International Congress for Ataxia Research

ICAR 2024

Abstract Book

12-15 November, 2024
Leonardo Royal Hotel London Tower Bridge
London, UK

A Biogen-sponsored symposium at the International Congress for Ataxia Research 2024

Translating research to practice: Omaveloxolone▼ as a novel treatment for Friedreich ataxia

13 November 2024
07:45–08:45 GMT

Trinity & Goodmans Suite,
Leonardo Royal Hotel London
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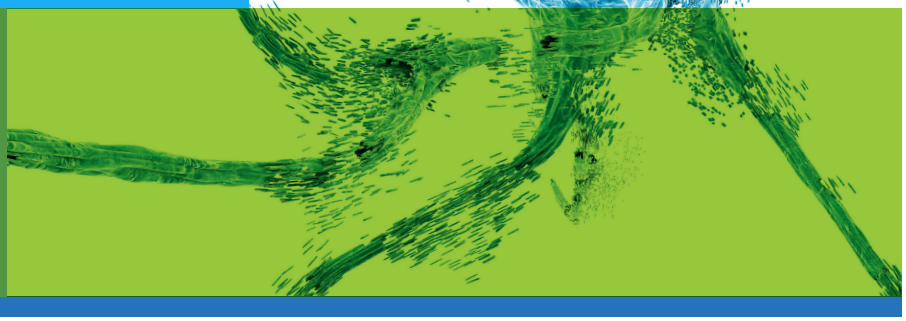

Join us for a deep dive into omaveloxolone, exploring real-world experiences of its use in Friedreich ataxia.

Our expert faculty will share practical insights on the typical patient profile and provide guidance on the use of omaveloxolone in clinical practice.

Take a closer look at our symposium agenda below:

Session	Speaker
Welcome and introduction to omaveloxolone	Prof. Paola Giunti (Chair) UCL Queen Square Institute of Neurology, UK
From data to impact: The clinical significance of omaveloxolone for people living with Friedreich ataxia	Dr Claire Ewenczyk Paris Brain Institute, Sorbonne University, France
Omaveloxolone in practice: Expert insights on treatment initiation, maintenance and monitoring	Prof. Susan Perlman UCLA Health, USA
Q&A	All faculty
Summary and close	Prof. Paola Giunti UCL Queen Square Institute of Neurology, UK

Omaveloxolone is indicated for the treatment of Friedreich ataxia in adults and adolescents aged 16 years and older. Omaveloxolone is not licensed in Great Britain. Omaveloxolone is licensed in the European Union, United States of America, and several other countries. Registration conditions differ from country to country. Please refer to your local marketing authorisation and labelling for full information before prescribing. For further information, please ask at the Biogen stand at this conference or contact MedInfoUKI@biogen.com.

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This event is organised and funded by Biogen and is intended for healthcare professionals and prescribers from countries where omaveloxolone has received market authorisation.

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Keynote

The NINDS Research Investment in Ataxia

Tuesday, 12th November - 13:30: Keynote speaker (Trinity & Goodmans Suite) - Invited Speaker

Dr. Walter Koroshetz [1]

1. *National Institute of Neurological Disorders and Stroke*

The National Institutes of Neurological Disorders and Stroke (NINDS) funds research with the goal of reducing suffering caused by neurological disorders. Our strategy is multi-faceted, including programs to uncover the mysteries of how the brain develops, the molecular and circuit features that enable all human capabilities, and the biologic basis of neurological disorders. Furthermore, we strive to provide funding mechanisms that stretch from basic discovery neuroscience to therapy development to clinical trials. To advance effective therapies, we also need to spur the biotech and pharmaceutical industries to pursue targets and make the research investments needed to develop and deliver therapeutic agents. Equally important for NINDS is our effort to nurture the neuroscience workforce to ensure that the discoveries of today lead to the treatments of tomorrow. Most of NINDS-funded research is “investigator-initiated” grants submitted for peer review and paid according to their merit scores. Multiple grant mechanisms form chains to encompass the wide neuroscience landscape. Regarding Ataxia Research, there are two seismic changes that have occurred recently through the support of NINDS. One emanates from technologies used to map, monitor, and modulate neural circuits that were developed through the NIH Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Initiative. Of note the BRAIN Initiative has led to innovation in cerebellar stimulation and a greater understanding of cerebellar function. As an example, tools from BRAIN have recently identified a surprising role of the cerebellum in attenuating pain. The second major scientific shift at NINDS is innovations in genomic therapy, gene replacement, gene knockdown, and soon to come, gene editing. Combined with the cell-type specific genomic keys coming out of the BRAIN Initiative Cell Census Network (BICAN) project, genomic therapy offers the opportunity to obtain strong effect sizes without the off-target effects that limit small molecule (drug) therapeutics. The intellectual curiosity and cleverness of our grantees, armed with the new tools from genomic and circuit neuroscience, offer exciting possibilities to bring more effective treatments for persons with ataxia from multiple causes.

Parallel session: Disease mechanisms I

AAGGG/CCCTT repeat expansions trigger RFC1-independent synaptic dysregulation in human CANVAS Neurons

Tuesday, 12th November - 15:30: (Trinity & Goodmans Suite) - Oral Presentation

Dr. Connor Maltby [1], ***Ms. Amy Krans*** [1], ***Ms. Samantha Grudzien*** [1], ***Ms. Andrea Suarez*** [1], ***Dr. Melissa Asher*** [1], ***Ms. Sydney Willey*** [1], ***Dr. Andrea Cortese*** [2], ***Dr. Vikram Khurana*** [3], ***Dr. Sami Barmada*** [1], ***Dr. Anke Dijkstra*** [4], ***Dr. Peter Todd*** [1]

1. *University of Michigan,* ***2.*** *Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London, WC1N 3BG, UK.,* ***3.*** *Harvard Medical School,* ***4.*** *Amsterdam UMC*

Cerebellar ataxia with neuropathy and vestibular areflexia syndrome (CANVAS) is a recessively inherited, neurodegenerative disorder characterized by vestibular, cerebellar, and somatosensory impairments caused by intronic biallelic, non-reference AAGGG/CCCTT repeat expansions within *RFC1*. Despite clinical interest in CANVAS, the mechanism(s) by which this repeat expansion causes pathogenesis and neuronal death are unknown.

Methods:

To investigate pathogenic mechanisms underlying CANVAS, we generated CANVAS patient iPSCs from dermal fibroblasts and CRISPR corrected to a heterozygous state. iPSCs were differentiated to neurons (iNeurons) by dual-SMAD differentiation and analyzed through multiple functional neuronal assays, transcriptomic analyses, and in vitro toxicity investigations.

Results:

AAGGG/CCCTT expansions do not alter neuronal *RFC1* splicing, protein expression, or DNA damage repair pathway functions. In reporter assays, AAGGG repeats are translated into pentapeptide repeat proteins, which were also observed at significantly higher rates in CANVAS patient cerebellar granule cells compared to control brains. However, neither these proteins nor repeat RNA foci were detected in iNeurons, and overexpression of these repeats in isolation failed to induce neuronal toxicity. CANVAS iNeurons exhibit defects in neuronal development, synaptic gene expression, and diminished synaptic connectivity that is rescued by CRISPR deletion of a single expanded AAGGG/CCCTT allele. In contrast, loss of RFC1-expression fails to recapitulate these cellular and molecular phenotypes and ectopic expression of RFC1 in CANVAS iNeurons is insufficient to reverse pathologic cascades.

Discussion & Conclusions:

Our studies support a repeat-dependent mechanism of neuronal dysfunction in CANVAS operating outside of the canonical functions of RFC1-protein. This stands in contrast to clinical genetic studies pointing towards an RFC1 loss-of-function as a central contributor to the molecular etiology of CANVAS and suggests that boosting canonical RFC1 function would be ineffective as a therapeutic approach. Instead, our findings suggest that these repeats act through an as-yet undefined molecular mechanism that will likely have relevance beyond this condition.

Action potential propagation failures in Purkinje cell axons in a mouse model of ARSACS

Tuesday, 12th November - 15:45: (Trinity & Goodmans Suite) - Oral Presentation

Dr. Amy Smith-Dijak [1], ***Ms. Chloe Stewart*** [2], ***Ms. Ayesha Pointer*** [3], ***Ms. Caroline Pack*** [1], ***Ms. Chavy Dworkind*** [1], ***Ms. Chanelle Lawson-Lartego*** [1], ***Ms. Zainah Islam*** [1], ***Mr. William Mattana dos Santos*** [4], ***Dr. Brenda Toscano Marquez*** [1], ***Dr. Alanna Watt*** [1]

1. *Department of Biology, McGill University, Montreal,* ***2.*** *Integrated Program in Neuroscience, McGill University, Canada,* ***3.*** *University of Manchester,* ***4.*** *Federal University of Parana*

Background and Objectives: Autosomal recessive spastic ataxia of the Charlevoix-Saguenay (ARSACS) is an inherited ataxia primarily affecting Purkinje cells in the anterior cerebellar vermis. Purkinje cell firing deficits begin early in disease and are followed shortly thereafter by loss of Purkinje cell synapses in the cerebellar nuclei. We aimed to understand whether loss of synapses is preceded by axonal dysfunction.

Methods: We used a mouse model of ARSACS in which the gene *Sacs* had been knocked out (ARSACS mice) and their wild-type littermates (WT). We measured the propagation of action potentials in Purkinje cell axons using simultaneous dual electrophysiological recordings from the soma and axons of individual Purkinje cells. Structural changes were assessed using immunohistochemistry followed by confocal, two-photon and transmission electron microscopy.

Results: Our recordings reveal that action potential propagation in ARSACS axons is profoundly impaired early in disease (onset, ~p40) when motor deficits are mild. In many ARSACS Purkinje cell axons, action potential propagation failure increased steeply as we recorded at longer distances from the soma, suggesting that axonal propagation may be passive rather than active. We investigated factors likely to contribute to axonal impairment and found a reduction in the level of the sodium channel scaffolding protein FGF14 in the axon initial segment of ARSACS Purkinje cells.

Discussion: Disorganisation in the ARSACS Purkinje cell axon initial segment may lead to impaired action potential propagation down the axon. As FGF14 is known to be mutated in other ataxias, such as SCA27B, our findings may suggest a common mechanism in multiple ataxias.

Conclusion: Given the severity with which action potential propagation is affected early in disease progression, axonal dysfunction is likely an important contributor to ARSACS pathology since this will degrade cerebellar information processing. Understanding the underlying causes of this impairment may help us treat this and related diseases.

