patients were treated. Mean treatment exposure was 13.0 (6.4) months. At treatment initiation, mean age was 36.2 (15.6) years, mean SARA score was 21.9 (8.8) and 38.4% were non ambulatory. Mean change of SARA score was -0.03 (2.6, n=271) at 12 months and +0.3 (2.8, n=177) at 18 months. In patients aged ≤ 20 years at treatment initiation, mean change of SARA score was +0.73 (3.3, n=42) at 12 months and +1.0 (4.1, n=30) at 18 months. 17.9% of patients permanently discontinued omaveloxolone, mainly due to patient decision or adverse events suspected to be treatment-related by investigators. 279 individuals reported 714 adverse reactions, most were expected according to EU SmPC (56%) and non-serious (91%), with elevated transaminases, gastrointestinal symptoms, and fatigue most frequently observed.

The laboratory results for the Paris site, for AST, ALT, LDL-cholesterol, triglycerides and NT-proBNP at 12 and 18 months will be presented.

Discussion and Conclusion: Over 18 months, SARA progression remained within the lower range of values reported in natural history studies, including in younger patients (EFACTS annual progression of SARA score for patients <27 years at baseline: +1.31). This suggests an attenuation of disease progression in real world, but needs to be confirmed with a longer follow-up. Safety findings remained consistent with those of the Moxie2 study, despite the more advanced disease stage in the treated population. Single-center laboratory analyses confirm long-term changes in hepatic, lipid, and NT-proBNP parameters.

Funding Sources: EAP data collection funded by Biogen according to French law.

EC6. A Phase 3, Multinational, Randomized, Placebo-Controlled, Double-Blind, Crossover Trial (IB1001-304) Evaluating Levacetylleucine in CACNA1A

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Background and Objectives: CACNA1A-related disorders refer to a range of rare disorders with pathogenic mutations in the CACNA1A gene. This gene plays a critical role in calcium-ion signaling and release of neurotransmitters. Levacetylleucine enters enzyme-controlled pathways to correct metabolic dysfunction, improve energy production, normalize lysosomal calcium levels, thereby stabilizing inter-ion calcium signaling. IB1001-304 is a Phase 3 trial to assess the efficacy and safety of levacetylleucine in CACNA1A-related disorders.

Methods: IB1001-304 will enroll participants ≥ 4 years with a genetically confirmed diagnosis of CACNA1A-related disorders and with manifest cerebellar ataxia based on the Scale for the Assessment and Rating of Ataxia (SARA). Participants will be assessed during three study periods: a baseline period, Treatment Period 1, and Treatment Period 2. Participants will be randomized 1:1 to receive levacetylleucine or placebo for 12-weeks and will crossover to receive the opposite treatment for 12-weeks. An Extension Phase will be planned to assess long-term effects. Treatment will be administered orally for a total daily dose of 2-4 g/day based on weight-tiered doses.

Results: The primary efficacy endpoint will be the SARA. Secondary and exploratory endpoints include Spinocerebellar Ataxia Function Index, quality of life, Neurology Quality of Life-Upper Extremity Function, Clinical Global Impression of Improvement, Scale for Ocular Motor Dysfunction in Ataxia, and others. Safety assessments include adverse event incidence and severity.

Discussion: Multiple clinical trials with levacetylleucine in closely related cerebellar disorders, including Niemann-Pick disease type C, GM2gangliosidosis, and ataxia-telangiectasia demonstrated improved neurological signs and symptoms with a favorable benefit-risk profile. CACNA1A-related disorders are a group of neurological disorders with no approved treatments, therefore there is a strong need for development of effective therapies to treat these disorders.

Conclusion: IB1001-304 will evaluate the efficacy and safety of levacetylleucine in improving neurological symptoms, functioning, and quality of life in people with CACNA1A-related disorders.

Funding Sources: This trial is funded by IntraBio Inc.

EC8. Intravenous AAVrh.10hFXN (LX2006) Gene Therapy in Friedreich Ataxia: An Externally Controlled Open Label Study of Neurological Progression

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Background and objectives LX2006 is an intravenous cardiotropic AAVrh.10 vector delivering human FXN, developed to treat Friedreich ataxia (FRDA) cardiomyopathy — the leading cause of FRDA mortality. Preclinical models suggest the vector may also transduce other tissues, and open-label participants have shown apparent neurologic stabilization. We quantified whether mFARS trajectories in LX2006-treated participants deviate from natural-history progression in a propensity-score-matched comparison.

Methods mFARS data from 16 LX2006-treated adults across two open-label studies (SUNRISE-FA; Weill Cornell/NHLBI HL151355 first-in-human trial) were compared with propensity-score-matched controls from UNIFAI. Logistic propensity scores used age, age at onset, GAA1, sex, baseline mFARS, and ambulatory status; matching was 3:1 nearest-neighbour (caliper 0.25 SD). Concurrent SKYCLARYS use (open-label 6/16, plus 2 partial; controls 1/45) was not a matching covariate. Baseline-adjusted mixed-effects models estimated annual progression; the group-by-time interaction gave the slope difference. Robustness used an extended matching pool, inverse-probability weighting, and alternative model specifications. Primary outcome mFARS; secondary FARS B (upper limb coordination), FARS C (lower limb coordination), USS (upright stability score)

Results Sixteen treated participants were matched to 45 natural-history controls. Treated participants improved on mFARS (slope -1.37 points/year) while matched controls progressed (+0.83 points/year), a difference of 2.33 points/year (95% CI 0.82–3.84; p=0.003). The effect concentrated in upper-limb coordination (FARS B difference 1.98, p=0.004); lower-limbs (FARS C, 0.17) and upright stability (USS, 0.13) were not significant. Findings were consistent across extended-pool matching (2.45) and inverse-probability weighting (2.10).

Discussion and Conclusion LX2006-treated participants deviated from UNIFAI natural history progression and showed slower mFARS decline concentrated in upper-limb coordination. Beyond its cardiac intent, this supports potential neurologic benefit of LX2006. These results need to be carefully interpreted, due to a small sample, open-label design, use of skyclarys for some participants, absence of a concurrent control, and the susceptibility of upper-limb mFARS items to placebo and training effects.

Funding Sources: Lexeo Therapeutics

EC11. Response to Omaveloxolone in Friedreich's Ataxia patients over a 3 year period as measured by mFARS scores

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Background and objectives: Omaveloxolone is approved since 2023 for treatment of FRDA in patients >15 years. We assessed response to treatment with omaveloxone in clinical practice with modified Friedreich Ataxia Rating Scale (mFARS) scores.

Methods: We recorded baseline and follow up mFARS scores over 3 years in FRDA patients treated with omaveloxolone.

Results: We identified 261 FRDA patients who received omaveloxolone since it became available in July 2023. 229/261 had baseline mFARS scores before treatment initiation. 14 patients lacked follow up scores. 215 patients had a baseline score and at least one year follow up data. We observed 156/215 patients as responders who demonstrated either stabilization or improvement in mFARS scores. Among responders, 64/156 were male with average age of onset 12.5 yrs, GAA1repeat length 629 and age of starting omaveloxolone, 31.4 yrs. Among responders, the mean annual change compared to baseline in mFARS scores was -3.2 during the first year of follow-up (128/214 patients), -3.5 during the second year (94/168 patients) and -6.4 during the third year (6/15 patients). Among patients who did not respond to treatment, 31/59 were male with average age of onset 14.7 yrs, GAA1repeat length 643 and age of starting omaveloxolone, 30.6 yrs. The mean annual change compared to baseline in mFARS scores was +3.1 at first year follow-up (86 patients), +4.1 at second year follow-up (74 patients) and +4.1 at third year follow up (8 patients) amongst non-responders.

Discussion: Our findings are consistent with the MOXIe trial and found that treatment improved mFARS scores to a similar degree in the majority of treated patients over a course of 3 yrs. The response was consistent over the course of 2 years with limited data available for third year follow up.

Conclusion: In this study, we provide longitudinal evidence for real world benefit of omaveloxolone in FRDA.

Funding Sources: Friedreich Ataxia Global Clinical Consortium UNIFIED Natural History Study (UNIFAI)

EC12. Systematic Search for Cryptic Exons to n-of-1 Therapy

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Background and Objectives Biallelic loss-of-function variants in SETX, encoding the RNA/DNA helicase senataxin, cause Ataxia with Oculomotor Apraxia type 2 (AOA2), characterised by progressive cerebellar ataxia, oculomotor apraxia, peripheral neuropathy and elevated serum alpha-fetoprotein. A subset of patients with a convincing AOA2 phenotype carry only a single identifiable pathogenic SETX variant, or none, on standard testing, leaving the second disease allele unaccounted for. Deep intronic variants that activate cryptic splice sites and drive pseudoexon inclusion are a plausible but underexplored source of this missing heritability. This project aims to identify such events in SETX and validate them functionally.

Methods Whole-genome sequencing data from the Genomics England National Genomics Research Library was interrogated for participants with a phenotype consistent with AOA2, including those with monoallelic SETX variants. A bioinformatic pipeline screened SETX deep intronic regions for variants predicted to alter splicing using splice-prediction tools (e.g. SpliceAI) with conservation and population-frequency filtering. Prioritised candidates were taken forward for functional characterisation in patient-derived cell lines using RT-PCR and minigene splicing assays.

Results (work in progress) Screening identified a candidate deep intronic SETX variants predicted to generate cryptic splice sites or pseudoexons in ten participants. were prioritised for functional testing. Preliminary studies in patient cell lines explored aberrant splicing, including pseudoexon inclusion. Validation is underway.

Discussion and Conclusion Exploration of deep intronic cryptic splicing may resolve unsolved AOA2 cases. Functional confirmation is essential to establish pathogenicity. Pseudoexon-causing variants are attractive candidates for splice-modulating antisense oligonucleotide therapy.

Funding Sources: MRC Centre of Research Excellence in Therapeutic Genomics. University of Oxford & St John's College Clarendon Fund

EC13. Intravenous coenzyme Q10replacement in COQ8A-ataxia: first-in-disease evidence for disease modification by BPM31510from n-of-1 treatment programs

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Background and objectives: COQ8A-ataxia is a progressive early-onset multisystemic cerebellar ataxia caused by primary coenzyme Q10(CoQ10) deficiency due to biallelic pathogenic variants in COQ8A. Clinical response to oral CoQ10is often limited, likely due to poor bioavailability and insufficient central nervous system penetration. BPM31510, a novel intravenous lipid nanoparticle formulation of CoQ10, has demonstrated improved bioavailability, enhanced blood–brain-barrier penetration, and established clinical safety in oncology trials. This provides a molecular and clinical rationale for first-in-disease treatment in COQ8A-ataxia.

Methods: Two patients with genetically confirmed COQ8A-ataxia – a 9-year-old girl (P1) and 16-year-old boy (P2) – both previously treated with oral CoQ10, received weekly infusions of BPM31510(50 mg/kg) within a German named-patient treatment framework (“Individueller Heilversuch”). Treatment effects were assessed longitudinally using multimodal outcome measures, including clinician-, patient-, and caregiver-reported outcomes as well as performance outcomes.

Results: BPM31510was safe and well tolerated under concomitant vitamin K supplementation. After 12 months, P1showed substantial improvement in ataxia severity (SARA 11→8), upright stability (FARS-E 7→4), and activities of daily living (FARS-ADL 11→4), as well as in goal attainment scales, caregiver-reported global impression of change (including balance, dexterity, and speech domains), and a smoothness metric of a digital-motor drawing task. This clinical improvement was maintained also beyond 6 months, allowing extension to biweekly infusions without deterioration. In P2, a clearly progressive disease course over the preceding two years was stabilized under treatment, with mild improvement in SARA (12→10) and FARS-ADL (9→7). Following shared decision-making, treatment was paused after 10 months with continued monitoring for possible re-initiation in case of renewed progression.

Discussion and Conclusion: These first-in-disease observations suggest that optimized intravenous CoQ10delivery might exert disease-modifying effects in COQ8A-ataxia beyond conventional oral supplementation. BPM31510may represent a mechanistically targeted treatment strategy for COQ8A-ataxia and warrants prospective evaluation in formal clinical trials.

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EC14. Intake of Omaveloxolone, Intake-associated Factors and Intake-continuity in Patients with Friedreich Ataxia: Results of the PROFA Study

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Background and objectives: Friedreich ataxia (FA) is a rare movement disease, but the most common autosomal recessive ataxia. Core symptoms include ataxia, impaired coordination, and speech difficulties. Since 2023, the first FDA-approved FA medication, omaveloxolone, has been available. This study aimed to report evidence on intake, continuity, and intake associated factors.

Methods: This analysis included patients from Germany, France, and Austria who participated in the PROFA study (2022 – current), a European multicenter longitudinal study that combined an on-site study center baseline visit with a remote mobile assessments for 6 months, capturing various clinical, patient-reported, and psychosocial outcomes, and healthcare utilization and medication use. Omaveloxolone intake, intake-associated factors, and intake continuity were descriptively analyzed.

Results: Ninety-six patients (mean: age 35.4 [14.4]; GAA-repeat size 572 ; 53.1% women) were included. At baseline, n=33 patients (34.3%) were taking omaveloxolone. During the six-month study period, omaveloxolone intake increased to 69.8% (n=67). Those patients with earlier FA onset (16.9 vs 25.9 years, p=0.001) which is associated with accelerated disease progression, more likely receive omaveloxolone. Omaveloxolone intake was also associated with a higher Cerebellar Cognitive Affective Syndrome (failed CCAS: 62.9% vs. 37.0%, p=0.044), cardiomyopathy (14.3% vs. 0%, p=0.011), and higher self-reported health (EQ-5D-5L: 0.77 vs. 0.67, p=0.040). Omaveloxolone intake was rarely discontinued, with only n=2 patients (2.9%) reporting a discontinuity within the first two (n=1) or between months two and four (n=1).

Discussion and conclusion: Omaveloxolone uptake increased substantially during the study period, and treatment continuity was high. Patients receiving omaveloxolone tended to have an earlier disease onset, suggesting preferential use among individuals with a more severe or rapidly progressive disease profile. These

findings provide early evidence on real-world utilization patterns of omaveloxolone, highlighting the need for continued monitoring of treatment persistence, patient selection, and outcomes.

Funding Sources: Funding: European Joint Programme on Rare Diseases (EJP RD) (2022-2025) & Biogen Germany (2025 – today).

EC15. An intensive home-based balance training program for pre-ataxic mutation carriers and early symptomatic patients with Spinocerebellar Ataxia

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BACKGROUND AND OBJECTIVES: Spinocerebellar ataxias (SCAs) are characterized by progressive impairments in coordination and balance, with early symptoms including gait difficulties. While no pharmacological treatment is available, intensive physiotherapy has been shown to reduce symptoms in moderate and advanced disease stages. However, pre-ataxic mutation carriers and early symptomatic patients have not been systematically studied. These early stages represent a promising therapeutic window, as neurodegeneration remains limited and neural plasticity largely preserved. In animal models, early motor training reduced neurodegeneration and delayed disease onset. In pre-ataxic mutation carriers, subtle balance impairments during complex tasks have been reported, but preventive training approaches remain largely unexplored. This intervention study investigates the effects of an intensive 6-week home-based balance training program on balance performance, gait and turning behavior in real-life.

METHODS: Participants (n=30, SARAposture&gait≤5) complete an intensive home-based balance training for 6 weeks, at least 3 days/week for 30–40 minutes/session. Before and after the intervention, clinical assessments (SARA, FARS-E, mini-BEST, static and dynamic balance tests) and digital movement data from three wearable sensors in laboratory and real-world settings are collected. Subjective outcome measures include the ABC, Goal Attainment Scale (GAS), PROM-short, and PGI-C. We present the assessment and training concept, the training app, and preliminary analyses of 10 datasets.

RESULTS: The training concept includes three core activity exercises and four exercises focusing on axial stability. To ensure high motor complexity, exercises derived from sports rehabilitation were selected that specifically target all balance components (steady-state, proactive, reactive) while incorporating coordinative demands. To ensure training at the individual performance limit, a modular progression system allows each exercise to be performed at six difficulty levels, individually adjusted at baseline and throughout training.

DISCUSSION AND CONCLUSIONS: Preliminary pilot data indicate promising effects supporting the implementation of preventive interventions during the pre-ataxic and early symptomatic stages of SCA.

Funding Sources: Deutsche Heredo-Ataxie-Gesellschaft (DHAG)

**EC16. Long term daily nomlabofusp administration of nomlabofusp in patients with Friedreich's ataxia:
Interim observations from an ongoing open label study**

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Background/Objectives: Friedreich's ataxia (FRDA) is a neurodegenerative disease resulting from a deficiency in frataxin (FXN). Nomlabofusp is a recombinant fusion protein being developed to increase FXN levels in adults and children with FRDA. Interim data from an ongoing open label study were reviewed to assess changes in FXN concentrations, clinical measures, and safety in patients with FRDA receiving long term nomlabofusp treatment.

Methods: Enrolled subjects received daily administration of nomlabofusp. FXN concentrations and clinical outcomes (e.g. mFARS, FARS_ADL, 9HPT) were measured at baseline and every three months. Safety was assessed monthly either during in-person or remote study visits.

Results: Data from 41 subjects who received at least 1 dose of nomlabofusp were reviewed. Subjects received nomlabofusp treatment for up to 2 years. Mean age at screening and symptom onset were 29.0 and 12.4 years, respectively. Mean baseline results for mFARS, FARS_ADL, and 9HPT were 55.0 points, 17.1 points, and 93.8 seconds, respectively. Median baseline buccal and skin FXN levels were 18-20% of healthy controls. FXN levels increased through Month 6 and then plateaued. Skin FXN concentrations in almost all subjects at Months 6 and 12 were within the range expected in heterozygous carriers without phenotypic expression of disease. Mean clinical outcome values were at or below baseline at all timepoints through 2 years. Mild-moderate injection site reactions were the most commonly reported adverse events. Anaphylaxis was observed in some subjects; almost all cases occurred in subjects exposed to nomlabofusp during a prior study. Adverse event reports decreased after the first 3 months of treatment.

Discussion/Conclusion: In patients with FRDA, daily nomlabofusp resulted in increased FXN levels with concomitant improvements in clinical outcomes that were consistent across measures and sustained out to 2 years. There is a risk of anaphylaxis during early treatment, particularly in patients with prior nomlabofusp exposure. Long term treatment with nomlabofusp appeared to be well tolerated.

Funding Sources: Larimar Therapeutics, Inc.

EC17. Clinical response to treatment with Omaveloxone in Friedreich's Ataxia patients over 3 years

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Background and objectives: Omaveloxolone, approved for use in Friedreich's ataxia (FRDA) in patients over 16 yrs, has been reported to provide benefit in neurological function. We assessed long-term subjective outcomes in FRDA patients receiving omaveloxolone.

Methods: We reviewed symptoms in FRDA patients treated with omaveloxolone who experienced stabilization/improvement in modified Friedreich's Ataxia Rating Scale (mFARS) scores (responders) vs decline in mFARS (non-responders) over 3 years. Additionally, we reviewed ECHO data pre and post treatment for 1 year, adverse effects amongst non-responders and reasons for discontinuation of drug.

Results: Of 261 FRDA patients who received omaveloxolone since July 2023, 156 had improvement/stabilization in mFARS scores. 119/156 responders reported benefit, most often in fatigue (46/119), speech (45/119), strength (37/119), and fine-motor skills (33/119). Among non-responders, 40/59 reported improvement in fatigue (14/40) and speech (14/40), with fewer noting gains in strength (8/40) or fine-motor skills (6/40); 15 also reported adverse effects (GI issues 6/15, fatigue 3/14, disease progression 2/14). Cardiac measures were similar at baseline and largely stable at 1 year. Responders had mean EF 59.7% at baseline (123/156) and 56% at 1 year (61/156), with IVSTd 1.04 cm at baseline (118/156) and 1.05 cm at 1 year (61/156). Non-responders had mean EF 60.4% at baseline (48/59) and 58.6% at 1 year (13/59), with IVSTd 1.02 cm at baseline (45/59) and 1.02 cm at 1 year (13/59). Forty-seven patients discontinued omaveloxolone after a mean of 10.8 months (9 no perceived improvement, 2 cost, 24 other/unspecified reasons).

Discussion: Most responders and some non-responders reported symptom improvement over 3 years. Baseline EF and IVSTd were similar between groups. IVSTd was stable at 1 year and EF may decline slightly. Omaveloxolone was generally well tolerated, with few discontinuations due to side effects.

Conclusion: We present longitudinal data demonstrating clinical improvement and favorable safety profile of omaveloxolone, regardless of objective response.

Funding Sources: Friedreich's Ataxia Research Alliance

EC18. Implications of an open-label metformin clinical trial in C9 or f72ALS for the treatment of RAN-positive spinocerebellar ataxias

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Background: Microsatellite repeat expansions cause over 70 neurological disorders. At least 18 diseases, including SCA1, SCA2, SCA3, SCA6, SCA7, and SCA8, feature toxic RAN protein pathology, making this common disease hallmark a critical and potentially unifying therapeutic target. In SCA8 and C9orf72ALS mice, metformin reduces toxic RAN proteins, decreases neuroinflammation, and improves behavioral abnormalities. Driven by these preclinical findings, we conducted an open-label clinical trial to evaluate the safety and efficacy of metformin in patients with C9orf72ALS.

Methods: This open-label clinical trial evaluated metformin treatment over 24 weeks in 38 participants with C9orf72 ALS. Primary and exploratory outcomes were assessed using meso-scale discovery (MSD) and nucleic-acid-linked immuno-sandwich assays (NULISA) to measure toxic RAN proteins, neurodegenerative biomarkers, and systemic inflammation. Functional decline was monitored using the ALS functional rating scale revised (ALS-FRS-R) and compared to C9orf72 ALS natural history cohorts.

Results: This trial met its primary outcomes of safety and the reduction of RAN proteins. Poly(GP) RAN proteins were decreased by 25.38% in CSF ($p=0.03$) and 18% in serum ($p=0.03$), while serum poly(GA) decreased by 37% ($p=0.002$). MSD and NULISA assays showed reductions in markers of neurodegeneration and neuroinflammation, including NfL (-39%, $p=0.0036$), GFAP (-22%, $p=0.0133$), TNF- α (-18%, $p=0.0036$), IFN- γ (-15%, $p=0.0133$), and IL-6 (-13%, $p=0.039$). Clinically, there were no serious drug-related adverse events, and the decline in ALSFRS-R was dramatically slowed (-0.164 points/week) compared to two natural history studies (-0.414 and -0.428 points/week). Time-to-event modeling predicts a 2.7-year delay in reaching an ALSFRS-R of 10, at which there is a 15% chance of 9-month survival.

Conclusions: Metformin safely reduces RAN proteins, neurodegeneration markers, and the rate of functional decline in C9orf72 ALS patients. These findings support advancing metformin to placebo-controlled trials for C9orf72 ALS and for clinical testing in other "RANopathies," including SCA1, SCA2, SCA3, SCA6, SCA7, and SCA8.

Funding Sources: Clinical trial funding: Department of Defense funding and The ALS Association (ASLA)
SCA8 and other SCAs funding: National Ataxia Foundation (NAF) and National Institutes of Health (NIH)

EC19. Feasibility of a double blind crossover trial of the Exopulse Mollii suit in hereditary ataxia and spastic paraplegia

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Background and objectives: The Exopulse Mollii® suit is a wearable electrostimulation system designed to modulate muscle activity and improve movement¹. Despite increasing use, independent clinical evaluation in hereditary ataxia and hereditary spastic paraplegia (HSP) is limited². This study aims to evaluate the feasibility, acceptability, and safety of conducting a double-blind randomised crossover trial of Mollii suit use in these populations.

Methods: This mixed-methods feasibility study will recruit adults and young people (≥5 years) with hereditary ataxia or HSP. Participants will undertake a 14-week home-based protocol: two 4-week blocks of daily suit use (1 hour/day), separated by a 4-week washout period, followed by a 2-week follow-up. Participants will be randomised to receive therapeutic or non-therapeutic stimulation in block order, with participant and assessor blinding. Assessments will be conducted at baseline (home visit) and remotely at multiple time points. Outcome measures include SARA, mFIM, ARAT, Modified Ashworth Scale, INAS, and Spastic Paraplegia Rating Scale (SPRS), alongside EQ-5D and Fatigue Severity Scale. Daily diaries will monitor adherence, perceived benefit/burden, and adverse events. Exit interviews will explore acceptability and trial experience.

Results: Ethical approval is currently in progress, with recruitment imminent. Preliminary findings will focus on feasibility outcomes including recruitment rates, retention, adherence to daily use and remote assessments, completeness of outcome measures, and participant-reported burden. Planned qualitative analyses will explore acceptability, practicality of home-based delivery, and barriers and facilitators to participation.

Discussion and Conclusion: This study will provide critical evidence on the feasibility of conducting a rigorously controlled trial of wearable electrostimulation in hereditary ataxia and HSP. Findings will inform optimisation of study design, outcome selection, and remote delivery strategies. The results will directly support the development of a future definitive trial to evaluate clinical effectiveness.

¹ <https://www.ottobock.com/en-gb/orthotics/exopulse-mollii-suit>.

² <https://www.remotion.co.uk/evidence/>

Funding Sources: Ataxia UK, The Hereditary Spastic Paraplegia Group UK, Exopulse Mollii suit loan supplied by Ottobock

EC20. Improvement of Friedreich's Ataxia Cardiac Function with Omaveloxolone

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Objective: To determine the effect of omaveloxolone (Oma) on cardiac function in Friedreich's ataxia patients. **Background:** Friedreich's ataxia (FA) is an inherited neurodegenerative disorder that is frequently associated with cardiomyopathy. Oma, an activator of the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, has demonstrated clinical benefits in neurological function; however, its effects on cardiac manifestations of FA remain uncharacterized. **Methods:** We evaluated the impact of Oma on cardiac parameters in patients with FA in a retrospective chart review. Patients included in the analysis had pre- and post- Oma cardiac testing, including echocardiography and/or cardiac magnetic resonance imaging (MRI) measurements of left ventricular ejection fraction (LVEF), intraventricular septal thickness in diastole (IVSd), and left ventricular mass index (LVMI). **Results:** We evaluated 25 patients (taking Oma 150 mg/day) who underwent pre- and post-Oma cardiac testing (mean time from Oma initiation to most recent cardiac imaging was 25.1 months (SD 20.9, median 19 months)). The mean IVSd decreased from 11.17 mm (SD 2.41) pre-Oma to 9.94 mm (SD 2.27) post-Oma (~9.2%) (Wilcoxon signed-rank test ($p = 0.027$)). The mean LVMI fell from 88.92 g/m² (SD 24.24) pre-Oma to 77.60 g/m² (SD 25.48) post-Oma — a mean reduction of 11.32 g/m² (~10.8%). LVEF did not change significantly pre- or post-Oma treatment. In a control group of FA patients who had not taken Oma or another antioxidant for 2 years prior to their last Echo, LVMI increased by 3.88 ± 15.24 g/m², (Welch's test $p = 0.0141$), IVSD increased by 0.25 ± 2.45 (p = 0.044), and LVEF did not change significantly (p = 0.88). **Conclusions:** In this retrospective chart, Oma was associated with significant improvements in both IVSd and LVMI compared with the control group, while cardiac function (LVEF) remained stable. This suggests that Oma may exert a cardioprotective effect in FA.

EC22. Efficacy and Safety of Dimethyl Fumarate in Friedreich Ataxia: Primary Results From the phase 2, Randomized, Double-Blind, Placebo-Controlled DMF-FA-2

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OBJECTIVES Evaluate the efficacy and safety of Dimethyl fumarate (DMF) in patients with Friedreich Ataxia (FA). **BACKGROUND:** FA is an autosomal recessive neurodegenerative disease caused by the homozygote expansion of the GAA trinucleotide in the first intron of the FXN gene. Compared to controls, FXN gene expression is 20% in patients and 50% in healthy carriers. Treatment of multiple sclerosis patients with DMF is known to increase FXN expression by 70%. **METHODS** We conducted a randomized, placebo-controlled, Phase II trial to test the efficacy of DMF on FXN gene transcription in patients with FA. Secondary objectives were the increase in frataxin protein, stimulation of the nrf2 pathway and mitochondrial biogenesis, safety and tolerability of DMF, and impact on clinical measures of disease. The study consisted of two sequential phases of 12 weeks each: a core phase (patients were randomized to receive DMF or placebo) and an extension phase (all patients were treated with DMF). EudraCT number: 2021-006274-23. **RESULTS** In total, 40 patients (female, 57.5%; mean age, 36.1 [12.6] years; disease duration 16.8 [6.9] years), were randomized (DMF n=20, placebo n=20). At the end of the core phase, 33 patients continued to the open-label extension. DMF significantly increased FXN gene expression after 12 weeks of treatment (difference compared to placebo +119.0% 95% CI +55.3, +182.7, p=0.018). In the DMF-DMF group FXN expression increased to +205% after 24 weeks compared to baseline (p<0.001). Frataxin protein and some clinical measures showed a non-significant trend towards improvement after treatment. The most frequent adverse events were gastrointestinal (heartburn, nausea, vomiting, diarrhea, abdominal pain), flushing, eosinophilia, lymphopenia, and elevated liver enzymes. **CONCLUSIONS** DMF significantly increases transcription of the FXN gene, with long-term stability, to a degree that restores expression levels to those of healthy carriers. The drug appeared safe, with adverse events consistent with DMF pharmacovigilance.

Funding Sources: The study was supported through a grant from Agenzia Italiana del Farmaco (AIFA) TRS-2018-00001358 and a joint grant from the Friedreich Ataxia Research Alliance (FARA) and the Tristan Allamby Research Fund (TARfa).

EC23. ATI-105: A Clinical-Stage Oral Astaxanthin LNP Formulation Rescues Frataxin Deficiency and Improves Cardiac Function in Friedreich's Ataxia Models

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Introduction: Friedreich's Ataxia (FA) is an inherited frataxin deficiency that impairs biogenesis of iron-sulfur clusters and Nrf2-mediated antioxidant enzyme synthesis, leading to mitochondrial dysfunction, neuromuscular degeneration and lethal cardiomyopathy. Currently, there are no approved therapies that address multi-organ deterioration and cardiomyopathy. Astaxanthin is the most potent antioxidant found in nature, with the ability to modulate multiple signaling pathways to restore mitochondrial function, inhibit oxidative stress and chronic inflammation, regenerate damaged cells and tissues, and reverse fibrotic organ failure. However, prior nutraceutical formulations have suffered from poor bioavailability and limited bioactivity. Here, we evaluated ATI-105, a novel oral lipid nanoparticle astaxanthin formulation with optimized bioactivity and bioavailability, currently being developed for rare mitochondrial diseases.

Methods: Inhibition of ROS and prevention of ferroptosis were assessed by cell-based assays and microscopy in FA patient-derived and healthy control fibroblasts. THP1-Nrf2 and THP1-NFkB reporter cells were used to assess Nrf2 activation and inhibition of NFkB. Inhibition of oxidative stress and restoration of cardiac function were assessed in an iPSC-derived human cardiac organoid model of HFpEF.

Results: In FA patient-derived fibroblasts, ATI-105 treatment demonstrated complete inhibition of ROS and ferroptotic cell death. In human THP1 reporter cells, ATI-105 demonstrated unprecedented inhibition of NFkB and activation of Nrf2 compared to clinically tested compounds and FDA-approved omaveloxolone. In a human organoid HFpEF cardiomyopathy model, ATI-105 inhibited mitochondrial stress and reversed cardiac dysfunction. In IND-enabling toxicology studies in rat and dog, no adverse safety findings were reported, and uptake into all rat tissues, including heart and brain, was observed at all dose levels.