Neuroinflammatory responses in Gluten ataxia

Tuesday, 12th November - 16:00: (Trinity & Goodmans Suite) - Oral Presentation

Dr. Mara-Luciana Floare [1], ***Dr. Julie Simpson*** [1], ***Prof. Stephen Wharton*** [1], ***Prof. Daniel Aeschlimann*** [2], ***Prof. Marios Hadjivasssiliou*** [3]

1. *Sheffield Institute for Translational Neurosicence, The University of Sheffield,* ***2.*** *Matrix biology & Tissue Repair Research Unit, School of Dentistry, Cardiff University,* ***3.*** *Sheffield Ataxia Centre*

Introduction: Gluten ataxia (GA) is the primary neurological manifestation of gluten sensitivity, characterised by loss of Purkinje cells throughout the cerebellar cortex and rooted in autoimmunity to transglutaminase 6 (TG6). This study aims to provide an extensive histopathological characterization of GA post-mortem material and of duodenal biopsies to better define the cellular neuroinflammatory responses associated with GA and the origin of TG6 autoimmunity.

Materials and methods: We assessed TG6 expression and microglial immunoreactivity (MHC-II, Iba-1, CD68) in the cerebellum, pons, spinal cord, parietal cortex and thalamus from 4 GA patients, 5 ataxia controls and 8 neurologically healthy controls, using immunohistochemistry with quantification. Qualitative analysis of T-cell (CD3, CD4 and CD8) and B-cell (CD20) immunoreactivity was performed in 4 GA patients. Immunofluorescence was performed to determine the presence of TG6-plasma cells in duodenal biopsies from 17 patients with GA and 7 patients with Coeliac disease (CD).

Results: Severe cerebellar atrophy, $CD20^+$ and $CD8^+$ lymphocytic infiltration and a significant upregulation of MHC-II positive, activated microglia were observed in the cerebellar granular ($p=0.0095$) and molecular layers ($p=0.0325$) and the cerebellar white matter ($p=0.0288$) of patients with GA. Additionally, $CD20^+$ and $CD8^+$ lymphocytic infiltration was observed in the dorsal column of the spinal cord and superior cerebellar peduncles. TG6 expression showed no differences across experimental groups. TG6-specific plasma cells were observed in duodenal biopsies of both GA and CD patients.

Conclusions: In GA, the cerebellum has the highest degree of pathological burden, consistent with the primary clinical manifestation of ataxia. However, inflammatory infiltrates are not limited to the cerebellum. Immune-mediated processes in GA have unique features compared to genetic forms of ataxia and are characterized by a B cell response which could be rooted at the level of the gut.

Funding: This project was generously supported by NeuroCare and Ryder Briggs Memorial Foundation.

Early molecular and electrophysiological alterations of the calcium channel Cav2.1 precede Purkinje cells degeneration in the ARSACS mouse model

Tuesday, 12th November - 16:15: (Trinity & Goodmans Suite) - Oral Presentation

Dr. Erica Spirito [1], ***Dr. Fabiana Longo*** [2], ***Dr. Daniele De Ritis*** [1], ***Dr. Angela Bachi*** [3], ***Dr. Thierry Nieus*** [4], ***Dr. Stefano Taverna*** [5], ***Dr. Francesca Maltecca*** [1]

1. *Mitochondrial Dysfunctions in Neurodegeneration Unit, Division of Neuroscience, IRCCS Ospedale San Raffaele, Milan, Italy and Vita-Salute San Raffaele University,* ***2.*** *Mitochondrial Dysfunctions in Neurodegeneration Unit, Division of Neuroscience, IRCCS Ospedale San Raffaele, Milan, Italy,* ***3.*** *IFOM- FIRC Institute of Molecular Oncology, Milan, Italy,* ***4.*** *University of Milan, Milan, Italy,* ***5.*** *Neuroimmunology Unit, Division of Neuroscience & INSPE - Institute of Experimental Neurology, IRCCS Ospedale San Raffaele, Milan, Italy*

Background and Objective-Sacsin is a multimodular protein highly expressed in cerebellar Purkinje Cells (PCs). Loss-of-function mutations in the sacsin-encoding gene cause Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS), a yet incurable childhood-onset neurodegenerative disease characterized by progressive PC degeneration. The molecular mechanisms underlying ARSACS are currently poorly understood. In this work, we investigated the early events driving the onset of ataxia in *Sacs*$^{-/-}$ mice.

Methods-We performed Label-Free-Quantitative (LFQ) proteomics, Western blots (WBs), electrophysiological recordings and simulations with a publicly available realistic PC computational model, and endogenous sacsin immunoprecipitation.

Results-By subcellular fractionation of the mouse cerebellum, we discovered that sacsin is enriched in the membrane fraction, especially in the post-synaptic density. Interestingly, LFQ proteomics and WBs revealed increased levels of the Voltage-Gated Calcium Channel Cav2.1 at the plasma membrane in the *Sacs*$^{-/-}$ mouse compared to controls at pre-symptomatic stages (P30). The increased amount of Cav2.1 is not due to transcriptional upregulation, suggesting that the absence of sacsin may affect the forward trafficking and/or recycling of this channel. Consistently, we discovered that sacsin belongs to the Cav2.1 nano-environment and interacts with the Cav2.1 auxiliary subunit alpha2delta2, responsible for the Cav2.1 transport to the membrane. The physiopathological relevance of these findings is supported by electrophysiological recordings in mouse cerebellar slices, where we measured a significant increase in Cav2.1-mediated calcium currents and reduced spontaneous firing in *Sacs*$^{-/-}$ PCs at P30. Exploiting a PC computational model, we demonstrated that the PC-specific calcium-dependent potassium channel (SK) KCa2.2 is the most relevant player accounting for this effect, as it is overactivated by the augmented Cav2.1 conductance. As a proof of concept, the SK-specific inhibitor apamin increases the *Sacs*$^{-/-}$ PC firing frequencies.

Discussion and conclusions-Overall, our work sheds light on the early steps of ARSACS pathogenesis, identifying Cav2.1 and KCa2.2 as potential targets for therapy.

Fundings-ARSACS Foundation.

Deciphering the Molecular Mechanisms: Investigating Dysregulated Pathways in Frataxin-Deficient Proprioceptive Neurons

Tuesday, 12th November - 16:30: (Trinity & Goodmans Suite) - Flash Talk

Ms. Deepika Mokkachamy Chellapandi [1], ***Dr. Helene Puccio*** [2]

1. *Institut NeuroMyoGène (INMG), Laboratoire Pathophysiologie du Neurone et du Muscle PGNM, UMR5261, INSERM U1315, Université Claude Bernard Lyon I Faculté de médecine Rockefeller, Lyon 08, France,* ***2.*** *Institut Neuromyogène (INMG-PGNM), Inserm U1315, CNRS-Université Claude Bernard Lyon 1 UMR5261, 69008 Lyon, France*

Friedreich ataxia (FA) is a rare neurodegenerative disorder characterized by mixed spinocerebellar and sensory ataxia. Proprioceptive sensory neurons (pSNs) of the dorsal root ganglia (DRG) are one of the primarily affected neurons in the disease, but the exact nature of their selective degeneration remains poorly understood. Our study aims to uncover what are the molecular pathways in proprioceptive neurons in the absence of Frataxin. As a disease model, we used *Fxn*$^{L3/L-}$; Pvalb^{tm1}(Cre)$^{Arbr/J}$ conditional knock-out mouse model with a full depletion of frataxin in the pSNs. We used single-cell RNA sequencing (scRNA-seq) at both pre and post-symptomatic stages to determine the transcriptomic signature of the DRG. This allows us to concentrate our studies on the proprioceptive neurons selectively and gives an overview of the cell-nonautonomous behavior cells in the DRG.

Our in-silico analysis revealed dysregulation in several molecular pathways critical for cellular homeostasis in pSNs. These include downregulated pathways involved in glutathione metabolism, calcium signaling, mitochondrial function, and protein synthesis. Additionally, scRNAseq data suggests an increase in satellite Glial cell (SGC) activity in DRG in a cell non-autonomous manner. This was inferred from the upregulation of pathways including stress response, immune response, inflammation, and cellular oxidative stress.

The dysregulation observed in both pSNs and SGCs collectively suggests, signaling involved in the activation of membrane receptor expression Toll-like receptor 4 (TLR4). TLR4-enhanced expression in frataxin-deficient DRG was validated both *in vitro* and *in vivo,* corroborating its potential regulatory node in FA neuropathophysiology. Treatment with TLR4 inhibitor has shown a reduction in mitochondrial oxidative stress and an increase in survival of the cells in primary DRG culture with complete deletion of frataxin. These findings deepen our understanding of FA pathophysiology by identifying specific pathological signatures of pSNs in DRG, highlighting a new potential therapeutic target for this neurodegenerative disorder.

The Role of Astrocytes in Sca1 pathogenesis

Tuesday, 12th November - 16:37: (Trinity & Goodmans Suite) - Flash Talk

Dr. Caleb Smith [1], ***Dr. Chandrakanth Edamakanti*** [1], ***Dr. Deepak Kumar*** [1], ***Dr. Sampurna Datta*** [1], ***Dr. Michaela Novakovic*** [1], ***Dr. Haram Kim*** [1], ***Dr. Murali Prakriya*** [1], ***Dr. Marco Martina*** [1], ***Dr. Puneet Opal*** [1]

1. *Northwestern University*

Background and Objectives: Spinocerebellar ataxia type 1 (Sca1) is an autosomal dominant degenerative disorder caused by a polyglutamine expansion (>40) in the ataxin1 protein (Atxn1). Though Purkinje neurons of the cerebellum are most vulnerable to the insult of mutant ataxin1, increasing research in this field suggests that the other neuronal and non-neuronal cell populations, in particular astrocytes, also show pathogenic changes. We have previously shown inflammation of Bergmann glia, while velate astrocytes display a developmental reduction. We therefore wished to understand the consequences of the depletion of astrocytes as well as the changes in glial function on the cerebellar circuit.

Methods and Results. Using Sca1 knock-in mouse models and patient iPSC-derived astrocytes and neurons, we characterized Sca1 astrocytes. We found that Sca1 astrocytes display aberrant calcium dynamics and reduced event frequency. These changes are accompanied by a reduction of neurotransmitter transporters. In addition, these astrocytes lose their ability to support synapses in culture as measured by PSD95 staining, with a resulting reduction of neural interconnectivity and circuit activity.

Discussion and Conclusions: Our results show that SCA1 astrocytes show cell autonomous dysfunction that could impact the health of Purkinje and potentially other neurons in the cerebellar circuit.

From Molecular Mechanisms to Clinical Correlations: Advancing SCA48 Therapeutics

Tuesday, 12th November - 16:44: (Trinity & Goodmans Suite) - Flash Talk

Dr. Selin Altinok [1], Ethan Paulakonis [1], Isaac Hwang [1], Rebekah Sanchez-Hodge [1], Christina D'Ovidio [1], Thomas Irons [1], Elena Vargas [1], Dr. Matt Scaglione [2], Dr. Nicholas Brown [1], Dr. Jonathan Schisler [1]

1. *University of North Carolina at Chapel Hill,* ***2.*** *duke*

Background and Objectives: We studied the causal mechanisms of SCA48, a progressive neurodegenerative disorder characterized by cerebellar atrophy and diverse symptoms, including movement disorders, cognitive decline, and psychiatric issues. Understanding these mechanisms is crucial for developing effective treatments.

Methods: We investigated the biochemical alterations in CHIP (C-terminus of HSC70 interacting protein) function due to SCA48 mutations, focusing on protein solubility, proteasome-dependent degradation, and oligomerization. We assessed CHIP's ubiquitin ligase and co-chaperone activities and its role as a regulator of the heat shock response. Additionally, we explored the association between SCA48 mutations and clinical symptoms.

Results: SCA48 mutations decreased CHIP protein solubility and increased its degradation, altering subcellular localization and function. Mutations affected CHIP's oligomeric state, with Ubox mutations promoting non-discrete high-order oligomers, impacting ubiquitin ligase activity. TPR mutations hindered substrate recruitment, while Ubox mutations inactivated ligase activity. TPR mutations also reduced HSP70 binding and co-chaperone activity, affecting the refolding of heat-denatured proteins. SCA48 mutations increased HSF1 transcriptional activity upon heat shock, suggesting a gain of toxic function. Clinical correlations revealed that Ubox mutations were associated with later disease onset and milder symptoms, while TPR mutations correlated with upper motor neuron dysfunction.

Discussion: The study highlights the significance of CHIP's oligomeric state and domain-specific mutations in SCA48's pathogenesis. The findings suggest that CHIP mutations disrupt its function, contributing to the disease's clinical spectrum. These insights are vital for therapeutic development, emphasizing the need to consider mutation location and domain-specific changes in CHIP function.

Conclusion: The insights into CHIP function offer multiple therapeutic targets. Stabilizing CHIP levels or enhancing solubility could mitigate mutation effects. Tailoring therapies to mutation types, preventing dysfunctional heterodimers, and modulating the heat shock response are promising strategies. This study underscores the need for a multifaceted approach to therapy development, considering the complex biochemical and clinical landscape of SCA48.

CACNA1A haploinsufficiency leads to reduced synaptic function and increased intrinsic excitability

Tuesday, 12th November - 16:51: (Trinity & Goodmans Suite) - Flash Talk

Mrs. Marina Hommersom [1], ***Ms. Nina Doorn*** [2], ***Dr. Sofía Puvogel*** [1], ***Ms. Elly Lewerissa*** [1], ***Ms. Annika Mordelt*** [1], ***Dr. Monica Frega*** [2], ***Dr. Dirk Schubert*** [3], ***Prof. Hans van Bokhoven*** [1], ***Prof. Nael Nadif Kasri*** [1], ***Prof. Bart van de Warrenburg*** [4]

1. *Department of Human Genetics, Radboud University Medical Center, Donders Institute for Brain, Cognition, and Behaviour, 6500 HB Nijmegen, The Netherlands,* ***2.*** *Department of Clinical Neurophysiology, University of Twente, 7522 NB Enschede, The Netherlands,* ***3.*** *Department of Cognitive Neurosciences, Radboud University Medical Center, Donders Institute for Brain, Cognition, and Behaviour, 6500 HB Nijmegen, The Netherlands,* ***4.*** *Department of Neurology, Donders Institute for Brain, Cognition, and Behavior, Radboud University Medical Center*

Haploinsufficiency of the *CACNA1A* gene, encoding the pore-forming α1 subunit of P/Q-type voltage-gated calcium channels, is associated with a clinically variable phenotype ranging from cerebellar ataxia, to neurodevelopmental syndromes with epilepsy and intellectual disability.

To understand the pathological mechanisms of *CACNA1A* loss-of-function variants, we characterized a human neuronal model for *CACNA1A* haploinsufficiency, by differentiating isogenic induced pluripotent stem cell lines into glutamatergic neurons, and investigated the effect of *CACNA1A* haploinsufficiency on mature neuronal networks through a combination of electrophysiology, gene expression analysis, and *in silico* modeling.

We observed an altered network synchronization in *CACNA1A*$^{+/-}$ networks alongside synaptic deficits, notably marked by an augmented contribution of GluA2-lacking AMPA receptors. Intriguingly, these synaptic perturbations coexisted with increased non-synaptically driven activity, as characterized by inhibition of NMDA and AMPA receptors on micro-electrode arrays. Single-cell electrophysiology and gene expression analysis corroborated this increased intrinsic excitability through reduced potassium channel function and expression. Moreover, we observed partial mitigation of the *CACNA1A*$^{+/-}$ network phenotype by 4-aminopyridine, a therapeutic intervention for episodic ataxia type 2.

In summary, our study pioneers the characterization of a human induced pluripotent stem cell-derived neuronal model for *CACNA1A* haploinsufficiency, and has unveiled novel mechanistic insights. Beyond showcasing synaptic deficits, this neuronal model exhibited increased intrinsic excitability mediated by diminished potassium channel function, underscoring its potential as a therapeutic discovery platform with predictive validity.

Plenary session: Advances in Genetics and Diagnostics

How to solve the unsolved: Repeat expansions in ataxia, genetics, tools and new sequencing methods

Wednesday, 13th November - 09:00: (Trinity & Goodmans Suite) - Invited Speaker

Prof. Christel Depienne [1]

1. *) Institute of Human Genetics, University Hospital Essen, University of Duisburg-Essen, Essen, Germany*

Tandem repeats are a common and highly polymorphic class of variation in human genomes. When these repeats expand beyond a pathogenic threshold, they can cause various genetic disorders. Over 60 disorders linked to repeat expansions have been identified to date, with at least 15 repeat expansions underlying distinct subtypes of cerebellar ataxia. In recent years, advancements in technology have led to a resurgence in the discovery of repeat expansion disorders, resulting in the identification of over 20 novel conditions associated with these expansions. In this presentation, I will review strategies, tools, and methods for identifying new expansion disorders and/or efficiently detecting and characterizing known repeat expansions, using cerebellar ataxia as an example. Specifically, I will demonstrate how bioinformatics tools like STRling and ExpansionHunter can detect repeat expansions from short-read genome data using an outlier approach. The example of SCA27B, one of the most prevalent forms of late-onset cerebellar ataxia uncovered only in 2023, underscores the importance of assessing not just expansion length but also flanking regions and motif content for an accurate diagnosis. Sequencing repeat expansions can be achieved in various ways depending on the size and repeat motif, with long-read sequencing emerging as one of the most promising techniques.

A common flanking variant is associated with enhanced stability of the FGF14-SCA27B repeat locus

Wednesday, 13th November - 09:30: (Trinity & Goodmans Suite) - Oral Presentation

Dr. David Pellerin [1], ***Dr. Giulia Del Gobbo*** [2], ***Ms. Madeline Couse*** [3], ***Dr. Egor Dolzhenko*** [4], ***Dr. Sathiji K. Nageshwaran*** [5], ***Dr. Warren Cheung*** [6], ***Mr. Isaac Xu*** [1], ***Ms. Marie-Josée Dicaire*** [7], ***Ms. Guinevere Spurdens*** [1], ***Dr. Gabriel Matos-Rodrigues*** [8], ***Mr. Igor Stevanovski*** [9], ***Ms. Carolin K. Scriba*** [10], ***Dr. Adriana Rebelo*** [1], ***Dr. Virginie Roth*** [11], ***Dr. Marion WANDZEL*** [11], ***Dr. Céline Bonnet*** [11], ***Dr. Catherine Ashton*** [7], ***Mr. Aman Agarwal*** [12], ***Dr. Cyril Peter*** [13], ***Dr. Dan Hasson*** [12], ***Dr. Nadejda Tsankova*** [14], ***Dr. Ken Dewar*** [15], ***Dr. Phillipa Lamont*** [16], ***Dr. Nigel Laing*** [10], ***Dr. Mathilde Renaud*** [11], ***Prof. Henry Houlden*** [17], ***Prof. Matthis Synofzik*** [18], ***Dr. Karen Usdin*** [19], ***Dr. Andre Nussenzweig*** [8], ***Dr. Marek Napierala*** [20], ***Dr. Zhao Chen*** [21], ***Dr. Hong Jiang*** [21], ***Dr. Ira Deveson*** [9], ***Dr. Gianina Ravenscroft*** [10], ***Dr. Schahram Akbarian*** [13], ***Dr. Michael A. Eberle*** [4], ***Dr. Kym M. Boycott*** [2], ***Dr. Tomi Pastinen*** [6], ***Dr. Bernard C. Brais*** [7], ***Dr. Stephan Zuchner*** [1], ***Dr. Matt C. Danzi*** [1]

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Background: Spinocerebellar ataxia 27B (SCA27B) caused by an intronic GAA expansion in *FGF14* is one of the most common genetic causes of adult-onset ataxia. Initial observations suggest that intermediate and expanded alleles are highly unstable upon intergenerational transmission, although the underlying mechanisms driving this instability remain unknown. Here, we investigated if sequence variants near the *FGF14*-SCA27B repeat locus affect its stability.

Methods: We studied the *FGF14* repeat locus, flanking regions, and haplotypes by PacBio HiFi sequencing in 2,191 individuals and Sanger sequencing in 339 individuals. We performed a phylogenetic analysis of the *FGF14* locus using genome sequences from 79 great apes. Single molecule chromatin architectures were assessed in post-mortem

brains using Fiber-seq.

Results: We identified a non-reference common 5'-flanking 17-bp variant (5'-CFV) in 70.34% of alleles analyzed (3,463/4,923), which is uniquely associated with non-pathogenic alleles containing fewer than 30 GAA-pure triplets, represents the phylogenetically ancestral allele, and is present on all major haplotypes. Pathogenic expansions did not carry the 5'-CFV and were found on different haplotypes. The 5'-CFV was a significant predictor of intergenerational stability (t=14.94, p=0) when controlling for repeat length and purity. Fiber-seq revealed increased chromatin accessibility on both flanks of the repeat locus on fibers bearing the 5'-CFV relative to fibers carrying the reference sequence. Fibers carrying a degenerate flanking sequence containing fewer terminal cytosines showed an intermediate pattern of chromatin accessibility, suggesting that the four terminal cytosines of the 5'-CFV are important to stabilize the repeat locus.