Conclusion: Due to the life-threatening consequences of FA, it is critical to accelerate development of safe and effective therapies to reverse cardiomyopathy and extend lifespan. ATI-105 is initiating Phase 1 safety trials October 2026, with FA safety and efficacy trials planned for 2027 at multiple clinical sites.

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Emerging and Existing Therapeutic (Pre-Clinical Research)

EP1. Unlocking sulforaphane's potential in Friedreich ataxia: further evidence using sensory neurons derived from patient-induced pluripotent stem cells

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Background and Objectives: Friedreich ataxia (FRDA) is a neurodegenerative disorder typically caused by autosomal recessive inheritance of expanded guanine-adenosine-adenosine (GAA) repeats (> 56) in both alleles of the frataxin (FXN) gene, leading to FXN protein deficiency. Omaveloxolone (Oma) is the only approved therapy. Therefore, further therapeutic options are essential. Previously, we showed that sulforaphane (SF) increases FXN expression and modulates epigenetic, inflammatory, and oxidative stress pathways in sensory neurons from one patient-induced pluripotent stem cell (iPSC) line with 550 GAA1repeats (FA2). Here, we compared SF, Oma, and dimethyl fumarate (DMF) treatment in sensory neurons derived from three patient iPSC lines with varying GAA1repeats: FA1(867), FA2, and FA3(450).

Methods: Sensory neurons derived from FRDA patient-induced pluripotent stem cells, were treated with 100nM to 20μM sulforaphane for 24 hours. DMF at 30μM and 10nM to 100nM Oma were included as positive drug controls regulating inflammation and oxidative stress. Cell viability, gene expression and protein assays, and confocal microscopy were conducted.

Results: In FA1, SF treatment improved cell viability and reduced oxidative stress and inflammation. In FA3, SF increased cell viability, FXN protein levels, and gene and protein expression of redox markers, while targeting dysregulated epigenetic mechanisms and inflammation. All three lines showed SF's consistent anti-oxidant and anti-inflammatory effects. Responses to Oma and DMF varied across the FA lines with less pronounced effects than when treated with SF. Overall, SF was more effective than Oma and DMF in improving cell viability and regulating FXN expression and epigenetic, redox, and inflammatory pathways.

Discussion and Conclusion: These findings reveal variability in drug responses based on FRDA genetic profiles and position SF as a promising drug to address multiple pathological processes. Our preclinical data supports SF as a strong FRDA drug candidate. Clinical evaluation is warranted to confirm its full therapeutic potential.

Funding Sources: This study was funded by the School of Health Sciences at Swinburne University of Technology, Melbourne School of Health Sciences at the University of Melbourne, Friedreich ataxia Research Alliance, Medical Research Future Fund Stem Cell Therapies Mission and the University of Wollongong.

EP2. Drug repurposing for Friedreich Ataxia using a mitochondrial phenotype screening platform in patient-derived fibroblasts

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Background: Friedreich's Ataxia (FA) is caused by a repeat expansion in the FXN gene, leading to reduced levels of frataxin. Frataxin functions in the formation of iron-sulphur clusters which are integral to mitochondrial electron transport chain function. Frataxin deficiency therefore leads to mitochondrial dysfunction, and this work therefore aims to identify mitochondrial therapeutics which could benefit FA patients.

Methods: Fibroblasts from 7 donors with FA and 7 healthy age-matched controls were characterised using high content imaging and respirometry to assess mitochondrial morphology, mitophagy and mitochondrial function. Over 500 blood-brain-barrier penetrant drugs approved by the FDA for alternative indications were screened at 10 μ M in 2 lines. Hits were selected as compounds increasing mitochondrial number, lysosome number, cell size or the proportion of functional mitochondria by more than 1 standard deviation from the vehicle mean. Hits were validated in a 9-point dose response curve.

Results: FA patient cells were smaller, had fewer mitochondria and lysosomes even when adjusted for cell size, and a lower oxygen consumption rate compared to controls, although mitochondrial membrane potential was not different. 72 compounds were identified as hits in the primary screen and tested in dose response format. Of these 72, the top 12 performing were validated across the other 5 patient lines. We found the most robust effects across patient cells were on the proportion of functional mitochondria and the top 3 compounds, assessed by amplitude of effect and robustness across patient cell lines, are being investigated for mechanism of action and effect on oxygen consumption rate.

Discussion and Conclusion: This study has identified a mitochondrial phenotype in FA patient cells using high content imaging and applied high through drug screening to identify repurposing agents to correct this phenotype. The compounds identified in this work could hold therapeutic potential for FA patients.

Funding Sources: This research was supported by a philanthropic donation to the Sheffield Institute for Translational Neuroscience. LC is funded by an NIHR Academic Clinical Fellowship.

EP3. Revealing regional differences in cerebellar spike entrainment by neuromodulation in Spinocerebellar ataxia type 3

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Background: The cerebellum receives increasing attention as a target for therapeutic neuromodulation in ataxia. Spinocerebellar ataxia type 3 (Sca3) cerebellar neuromodulation studies have varied effectiveness and do not have a specific cerebellar target. The cerebellum exhibits diverse neuronal properties across mediolateral regions, but it is unclear whether this diversity affects Purkinje cell and nuclei cell susceptibility to neural stimulation. This project investigates the responsiveness of cerebellar Purkinje and nuclei cells to stimulation in Sca3. Knowing where cerebellar neurons are the most responsive to stimulation is critical for identifying an optimal neuromodulation target and improving effectiveness. We hypothesize there will be regional differences in the ability to modulate cerebellar neuron activity.

Methods: In vivo, extracellular Purkinje (PC) and nuclei (NC) cell electrophysiology recordings will be collected from three medio-lateral cerebellar zones (vermis, paravermis, hemisphere) in anesthetized control and Sca3mice. Later, PCs will be optogenetically stimulated while that PC or an NC is being simultaneously recorded. PC and NC recordings will be analyzed to determine whether there are differences in their baseline spike activity across cerebellar regions. Different parameters measuring spike activity will be analyzed. In optogenetic recordings, PCs and NCs' spike activity will be analyzed to see if there are regional differences in the ability to use optogenetic stimulation to modulate their spike activity.

Results: Preliminary results show regional differences in PC and NC spike patterns between medio-lateral cerebellar zones at baseline. Specifically, parameters measuring firing irregularity have shown significant differences between the medial and lateral regions in PC and NC recordings.

Discussion & Conclusion: Because of PC and NC diversity, there may be regional differences in the ability to modulate their activity. This project begins to indicate where cerebellar neurons are more responsive to stimulation, helping to identify an optimal therapeutic target and improve effectiveness in Sca3cerebellar neuromodulation.

Funding Sources: National Ataxia Foundation, National Institute of Neurological Disorders & Stroke, Virginia Tech Fralin Biomedical Research Institute Start-up Funds & Red Gates Foundation

EP5. Dimethyl Fumarate as a Therapeutic in Spinocerebellar Ataxias

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Background and objectives: SCAs are incurable neurodegenerative diseases characterized by loss of coordination and motor skills, impaired speech and swallowing, and premature death. An alternative to targeting each unique mutation separately is to engage downstream molecular pathways common to multiple SCAs. Neuroinflammation and oxidative stress are key contributors to degenerative cerebellar ataxias. Here, we chose to repurpose the FDA-approved NRF2-activator, dimethyl fumarate (DMF), currently used to treat multiple sclerosis. While DMF improves ataxia in mouse models of Friedreich's Ataxia, its role in SCA therapy has not been examined.

Methods: Wild type and SCA1knock-in (Atxn1154Q/2Q) mice were dosed with DMF via drinking water from 4 to 20 weeks of age. Motor ataxia was assessed via balance beam and rotarod. Immunofluorescence (IF) staining was used to assess degeneration and neuroinflammation. We then performed transcriptional analysis using dedicated qPCR and bulk RNA sequencing with splicing analysis.

Results: SCA1mice showed a significant improvement in balance and coordination in response to DMF treatment compared to sucrose, though no rescue of degeneration. DMF significantly reduced GFAP and IBA1levels in SCA1mice compared to untreated controls. Interestingly, we did not find significant changes in gene expression of NRF2pathway targets by either qPCR or RNA-seq. RNA-seq analysis instead revealed significant changes in alternative splicing. Future studies will explore the cell specificity of these events, including immunophenotyping of cerebella and blood.

Discussion and Conclusion: Repurposing DMF may represent a viable and efficient approach to offering therapy to SCA patients. Further, our data suggests that its benefits may proceed through NRF2-independent pathways and impinge on alternative splicing, which is an early transcriptional feature in SCA pathology. Exploring DMF's immunomodulatory features is a critical next step in uncovering additional novel targets.

Funding Sources: NINDS K08NS13462402, NAF AWD032023

EP6. SNP-guided targeting strategy in spinocerebellar ataxia type 3 for the development of allele-specific antisense oligonucleotides

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Background and Objectives: Spinocerebellar ataxia type 3 (SCA3), or Machado-Joseph disease, is the most common autosomal dominant cerebellar ataxia and is caused by a pathogenic CAG repeat expansion in ATXN3. Current therapeutic approaches do not selectively target the mutant allele and may therefore compromise the neuroprotective functions of wild-type ataxin-3. This study aimed to identify single nucleotide polymorphisms (SNPs) linked to the pathogenic CAG expansion and evaluate their potential for allele-specific therapeutic silencing.

Methods: We performed allele-specific, targeted, multiplexed nanopore sequencing in 79 patients with SCA3 from German, UK, and Portuguese subcohorts. Identified polymorphisms were analyzed for linkage with the expanded allele and grouped into haplotype blocks based on linkage disequilibrium. Candidate SNPs were then assessed as targets for allele-specific antisense oligonucleotides (ASOs). Seventeen ASOs were evaluated, including candidates targeting exonic and intronic SNPs, and selected ASOs were tested in SCA3 iPSC-derived neurons. Toxicity and transcriptomic effects were examined using cellular assays and RNA sequencing.

Results: Nearly 200 polymorphisms were identified and organized into three haplotype blocks. SNPs within these blocks were strongly associated with the pathogenic CAG expansion. Targeting only two SNPs from different blocks could potentially enable treatment of approximately ~70% of European patients with SCA3. In patient-derived neurons, a single ASO application reduced mutant ATXN3 protein levels by up to 90%. Toxicity assessments and RNA sequencing showed no evidence of relevant toxicity or widespread transcriptional disruption.

Discussion and Conclusion: These findings demonstrate that haplotype-informed SNP targeting can support highly selective allele-specific therapies for SCA3. The proof-of-principle ASO experiments confirm the feasibility of selectively silencing mutant ATXN3 while expanding the range of therapeutic target sites for future precision treatments.

Funding Sources: Ataxia UK - Research Grant 2025. Ataxia UK - Small Grant 2023

EP7. SRP-1004, an Investigational Lipid-Conjugated siRNA Therapy for Patients With Spinocerebellar Ataxia Type 2 (SCA2): Preclinical Findings

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Background and Objectives: Spinocerebellar ataxia type 2 (SCA2) is a progressive cerebellar ataxia due to CAG repeat expansion in the Ataxin-2 (ATXN2) gene, resulting in mutant ATXN2proteins. SRP-1004, a lipid-conjugated small interfering RNA (siRNA), silences ATXN2mRNA in the central nervous system (CNS) through the RNA interference pathway. Here, we present preclinical pharmacology and toxicology assessments of SRP-1004, supporting the ongoing first-in-human (FIH) study in patients with SCA2.

Methods: Studies included BAC-Q22mice, rats, and non-human primates (NHPs). SRP-1004 was administered at varying doses intrathecally in rats/NHPs (rats: 0.8-3.2 mg; NHPs: 5-60 mg) or intracerebroventricularly in mice (10-300 µg). ATXN2mRNA and protein knockdown were assessed in the cerebellum and spinal cord (mice/NHPs) and brainstem and cortex (NHPs) up to day 253. Safety and tolerability of SRP-1004 were evaluated with Good Laboratory Practice (GLP) toxicology studies (rats/NHPs).

Results: SRP-1004 demonstrated potent in vivo hATXN2mRNA and protein knockdown in the cerebellum of BAC-Q22mice. In NHPs, a single 60-mg SRP-1004 dose resulted in a significant reduction in ATXN2mRNA in Purkinje cells and exhibited a half-life of ~40-90 days across the CNS. Following single doses of 5-60 mg, SRP-1004 produced a dose-dependent decrease in ATXN2protein across disease-relevant brain regions, with ≥50% knockdown at 3 months after the 60-mg dose. All chronic dosing administered every 3 or 6 months (15-150 mg/dose) achieved ≥75% ATXN2protein reduction (day 253), further enhancing pharmacodynamic effects. In a GLP toxicology study, the highest evaluated dose in both rats (3.2-mg) and NHPs (180-mg) was selected as the No Observed Adverse Effect Level (NOAEL).

Discussion and Conclusions: SRP-1004 demonstrated efficient CNS delivery, robust knockdown of ATXN2mRNA and protein, and NOAEL doses exceeding those required for substantial knockdown. Together, these findings support clinical translation, and an FIH study in patients with SCA2is ongoing.

Funding Sources: Sponsorship: Sarepta Therapeutics, Inc.

EP8. CRISPR/hfCas12 Max-Mediated DNA Editing Therapy for Spinocerebellar Ataxia Type 3

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Objective: To develop a CRISPR-based therapeutic strategy for spinocerebellar Ataxia Type 3 (SCA3). This study adopts adeno-associated virus (AAV) to package high-fidelity Cas12Max (hfCas12Max) targeting the polyglutamine (polyQ) exon of ATXN3 gene. It takes advantage of the compact size of hfCas12Max to realize single-vector delivery, and utilizes self-processing pre-crRNA to achieve efficient genome editing.

Method: DNA editing therapy spinocerebellar Ataxia Type 3 (SCA3) via a single AAV vector.

Results:

hfCas12Max realizes efficient and specific genome editing of ATXN3 in vitro, and in vitro screening of hfCas12Max-mediated editing is conducted at the ATXN3 locus.

hfCas12Max achieves efficient ATXN3 gene editing in vivo. Intracisternal magna (ICM) delivery of all-in-one packaged hfCas12Max edits ATXN3 gene and decreases mutant ATXN3 (mutATXN3) protein levels in the cerebellum and brainstem of newborn humanized ATXN3(84Q/84Q) transgenic (TG) mice.

ICM delivery of all-in-one packaged hfCas12Max maintains neuronal integrity in humanized ATXN3(84Q/84Q) TG mice.

ICM delivery of all-in-one packaged hfCas12Max improves motor function and inhibits weight loss in newborn humanized ATXN3(84Q/84Q) TG mice.

Conclusions

hfCas12Max combined with ATXN3gRNA can edit ATXN3 DNA efficiently and specifically both in vitro and in vivo.

The all-in-one packaged hfCas12Max system reduces mutATXN3 protein expression in cerebellum (CB) and brainstem (BS), and improves motor function in humanized ATXN3(84Q/84Q) mice.

Funding Sources: Research on the progression and therapeutic intervention of neurodegeneration mediated by abnormal protein aggregation in polyglutamine (polyQ) diseases (Grant No. 82230039); Mechanistic study of autophagy-mediated degradation of mutant spinocerebellar ataxia type 3 (SCA3) protein by specific short peptides (Grant No. 82371879).

EP9. SRP-1006, a Transferrin Receptor–Targeting Fab Conjugated siRNA Therapy for Patients With Spinocerebellar Ataxia Type 3: Preclinical Findings

Paloma Martinez Redondo¹; Phillip Lazzara¹; Emily Thompson²; Gregory Baker³; Ping Jin¹; Szymon Klossowski¹; Tao Niu³; Duc Quach¹; Oliver Rogers⁴; Jingdong Ye¹; Lilly East³; Rachael Potter³

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Background and Objectives: Spinocerebellar ataxia type 3 (SCA3) is a dominantly inherited progressive cerebellar ataxia due to CAG repeat expansion in the Ataxin-3 (ATXN3) gene, resulting in expression of mutant ATXN3protein. SRP-1006, an innovative small interfering RNA (siRNA), silences ATXN3mRNA through the RNA interference pathway using a subcutaneous route with high delivery to the central nervous system (CNS), including in the cerebellar and brainstem regions—the primary drivers of SCA3, via conjugation to a TfR1-targeting antibody fragment. We present SRP-1006 preclinical pharmacology and preliminary safety assessments.