Discussion and Conclusion: We provide the first evidence of a stabilizing variant flanking the *FGF14*-SCA27B repeat locus. This finding may yield further insight into the mechanisms underlying tandem repeat expansions and facilitate the identification of similar sequence variants at other known pathogenic repeat loci.

Long-read genomic sequencing reveals expanded GAA-GGA chimeric alleles in Friedreich ataxia

Wednesday, 13th November - 09:45: (Trinity & Goodmans Suite) - Oral Presentation

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

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Background & Objective: Friedreich ataxia (FRDA) is the most prevalent inherited ataxia. It is typically caused by inheriting an expanded GAA triplet-repeat in intron 1 of the *FXN* gene from both parents. Expanded alleles range in size from 100 to 1500 triplets, and most alleles have >500 triplets. 15-20% of FRDA patients are genetically diagnosed as having expanded alleles of the same size (e.g., 800 and 800 triplets), because only a single expanded allele is visualized. We hypothesized that such patients may be hemizygous, i.e., for some reason only one *FXN* allele is being detected.

Methods: Long-read genomic sequencing (Oxford Nanopore, PromethION) of high molecular weight DNA.

Results: In a prospective cohort of 105 FRDA patients, we identified 21 (20%) unrelated individuals with a single expanded allele via two different long-range PCR amplicons. Long-read genomic sequencing in 12 of these patients revealed proximal *FXN* gene deletions in two. However, in the remaining 10 patients we discovered an expanded GAA-GGA chimeric repeat ranging from 800-1100 triplets. The conventional expanded GAA repeat was also detected in all patients, which matched the single expanded allele detected by long-range PCR. All 10 expanded GAA-GGA chimeric repeats were resistant to PCR amplification by the standard long-range PCR test.

Discussion & Conclusion: The current assumption that visualizing a single expanded allele is tantamount to a patient being homozygous for the same sized allele is frequently incorrect. Approximately 16% of FRDA patients have a new class of pathogenic allele that has been (and continues to be) missed by standard genetic diagnostic testing. Such individuals will have relatives whose heterozygous carrier status has been mis-assigned. Proximal *FXN* deletions are also likely under-diagnosed in FRDA.

Spinocerebellar ataxia type 4: a novel polyglycine disorder caused by GGC repeat expansion in ZFHX3.

Wednesday, 13th November - 10:00: (Trinity & Goodmans Suite) - Oral Presentation

Prof. Stefan Pulst [1], ***Ms. K.P. Figueroa*** [1], ***Dr. Caspar Gross*** [2], ***Dr. Elena Buena-Atienza*** [3], ***Dr. Sharan Paul*** [1], ***Dr. Mariana Gandelman*** [4], ***Dr. Naseebkhan Kakar*** [5], ***Dr. Nicolas Casadei*** [6], ***Dr. Jakob Admard*** [7], ***Dr. Joohyun Park*** [8], ***Prof. Christine Zuehlke*** [5], ***Dr. Yorck Hellenbroich*** [9], ***Dr. Jelena Pozojevic*** [9], ***Dr. Saranya Balachandran*** [10], ***Dr. Kristian Haendler*** [10], ***Prof. Simone Zittel*** [11], ***Prof. Dagmar Timmann*** [12], ***Dr. Friedrich Erdlenbruch*** [13], ***Dr. Laura Herrmann*** [13], ***Dr. Thomas Feindt*** [14], ***Dr. Martin Zenker*** [15], ***Dr. Claudia Dufke*** [16], ***Dr. Arnulf Koeppen*** [17], ***Prof. Thomas Klopstock*** [18], ***Dr. Marc Sturm*** [16], ***Prof. Stephan Ossowski*** [6], ***Prof. Malte Spielmann*** [10], ***Dr. Tobias Haack*** [16], ***Prof. Daniel Scoles*** [1], ***Prof. Olaf Rieß*** [6]

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Background: SCA4 is an autosomal dominant disease, characterized by sensory and cerebellar ataxia, and originally described in a Utah pedigree. Although mapped to 16q in 1996, the mutation has escaped identification for 3 decades.
Methods: Genetic linkage analysis followed by single strand, high fidelity long-read genomic sequencing (LR-GS), characterization of nuclear aggregates in SCA4 brain, in vitro analysis of ZFHX3 and autophagic flux.
Results: Using LR-GS, we identified a heterozygous GGC-repeat expansion (RE) in Utah pedigrees coding for polyglycine (polyG) in ZFHX3/ATBF1. A query of 6,495 GS datasets identified the RE in a further 7 pedigrees. The most common alleles contained 21 repeats, often twice interrupted at the DNA level. Pathologic expansions ranged from 44 to 70 perfect GGC repeats with GGC number explaining ~50% of age-of-onset variance. RE-adjacent rare DNA variants indicate a common distant founder event in Sweden. The SCA4 phenotype ranges from cerebellar and sensory ataxia, to autonomic dysfunction and chronic cough. Intranuclear aggregates staining for ZFHX3, p62, and ubiquitin were abundant in SCA4 basis pontis neurons. In SCA4 fibroblasts, we detected normal and polyG-expanded ZFHX3 consistent with a gain of function. In fibroblast and iPS cells from SCA4 patients, GGC expansion leads to increased ZFHX3 protein levels and is associated with markers of abnormal autophagy. Levels of mTOR, p62, and LC3-II were normalized with siRNA-mediated *ZFHX3* knockdown.
Discussion: SCA4 now joins neuronal intranuclear inclusion disease, some forms of oculopharyngeal muscular dystrophy, and FXTAS as a polyglycinopathy. The coding GGC-RE in an extremely GC-rich region was not detectable by short-read whole-exome sequencing. We describe a novel polyG cellular phenotype with impaired autophagy and neuronal intranuclear inclusions. Improving autophagy by targeting ZFHX3 mRNA points to a therapeutic avenue for this novel polyG disease.

Population analysis of repeat expansions indicates increased frequency of pathogenic alleles disease across different populations

Wednesday, 13th November - 10:15: (Trinity & Goodmans Suite) - Oral Presentation

Dr. Arianna Tucci [1], ***Dr. Kristina Ibanez*** [2], ***Dr. Matteo Zanovello*** [3], ***Dr. Christopher Clarkson*** [2], ***Dr. Delia Gagliardi*** [3], ***Dr. Aupriya Dalmia*** [4], ***Dr. Davina Hensman Moss*** [5], ***Dr. Bharati Jadhav*** [6], ***Dr. Andrea Cortese*** [7], ***Prof. Henry Houlden*** [8], ***Dr. Andrew Sharp*** [6]

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o Background and objectives

Repeat expansion disorders (REDs) include some of the most common inherited neurological diseases, including ataxia. REDs have a worldwide distribution and are estimated to affect ~1 per 3,000 individuals. Prevalence studies of REDs are hampered by heterogeneous clinical presentation leading to under-ascertainment, variable geographic distributions, and by technological limitations for screening large numbers of individuals.

o Methods

We here address the difficulty of meaningful REDs prevalence estimates using a genomic-first approach, by analyzing over 82,000 whole genome sequencing from two large-scale genomic dataset: TopMed and Genomics England. We estimate RED prevalence globally and among different genetic ancestries, and analyze the population-specific distribution of different allele lengths in Europeans, Africans, South Asians, Americans and East Asians.

o Results

We found an overall disease allele frequency of REDs of ~1 in 300 individuals. Modelling disease prevalence using genetic data, age at onset and survival (using EUROSCA registries for examples), we show that the expected number of people with REDs is two to three times higher than currently reported figures, indicating under-diagnosis and/or incomplete penetrance. While some REDs are population-specific, e.g. Huntington's disease type 2, most REDs are represented in all broad genetic ancestries, challenging the notion that some REDs are found only in specific populations

The finding that a much larger number of people in the general population carry pathogenic alleles of REDs has important implications both for diagnosis and genetic counseling of RED. For diagnosis, when a patient presents with symptoms compatible with a RED, clinicians should have a higher index of suspicion of these diseases, and clinical diagnostic pathways should facilitate genetic testing for REDs. As for genetic counsellng, when a RED expansion is identified in an individual clinically unaffected, it incomplete penetrance of the repeat should be discussed.

Parallel session: Cellular and Animal Models

A missense mutation in the CCDC88C gene induces cerebellar neurodegeneration and activation of mixed lineage kinase in a knock-in mouse model of SCA40

Wednesday, 13th November - 11:00: (Trinity & Goodmans Suite) - Oral Presentation

Edwin Chan [1], ***Huan Yang*** [2], ***Sum Yi Ma*** [2], ***Jacquelyne Ka-Li Sun*** [2], ***Agnieszka Charzewska*** [3], ***Urszula Fiszer*** [4], ***Dorota Hoffman-Zacharska*** [3], ***Marta Leńska-Mieciek*** [4], ***Hei Man Chow*** [5], ***Kin Ming Kwan*** [2]

1. *School of Life Sciences, The Chinese University of Hong Kong, Shatin N.T., Hong Kong SAR, China, Gerald Choa Neuroscience Institute, The Chinese University of Hong Kong, Shatin N.T., Hong Kong SAR, China,* ***2.*** *School of Life Sciences, The Chinese University of Hong Kong, Shatin N.T., Hong Kong SAR, China,* ***3.*** *Department of Medical Genetics, Institute of Mother and Child, Warsaw, Poland,* ***4.*** *Department of Neurology and Epileptology, Centre of Postgraduate Medical Education, Warsaw, Poland,* ***5.*** *Gerald Choa Neuroscience Institute, The Chinese University of Hong Kong, Shatin N.T., Hong Kong SAR, China*

Spinocerebellar ataxias (SCAs) are a group of heterogeneous dominantly hereditary disorders that cause progressive deterioration of the cerebellum in the patient's brain. Mutations in the *Coiled-Coil Domain Containing 88C (CCDC88C)* gene have been reported to cause SCA subtype 40 (SCA40). Previous studies have found that mutations in the *CCDC88C* gene induces hyperphosphorylation of c-Jun N-terminal kinase (JNK), leading to caspase-3 activation. However, the detailed mechanisms of disease progression have not been fully elucidated. In this study, a knock-in mouse model was established. Behavior tests revealed deteriorated movement and balance ability in mutant disease mice. Hematoxylin-eosin and immunofluorescence staining reflected morphological changes and cellular degeneration. Additionally, apoptosis and phosphorylation of proteins in the mitogen-activated protein kinases (MAPK) pathway were observed in the cerebellum area of mutant mice. Further biochemical experiments indicated that the mutant CCDC88C protein caused synaptic loss, and induced JNK phosphorylation by activating the mixed lineage kinase (MLK)-mediated MAPK pathway. These pathogenic events were found to be rescued after the treatment of a MLK inhibitor. Our findings link up *Ccdc88c* mutation with MLK-mediated apoptosis, and cerebellar degeneration in an in vivo mouse model of SCA40. Our MLK mechanistic study opens up a potential direction for alleviating the disease progression.

Phosphodiesterase inhibitors improve Friedreich's Ataxia conditions by correcting cofilin pathway and mitochondrial distribution in Drosophila models

Wednesday, 13th November - 11:15: (Trinity & Goodmans Suite) - Oral Presentation

Mr. Alexandre Llorens Trujillo [1]***, Dr. María Dolores Moltó*** [2]***, Dr. Juan Antonio Navarro Langa*** [3]

1. *1-INCLIVA, Biomedical Research Institute, Valencia, Spain. 2-Department of Genetics, Universitat de València, Valencia, Spain.,* ***2.*** *1-INCLIVA Biomedical Research Institute. 2-Department of Genetics, Universitat de València, Valencia, Spain. 3-Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Madrid, Spain,* ***3.*** *1-INCLIVA Biomedical Research Institute. 2-Department of Genetics, Universitat de València, Valencia, Spain. 4-Centro de Investigación Biomédica en Red de enfermedades raras (CIBERER), Madrid, Spain*

Introduction: Friedreich's ataxia (FRDA) is a neurodegenerative disorder caused by lack of the protein frataxin without any current effective treatment. The reduction of frataxin levels impairs mitochondrial function leading to multiple cellular defects Alterations in the stability of the actin cytoskeleton are among the most prominent ones. Defects related to the regulation of cofilin activity have been associated with the lack of frataxin. Importantly, Class IV and V phosphodiesterase inhibitors have been shown to act upstream of the cofilin pathway and impact the actin scaffold in cellular models of the disease.

Objectives: We aim to carry out the first in vivo evaluation of the therapeutic impact of two phosphodiesterase inhibitors using *Drosophila* models as a preclinical multi-cellular system.

Methods: Fly frataxin was downregulated in *Drosophila* tissues using the UAS/GAL4 system and drugs were incorporated in the fly food. Impact on frataxin deficient phenotypes was studied at molecular, cellular, and physiological levels

Results: Our experiments show that phosphodiesterase inhibitors significantly improve the longevity and locomotion of FRDA flies whilst decreasing neurodegeneration. Remarkably, this recovery is not accompanied by improvements in the mitochondrial function. Interestingly, both drugs were able to revert molecular defects linked to cofilin pathway and correct in vivo axonal structure along with abnormal mitochondrial distribution within the axon. Using genetic interactions, we proved phosphodiesterase inhibitors mostly act through cofilin.

Discussion: Our results clearly indicate that correcting a defective cytoskeletal function is sufficient to alleviate physiological disease's symptoms without correcting mitochondrial function. Furthermore, our results highlight the role of the cofilin pathway in the pathology and suggest a link between the modulation of actin dynamics and the mitochondrial localization leading to more efficient energy distribution.

Conclusion: Phosphodiesterase Inhibitors show a strong potential as a therapeutic avenue for Friedreich's Ataxia.

Unraveling the cause of phenotypic heterogeneity in spinocerebellar ataxia-type 47 (SCA47): distinct mutations, distinct mechanisms

Wednesday, 13th November - 11:30: (Trinity & Goodmans Suite) - Oral Presentation

Mr. Maximilian Cabaj [1], ***Dr. Vincenzo Gennarino*** [1], ***Dr. Nicola de Prisco*** [1], ***Dr. Mu Yang*** [1]

1. *Columbia University Irving Medical Center*

Like several non-repeat spinocerebellar ataxias, SCA47 is quite phenotypically heterogeneous. With a cohort of just over 100 patients, we have identified 41 unique variants in the RNA-binding protein Pumilio1 (PUM1) that cause at least five distinct phenotypes. The two most common variants are PUM1-R1147W (n=10) and PUM1-T1035S (n=11). R1147W causes small size at birth, motor and cognitive delays, hyperactivity, seizures, and ataxia, whereas T1035S causes a pure cerebellar ataxia that typically develops in the fifth decade of life.

Curiously, T1035S abolishes PUM1's ability to bind its mRNA targets yet causes a much milder disease than R1147W, which retains the ability to bind PUM1 targets. We therefore hypothesized that the mild disease is caused by loss of target binding, while the severe disease is caused primarily by aberrant PUM1 protein-protein interactions. To test this hypothesis, we developed a PUM1 interactome in the mouse brain and found that R1147W does indeed disrupt PUM1's interactions with other proteins, including other RNA-binding proteins, while T1035S does not (*EMBO J*, 2023). We have generated and characterized mouse models for each of these two variants to investigate the molecular pathogenesis *in vivo*.

The two mouse models recapitulate key features of the human phenotypes. R1147W mice are born small and exhibit cognitive impairment, hyperactivity, seizures, and ataxia. T1035S mice, on the other hand, are indistinguishable from wild-type littermates until 52 weeks, when ataxia emerges; high-resolution 3D immunofluorescence reveals cerebellar degeneration in the aged mice. We have harvested brains for RNA-seq, quantitative proteomics, and IP-LC/MS to identify and compare changes to protein-protein interactions and the PUM1 targetome in both mouse lines; furthermore, cell culture studies using the remaining 39 variants are shedding light on the cellular biology of SCA47.

Time-specific inactivation of FXN gene reveals its essential early post-development role: insights from a new mouse model and human DRG organoids.

Wednesday, 13th November - 11:45: (Trinity & Goodmans Suite) - Oral Presentation

Dr. Agostina Di Pizio [1], ***Ms. Elena Melacini*** [1], ***Ms. Margherita Rossi*** [1], ***Dr. Vania Broccoli*** [1]

1. *IRCCS San Raffaele Hospital*

Friedreich's ataxia (FA) is a neurodegenerative disorder associated with additional peripheral symptoms caused by FXN gene silencing. The precise pathophysiological consequences caused by FXN gene loss remain not fully understood, and several open questions persist. For instance, FA predominantly affects proprioceptive dorsal root ganglia (DRG) neurons, while other sensory neuron populations remain unaffected. The reasons for this specific vulnerability are not yet fully understood. Furthermore, the classical presentation of FA begins in adolescence, with the reasons for this age-specific onset remaining also unknown.

To address these questions, we have generated a novel mouse model for the time-specific FXN gene inactivation by crossing Fxn-floxed with tamoxifen-inducible UBC-CreERT2 mice. This model enables the constitutive FXN deletion at any desired time-points to address the role of FXN at specific developmental stages.

Our results show that FXN deletion in P14 mice results in rapid and dramatic deficits that lead animals to death within two weeks. However, the FXN gene inactivation in adult animals results in much milder symptoms, with modest weight loss and prolonged survival.

To complement the in vivo models, we generated DRG organoids derived from FA patients, which showed impaired axonal growth compared to DRG derived from healthy donors. This impairment was particularly evident in the early stages of growth, aligning with results obtained from the mouse models.

These findings suggest that FXN plays a crucial role during early development, potentially offering key insights into the molecular mechanisms underlying the disease.

Moreover, the development of gene therapy has been hindered by the lack of a mouse model that accurately replicates the human disease, given the early lethality of mice with full FXN depletion. We propose that our model serves as a promising and novel tool for testing gene therapy therapeutic relevance in a living FXN mutant mouse model.

*Funding: GoFAR Italian Association

Modelling Spinocerebellar Ataxia Type 29 (SCA29) in Cerebellar Organoids with Loss-of-Function and Gain-of-Function Variants in the ITPR1 gene

Wednesday, 13th November - 12:00: (Trinity & Goodmans Suite) - Flash Talk

Dr. Jussi-Pekka Tolonen [1], ***Dr. Elizabeth Apsley*** [2], ***Dr. David Sims*** [3], ***Dr. Salla Kangas*** [1], ***Dr. Sally Cowley*** [3], ***Dr. Joey Riepsaame*** [3], ***Prof. Johanna Uusimaa*** [4], ***Prof. Esther B. E. Becker*** [3]

1. *University of Oulu,* ***2.*** *King's College London,* ***3.*** *University of Oxford,* ***4.*** *Oulu University Hospital and University of Oulu*

Background and Objectives

Spinocerebellar ataxia type 29 (SCA29), an early-onset ataxia disorder with cognitive deficits, is caused by pathogenic missense variants in the *ITPR1* gene. *ITPR1* encodes the type 1 inositol 1,4,5-triphosphate (IP_3) receptor (IP_3R1), a calcium channel predominantly expressed in cerebellar Purkinje cells. Our objective was to model SCA29 in human induced pluripotent stem cell (hiPSC)-derived cerebellar organoids to identify potential disease mechanisms downstream of dysregulated calcium signaling.

Methods

Isogenic hiPSC lines with loss-of-function (LOF; p.Thr267Met) and gain-of-function (GOF; p.Arg36Cys) variants in the *ITPR1* gene were generated from a control hiPSC line (AH0-17-3) through CRISPR/Cas9-mediated genome editing. An additional patient-derived hiPSC line with the GOF variant p.Arg36Cys was used for comparison. Three batches of cerebellar organoids were produced using established protocols, including quality control (QC) steps at 21 and 35 days in vitro (div). Organoids were characterized using calcium imaging at 63 div (Caged-IP_3; Calbryte™ 590 as calcium indicator) and bulk RNA sequencing at 90 div.

Results

Organoids from all hiPSC lines demonstrated similar growth rates with comparable expression of QC marker genes at 21 div (*GBX2* and *EN1*) and 35 div (*ATOH1* and *KIRREL2*). At 63 div, IP_3 stimulation of cerebellar organoid-derived 2D neuronal cultures produced altered calcium signals in line with mutation status. A comparison of control vs. mutant organoids by RNAseq at 90 div revealed ~2000 differentially expressed genes, most of which were downregulated in the *ITPR1* mutant organoids. A subset of differentially expressed genes, including genes associated with cerebellar neuron function, were shared by both LOF and GOF organoids.

Discussion and Conclusion

Cerebellar organoids are an accessible platform to study the function of the IP_3R1 calcium channel at endogenous expression levels. Our calcium imaging and RNAseq data reveal new insights into the disease mechanisms of SCA29, providing a reproducible system for testing future treatment modalities.