Methods: Studies were conducted in SCA3-YAC-Q84and hTFR1transgenic mouse models and nonhuman primates (NHPs). Following SRP-1006 administration (mice: 2 or 4 consecutive days; NHPs: 3 mg/kg 3 times/wk), ATXN3mRNA and protein reduction were assessed at day 29 in mice and day 43 in NHPs; phenotypic rescue in SCA3-YAC-Q84mice was assessed up to 12 weeks post final dose and preliminary safety in hTFR1mice out to 29 days following 3 weekly doses (up to 30 mg/kg).

Results: SRP-1006 demonstrated knockdown of hATXN3mRNA (~60% reduction) and protein (~40% reduction) in SCA3-YAC-Q84mice, which was sufficient to improve impaired oligodendrocyte maturation and motor coordination in this SCA3disease model. In NHPs, SRP-1006 administration resulted in a substantial knockdown in ATXN3mRNA (~50%) across CNS tissues, including >60% knockdown in cerebellar and brainstem neurons. Consequently, a corresponding ~40%-50% decrease in ATXN3protein was observed across disease-relevant brain regions, including the cerebellum and brainstem. Preliminary safety evaluations in hTFR1-expressing transgenic mice suggest that SRP-1006 is well tolerated following 3 weekly administrations.

Discussion and Conclusion: SRP-1006 demonstrated substantial reduction of ATXN3mRNA and protein in disease-relevant brain regions in multiple animal models. SRP-1006 improved motor coordination impairment and oligodendrocyte maturation deficits in the SCA3-YAC-Q84disease mouse model, supporting its therapeutic potential for SCA3.

Funding Sources: Sponsorship: Sarepta Therapeutics, Inc.

EP12. Novel gene therapy strategy to restore full-length ATM expression in ataxia-telangiectasia

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Introduction and objectives Ataxia-telangiectasia (A-T) is a rare autosomal recessive disorder caused by mutations in the ATM gene, resulting in progressive cerebellar neurodegeneration, immunodeficiency, and increased cancer predisposition. The development of effective gene therapies for A-T has been limited by the large size of the ATM coding sequence and the need to target both hematopoietic and neuronal compartments. This study aimed to develop split-vector strategies capable of restoring full-length ATM levels and functionality. **Methods** We developed a novel gene therapy strategy to restore full-length ATM levels using an intein-based protein trans-splicing approach. In this system, the ATM gene is split into separate fragments that are reassembled into a functional protein after translation through precise intein-mediated self-excision. In particular, we developed both lentiviral (LV) and AAV-compatible systems.

Results Using a dual LV system, we achieved efficient reconstitution of full-length ATM in HEK293cells, restoring DNA repair activity following X-ray-induced damage. This single-split strategy is being developed for ex vivo correction of the immunological phenotype in Atm knock-out mice through transplantation of genetically corrected hematopoietic stem cells (HSCs). Preliminary data demonstrated successful ATM reconstitution in mutant HSCs and recovery of impaired cellular growth. To address cerebellar degeneration, the LV system was pseudotyped to enable retrograde transport to Purkinje neurons after injection into the deep cerebellar nuclei, where their axonal terminals are confined. This strategy will be evaluated in a novel A-T mouse model characterized by progressive cerebellar degeneration and ataxia. In parallel, we developed an AAV-compatible double-split system enabling efficient ATM reconstitution in vitro.

Discussion and conclusion Our findings demonstrate that split-intein strategies can overcome ATM vector size limitations while preserving protein functionality. Dual LV delivery represents a promising platform for hematopoietic and neuronal gene therapy. Together, LV- and AAV-based split systems provide a versatile framework for gene therapy applications in A-T.

Funding Sources: A-T Children's project; Associazione nazionale atassia telangiectasia (italian association).

EP13. Excision of the pathogenic GAA repeats from intron 1 of the FXN gene completely alleviates phenotypes of FRDA mouse in vivo

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Background: Friedreich's ataxia (FRDA) is a disorder caused by GAA expansion in the first intron of the frataxin (FXN) gene. Currently, there is no cure for FRDA. We have previously demonstrated that deletion of the GAA repeats (GAAR) from FXN upregulated frataxin expression and reversed gene expression signatures of FRDA cardiomyocytes. This result highlights the potential of this approach as a future treatment for FRDA. The Fxnnull::YG8s(GAA)>800 (YG800) humanized transgenic mouse carries a large repeat expansion of ~800 GAAs and develops a progressive phenotype. We utilized the CRISPR/Cas9 system to excise the fragment of FXN intron 1 containing ~800 GAAs to generate the new YG-del.800 isogenic strain. In this study, the new model serves to validate the efficiency and safety of our gene editing approach in a disease-relevant mouse.

Methods: YG800 zygotes were electroporated with CRISPR/Cas9 components to delete the expanded GAAR from the FXN transgene. Embryos were transferred to pseudo-pregnant female mice. A male YG-del.800 founder was selected and crossed with B6 wild-type females to minimize potential off-target effects. DNA sequencing was performed, followed by quantification of frataxin mRNA and protein levels across different tissues. We then used various functional tests to study the behavioral characteristics of the YG8-del.800 strain.

Results: PCR products of the GAA repeat region in different tissues collected from YG800 and YG-del.800 mice demonstrated that all YG-del.800 mice carried edited alleles. RNA and protein analyses demonstrated significant upregulation of frataxin expression after excision of the GAAR compared to the YG800 strain. Notably, locomotor deficits exhibited by YG800 mice were reversed in the YG-del.800 mice, as shown by their improved performance in neurobehavioral tests.

Conclusions: This new isogenic model is proof of concept that the deletion strategy is safe and efficient. It can serve as a control for studying pathological mechanisms and treatments of FRDA.

Funding Sources: FARA

EP14. From safety to long-term efficacy: preclinical evaluation of sphingolipid-metabolising enzymes in Friedreich's ataxia (FRDA)

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Background and Objectives: Dysregulation of sphingolipid metabolism is increasingly recognised as a contributor to neurodegenerative diseases, including Friedreich's ataxia (FRDA). Bioactive sphingolipids form an interconnected signalling network that regulates cellular survival, redox balance and mitochondrial homeostasis. Although altered sphingolipid profiles have been described in FRDA, the role of enzymes governing sphingolipid interconversion remains poorly defined. This study aims to evaluate the therapeutic potential of targeting sphingolipid metabolism as a disease-modifying strategy in FRDA.

Methods: We investigated the expression and activity of key sphingolipid regulators across multiple FRDA cellular models and mouse tissues. Pharmacological modulators were used to restore sphingolipid balance, and downstream effects were assessed using biochemical, molecular and imaging approaches. Short-term in vivo studies evaluated safety and biological activity, while ongoing experiments in validated FRDA mouse models assess long-term efficacy through integrated behavioural, biochemical, histological and lipidomic analyses.

Results: We identified consistent dysregulation of sphingolipid regulatory pathways, driving a shift towards stress-associated lipid signalling. Pharmacological modulation restored lipid homeostasis and significantly reduced oxidative stress. This was accompanied by marked improvements in mitochondrial function, including membrane potential, mass, mitochondrial DNA copy number and enzymatic activity. Treatment also normalised iron handling and increased frataxin expression. Notably, sphingolipid modulation activated adaptive stress response pathways involving both redox-associated and complementary signalling mechanisms. In vivo studies demonstrated favourable safety, tolerability and evidence of biological engagement.

Discussion and Conclusion: These findings identify sphingolipid metabolism as a critical upstream regulator of mitochondrial dysfunction and oxidative stress in FRDA. Targeting this pathway offers a novel and mechanistically distinct therapeutic strategy with disease-modifying potential. Ongoing studies will define treatment durability, mechanistic underpinnings and translational relevance. This work provides strong preclinical justification for the development of sphingolipid-targeted therapies in FRDA.

Funding Sources: This work was supported by funding from the Friedreich's Ataxia Research Alliance (FARA), Ataxia UK, the Association Française Ataxie de Friedreich (AFAF) and the Foundation For Rare Diseases.

EP15. Optimising viral vector mediated gene replacement therapy for Christianson syndrome-associated motor dysfunction

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Background: Christianson syndrome (CS) is a rare X-linked neurodevelopmental disorder caused by mutations in SLC9A6, encoding the endosomal Na⁺/H⁺ exchanger NHE6. Loss of NHE6 disrupts endolysosomal pH regulation, impairs autophagy, and drives progressive Purkinje cell dysfunction and loss. Thus, patients with CS experience cerebellar atrophy, progressive gait ataxia, treatment-refractory epilepsy, severe intellectual disability, and many other symptoms. No effective therapies currently exist.

Methods: This study evaluated the therapeutic efficacy of AAV9-mediated SLC9A6 gene replacement in the shaker rat, a naturally occurring model of CS. We assessed promoter and dose effects using ubiquitous CAG and neuron-specific SYN1 promoters at 1E10 and 1E11 vg. Subsequently, a treatment-timing study compared the effect of dosing at timepoints ranging from early postnatal to advanced disease timepoints. AAV9 was delivered via intracerebroventricular administration, followed by longitudinal motor assessment using force plate actometry up to 25 weeks, behavioural testing, structural MRI, and molecular analyses.

Results: The neuron-specific SYN1 produced the strongest therapeutic benefit, in a dose-dependent manner, significantly improving gait coordination and rescuing of cerebellar atrophy relative to both untreated controls and other treatment groups. Notably, early intervention at postnatal day 4 (P4) resulted in complete prevention of gait ataxia across the longitudinal assessment period, statistically indistinguishable from wild-type performance. Molecular analysis confirmed robust NHE6 expression in the early P4 treatment group, averaging approximately double that of wild-type controls in the cerebellum without obvious signs of toxicity.

Conclusion: These findings demonstrate that AAV9-mediated SLC9A6 gene replacement can treat motor symptoms in a preclinical model of CS. Promoter selection and therapeutic timing were critical determinants of efficacy, with early neuron-targeted delivery producing the most robust motor benefit. This work supports early recombinant AAV gene therapy as a promising strategy to prevent progressive gait ataxia and cerebellar degeneration in Christianson syndrome.

Funding Sources: RTW Charitable Foundation. University of Sydney

EP16. Leriglitazone alleviates oxidative stress and iron dysregulation in Friedreich Ataxia

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Introduction: Friedreich Ataxia (FA) is a rare inherited neurodegenerative disease characterized by gait and limb ataxia, dysarthria, and loss of lower limb reflexes. Most patients are homozygous for the GAA triplet expansion in the frataxin gene (FXN), which results in reduced levels of frataxin, a mitochondrial protein. FA is considered a multisystem disorder affecting several organs, particularly the nervous system and heart.

Objectives: (i) To test the effectiveness of leriglitazone, a novel brain-penetrant, full, and selective PPAR γ agonist, as a standalone therapeutic approach in reversing key altered biomarkers of FA. (ii) To assess whether the combination of leriglitazone and omaveloxolone (an NRF2activator) can achieve similar beneficial effects with the use of lower doses of each compound, as a complementary therapeutic strategy.

Methods: Dorsal root ganglia (DRG) neurons from neonatal rats with reduced FXN levels by shRNA lentivirus transduction were cultured. Cells were treated with leriglitazone alone or in combination with omaveloxolone, and compared with vehicle-treated controls. In parallel, the therapeutic effects of leriglitazone were also evaluated in the FXN151F mouse model of FA.

Results and Discussion: Leriglitazone improved most of the analyzed parameters, including cell survival, mitochondrial respiratory activity, iron homeostasis, and oxidative stress. Moreover, increased lipid peroxidation, a key marker of ferroptosis, was almost completely rescued by leriglitazone. Interestingly, the combination of leriglitazone and omaveloxolone, rescued both cell survival and mitochondrial function at reduced doses of each compound. In the FXN151F mouse model, preliminary results also indicate some beneficial effects of leriglitazone on FA-related alterations.

Conclusion: Leriglitazone may be a promising therapeutic strategy for FA. Likewise, a combination therapy with omaveloxolone could be an alternative approach for FA patients with reduced side effects.

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EP17. Protective Effects of SBT-589 Across Cardiac and Neurological Models of Friedreich's Ataxia

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Background/Objectives: Friedreich's ataxia (FA) is a multisystem disorder that is caused by reduced expression of frataxin (FXN), a mitochondrial protein involved in the assembly of the iron-sulfur complexes within the electron transport chain (ETC). Reduced FXN levels result in iron dysregulation and impaired mitochondrial bioenergetics, ultimately leading to cell death. SBT-589 is a novel, brain-penetrant small molecule that acts by reducing iron-mediated cell death and overcoming deficits in complex I to restore bioenergetics. In these studies, we determined the potential protective effects of SBT-589 against the cardiac and neurological manifestations of FA using several distinct mouse models of FA.

Methods: Fxn^{flx}/nullMCK-cre mice were treated with SBT-589 for 8 weeks. Survival was monitored daily and heart tissue samples were collected at study endpoint to assess cardiac hypertrophy, mitochondrial morphology, and iron deposition. Fxn^{flx}/nullPvalb-cre mice were treated with SBT-589 for 9 weeks, after which motor coordination was assessed by open field test. Frataxin knock-in/knockout (KIKO) mice were treated with SBT-589 for 60 days. Cerebellum samples were collected and assessed for neurodegeneration and ETC complex activities.

Results: SBT-589 protected against cardiomyopathy in Fxn^{flx}/nullMCK-cre mice by delaying the onset of mortality, reducing cardiac hypertrophy and iron deposition, and normalizing mitochondrial morphology. Fxn^{flx}/nullPvalb-cre mice showed signs of ataxia with impaired hind limb rearing, a motor deficit that was improved following treatment with SBT-589. KIKO mice displayed increased neurodegeneration and impaired activities of ETC complexes I, II, and IV, both of which were mitigated by treatment with SBT-589.

Conclusions: These data from three distinct FA mouse models highlight the potential of targeting mitochondria to address both the cardiac and neurological manifestations of FA and provide promising support for further development of SBT-589 as a disease-modifying therapy in FA. These studies were funded in part by grants from the Friedreich's Ataxia Research Alliance (FARA).

Funding Sources: FARA, Stealth BioTherapeutics

EP18. Pesampator rescues cerebellar Purkinje cell AMPAR-mediated currents in a Friedreich's ataxia (FA) mouse model

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Background & Objectives: Friedreich's ataxia knockdown (FRDAkd) mice develop a doxycycline (DOX)-induced frataxin deficiency. We previously demonstrated that these mice exhibit decreased AMPAR subunit GluR2 protein levels, and impaired synaptic transmission in cerebellum. In this study, we evaluated whether pesampator, a positive allosteric modulator of the AMPAR, could rescue AMPAR-mediated synaptic deficits.

Methods: Whole-cell patch clamp recording was performed in Purkinje cells from FRDAkd mice. Frataxin knockdown was induced by DOX administration for 8wk, starting at age 16wk. Total AMPAR currents (both synaptic and extra-synaptic) were measured by bath application of AMPA (2.5 μ M). To selectively evaluate synaptic AMPAR-mediated responses, excitatory postsynaptic currents were evoked by electrical stimulation of the parallel fibers (0 to 250 μ A) in the presence of NMDAR and GABAR blockers. Current amplitudes were compared in the presence and absence of pesampator (300 μ M).