A novel transgenic mouse model of spinocerebellar ataxia type 2 bearing 129 CAG repeats: neuropathologic and phenotypic characterization

Wednesday, 13th November - 12:07: (Trinity & Goodmans Suite) - Flash Talk

Ms. Rebekah Koppenol [1], Mr. André Conceição [1], Mrs. Inês Afonso [1], Ms. Cristiana Madeira [1], Mr. Lorenzo Mirapalheta [1], Prof. Carlos Matos [2], Prof. Clévio Nóbrega [3]

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Background and objectives: Polyglutamine diseases are caused by mutation of nine unrelated genes, arising when CAG repeat tracts therein contained are expanded beyond a critical threshold. Out of the 9 known polyglutamine disorders, 7 involve a progressively ataxic phenotype resulting from cerebellar neurodegeneration. Spinocerebellar ataxia type 2 (SCA2) is associated with expansions of 32-200 CAG repeats in the *ATXN2* gene, which codifies the ubiquitous RNA-binding protein ataxin-2. Currently, there is no disease-modifying therapy available to SCA2 patients. Animal models constitute a vital platform to better understand the molecular mechanisms underlying SCA2 and test novel therapeutic routes. Though several SCA2 rodent models have been developed, all present important limitations, including absent-to-mild motor phenotypes and limited availability. The aim of this work was to characterize a novel transgenic mouse that expressed human ATXN2 cDNA with 129 CAG repeats, the longest repeat ever used in SCA2 mouse model generation.

Methods: The novel model expresses human ATXN2 cDNA under the control of the L7-6 promoter, which directs expression to cerebellar Purkinje cells (PCs), the cell type that is particularly affected in SCA2. We characterized the animals through a set of tests that evaluate motor performance and balance and assess their neuropathologic phenotype through immunohistochemistry.

Results: SCA2 transgenic animals display motor impairments that include gait abnormalities, loss of motor coordination and involuntary limb contraction. Importantly, mice also mice display ataxin-2 aggregates in PCs and loss of PCs.

Discussion and Conclusion: This novel transgenic mouse model recapitulates crucial aspects of SCA2 clinical presentation. Its neuropathological phenotype predates the establishment of the motor phenotype, something that is increasingly admitted to take place in human patients. The fact that both manifest at a relatively early time point (4 and 12 weeks, respectively) makes this model adequate to study novel disease-modifying therapies in a practicable timeframe.

Comprehensive Analysis of the CACNA1A SCA6 protein, a1ACT: Insights from Transgenic Mouse Models and Multi-Omics Approaches for SCA6 Pathogenesis

Wednesday, 13th November - 12:14: (Trinity & Goodmans Suite) - Flash Talk

Dr. Xiaofei Du [1], Mr. Eric Gama [1], Mr. Cenfu Wei [2], Dr. Juan Sun [1], Dr. Kellie Benzow [3], Dr. Michael Koob [3], Dr. Christopher M. Gomez [4]

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The presence of an expanded polyglutamine (polyQ) tract encoded by exon 47 of the *CACNA1A* gene is associated with spinocerebellar ataxia type 6 (SCA6). a1ACT, a transcription factor and the second gene product of *CACNA1A*, bears this polyQ. Overexpression of expanded polyQ(Q33) a1ACT in cells causes cell death. To delineate the progressive disease caused by expanded polyQ a1ACT, we engineered a mouse model utilizing a bacterial artificial chromosome (BAC) system that expresses a1ACTQ33 ($a1ACT_{SCA6}$) under the control of the PCP2 promoter. PC-BAC $a1ACT_{SCA6}$ mice develop normally, but from 3 to 9 months, they manifest progressive motor deficits, Purkinje cell loss, and cerebellar atrophy, predominantly in the anterior lobe, mirroring SCA6 pathology. Transcriptional profiling of $a1ACT_{SCA6}$ via RNA-seq revealed its involvement in neurodegeneration and DNA damage pathways. Proteomics analysis of extracted cerebellar proteins identified an age-depended protein *decrease* in PC-BAC $a1ACT_{SCA6}$, differences in the co-transcription factor/protein partner network between of $a1ACT_{SCA6}$ and $a1ACT_{wt}$, and chaperones associated with nuclear aggregation in $a1ACT_{SCA6}$. miRNA/mRNA analysis pinpointed miRNAs affected by the polyQ expansion. These integrated multi-omics profiles underscored the crucial role of $a1ACT_{SCA6}$ in cerebellar degeneration, emphasizing both spatial and temporal aspects of its impact. These studies provide compelling evidence for the critical role of $a1ACT_{SCA6}$ in SCA6 progression. Taken together, PC-BAC $a1ACT_{SCA6}$ mouse model along with multi-omics analyses, provide valuable insights into SCA6 pathogenesis, biomarker identification, and gene-targeting therapeutic strategies.

Unraveling Peripheral Neuropathy in Spinocerebellar Ataxia Type 3: Insights from a Mouse Models

Wednesday, 13th November - 12:21: (Trinity & Goodmans Suite) - Flash Talk

Mr. Juan Mato [1], Mr. John Hayes [1], Mr. Arsal Naeem [1], Mr. Jacen Emerson [1], Dr. Eva Feldman [1], Dr. Hayley McLoughlin [1]

1. *University of Michigan*

Background and Objectives: Spinocerebellar ataxia type 3 (SCA3) is the most prevalent dominantly inherited ataxia, marked by a lack of effective treatments. While research has predominantly targeted the central nervous system, over half of SCA3 patients display peripheral neuropathy. This study seeks to understand the progression of SCA3-associated peripheral neuropathy, yielding potential therapeutic insights for addressing this and similar neurological disorders.

Methods: Utilizing two established SCA3 mouse models (YAC Q84 transgenic mice and Knock-In Q300 mice) relative to wildtype littermate controls and Atxn3 Knock-out mice, we comprehensively examined sensory and motor behavioral, electrophysiological, and histological features of peripheral neuropathy across the disease's progression.

Results: The YAC Q84 and KI Q300 mouse models recapitulate sensory nerve action potential and sensorimotor nerve conduction velocity reductions reported in SCA3 patients. ATXN3 loss of function does not seem to affect nerve conduction. In at least one mouse model, the pathological disease hallmark of nuclear accumulation of ATXN3 is evident in sensory and motor neurons and Schwann cells. Ultrastructural analysis of peripheral nerves showed myelinated fiber density loss, large axon caliber composition reductions, and demyelination in SCA3 that reflect electrophysiological changes."

Conclusions: The observed behavioral, electrophysiological, and pathological abnormalities in SCA3 mice confirm the peripheral nervous system's role in SCA3 disease and underscore the progressive nature of peripheral neuropathy in this condition. These results pave the way for the future identification of biomarkers and the development of targeted therapies, offering hope for therapeutic interventions in SCA3 and other related neurodegenerative diseases.

Parallel session: Biomarkers and clinical outcome measures I

Predictive models for ataxia progression and conversion in SCA1 and SCA3

Wednesday, 13th November - 14:00: (Trinity & Goodmans Suite) - Oral Presentation

Mr. Emilien Petit [1], ***Dr. Thomas Klockgether*** [2], ***Prof. Alexandra Durr*** [3], ***Dr. Henry Paulson*** [4], ***Dr. Gulin Oz*** [5], ***Dr. Tetsuo Ashizawa*** [6], ***Dr. Sophie Tezenas du Montcel*** [7]

1. *Sorbonne Université, Paris Brain Institute, Inserm, INRIA, CNRS, APHP, 75013 Paris, France,* ***2.*** *German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany,* ***3.*** *Sorbonne University, Paris Brain Institute, Pitié-Salpêtrière Hospital,* ***4.*** *University of Michigan,* ***5.*** *University of Minnesota,* ***6.*** *The Houston Methodist Research Institute, Houston, TX 77030, USA,* ***7.*** *ARAMIS, Sorbonne Université, Institut du Cerveau-Paris Brain Institute-ICM, CNRS, Inria, Inserm, AP-HP, Groupe Hospitalier Sorbonne Université, Paris, France*

Background and objectives: The READISCA study aims to prepare for clinical trials in SCA1 and SCA3. Hence, we are searching for predictive variables of conversion and progression.

Methods: Individuals with SCA1 or SCA3 and controls were enrolled from 2018-2020 in US and Europe. Clinical scores, MRI measures (in ~half of the cohort), and NfL levels were assessed annually for 5 years. In the pre-ataxic group at baseline, we compared phenoconverters with non-converters. A Bayesian mixed model was used to model the longitudinal progression of clinical scores and NfL levels. The impact of previously selected baseline variables (demographic, clinical, MRI) on the expected SARA progression was tested.

Results: 43 controls, 55 SCA1 and 124 SCA3 were included. Converters represented 5/22 (22%) and 12/38 (32%) of the pre-ataxic participants for SCA1 and SCA3 respectively. Converters were more depressed (PHQ9 median [Q1;Q3] : 3 [2;7] vs 1 [0;4], p = 0.04), had higher NfL levels (17.8 $pg.mL^{-1}$ [13.5;21.6] vs 9.7 [6.8;14.5], p<0.0001) and more INAS signs (2 [1;3] vs 1 [0;1], p = 0.002). All clinical scores except the CCAS were significantly increasing during the study. NfL levels were significantly increasing in non-converters and SCA3 progressors (ataxic+phenoconverters) (1.06±0.33 $pg.mL^{-1}$/year, p=0.002 and 0.57±0.21, p=0.01) but not in controls and SCA1 progressors (0.31±0.26 p=0.24 and 0.26±0.42, p=0.55). In the best predictive model of SARA progression after 1 year (R^2=0.3), factors linked with faster progression were the absence of lower motoneuron signs (p=0.02), longer CAG repeat length (p=0.01), lower total NAA concentration in pons (p=0.03) and lower inferior cerebellar peduncle fractional anisotropy (p=0.03).

Discussion: Factors significantly linked to conversion are different from the ones linked to the later progression rate of the disease. For future cohort enrichment, NfL levels and INAS could be used as predictors of conversion and MRI variables as ataxia progression predictors.

Genotype-specific Spinal Cord Damage in Spinocerebellar Ataxias: an ENIGMA-Ataxia Study

Wednesday, 13th November - 14:15: (Trinity & Goodmans Suite) - Oral Presentation

Dr. Thiago Rezende [1], ***Prof. Marcondes França*** [2], ***Dr. Ian Harding*** [3], ***Mr. . Enigma Ataxia Working Group*** [4]

1. *University of Campinas,* ***2.*** *Department of Neurology, University of Campinas, Campinas, Sao Paulo,* ***3.*** *Monash University, Melbourne and QIMR Berghofer Medical Research Institute, Brisbane,* ***4.*** *Enigma Ataxia*

Background/Objective: Spinal cord damage is acknowledged in several spinocerebellar ataxias (SCAs), yet thorough in vivo studies are still lacking, leaving the connections with disease severity and progression ambiguous. In this study, we aim to characterize morphometric abnormalities in the cervical spinal cord in SCA1, SCA2, SCA3, and SCA6 using data from a extensive multisite MRI database.

Materials and Methods: A cross-sectional analysis focusing on the cross-sectional area (CSA) and eccentricity of the upper spinal cord (C1 to C4) was carried out using MRI data obtained from nine sites collaborating within the ENIGMA-Ataxia consortium. This included 364 individuals with SCA, 56 preataxic individuals, and 394 nonataxic individuals. Correlations and subgroup analyses were conducted within the SCA cohorts based on disease duration and severity of ataxia.

Results: Individuals with SCA1, SCA2, and SCA3 exhibited significantly decreased CSA and increased eccentricity compared to nonataxic participants across all levels examined. The CSA differences showed large effect sizes ($d>2.0$) and correlated strongly with ataxia severity ($r<-0.43$) and disease duration ($r<-0.21$). Eccentricity was only significantly correlated with ataxia severity in SCA2 ($r=0.28$). However, no significant effects were observed in SCA6. Notably, CSA was notably reduced in preataxic individuals with SCA2 ($d=1.6$) and SCA3 ($d=1.7$), and only the SCA2 preataxic group showed increased eccentricity ($d=1.1$) relative to nonataxic individuals. Subgroup analyses revealed that abnormalities in CSA and eccentricity are present in the early stages of the disease in SCA1, SCA2, and SCA3. Additionally, CSA decreased with disease progression, while eccentricity only progressed in SCA2.

Discussion/Conclusion: Interpretation: Spinal cord abnormalities manifest early in SCA1, SCA2, and SCA3, progress through disease stages, and are detectable through quantitative MRI techniques.

Delineating the phenotypic spectrum and FGF14 GAA repeat size pathogenic threshold in a large French-Canadian SCA27B cohort

Wednesday, 13th November - 14:30: (Trinity & Goodmans Suite) - Oral Presentation

Dr. Catherine Ashton [1], Dr. David Pellerin [2], Dr. Felipe Villa [2], Dr. Matt C. Danzi [3], Dr. Mathilde Renaud [4], Ms. Marie-Josée Dicaire [1], Dr. Rami Massie [1], Dr. Colin Chalk [1], Dr. Anne-Louise La Fontaine [1], Dr. Francois Evoy [5], Dr. Marie-France Rioux [5], Dr. Kym M. Boycott [6], Prof. Henry Houlden [7], Prof. Matthis Synofzik [8], Dr. Roberta La Piana [9], Dr. Stephan Zuchner [3], Dr. Antoine Duquette [10], Dr. Bernard C. Brais [1]

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Background: Spinocerebellar ataxia 27B (SCA27B) due to an autosomal dominant intronic (GAA)•(TTC) repeat expansion in *FGF14* is an increasingly recognised common cause of adult-onset cerebellar ataxia. We performed in-depth phenotyping of an expanding cohort of French-Canadian patients with SCA27B, including reassessment of younger subjects from several large families to delineate the early clinical manifestations and reassess the pathogenic threshold.

Methods: All individuals carrying an *FGF14* allele $(GAA)_{\geq 200}$ were clinically reassessed where possible Those with alternative causes for ataxia were excluded. Segregation studies of individuals with $(GAA)_{200\text{-}250}$ were conducted in larger families. Brain MR imaging was re-evaluated where available.

Results: Data from 103 individuals from 54 families were assessed: 84 with permanent ataxia (82%). Most (79/103, 77%) reported initial episodic symptoms at mean age 53±12.7y. Six persons reported episodes of speech arrest and 11 individuals had severe episodes presenting to hospital with stroke-like episodes. Permanent ataxia began at mean age of 59±11y. Cerebellar features on examination included: axial ataxia (79/84, 94%), appendicular ataxia (72/84, 86%), dysarthria (45/84, 54%), downbeat nystagmus (51/84, 60%), horizontal nystagmus (44/84, 5%). Average SARA scores (n=54) were 8.2 after mean disease duration of 13 years. Cerebellar atrophy was present in 26/27 (96%) brain MRIs and the superior cerebellar peduncle (SCP) sign was visualised in 16/26 (62%). Alleles between $(GAA)_{219}$ and $(GAA)_{247}$ were found in 11 individuals, six with confirmed segregation from large family studies: seven had ataxia on examination, two had episodic symptoms only, 2/2 had the SCP sign on MRI.

Discussion and conclusion: SCA27B is associated with a prodromal episodic syndrome occurring about a decade prior to onset of permanent ataxia that can lead to severe acute presentations. Segregation studies showed that expansions as small as $(GAA)_{219}$ were associated with disease in affected families. Our results call for further reassessment of the pathogenic threshold.

Preliminary natural history data in Spinocerebellar Ataxia type 44 (SCA44) reveals marked speech impairment compared to other metrics

Wednesday, 13th November - 14:45: (Trinity & Goodmans Suite) - Oral Presentation

Prof. Helen Dawes [1]***, Prof. Esther B. E. Becker*** [2]***, Dr. Patrick Esser*** [3]***, Dr. Mario De Oliveira Inacio*** [3]***, Prof. Andrea Nemeth*** [4]

1. *University of Exeter, UK,* ***2.*** *University of Oxford,* ***3.*** *Oxford Brookes University,* ***4.*** *Nuffield Department of Clinical Neurosciences, University of Oxford*

Background and Objectives: SCA44 is a very slowly progressive cerebellar ataxia, caused by gain-of-function missense mutations in the metabotropic glutamate receptor type1 (mGluR1). A recently generated murine model offers prospects for pharmacological or other interventions.

mGluR1 signalling plays a critical role in cerebellar motor learning and motor control, therefore data on human SCA44 will provide valuable assessment and therapeutic strategies for multiple cerebellar ataxias, and provide a platform for rapid translation from murine model to human clinical trials. Here we report the first 3 years of natural history data on clinical and digital outcome measures of ataxia.

Methods: Clinical data was obtained at yearly intervals on 2 original index families, plus a newly identified case. Data included neurological exam, plus SARA, Functional and ADL Scores.

Digital gait data was obtained using an inertial measurement unit and participants performed a 10m walking test. Time to completion as well as 15 spatial/temporal gait and variability parameters were measured, and compared to 60 controls.

Results: Cases remained ambulant despite long disease duration, with no clear relationship between severity of gait impairment and disease duration. Speech scores were the highest contributor to total SARA scores, but gait was also abnormal. Functional and ADL scores showed mild impairment.

SARA gait scores remained stable over 3 years. However, digital gait analysis detected increased overall temporal variability with disease duration. There was more yearly session to session variability with less severity.

Discussion and Conclusions: SCA44 cases have disproportionate speech impairment compared to gait and other metrics. Digital speech monitoring may be useful for clinical trials. Digital gait metrics were more responsive to change over time than SARA gait scores, but more frequent testing intervals than 1 yearly may improve precision. Comparison with the murine model may allow anatomical and functional correlations to be made.

Longitudinal analysis of clinical outcomes and plasma NfL, total tau, GFAP and UCHL1 in spinocerebellar ataxia type 3/Machado-Joseph disease.

Wednesday, 13th November - 15:00: (Trinity & Goodmans Suite) - Flash Talk

Dr. Hector Garcia-Moreno [1], ***Prof. Douglas R Langbehn*** [2], ***Dr. Cristina Gonzalez-Robles*** [1], ***Dr. Amanda Heslegrave*** [3], ***Prof. Henrik Zetterberg*** [4], ***Dr. Magda Santana*** [5], ***Prof. Luis Pereira de Almeida*** [6], ***Dr. Mafalda Raposo*** [7], ***Prof. Manuela Lima*** [7], ***Dr. Jennifer Faber*** [8], ***Dr. Thomas Klockgether*** [8], ***Prof. Ludger Schöls*** [9], ***Prof. Matthis Synofzik*** [9], ***Dr. Jeannette Hübener-Schmid*** [10], ***Dr. Jon Infante*** [11], ***Prof. Bart van de Warrenburg*** [12], ***Prof. Kathrin Reetz*** [13], ***Prof. Paola Giunti*** [1]

1. *Ataxia Centre, Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, London,* ***2.*** *Department of Psychiatry, University of Iowa Carver College of Medicine, Iowa City, Iowa,* ***3.*** *Department of Neurodegenerative Disease, UCL Queen Square Institute of Neurology, London. UK Dementia Research Institute at UCL, London,* ***4.*** *Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal. Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal,* ***5.*** *Center for Neuroscience and Cell Biology, University of Coimbra,* ***6.*** *Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra,* ***7.*** *Faculdade de Ciências e Tecnologia, Universidade dos Açores, Ponta Delgada. Instituto de Biologia Molecular e Celular (IBMC), Instituto de Investigação e Inovação em Saúde (i3S), Universidade do Porto, Porto,* ***8.*** *Department of Neurology, University Hospital Bonn, Bonn. German Center for Neurodegenerative Diseases (DZNE), Bonn.,* ***9.*** *Department for Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research and Center for Neurology, University of Tübingen. German Center for Neurodegenerative Diseases (DZNE), Tübingen,* ***10.*** *Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen,* ***11.*** *Neurology Service, University Hospital Marqués de Valdecilla-IDIVAL, University of Cantabria, Centro de Investigación en Red de Enfermedades Neurodegenerativas (CIBERNED), Santander,* ***12.*** *Department of Neurology, Donders Institute for Brain, Cognition, and Behaviour, Radboud University Medical Center, Nijmegen,* ***13.*** *Department of Neurology, RWTH Aachen University, Aachen. JARA-BRAIN Institute Molecular Neuroscience and Neuroimaging, Forschungszentrum Jülich, RWTH Aachen University, Aachen*

Background and objectives. Clinical trials in SCA3 will require outcome measures and biomarkers that can track disease progression with enough sensitivity. Neurofilament light chain (NfL), total tau (t-tau), glial fibrillary acidic protein (GFAP) and ubiquitin carboxy-terminal hydrolase L1 (UCHL1) have shown a role as fluid biomarkers in different neurological conditions. We aim to study longitudinal changes in clinical outcomes and these plasma biomarkers in a European SCA3 cohort.

Methods. Participants underwent a standardised protocol. Plasma NfL, t-tau, GFAP and UCHL1 were measured with the Neurology 4-Plex "A" kit in the Simoa HD-X analyser.