Results: Total AMPAR currents showed a trend toward smaller amplitude in FRDAkd mice compared to WT (WT 651 ± 85.4 pA, n=15 vs FRDAkd 420 ± 49.9 pA, n=11). Pesampator significantly increased these currents in FRDAkd mice (1422 ± 133.9 pA, n=9, p<0.0001). Synaptic AMPAR currents were significantly decreased in FRDAkd (to 250 μ A stimuli, WT 610 ± 134.7 pA, n=15, FRDAkd 297 ± 49.3 pA, n=21, p<0.0001). Importantly, pesampator reversed this synaptic impairment, bringing the current amplitudes to WT levels (792 ± 153.0 pA, n=21).

Discussion & Conclusion: We demonstrated that Purkinje cells of FRDAkd mice exhibit impaired AMPAR-mediated electrophysiological responses. Importantly, pesampator restored this functional deficits, suggesting therapeutic potential for treatment of FA.

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EP19. Protective effects of omaveloxolone on oxidative imbalance and iron dysregulation in Friedreich Ataxia

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Introduction: Friedreich Ataxia (FA) is a rare inherited neurodegenerative disease characterized by gait and limb ataxia, dysarthria, and loss of lower limb reflexes. Most patients are homozygous for the GAA triplet expansion in the frataxin gene (FXN), resulting in reduced levels of frataxin, a mitochondrial protein. FA is considered a multisystem disorder affecting several organs, particularly the nervous system and heart.

Objectives: On one hand, to test the effectiveness of omaveloxolone (an NRF2activator) in reversing some altered biomarkers of FA. On the other hand, to assess whether combining omaveloxolone and honokiol allows achieving similar beneficial effects with lower doses of each compound.

Methods: Dorsal root ganglia (DRG) neurons and cerebellar granule neurons (CGN) from neonatal rats were cultured and frataxin levels were reduced by shRNA lentivirus transduction. Cells were treated with omaveloxolone (alone or in combination with honokiol) and compared with vehicle-treated controls.

Results and Discussion: Omaveloxolone improved most of the analyzed parameters, including mitochondrial respiratory activity, iron homeostasis, and oxidative stress. Moreover, increased lipid peroxidation, a key marker of ferroptosis, was almost completely rescued by the treatment. Interestingly, the combination of omaveloxolone and honokiol was able to rescue both cell survival and mitochondrial function using reduced doses of each compound.

Conclusion: Combination therapy with omaveloxolone and honokiol may represent a promising strategy for FA patients, potentially reducing side effects through dose minimization.

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EP20. ASO-induced exon 10 skipping of Ataxin-3: assessing the functionality of the truncated Δ polyQ ataxin-3 protein isoform

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Background: Spinocerebellar Ataxia type 3 (SCA3) is a neurodegenerative disease caused by a dominant CAG repeat expansion mutation in exon 10 of ataxin-3 (ATXN3) that codes for an ATXN3 protein with a toxic expanded polyglutamine (polyQ) tract. To treat SCA3, several antisense oligonucleotide (ASO) therapies are being developed. Both VO659, a CAG repeat-targeting ASO developed by VICO Therapeutics B.V., and 10.a, an ASO developed by the LUMC, bind to exon 10 in ATXN3 pre-mRNA, inducing exon-skipping. The resulting shortened mRNA codes for a truncated ATXN3 protein known as Δ polyQ that lacks the polyQ domain and one of three ubiquitin-interacting motifs (UIMs). Because limited information on the effect of the truncation on ATXN3 protein function is available, the aim of this study is to examine the functionality of Δ polyQ.

Methods: Control induced pluripotent stem cells (iPSCs) were gene-edited with Crispr/Cas9 to delete ATXN3 exon 10 or exon 7 to generate iPSCs that express Δ polyQ or to knock out (KO) ATXN3, respectively. The Crispr-edited iPSC clones were then differentiated into cerebral organoids. Additionally, the deubiquitinating (DUB) function of Δ polyQ was assessed by treating ubiquitin chains with recombinant Δ polyQ in vitro, followed by quantification using Capillary Western Blot (Wes).

Results: Recombinant Δ polyQ exhibited DUB activity on ubiquitin chains. Pure iPSC clones with the ATXN3 exon 10 or exon 7 deletion showed expression of Δ polyQ and KO of ATXN3, respectively. Cerebral organoids were successfully generated from control as well as Δ polyQ and ATXN3KO iPSC clones.

Discussion: Recombinant Δ polyQ proteins exhibited DUB activity, indicating that Δ polyQ may retain wild type-like ATXN3 function. To further investigate this, the phenotype of the Δ polyQ and ATXN3KO cerebral organoids will be compared to controls using 3D imaging and RNA sequencing. Using this approach, this study will improve understanding of the therapeutic potential of exon-skipping ASOs for SCA3.

Funding Sources: This research is a collaboration between the Leiden University Medical center (LUMC) and VICO Therapeutics B.V. and was funded by VICO Therapeutics B.V..

EP22. Ataxia Research Resource Database (ARRD): A novel open-access centralized database for ataxia research resources

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Background and objectives: Ataxias comprise a large group of degenerative diseases affecting the cerebellum, which can have a genetic or sporadic cause. Individuals with ataxia show progressive difficulty with coordination and movement, eventually leading to disability and, in some cases, premature death. Recent advances and ongoing efforts in ataxia research have resulted in the development of a wide range of ataxia research tools. To gain insight in the currently available research resources for ataxia, an overview of these resources for the majority of ataxia types was compiled.

Methods: These resources include (patient-derived) cell lines, animal models and human brain tissue samples obtained from a systematic PubMed search, online repositories, and a resource availability survey sent out to ataxia researchers worldwide.

Results: Identified resources were integrated into an open-access, user-friendly online database, designed to create a single, reliable, centralized platform where researchers can find, compare, and share validated resources.

Discussion and conclusion: To expand the current repository of ataxia research tools, all ataxia researchers are invited to provide information, data and models for ataxia research to database@ataxia.org. This database aims to advance ataxia research by identifying existing research gaps, fostering collaboration and preventing duplicate research efforts in the diverse field of ataxias.

Funding Sources: This project is funded by the National Ataxia Foundation.

EP23. Developing an engineered miniaturized saccin protein for gene therapy of Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS)

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Background and Objectives: Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS) is a yet incurable childhood-onset cerebellar ataxia caused by loss-of-function mutations in the SACS gene. Purkinje cells (PCs) degeneration is the earliest feature of ARSACS, both in patients and in the Sacs^{-/-} mouse model. SACS encodes for saccin, a 520 kDa protein with multimodular domains. We demonstrated abnormal bundling of vimentin in ARSACS fibroblasts and of neurofilaments (NFs) in Sacs^{-/-} mice, implicating saccin in intermediate filament dynamics. ARSACS is suitable for gene replacement therapy, as saccin is totally or strikingly reduced in patients, regardless of the mutation type. Adeno-associated viruses (AAV) are promising clinical vectors for gene therapy, but their genome size (4.7kb) is limited for saccin cDNA (15kb). We aimed to engineer a miniaturized version of saccin ("minisaccin") that retains saccin function while fitting AAV packaging capacity.

Methods: Minisaccin was designed by bioinformatics analyses and cloned into pAAV vectors. It was characterized biochemically and functionally in human SACS^{-/-} SH-SY5Y cells and primary Sacs^{-/-} PCs cultures. For in vivo proof-of-concept, AAVs carrying minisaccin were produced and administered intracerebroventricularly (ICV) at P0 in Sacs^{-/-} mice.

Results: Essential saccin domains were defined by integrating clinical, structural and AlphaFold data. Minisaccin was cloned by combining selected domains, and the construct showed stable expression with subcellular localization comparable to endogenous saccin, both in immortalized human cells and primary PCs. Minisaccin overexpression significantly ameliorated NF bundling in SACS^{-/-} SH-SY5Y cells and cultured Sacs^{-/-} PCs. In vivo, minisaccin was detectable in cerebellum and brain following AAV injection, although with variable expression levels.

Discussion and Conclusion: Current efforts focus on optimizing viral production and expression levels. While functional rescue remains to be established in vivo, these results are valuable to pave the way toward a rationally designed gene therapy candidate for ARSACS

Funding Sources: Fondazione Cariplo Bando Giovani Ricercatori 2024

EP24. Efficacy and Safety of a Novel Investigational AAV FXN Gene Therapy (SGT-212) for the Treatment of Friedreich's Ataxia

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Background: Friedreich's ataxia (FA) is an autosomal recessive neurodegenerative disorder caused by an abnormal GAA repeat expansion within the frataxin (FXN) gene, which encodes a mitochondrial protein essential for energy production. FA is a multi-system disorder marked by progressive ataxia, sensory neuropathy, and cardiomyopathy. There is significant unmet need for disease-modifying therapies that address the cardiomyopathy, neurologic and systemic manifestations.

Objective: Develop an AAV-mediated gene replacement therapy to safely increase FXN expression in disease-relevant tissues and modify the course of FA.

Methods: SGT-212, Solid Biosciences' investigational AAV gene therapy for FA, expresses human FXN under the control of a ubiquitous promoter. Preclinical evaluation of SGT-212 assessed neurologic and cardiac outcomes in conditional Fxn knockout mouse models targeting the nervous system (nKO) and heart (cKO). Long-term safety and biodistribution were assessed in non-human primates (NHPs) using a dual route of administration via intraparenchymal dentate nucleus and intravenous (IDN-IV) infusions.

Results: In Fxn nKO mice, SGT-212 IV administration resulted in increased number of frataxin+ neurons and dose-dependent improvements in lifespan and sensorimotor function. In Fxn cKO mice, IV administration led to increased frataxin expression in cardiac tissue, prolonged survival, and dose-dependent improvements in cardiac function and structure. In NHPs, dual IDN-IV administration was well tolerated at the clinically proposed doses throughout the study duration. No adverse findings were observed across multiple dose levels, while achieving robust frataxin expression in key FA target tissues including the dentate nucleus, dorsal root ganglion, and myocardium.

Discussion and Conclusion: These nonclinical studies demonstrated that SGT-212 increased frataxin expression in disease-relevant tissues, improved neurologic and cardiac outcomes in mouse models, and was well tolerated in NHPs. Altogether, this nonclinical data package supports the Phase 1b FALCON trial (NCT07180355), which is actively enrolling and dosing participants with FA to evaluate the safety and tolerability of SGT-212.

Funding Sources: This study was sponsored by Solid Biosciences Inc. (Charlestown, MA, USA).

EP25. A modified form of DNA Ligase 3 (LIG3) lacking Exon17 repairs AMPylated DNA strand breaks found in ataxia-oculomotor apraxia type 1 (AOA1)

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Background and objectives: Ataxia with oculomotor apraxia (AOA1) is a rare, autosomal recessive disease caused by mutations in the APTX gene encoding aprataxin. During the final ligation steps of DNA break repair, abortive ligation can result in the formation of adenosine monophosphate (AMP) intermediates on 5' DNA ends. These must be removed by aprataxin to facilitate completion of DNA break repair. Loss of functional aprataxin protein in AOA1 patients means that AMPylated DNA intermediates cannot be removed and DNA breaks persist. Here we summarize pre-clinical data providing proof of concept for the ability of modified DNA ligase 3 (LIG3) protein to ligate and repair AMPylated DNA in cells lacking functional aprataxin.

Methods: We have generated LIG3 Δ EX17 (LIG3 lacking Exon17) recombinant protein and cell lines overexpressing or expressing a single mutant allele of LIG3. LIG3 Δ EX17 adenylation status was determined through an AMP-Glo assay. The ability of LIG3 Δ EX17 to ligate AMPylated DNA biochemically was assessed on SDS-PAGE. The ability of LIG3 Δ EX17 to ligate DNA breaks in cells was assessed using the COMET assay. For epidemiology, individuals carrying deletions and predicted splice variants were identified using gnomAD and All of Us.

Results: Recombinant LIG3 Δ EX17 does not undergo auto-adenylation but catalyzes ligation of AMPylated DNA ends. In COMET assays, LIG3 Δ EX17 decreased accumulation of DNA damage in cells lacking functional aprataxin, reducing tail moments by ~45% compared to controls. We identified individuals carrying LIG3 genetic variants predicted to cause deletion or skipping of Exon17 (n<20) who did not appear to have any significant increase in comorbidity compared to the general population in digital health records.

Discussion and conclusion: Here we showed that LIG3 Δ EX17 is competent in repair of AMPylated DNA damage accumulation that is characteristic of AOA1. The presence of LIG3 variants likely lacking Exon17 in individuals without increased comorbidity further supports LIG3 Δ EX17 therapeutic potential.

Funding Sources: This study was funded by Insmed.

EP26. Route of Administration and Systemic Immunity Differentially Influence Cerebellar Transduction by AAVrh.10hFXN

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Background and Objectives. Friedreich ataxia (FA) involves progressive cerebellar degeneration in addition to the cardiac manifestations, creating a need for efficient CNS-directed gene therapy. AAVrh.10hFXN is a candidate vector for cerebellar delivery, but the optimal route of administration and the impact of systemic anti-AAV immunity, particularly relevant for patients previously treated systemically for cardiomyopathy remain key considerations.

Methods. Nonhuman primates (NHPs) received AAVrh.10hFXN-HA (an adenoassociated virus serotype rh.10 coding the normal human frataxin coding sequence with a hemagglutinin tag) either by direct intraparenchymal cerebellar injection (3.2x10¹⁰vg, divided bilaterally) or by cerebrospinal fluid (CSF) administration at a dose of 5x10¹³ vg. Cerebellar vector genome levels were quantified six weeks post-delivery. A subset of NHPs was rendered systemically immune through prior intravenous vector exposure, generating circulating anti-AAVrh.10 IgG antibodies. Vector genome levels were compared across delivery routes and immune conditions.

Results. Direct cerebellar injection yielded a mean of 2,641 vg/μg genomic DNA, with 46.5% of cerebellar cubes above background and 25.9% exceeding 1,000 vg/μg. CSF administration in a non-immune NHP produced 35,940 vg/μg DNA, with 100% of cubes above background and 92% above 1,000 vg/μg, a 13.6-fold increase over that achieved by the direct cerebellar route. Prior intravenous exposure induced systemic anti-AAVrh.10 IgG (1.2x10³ ± 1.4x10³). In immune NHPs, these antibodies did not reduce cerebellar vector genome levels following CSF delivery (p > 0.4) but significantly decreased levels after direct cerebellar injection (p < 0.03).

Discussion and Conclusion. CSF administration enables higher, safer dosing and results in superior cerebellar transduction compared with dose-limited direct injection. Systemic anti-AAV immunity impairs direct cerebellar delivery but does not diminish CSF-mediated transduction. These findings support CSF administration as the preferred route for cerebellar gene therapy in FA, particularly in patients with prior systemic AAV exposure.

Funding Sources: LEXEO Therapeutics, Inc

EP27. Dimethyl fumarate rescues CGG repeat induced phenotypes in models of Fragile X-Associated Tremor/Ataxia Syndrome

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Background and objectives: Fragile X-associated tremor/ataxia syndrome (FXTAS) is a neurodegenerative disease caused by a 55–200 CGG repeat expansion in the FMR1 gene. Pathological analysis reveals intranuclear inclusions triggered by Repeat associated non-AUG (RAN) translation as well neuroinflammation and mitochondrial dysfunction. Dimethyl fumarate (DMF) is an FDA approved antioxidant drug that activates NRF2 and immune suppression. We assessed whether DMF treatment might mitigate key pathological and behavioral features observed in drosophila and mouse models of FXTAS.

Methods: We used cell-based measures of CGG repeat induced mitochondrial dysfunction in patient fibroblasts to assess the impact of DMF on disease relevant phenotypes. We used an established FXTAS drosophila model treated with DMF at various concentrations. We generated a CGG100 repeat overexpression mouse model of FXTAS and assessed the impact of adding 100mg/kg of DMF to the drinking water starting at 2 months of age followed by behavioral, molecular, and histological/pathological analysis at 6 months of age.

Results: In FXTAS patient fibroblasts, elevated mitochondrial superoxide that was suppressed by DMF treatment. In Drosophila, DMF partially rescued a rough eye phenotype in flies and decreased polyglycine aggregates. In FXTAS model mice, DMF treatment induced an upregulation of the Nrf-2 protein, activation of superoxide dismutase, reduced mitochondrial P62/SQSTM1, and an increase in ATP production. DMF treatment improved motor deficits and coordination on a rotarod treadmill assay, and reduced P62/SQSTM1 inclusions in cortex and hippocampus. These behavioral and pathological phenotypes further correlated with a reduction of reactive astrocytes and microglia in DMF treated mouse cortex.

Discussion: We demonstrated the potential therapeutic benefits of DMF in two FXTAS disease models and patient cells. DMF was particularly effective against multiple key features of FXTAS pathology, including intranuclear inclusion formation and neuroinflammation and mitochondrial dysfunction.

Conclusion: DMF may constitute a promising therapeutic for the treatment of FXTAS.

Funding Sources: National Institute of Health (NIH) R01NS086810, and P50HD104463

EP28. Loss of SARM1 Improves Phenotypes in a Mouse Model of Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay

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Background and Objectives Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS) is a neurodegenerative disease caused by pathogenic variants in SACS, characterized by mitochondrial abnormalities and disruptions of the neurofilament cytoskeleton. These features have been linked to activation of Sterile Alpha and TIR Motif Containing 1 (SARM1), an enzyme that triggers axon degeneration and neuronal death. SARM1 inhibition is an attractive therapeutic strategy because SARM1 is inactive in healthy cells and its knockout has little or no deleterious effects. We asked whether SARM1 activity contributes to Purkinje cell degeneration and motor defects in a *Sacs*^{-/-} mouse model of ARSACS.

Methods We studied 4 cohorts of mice: (1) *Sacs*^{-/-}; *Sarm1*^{+/+}; (2) *Sacs*^{+/+}; *Sarm1*^{+/+}; (3) *Sacs*^{-/-}; *Sarm1*^{+/+}; and (4) *Sacs*^{-/-}; *Sarm1*^{-/-}. In 9-month-old mice, we analyzed protein markers of Purkinje cells (n = 3–4 per genotype), counted surviving Purkinje cells in folia III, IV, and VIII of cerebellar sections (n = 3 per genotype, 6 sections per mouse), and tested motor function at 3, 6, and 9 months by quantifying gait parameters (DigiGait) and coordination and balance (Rotarod) (8 male, 8 female mice per genotype).

Results Cerebellar extracts showed that Purkinje cell protein markers Calbindin-1, RGS8, and PCP2 were decreased in *Sacs*^{-/-} mice but restored to normal levels in *Sacs*^{-/-}; *Sarm1*^{-/-} mice. Purkinje cell loss was most prominent in anterior folia. *Sarm1* loss partially mitigated Purkinje cell death in folium III of 9-month-old *Sacs*^{-/-} mice. Longitudinal behavioral assessment showed that gait disturbances (slower cadence, prolonged swing, and stance phases) were partially alleviated. Rotarod results were more ambivalent, as homozygous *Sarm1* loss was less effective than heterozygous loss in ameliorating the *Sacs*^{-/-} phenotype.

Discussion and Conclusion SARM1 contributes to neurodegeneration in ARSACS, and its downregulation or inhibition represents a significant potential therapeutic strategy for this disease.

Funding Sources: R01NS107450, R35NS127253

EP29. Epigenetic Editing Restores Frataxin Expression to Physiological Levels in Friedreich's Ataxia Cells

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Introduction: Friedreich's Ataxia (FRDA) is an autosomal recessive multisystem disorder caused by epigenetic silencing of the frataxin gene, and subsequent protein loss. Carriers of a single diseased FXN allele have at least 30% expression of frataxin relative to non-carriers and are asymptomatic. Mouse experiments have shown that FXN protein levels 20x physiological levels are toxic. This establishes a "goldilocks zone" of FXN protein restoration between 0.3x and 20x healthy levels.

Methods: We have developed dCasONYX, a hypercompact catalytically dead Cas variant that, when fused with selected epigenetic modulators and targeting gRNA, comprise Epicrispr's Gene Expression Modulation System (GEMS): a CRISPR-based epigenetic editing platform currently being clinically evaluated (NCT06907875). We hypothesize that GEMS can be harnessed to correct the repressive epigenetic marks at the mutant frataxin locus. To test this hypothesis, we screened 40 combinations of GEMS for their ability to restore frataxin expression in FRDA patient-derived iPSCs within the goldilocks zone.

Results: Screening identified 10 candidate GEMS that activate frataxin mRNA in FRDA cells. Top GEMS candidates were delivered as a single cargo copy to a patient iPSC line with the lowest FXN protein levels (5% of healthy) and were found to be capable of restoring FXN above the 30% threshold. Candidates were then studied under high copy number delivery conditions by nucleofecting into the patient iPSC line with the highest FXN protein levels (22% of healthy) and were found to restore FXN to approximately 100% of WT levels.

Conclusions: Epigenetic reactivation of the mutant frataxin locus effectively restores frataxin protein levels and promisingly remains within the therapeutically acceptable "goldilocks zone" in our studies. Our compact GEMS platform is uniquely positioned to provide therapeutic levels of frataxin from a single AAV vector, leveraging our clinically-derisked dCas molecule to further meaningful drug development for FRDA.

Funding Sources: This work was generously funded and guided by the FARA foundation.

EP30. Do Sacsin Fragments Retain Residual Function? A Strategy to Address ARSACS

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Background and Objectives: Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a rare neurodegenerative disease caused by mutations in the SACS gene, which encodes sacsins, a large 520-kDa multidomain protein. Sacsins contain several regions, including ubiquitin-like, Hsp90-like, J-domain and HEPN domains, suggesting roles in protein quality control, mitochondrial homeostasis and cytoskeletal organization. However, in vitro studies of sacsins, including recombinant expression and characterization, are hampered by its large size and complex architecture. In ARSACS, pathogenic sacsins are thought to undergo cotranslational degradation, resulting in very low protein levels. This project aims to bypass full-length protein degradation by producing cell-penetrating Trojan sacsins fragments, enabling the study of sacsins domains and their potential ability to partially rescue disease-associated cellular phenotypes.

Methods: Trojan sacsins fragments were designed based on predicted domain architecture and structural information. Recombinant fragments were expressed in *E. coli* and purified. Protein purity and identity were assessed by SDS-PAGE, HPLC, Western blotting and mass spectrometry. Cellular internalization was evaluated in human cell lines. Biophysical characterization was performed using circular dichroism and fluorescence-based approaches to assess conformational properties.

Results: Four sacsins fragments were designed to represent selected regions of interest within the protein. Two recombinant Trojan fragments were produced as soluble proteins, purified and their identity was validated. Preliminary cellular internalization assays were performed for selected fragments. Biophysical analyses provided initial evidence regarding the structural organization of these fragments.

Discussion and Conclusion: This fragment-based strategy provides a practical approach to study sacsins regions that are otherwise difficult to analyze in the context of the full-length protein. By bypassing cotranslational degradation, Trojan fragments may help dissect domain-specific functions of sacsins and evaluate their capacity to modulate ARSACS-related cellular alterations. These results establish a platform for future functional rescue assays and may contribute to the development of novel therapeutic-oriented strategies for ARSACS.

Funding Sources: The Ataxia Charlevoix-Saguenay Foundation, CONICET and Universidad de Buenos Aires.

EP31. Protocatechuic acid (PCA): a potential therapeutic and a new tool to study FRDA cardiomyopathy

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Background and objectives: Cardiomyopathy is the leading cause of premature death in FRDA. Autopsy tissue shows infiltration of macrophages in the myocardium, but the inflammatory component of FRDA cardiomyopathy remains largely unexplored. Recently, we purified PCA from an aqueous extract of chestnut shells and demonstrated anti-ferroptotic activity. PCA also has anti-inflammatory properties, which we explored further. We aimed: 1) to set up a cellular system to study the interactions between macrophages and heart-cell types and 2) to test whether PCA could modulate these interactions.

Methods: We used human monocytic cell lines, THP1 and U937, which can be matured into macrophages (M0) and further polarized into pro-inflammatory (M1) or anti-inflammatory (M2) subtypes, each of which up-regulates specific sets of cytokines. Using fluorescently labeled monocytes, we developed a cellular assay to quantify changes in cell migration in response to frataxin loss.

Results: Migration of monocytes toward cardiac fibroblasts in which frataxin was knocked down (c-Fibro-fxn-kd) increased 1.8-fold on average compared to monocytes exposed to control fibroblasts. Addition of 0.5 μ M PCA decreased migration by 25%. When THP1 were polarized to M1 in the presence of c-Fibro-fxn-kd conditioned medium, IL1- β and TNF- α were further upregulated (2.2-fold and 1.3-fold, respectively). Polarization to M1 in the presence of 1 μ M PCA resulted in decreased expression of IL-1 β (-30%) and TNF- α (-20%).

Discussion and Conclusion: Macrophages are known to contribute to cardiomyopathy and cardiac fibrosis. Our results show that acute loss of frataxin in cardiac fibroblasts is sufficient to increase monocyte recruitment and suggest that polarization to M1 would be enhanced and/or that specific cytokine expression could be further upregulated. As PCA can modulate both phenotypes, it has potential as a therapeutic and can be used as a tool to study these cell-to-cell interactions. Further investigation is needed to characterize how these interactions contribute to FRDA cardiomyopathy.

Funding Sources: The Friedreich's Ataxia Research Alliance and the Hamilton and Finneran Family Foundation.

EP33. Absence seizure phenotype in the Shaker rat model of Christianson Syndrome

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Background and Objectives: Christianson syndrome (CS) is a developmental and epileptic encephalopathy caused by the mutation of the SLC9A6 gene; affected children have intellectual disability, epilepsy, and degenerative ataxia. The shaker rat has a naturally occurring Slc9a6 mutation with profound Purkinje cell loss; the model recapitulates degenerative ataxia symptoms and cognitive deficits, and this study will characterize the epilepsy phenotype of this model.

Methods: In this study, 12 rats (6 Shaker, 6 WT) underwent surgery to implant a cortical EEG electrode and a wireless telemetry system in the abdomen. Chronic EEG was recorded for 60 days, and manually reviewed for epileptic abnormalities. In a blinded and randomised fashion, 3 reviewers (including a clinical epileptologist) determined whether EEG abnormalities with the accompanying behavioral video were epileptic and, if so, the seizure subtype.

Results: Seizures were identified in 5/5 shaker rats with functioning recording hardware. All shaker rats experienced regular absence seizures (mean: 300.2, min: 21, max: 629). Absence. Seizures had a strong spectral power at 8 Hz and exhibited clustering and circadian rhythmicity with a majority occurring during the awake period for rodents. 1 WT rat experienced absence seizures (363 total). Other seizure types were rare but present, including rare myoclonic seizures in 2 shaker rats and 1 generalised tonic-clonic seizure in one shaker rat. Machine learning achieved 83% accuracy in automating absence seizures detection compared to labelled data, serving as an additional screen to identify events missed during manual review.

Discussion and conclusion: The shaker rat has an epilepsy phenotype resembling reports of children with CS. Absence seizures were the most common seizure subtype and may be a valuable biomarker to evaluate drug efficacy. Remaining study will determine whether machine learning may be used to forecast seizure risk in CS as managing seizures is a high priority for patient families.

Funding Sources: RTW Charitable Foundation Research Grant x3. National Ataxia Foundation Junior Investigator Award. Rare Village Foundation Research Grant.

EP34. ApoE-FXN construct as a candidate for protein replacement therapy in Friedreich's ataxia

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Background and objectives: The development of an effective protein replacement therapy for the treatment of Friedreich's Ataxia (FRDA) is hindered by the presence of the blood–brain barrier (BBB) and the lack of efficient protein delivery systems to the central nervous system. To address this, we engineered a FXN derivative incorporating a BBB-shuttle peptide to allow targeted delivery of frataxin (FXN) and restore the disease phenotype (patent application nº EP26382170.4).

Methods: ApoE-FXN was expressed and characterized by biochemical and biophysical techniques. Its activity was evaluated in various FXN-deficient in vitro models, including patient-derived fibroblasts, primary cultures of dorsal root ganglia neurons (nDRG), primary cultures of cerebellar granule neurons (CGN) and primary cultures of cardiomyocytes. Internalization efficiency and subcellular localization were evaluated using flow cytometry and confocal microscopy. FXN levels were analyzed by Western blot, and mitochondrial function by Seahorse analysis.

Results: ApoE–FXN is correctly folded and structurally comparable to native frataxin, maintaining overall conformational integrity and similar chemical stability. Importantly, ApoE–FXN was efficiently internalized in FXN-deficient models and exhibited preferential mitochondrial localization, outperforming other peptide derivatives. Moreover, treatment with ApoE–FXN restored both FXN and NRF2 protein levels in frataxin-deficient cells to values comparable to unaffected controls. This recovery was accompanied by a significant improvement in mitochondrial function, as ApoE–FXN effectively rescued bioenergetic parameters, demonstrating superior performance over other cell-penetrating peptide constructs.

Discussion and conclusion: We developed and characterized an ApoE-FXN derivative with promising therapeutic potential for FRDA. ApoE–FXN treatment in frataxin-deficient models enables mitochondrial targeting, restoring FXN levels and mitochondrial function supporting its application in protein replacement strategies for neurodegenerative diseases.

Funding Sources: We thank Friedreich's Ataxia Research Alliance (FARA), Association Française de l'Ataxie de Friedreich (AFAF), Federación Española de Ataxias (FEDAES), Asociación granadina de Ataxia de Friedreich (ASOGAF) and the Associació Catalana d'Àxies Hereditàries (ACAH) for their support.

EP35. Therapeutic Manipulation of the Gut-Brain Axis in a Mouse Model of Spinocerebellar Ataxia Type 3

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Background and objectives: Spinocerebellar Ataxia Type-3 (SCA3, also known as Machado-Joseph disease, MJD) is a fatal neurodegenerative disease characterised by impaired movement, speech, and swallowing. SCA3 is caused by the inheritance of an expanded trinucleotide repeat region within the ATXN3 gene, leading to an expanded polyglutamine (polyQ) tract within the ataxin-3 protein. We have recently identified that SCA3 mice have significantly different fecal microbiome communities compared to littermate wild-type mice, with changes present as early as 5-weeks-old, prior to presentation of motor phenotypes in the mice.

Methods: We examined butyrate levels in brain samples from transgenic SCA3 (CMVMJD135) mice. We also compared strategies to increase gut microbial butyrate production and assessed their effects on relevant SCA3 disease phenotypes.

Results: We identified that 15-week-old male CMVMJD135 mice had decreased butyrate levels within brain samples compared to littermate controls. We further demonstrated that acarbose was the most effective intervention tested for increasing microbiome-derived butyrate production. Acarbose is an FDA-approved α -glucosidase inhibitor that delays carbohydrate digestion, increasing the delivery of fermentable substrates to the gut microbiota. We examined the effect of administering an acarbose-containing diet to CMVMJD135 mice, identifying improved rotarod running in the acarbose-treated mice.

Discussion and Conclusion: Building on our previous findings of early alterations in the fecal microbiome of CMVMJD135 mice, we identified reduced brain butyrate levels in male CMVMJD135 mice. We further demonstrate that an acarbose-containing diet warrants further investigation as a potential therapeutic strategy for SCA3.

Funding Sources: This research was supported by the National Ataxia Foundation (Research Seed Money Grant), MJD Foundation, and NHMRC (grant ID APP2012895), Australia. PC was supported by a Macquarie University Research Excellence Scholarship.

EP36. RNA trans-splicing targeting Ataxin-1 phosphorylation is associated with improved phenotypes in a mouse model of spinocerebellar ataxia type 1

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Background and objectives: RNA-trans-splicing creates a reprogrammed mRNA through a trans-splicing reaction between a target pre-mRNA and an exogenous RNA trans-splicing molecule (RTM). Our objective was to determine whether the pathogenic serine phosphorylation of Ataxin-1 in spinocerebellar ataxia type 1 (SCA1) can be converted to alanine using RNA trans splicing.

Methods: Human and mouse FLAG-tagged RTMs were designed to replace S776(human) or S751(mouse) with alanine. RTMs were delivered via lentiviral vectors in cells or AAV2/8 vectors in Sca1154Q/2Q knock-in mice (intracerebroventricular injections at P0–2). Ataxin-1 reprogramming, S776/S751phosphorylation and aggregation were assessed using qRT-PCR, immunoprecipitation, western blotting, filter-retardation and Thioflavin T. Mice were monitored weekly (n=70) for weight and leg-splaying. Motor performance was assessed every 15 days using accelerating rotarod and mice perfused at P150for immunohistochemistry or P180for molecular analyses. Stereotaxic cerebellar injections were also performed at symptomatic stage P100in naïve mice (n=24), with rotarod testing at P120, P150and P180.

Results: qRT-PCR indicated successful trans-splicing in cell and mouse models and immunohistochemistry for FLAG and calbindin at P150in cerebellar lobules IX–X showed efficient in-vivo transduction of Purkinje cells. Western blot analysis suggests reduced S776/S751phosphorylation with RTM S776A/S751A treatment, reaching significance in cell models (40% reduction, p<0.005). Filter-retardation and Thioflavin T assays of P180cerebellar lysates showed a trend toward reduced Ataxin-1 aggregation in the RTMS751A group. Confocal imaging at P150showed preserved synaptic integrity with maintained excitatory and inhibitory Purkinje inputs (p<0.05). Rotarod and leg-splaying tests showed significant preservation of motor function and coordination (p<0.0001). Therapeutic delivery at a symptomatic stage also produced significant functional improvement (p<0.01).

Discussion and Conclusion: RNA trans-splicing targeting S776/S751is consistent with reduced phosphorylation and aggregation, alongside improved motor outcomes. These data support further evaluation of RNA trans-splicing as a potential therapeutic strategy for SCA1.

Funding Sources: This work has been part-supported by research grants from Ataxia UK.

EP37. Targeting Endoplasmic Reticulum Stress as a Therapeutic Strategy in Spinocerebellar Ataxia Type 6 (SCA6) Mouse Models

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Background: Spinocerebellar ataxia type 6 (SCA6) is caused by a polyglutamine (polyQ) repeat expansion in the P/Q-type voltage-gated calcium channel, Cav2.1. Our previous work suggests that misfolded Cav2.1 channels induce endoplasmic reticulum (ER) stress in vulnerable Purkinje cells (PCs). This ER stress is mitigated by activation of the unfolded protein response (UPR), which may contribute to the late-onset nature of SCA6, and results in firing abnormalities that lead to motor dysfunction.

Methods: A computational protein modeling tool, Boltz-2, is being used to identify potential small-molecule compounds that bind with high affinity to Cav2.1. Changes in PC firing are being analyzed using patch-clamp electrophysiology in 19-month acute cerebellar slices which are pre-incubated with identified compounds. Mitigation of the activation of UPR pathways is being used as a readout for the relief of ER stress in 6-month SCA6mice. We are also using spatial transcriptome analysis to determine whether specific UPR-dependent chaperones are no longer utilized in PCs to mitigate polyQ Cav2.1-dependent ER stress. Then, to examine whether the improvement in PC firing also improves motor dysfunction, we are performing a rotarod behavioral assay with systemically administered compounds.

Results: Using Boltz-2, we identified several FDA-approved agents used for the treatment of cystic fibrosis, including lumacaftor and elexacaftor, that bind with high affinity to Cav2.1. In preliminary results, 2 μ M lumacaftor did not significantly affect PC firing, whereas 2 μ M elexacaftor significantly improved PC firing regularity in cerebellar slices in SCA6mice. In addition, our data suggests that elexacaftor alleviates ER stress.

Discussion/Conclusion: Our findings indicate that elexacaftor, at low micromolar concentrations, improves the aberrant PC spiking in SCA6mice in vitro and can relieve ER stress. Further studies will determine whether elexacaftor is able to improve both motor function and markers of neurodegeneration in SCA6mice.

Funding Sources: Raynor Cerebellum Project, NIH

EP38. Targeting the neuroinflammatory cascade in ARSACS with dimethyl fumarate (DMF) reveals sex-specific effects in the therapeutic response

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Background: In addition to cerebellar degeneration, ARSACS mice exhibit changes in mitochondrial function, oxidative stress, and neuroinflammation. We observed abnormal activity of the antioxidant/anti-inflammatory Nrf2 pathway in male ARSACS mice, which can be targeted by the FDA-approved compound dimethyl fumarate (DMF). Therefore, we treated ARSACS mice with DMF to test whether targeting neuroinflammation can ameliorate disease progression. **Methods:** Post-symptomatic wild-type (WT) and Sacs knockout (KO) mice received DMF (15 or 50 mg/kg). Motor function was assessed by rotarod, beam, and inverted grid over 10 weeks. We used qPCR for Nrf2-axis genes and bulk RNA-seq to quantify directional concordance between disease and drug signatures and neuroinflammatory reversibility. To localize the disease signature, we integrated bulk results with unpublished single-nucleus RNA-seq of the cerebellum, projecting disease signatures onto annotated cell types and microglial subtypes. **Results:** The male KO transcriptional signature was broad, spanning neuroinflammatory, mitochondrial, and neuronal genes, whereas the female signature was narrower and inflammation-dominated. High-dose DMF reversed the male signature toward WT (70% of disease genes), but not the female signature, which was unchanged or, at low dose, shifted further from WT. qPCR revealed a sex difference in the Nrf2-axis: female KO cerebellum showed elevated baseline Gclc/Nfe2l2, consistent with endogenous compensation, whereas in males Gclc was DMF-responsive. Behavioral improvement was modest and non-significant, most evident in male rotarod. **Discussion and Conclusion:** To our knowledge, this is the first report of sex-specific differences in the neuroinflammatory signature. Both sexes shared a concordant core of microglial genes but differed in breadth: the male signature was substantially broader and largely DMF-reversible, while the female signature was a narrower, inflammation-predominant subset with intrinsic Nrf2 compensation and limited drug response. DMF ameliorated molecular signatures without substantial motor improvement, underscoring sex as a biological variable and that molecular rescue need not translate to phenotypic benefit.

Funding Sources: Fondation de l'Ataxie Charlevoix-Saguenay (Ataxia Charlevoix-Saguenay Foundation) — "Dimethyl Fumarate Tested on the ARSACS Mouse Model," Drs. Justin Wolter & Huaxia Wang (Co-PIs), 2024–2025

EP39. Development of a Transgene Expression Control Switch Targeting the CNS for Friedreich Ataxia Gene Therapies

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Friedreich ataxia (FA) is caused by a trinucleotide repeat expansion in the frataxin (FXN) gene, resulting in decreased FXN expression. Currently, no curative treatment is available, highlighting the need for novel therapeutic strategies for FA. Gene replacement therapy using AAV vectors to restore FXN expression represents a promising approach. However, FXN overexpression results in cytotoxic effects in cardiomyocytes, while CNS overexpression thresholds remain undefined, posing a critical safety barrier for clinical translation. We hypothesize that a tunable RSwitch FXN transgene expression system, developed by Rgenta Therapeutics, will achieve optimal FXN levels in disease-relevant CNS tissues, improving the safety and efficacy of AAV-FXN gene therapy for FA.

We will perform a series of in vivo rodent studies in the Fxn:PV-Cre conditional frataxin knock-out model of FA. We will first characterize PHP.eB capsid-based RSwitch-FXN vectors to identify a lead construct that efficiently regulates hFXN levels in the CNS. The lead candidate will then be used to establish a PK/PD relationship between FXN transgene expression and RDrug dose across disease-relevant CNS tissues. Finally, the optimized RSwitch-FXN vector and RDrug dosing regimen will be assessed for amelioration of FA phenotypes and molecular signatures of frataxin dysfunction. Screening of PHP.eB RSwitch-FXN vectors in vivo will identify a lead candidate with minimal hFXN baseline expression in the absence of RDrug. The lead RSwitch-FXN candidate will be advanced into RDrug dose-response studies to establish the PK/PD relationship for fine-tuned FXN expression across disease-relevant tissues.

If successful, this work will provide proof-of-concept for RSwitch-mediated regulation of FXN gene therapy in the CNS, demonstrating that tunable transgene expression can safeguard against overexpression toxicity – a critical barrier facing current AAV-based therapies. Beyond FA, this RSwitch technology could be broadly applied to other gene therapies, including CRISPR-based approaches where transient transgene expression is necessary to minimize off-target effects.

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EP40. Human neuronal networks on micro-electrode arrays as a tool to assess genotype-phenotype correlation in CACNA1A-related disorders

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CACNA1A-related disorders constitute a diverse group of neurological conditions, including ataxia, migraine, and epilepsy. Despite extensive genetic studies, clear genotype-phenotype correlations remain elusive. Moreover, next generation sequencing has identified many variants of uncertain significance (VUS). Here, we leveraged patient-derived and CRISPR/Cas9-engineered human neuronal networks to explore relationships between CACNA1A variants and neurophysiological activity. CACNA1A haploinsufficiency induced subtle alterations in glutamatergic network activity, whereas missense variants had a more pronounced effect on overall network function. Network fingerprints were most affected from patients where ataxia co-occurred with migraine or epilepsy. Furthermore, we analysed the impact of CRISPR/Cas9-induced VUS on network developmental trajectories. Although functional changes could not be directly linked to clinical phenotypes, all tested variants induced measurable alterations in neuronal network function, supporting their classification as likely pathogenic. These findings highlight the potential of human neuronal networks as a translational model for evaluating CACNA1A variant effects and improving clinical variant interpretation.

Funding Sources: This work was supported by a grant from the Radboud university medical center and Donders Institute for Brain, Cognition and Behaviour and a grant from the CACNA1A Foundation.

EP41. Putting Veterans at the Center: A Retrospective Look at Interdisciplinary Care for Ataxia in a Veterans Administration (VA) Movement Disorders Clinic

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Backgrounds & Objectives: Hereditary spinocerebellar ataxias (SCAs) are progressive neurologic disorders that lead to varying impairment in coordination, balance, gait, speech, vision and cognition. Since 2022, the Richmond Veteran's Administration (VA) Parkinson's Disease Research Education and Clinical Center (PADRECC) has operated a nurse-driven, interdisciplinary team (IDT) clinic dedicated to the care of Veterans with movement disorders. This comprehensive program brings together physical therapy (PT), occupational therapy (OT), speech (SLP), assistive technology (AT), social work (SW), nursing, vision rehabilitation and a clinical pharmacist, to offer coordinated evaluations and annual follow-up. The objective was to examine the recommendations provided by this well-established team for hereditary ataxia. **Methods:** This retrospective chart review included Veterans evaluated by this IDT clinic since 2022. Electronic health record data were compiled with diagnosis, dates of eval, and recommendations from each care plan. **Results:** To date, this cohort included 9 Veterans with confirmed or suspected hereditary ataxias with 18 cumulative IDT evaluations. The ataxia diagnoses included: SCA2, SCA7, SCA8, SCAR10, and some unknown. Half of the Veterans had been through IDT 3 times, 2 had been through twice and 3 had only been seen once. 50% were seen in-person, 50% were virtual. 100% received follow-up interventions with vision rehab, while 89% were offered intervention with PT, SLP and OT. Social work follow-up, clinical pharmacist intervention and social or recreation-based groups were recommended in 67% of Veterans and AT interventions were recommended in 56%. Other recommendations included palliative care, music therapy, neurosych testing, or consults in sleep, nutrition, driving, and wheeled mobility. No Veterans were lost to follow-up. **Discussion and Conclusion:** While hereditary ataxia may not have any cure or disease-modifying therapies established, there is much that can be done to support these patients and their families, maintain independence and support quality of life. Interventions varied widely by patient, showing that a patient-centered approach is necessary. The VA is a unique health system well-equipped to deliver high quality comprehensive care for these patients and their families. Improved awareness and standardized interdisciplinary care for hereditary ataxias could lead to improved quality of life for these patients.

EP42. Novel ROCK2 inhibitor increases expression of frataxin in FRDA-derived cells

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Friedreich Ataxia (FRDA) is the most common hereditary ataxia. The pathology includes neuroinflammation, nociceptive neuron death, hypertrophic and fibrotic cardiomyopathy, and neurodegeneration in dentate nuclei of the cerebellum. It is now known that the disease is caused by insufficient expression of frataxin protein in patients due to the gene-silencing effect of expanded GAA repeats in the first intron. Elevation of ROCK1/2 activity has been observed in a variety of diseases; consequently, ROCK inhibitors have been shown to be beneficial in suppressing disease progression. A recent study suggests that ROCK activity plays a role in regulating the accessibility of the frataxin gene, as shown by the usage of the ROCK2-specific inhibitor, Belumosudil. Thus, we investigated whether our ROCK2 inhibitor, GV101, increases frataxin expression in FRDA-derived B lymphoblastoids and primary fibroblasts.

B lymphoblastoids obtained from Coriell Institute and primary fibroblasts from Friedreich Ataxia Cell Line Repository were used as the in vitro FRDA model. Cells were cultured according to the protocols from both organizations. Cells were treated with GV101 or vehicle (DMSO) for different periods of time. After treatment, cells were harvested and lysed for total RNA extraction to perform quantitative RT-PCR or for lysate preparation to perform ELISA to measure the amount of frataxin protein.

After being treated with GV101 for 7 days, primary fibroblasts derived from a mild case of FRDA displayed a significant increase of mRNA and protein. Remarkably, the amount of frataxin also observed to reach the level of a healthy individual. Using B lymphoblastoids and primary fibroblasts derived from the moderate or severe cases of FRDA patients, the treatment of GV101 also resulted in a significant increase of frataxin in both levels of mRNA and proteins. Thus, we believe that GV101 can be the potential cure for FRDA.

EP43. New molecules harnessing targeted protein degradation for the treatment of Machado-Joseph disease

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Machado–Joseph disease (MJD) is a genetically determined spinocerebellar ataxia caused by an expansion of a polyglutamine tract in the ataxin-3 (ATXN3) protein. This mutant protein is prone to self-association and aggregation, leading to neuronal dysfunction. Targeted protein degradation strategies have recently emerged as potential therapeutic strategies, employing bifunctional small molecules that recruit simultaneously the wrongful protein and endogenous degradation pathways to selectively eliminate disease-causing proteins. This study aims to develop novel proximity-inducing degraders targeting mutant ATXN3(mATXN3) by combining ATXN3-binding moieties with ligands engaging E3ubiquitin ligases or the autophagy machinery, as innovative therapeutics for MJD.

To identify novel mATXN3-targeting compounds, a first-generation mATXN3-specific druggable pocket was developed using chemoinformatics approaches, and used to evaluate the binding of three previously reported ATXN3degraders, here used as reference compounds. Next, we conducted a virtual screening comprising >90,000 compounds and identified approximately 150 candidate mATXN3-specific binders. This screening provides a foundation for selecting the most promising compounds for subsequent validation in cellular and animal models of the disease. We are currently validating the binding capacity and selectivity of the candidate warheads in vitro, using Thermal Shift Assay (TSA) and Microscale Thermophoresis (MST). In parallel, we proceeded to validating the binding to wild-type and mATXN3(in vitro) and the therapeutic potential (in vivo) of two of the reference compounds (AN1and AN2), developed for mutant huntingtin but reported to promote mATXN3degradation. Although binding of AN1and AN2to mutant or wild-type ATXN3was not detected using TSA, perhaps due to lack of sensitivity of the technique, both compounds lead to an improved locomotor performance in *Caenorhabditis elegans* models of MJD.

Overall, these results support the feasibility of targeting mATXN3through selective protein degradation and provide a basis for the identification of novel therapeutic candidates for MJD.

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EP44. Nanobody-Based Approaches to Address Fe-S Cluster Biogenesis

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In Friedreich Ataxia (FRDA), reduced levels of frataxin (FXN) impair Fe-S cluster production via the ISC machinery (NFS1/ACP-ISC11/ISCU2/FXN)². Nanobodies (NBs) are powerful tools for dissecting, modulating, and studying protein-protein interactions. Building on previous work with FXN-specific NBs and a cell-penetrating peptide (CPP) NB construct, we expanded this strategy for other core components ((NFS1/ACP-ISC11)² and ISCU2) and also AI-driven designed new ones for the electron donor FDX2. We present advances on the integral project of quaternary addition of NBs targeting the ISC machinery to modulate its function and dissect in-cell interactions.

Interactions were assessed using SEC-FPLC, fluorescence anisotropy, bilayer interferometry (BLI), and ITC. In-cell interactions were evaluated by proximity ligation assay (PLA). An anti-FXN CPP-NB (CAMP4A7) was purified from *E. coli*, characterized (DLS, CD, BLI), and tested for immunogenicity (mice) and cytotoxicity. Transduction effects on FXN levels and mitochondrial activities were analyzed in FRDA fibroblasts, lymphoblasts and control hiPSC derived cardiomyocytes.

NB4A7 and FXN interacted in the context of ISC as shown by BLI and anisotropy. Interaction was confirmed in HeLa Kyoto cells by PLA after NB transfection; NB4A7 and NFS1 were found in close proximity. CAMP4A7 showed no cytotoxicity, low immunogenicity in mice, and successfully entered the different cell types. Unexpectedly, FXN levels and SDH activity were slightly reduced in FRDA fibroblasts and lymphoblasts, after 48h treatment. Regarding other ISC targets, NBs against ISCU2 and NFS1/ACP-ISC11 bound their targets and displayed a broad range of effects on Cys desulfurase activity.

NB4A7 successfully interacts with frataxin within the cellular ISC machinery without impairing the FXN-NFS1 interaction, validating the quaternary addition strategy. Although transduced CAMP4A7 did not rescue FXN levels or improve Fe-S-related activities—highlighting the need to explore alternative candidates—the newly developed NBs against NFS1/ACP-ISC11, ISCU, and FDX2 offer powerful tools to dissect the ISC-machinery configuration across diverse cellular contexts

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EP47. Towards a Small-Molecule Combination Therapy for Machado–Joseph Disease

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Machado–Joseph disease (MJD), also known as spinocerebellar ataxia type 3 (SCA3), is the most common dominantly inherited ataxia and is caused by an expanded CAG repeat in the coding region of the ATXN3 gene. Although multiple pathogenic pathways have been implicated in MJD, no disease-modifying therapy is currently available to halt or delay disease progression.

Using chemical genetic approaches, we previously demonstrated that modulation of serotonin signalling with selective serotonin reuptake inhibitors (SSRIs) suppresses mutant ATXN3 aggregation and improves motor dysfunction in animal models. SSRIs inhibit serotonin reuptake through the serotonin transporter SERT at the presynaptic neuron, thereby increasing serotonin availability in the synaptic cleft. In parallel, we screened several bile acids for therapeutic potential and identified tauroursodeoxycholic acid (TUDCA) as the most effective candidate. In a preclinical trial using transgenic MJD mice, TUDCA induced robust phenotypic rescue, accompanied by attenuation of neuronal loss and neuroinflammation. Transcriptomic and chemical genetic analyses further suggested that these beneficial effects depend on neuronal glucocorticoid receptor signalling. Given the broad and potentially complementary neuroprotective actions of SSRIs and TUDCA, we performed bulk RNA-seq analysis to identify shared and distinct molecular pathways modulated by citalopram and TUDCA in MJD mouse brains. Unexpectedly, molecular profiling revealed a striking modulation of the serotonergic system in TUDCA-treated MJD mice, with serotonin biosynthetic enzymes and SERT among the top differentially expressed genes. These findings support the rationale for testing a combined SSRI and bile acid therapeutic strategy in MJD preclinical models. Preliminary data indicate that co-administration of citalopram and TUDCA is safe in *C. elegans*.

Overall, our results suggest that combined modulation of serotonergic and bile acid-related pathways may represent a promising therapeutic approach for MJD, with the potential to achieve enhanced efficacy for patients. Funding Sources: National Ataxia Foundation (NAF)

EP48. SCA3 mitochondrial phenotype is rescued by an antisense oligonucleotide targeting expanded CAG repeats in Ataxin-3 pre-mRNA

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Background and Objectives. Spinocerebellar ataxia type 3 (SCA3), also known as Machado-Joseph Disease (MJD), is the most common autosomal, dominantly inherited ataxia worldwide, caused by polyglutamine (polyQ) expansion. It is a progressive neurodegenerative disorder resulting in a loss of coordination and balance caused by a CAG repeat expansion in exon 10 of the Ataxin-3 (ATXN3/MJD1) gene. This results in an aberrantly long polyQ tract in the encoded protein, Ataxin-3 (ATXN3), which drives the pathogenesis of the disease. Mitochondrial dysfunction is reported to play a crucial role in neuronal death in the SCA3affected brain regions, mostly the cerebellum, brainstem, and spinal cord.

Methods. In this study, we evaluated mitochondrial pathophysiology, using functional microscopy assays at single-cell resolution, as a readout of the therapeutic efficacy of a CAG repeat targeting antisense oligonucleotide (ASO). For this purpose, SCA3patient fibroblasts were transfected with the ASO to evaluate its efficacy in reducing mutant ATXN3protein levels and its impact on mitochondrial health, including the potential prevention of oxidative stress.

Results. The results demonstrate that SCA3patient fibroblasts exhibit mitochondrial abnormalities and increased oxidative stress. Targeting the CAG repeat expansion in ATXN3with an ASO lowers mutant protein levels and alleviates its pathological impact, restoring mitochondrial Complex I activity.

Discussion and Conclusion. These findings highlight the therapeutic potential of ASO-mediated suppression of the toxic CAG repeat expansion in rescuing mitochondrial function in SCA3.

Funding Sources: Vico Therapeutics.

EP49. An optimized system for small molecule-regulated transgene expression for Ataxia gene therapies

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Introduction: Precise control of transgene expression is critical for the safety and efficacy of viral-based therapies. However, existing control technologies fall short due to size, leakiness, limited dynamic range, and reliance on exogenous cofactors. To overcome these limitations, we developed a synthetic RNA regulatory module termed “RSwitch.” Placement of RSwitch upstream of a transgene makes expression dependent on an orally bioavailable small molecule modulator (“RDrug”). Without RDrug treatment, the mature transgene mRNA lacks a start codon, preventing its translation. Upon RDrug administration, a start codon-containing exon is spliced into the transcript, permitting expression. As gene therapy is a promising therapeutic modality for human ataxias, we foresee that RSwitch is valuable in development of successful treatments.

Methods: To systematically optimize RSwitch performance, we developed a high-throughput platform that integrates large-scale in vitro splicing assays with deep learning. The goal is to enable the rapid discovery of novel RSwitch cassettes and to improve the response characteristics of existing switches to given RDrug molecules. The methodology enables the evaluation of 20,000+ computationally designed, RSwitch variants in cells using RNASeq to quantify RDrug-induced splicing. These data are used to train neural networks that predict RDrug-dependent splicing behavior directly from sequence.

Results: Through iterative use of our platform, we generated unique RSwitch backbones with distinct performance characteristics, including ultra-low basal activity and a broadened induction range, while reducing switch lengths to 235 or fewer nucleotides. In cell lines harboring a single copy of an RSwitch-transgene, optimized RSwitch variants achieve >8,000-fold protein induction upon RDrug treatment.

Conclusion: These results demonstrate a method for systematic, sequence-level optimization of RNA switch performance. In applying the platform, we engineered compact RSwitch variants with robust RDrug response, low basal expression, and highly RDrug inducible expression. The optimized constructs are well suited for therapeutic applications across the spectrum of human ataxias.

Funding Sources: Research was funded by Rgenta Therapeutics and a grant from the Friedreich's Ataxia Research Alliance.

EP50. Beyond Iron: Copper Deficiency Emerges as a Therapeutic Target in Friedreich's Ataxia Cardiomyopathy

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Background/Objectives: Friedreich's ataxia (FA) is a neurodegenerative disorder caused by mutations in the frataxin (FXN) gene, resulting in reduced mitochondrial FXN expression. Loss of FXN disrupts iron-sulfur cluster biogenesis, promotes mitochondrial iron accumulation, and increases reactive oxygen species production. These mitochondrial abnormalities contribute to progressive cardiac pathology, including fatal cardiomyopathy. Although FA research has primarily focused on iron dysregulation, emerging evidence suggests that copper levels are significantly reduced in the hearts of FA patients and may contribute to disease progression. This study investigated whether copper deficiency contributes to the development of cardiomyopathy in FA.

Methods: Cardiac-specific MCK-Cre frataxin knockout (FXN-cKO) mice were used to model FA cardiomyopathy. FXN-cKO mice received daily treatment with copper gluconate (CuGlu; 20 mg/kg) or vehicle for 5 weeks. Cardiac function was assessed by echocardiography in control (CTRL), FXN-cKO, and FXN-cKO+CuGlu mice. Following study completion, cardiac tissue was collected for biochemical analyses.

Results: Copper concentrations in plasma and cardiac tissue were significantly reduced in FXN-cKO mice compared with CTRL mice ($p \leq 0.01$). Left ventricular (LV) diameter was significantly increased in FXN-cKO mice (+36%, $p \leq 0.0001$) and was significantly reduced following CuGlu treatment (-27% vs. FXN-cKO, $p \leq 0.0001$). Similarly, LV volume was increased in FXN-cKO mice (+93%, $p \leq 0.0001$) and decreased following CuGlu treatment (-54%, $p \leq 0.0001$). Ejection fraction was significantly reduced in FXN-cKO mice ($p \leq 0.001$) and significantly improved with CuGlu treatment ($p \leq 0.0001$). Ongoing studies in additional cohorts will further evaluate the cardiac efficacy of copper supplementation.

Discussion/Conclusion The FXN-cKO mouse model exhibits copper depletion in both plasma and cardiac tissue, consistent with observations reported in FA patients. Copper supplementation significantly improved cardiac structure and function, including key left ventricular parameters. These results identify copper deficiency as a previously underappreciated contributor to FA cardiomyopathy and support further investigation of copper restoration therapies as a novel treatment for cardiac health in FA.

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EP51. SCA27B Patient-Reported and Ranked Symptoms and their Response to 4-Aminopyridine

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Background & Objectives: SCA27B was genetically identified in late 2022 and is recognized as a common cause of late-onset hereditary cerebellar ataxia. It is caused by a GAA repeat expansion in FGF14. The drug, 4-aminopyridine (4-AP), has been widely reported to provide symptomatic relief for SCA27B patients. The SCA27b Ataxia Foundation was formed to support and educate patients, inform medical providers about this patient population, and promote research on SCA27B. The foundation surveyed affected members to determine the patients' most bothersome symptoms and report their experience with 4-AP.

Methods: The survey was emailed to foundation members, and data was collected on age, sex, nationality, GAA repeat length, most bothersome and frequency of symptoms, experience with formulations of 4-AP, symptoms alleviated by 4-AP, side effects from the drug, dosing schedule of the drug, and characteristics of the drug they wish could be improved.

Results: Survey results were collected anonymously from 235 members. Of those who completed the survey, most patients were in their 70's and 81% lived in the United States. Of their top three most bothersome symptoms, 83% included poor balance, 63% difficulty walking, 40% diplopia/double vision, 39% nystagmus, 38% fatigue, 26% dizziness/vertigo, and 20% speech. Of those surveyed, 86% had tried a form of 4-AP. Patients who had taken the drug reported its effectiveness as: 27% VERY effective, 27% effective, 20% moderately effective, 12% a little effective, 15% not effective. The most improved symptoms were nystagmus, balance, speech, and gait. The least improved symptoms were double vision and fatigue. The most common reported side effect was insomnia.

Discussion & Conclusion: Patients would like to have more flexible dosing options for 4-AP, insurance coverage for the drug, and availability of it in all countries. A clinical trial is needed for regulatory approval of 4-AP for SCA27B patients.

Funding Sources: SCA27b Ataxia Foundation

EP54. Comparative analyses of Omaveloxolone and Idebenone treatments in Friedreich ataxia dorsal root ganglia sensory neurons

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Background and objectives: Omaveloxolone (Oma), a NRF2 agonist, is the sole drug approved for Friedreich ataxia (FRDA) which is believed to safeguard cells from oxidative damage. Idebenone, a compound that can rescue mitochondrial dysfunction by bypassing complex I activity is considered one of the potential therapies in FRDA. Our study aimed to determine whether combining Oma with Idebenone significantly augment each drug's therapeutic efficacy.

Methods: We treated two genetically different FRDA iPSC-derived sensory neurons (iSNs) with Oma alone or in combination with Idebenone. Biological readouts included analyses of cellular reactive oxygen species (ROS), ATP levels, cell viability and transcriptional alterations via RNA sequencing.

Results and Discussion: Our study confirmed that Oma induces NQO1 and NRF2 expressions in FRDA iPSC-derived SNs with a corresponding decrease in cell ROS levels. Additionally, gene set enrichment analysis of RNA sequencing data revealed that Oma regulates pathways-related to endoplasmic reticulum (ER) stress in FRDA iSNs. Surprisingly, combining Idebenone with Oma failed to improve ATP levels, however, increased cell viability was observed with the higher doses of Oma.

Conclusions: Collectively, our results demonstrate the potential of combining Oma with mitochondria targeting therapies to improve disease conditions synergistically in FRDA iSNs. Further, this study reveals pathways-related to ER stress as a new potential therapeutic target for FRDA.

Funding Sources: This work is funded by Friedreich's Ataxia Research Alliance (FARA).

EP56. Reversing the Pathophysiology of Friedreich's Ataxia: *Camellia sinensis* Rescues Developmental and Locomotor Defects in *Drosophila* models

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Background and Objectives: Friedreich's ataxia (FRDA) results from frataxin deficiency, leading to mitochondrial dysfunction, oxidative stress, and impaired movement. Its complex pathophysiology has limited effective treatments to halt or reverse disease progression. New therapies are needed to alleviate symptoms and improve disease management. This study evaluates the therapeutic efficacy of *Camellia sinensis* (CS) in rescuing FRDA-associated phenotypes using a *Drosophila melanogaster* model.

Methods: Wild-type and FRDA *Drosophila* models (EMS-induced S136R mutation in the *fh* gene, #67161) were obtained from the Bloomington *Drosophila* Stock Centre. To assess frataxin deficiency pathophysiology, larval phenotypes were analyzed for lethality, developmental delay, locomotion, and thermal sensitivity. CS was collected from the Himalayan range, India. Leaves were air-dried, crushed, and aqueous extracts prepared and stored at 4°C. Extracts (2.5 and 5 mg/ml) were mixed with corn-flour fly food. First-instar larvae collected 24 h after egg laying were fed this diet, with a second treatment after 48 h, and maintained until the third-instar wandering stage for assays.

Results: In *Drosophila* FRDA models, frataxin deficiency caused 100% pupal lethality, 65% larval lethality, and only 35% larvae pupariated. Larval locomotion and temperature sensitivity issues were observed. CS treatment improved pupariation (73% vs 35%) and partially rescued lethality, indicating it reduces developmental defects. It did not harm control flies. CS also rescued delayed pupariation (38% vs 20% at 168 hr AEL) and improved development. Frataxin-deficient larvae showed poor crawling (3.2 cm/min) versus WT (6.8 cm/min). CS treatment improved crawling (upto 5.4 cm/min) and increased larvae reaching periphery (from 18.33% to 68%). CS treatment also upregulated aconitase transcripts in frataxin-deficient flies.

Discussion and Conclusion: CS partially rescued developmental, locomotor, and molecular defects associated with frataxin deficiency, suggesting its potential to ameliorate FRDA-associated phenotypes. These findings support further investigation of *Camellia sinensis* as a potential therapeutic strategy for FRDA.

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