Results. We included 181 ataxic patients, 33 preataxic carriers and 92 control participants. Clinical outcomes showed worse scores in ataxic patients, compared to preataxic carriers and controls. Significant progression was detected in ataxic patients for SARA (1.1 points/year), ADL (1.0 point/year), and SCAFI-9HPT (-0.0009 s^{-1}/year). Adjusted plasma NfL was significantly higher in ataxic patients (3.28 logpg/mL), compared to preataxic carriers (2.70 logpg/mL, p<0.001) and controls (1.69 logpg/mL, p<0.001). Levels in preataxic carriers were also higher compared to controls (p<0.001). Significant progression in plasma NfL was not detected in any of the groups. Higher baseline NfL was associated with worse scores in clinical outcomes. Plasma t-tau, GFAP and UCHL1 did not differ among groups, and progression in their concentrations was not observed.

Discussion and Conclusion. Clinical outcomes differentiate ataxic patients from non-ataxic participants, but pro-

gression rates are of small magnitude. Plasma NfL can be implemented as a prognostic biomarker in clinical trials in SCA3. Further studies in CSF and blood with different assays will be necessary to clarify the role of t-tau, GFAP and UCHL1 as fluid biomarkers in SCA3.

Funding sources. Federal Ministry of Education and Research (BMBF), The Netherlands Organisation for Health Research and Development, Fundação para a Ciência e a Tecnologia (FCT), Medical Research Council.

Comparison of Two Matching Methods to Assess Effectiveness of Troriluzole versus Untreated Natural History Cohort in Spinocerebellar Ataxia

Wednesday, 13th November - 15:07: (Trinity & Goodmans Suite) - Flash Talk

Ms. Basia Rogula [1], Ms. Lauren Powell [1], Dr. Michele Potashman [2], Ms. Victoria Wirtz [2], Dr. Melissa Wolfe-Beiner [2], Prof. Jeremy D. Schmahmann [3], Dr. Susan Perlman [4], Dr. Vlad Coric [2], Dr. Gilbert L'Italien [2]

1. *Broadstreet HEOR,* ***2.*** *Biohaven Pharmaceuticals, Inc.,* ***3.*** *Department of Neurology, Massachusetts General Hospital and Harvard Medical School,* ***4.*** *University of California at Los Angeles*

Background/Objectives: BHV4157-206 (NCT03701399) is a pivotal efficacy trial examining troriluzole in patients with spinocerebellar ataxia (SCA), consisting of a randomization period followed by open-label extension. The objective of this analysis was to estimate the effectiveness of troriluzole vs an external natural history (NH) control group over 3-years, comparing results obtained using matching-adjusted indirect comparison (MAIC) and propensity score matching (PSM) methodologies.

Methods: A MAIC was conducted by selecting and weighting individual patient-level NH data to create a cohort matched to troriluzole-treated subjects based on several key characteristics: modified-functional Scale for the Assessment and Rating of Ataxia (f-SARA) score, genotype, sex, age, and age of symptom onset. For PSM, the same characteristics were analyzed via logistic regression to estimate a propensity score for each patient that was then used to match NH to troriluzole treated patients at a ratio of 3 to 1. The between-group least squares (LS) mean change from baseline (CFB) differences on f-SARA were derived at years 1, 2, and 3 to estimate troriluzole effectiveness.

Results: A total of 96 troriluzole-treated subjects and 611 untreated NH subjects were the basis for the analysis. Using MAIC, LS mean change differences in f-SARA were -0.64, -1.16, and -1.34 at years 1, 2, and 3, favoring troriluzole (p=0.0008, <0.0001, and <0.0001), respectively. Using PSM, LS mean change differences in f-SARA were -0.63, -1.13, and -1.30 at years 1, 2, and 3, favoring troriluzole (p=0.0051, <0.0001, and <0.0001), respectively. Comparison with MAIC or PSM both demonstrated greater ataxia-related impairment and clinically decline amongst the NH cohort when compared to troriluzole-treated subjects.

Conclusions: Compelling and sustained treatment effects over 3 years were observed when troriluzole-treated subjects were compared to an untreated matched NH cohort. Results were consistent across MAIC and PSM methodologies.

Study funded by Biohaven Pharmaceuticals, Inc.

Measuring Friedreich Ataxia in children – exploring how typically developing children perform on clinical rating scales.

Wednesday, 13th November - 15:14: (Trinity & Goodmans Suite) - Flash Talk

Dr. Louise A Corben [1], ***Prof. Malcolm Horne*** [2], ***Prof. Pubudu Pathirana*** [3], ***Prof. Martin Delatycki*** [4], ***Ms. Jennifer Farmer*** [5], ***Prof. Nellie Georgiou-Karistianis*** [6], ***Prof. Kathrin Reetz*** [7], ***Dr. Imis Dogan*** [7], ***Prof. Pierre-Gilles Henry*** [8], ***Prof. David Lynch*** [9], ***Dr. Christian Rummey*** [10]

1. *Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute; University of Melbourne; Monash University.,* ***2.*** *Bionics Institute,* ***3.*** *School of Engineering, Deakin University,* ***4.*** *Monash University; Murdoch Children's Research Institute; University of Melbourne; Victorian Clinical Genetics Service,* ***5.*** *Friedreich's Ataxia Research Alliance, Downingtown, PA,* ***6.*** *Monash University,* ***7.*** *Department of Neurology, University of Aachen,* ***8.*** *University of Minnesota,* ***9.*** *Children's Hospital of Philadelphia,* ***10.*** *Clinical Data Science GmbH*

Background

Accurate measurement of clinical progression in Friedreich ataxia (FRDA) in pre-teen children necessitates untangling the interaction of the maturation of the cerebellum, peaking around 12 years, and neurodegeneration related to FRDA. It remains unclear if current clinical rating scales can accommodate this interaction and the normal individual variability in maturation of age-related motor control.

Methods

We aimed to evaluate the effect of age and neuromechanical parameters on scores from the modified Friedreich Ataxia Rating Scale (mFARS) in typically developing children aged ≤12 years. We collected data related to age, height and weight and as well as scores on the mFARS and subscales: Upper Limb (UL), Lower Limb (LL) and Upright Stability (US).

Results

Thirty-two typically developing children ($\bar{x}$ age =9.8, SD=2.0, range= 5-12) were recruited from four sites with recruitment ongoing. Scores on the mFARS Total ($\bar{x}$ =3.1, SD=4.2) ranged from 0-18. UL scores ($\bar{x}$ =2.1, SD=2.8) ranged from 0-11; LL scores ($\bar{x}$ =0.1, SD=0.4) ranged from 0-2, whereas US scores ($\bar{x}$ =0.9, SD=1.8) ranged from 0 - 4.9. The UL subscale demonstrated considerable (developmental) variability however, still drove the higher mFARS scores in younger participants. The US subscale provided a less variable, more stable measure reflective of mastery of stability with cerebellar maturation.

Discussion

Typically developing children demonstrated age related performance on the mFARS driven largely by the UL subscale. Likewise, a recent study identified the inability of the overall mFARS score to reflect change accurately in young children due to the erratic and unstable nature of performance on the UL subscale, proposing the US subscore may ensure a more stable measure that translates across childhood to adulthood.

Conclusion

This study is ongoing and will provide significant guidance in the use of the mFARS in clinical trials involving pre-teen children with FRDA.

Funding: FARA General Research Grant.

In-clinic Eye Tracking during Passage Reading Supports Precise Assessment of Oculomotor Signs of Ataxia

Wednesday, 13th November - 15:21: (Trinity & Goodmans Suite) - Flash Talk

Dr. Brandon Oubre [1], ***Ms. Faye Yang*** [2], ***Ms. Anna Luddy*** [2], ***Mr. Rohin Manohar*** [2], ***Dr. Nancy Soja*** [2], ***Dr. Christopher D. Stephen*** [1], ***Prof. Jeremy D. Schmahmann*** [1], ***Dr. Divya Kulkarni*** [1], ***Mr. Lawrence White*** [2], ***Dr. Siddharth Patel*** [1], ***Dr. Anoopum Gupta*** [1]

1. *Department of Neurology, Massachusetts General Hospital and Harvard Medical School,* ***2.*** *Department of Neurology, Massachusetts General Hospital*

Background and Objective: Clinical assessments of oculomotor signs of ataxia lack precision, limiting sensitivity to capture progression. Quantitative analysis of eye movements during natural tasks could enable precise digital oculomotor assessments.

Method: A total of 95 individuals with ataxia (1 SCA-1, 6 SCA-2, 15 SCA-3, 7 SCA-6, 7 other SCAs, 11 Friedreich's ataxia, 7 CANVAS, 5 MSA-C, 4 HSP-7) and 70 healthy controls participated in the Neurobooth study. Longitudinal data was present in 28 individuals with ataxia. Each participant read aloud the Bamboo passage. Binocular gaze was sampled at 1000 *Hz* using an EyeLink Portable Duo in the head-free configuration. Saccades were categorized as regressions, return sweeps, or rightward saccades based on their direction and displacement. Saccade and fixation kinematics were summarized using 28 statistical features. Linear models were trained to 1) distinguish between ataxia participants and controls, and 2) estimate the total Brief Ataxia Rating Scale (BARS).

Results: With increasing severity, ataxia participants produced more saccades with a higher proportion of regressions and had longer fixations with greater displacement. Logistic regression distinguished between ataxia participants and controls with an area under the receiver operating characteristic curve (AUROC) of 0.88. Model probabilities demonstrated strong agreement between eyes (ICC=0.88, $p<0.001$) and captured progression longitudinally ($p=0.017$). Lasso regression estimates strongly correlated with total BARS ($r=0.80$, $p<0.001$) and demonstrated strong agreement between eyes (ICC=0.93, $p<0.001$), but longitudinal change did not reach significance ($p=0.063$).

Discussion: Eye movements during a short passage reading task are highly informative of ataxia presence and severity and may capture change over time. Sophisticated modeling of temporal patterns could lead to further improvements.

Conclusion: Eye tracking during natural tasks could be feasibly deployed in clinical settings to enable precise and reliable assessments. Accumulating longitudinal data could inform more sensitive measures of progression.

Funding: Massachusetts Life Sciences Center, NIH NS117826, Biogen

Parallel session: Imaging

Neuroimaging biomarkers of hypoplasia and disease progression in Friedreich Ataxia: preliminary 12-month longitudinal results from TRACK-FA

Wednesday, 13th November - 16:00: (Trinity & Goodmans Suite) - Oral Presentation

Prof. Nellie Georgiou-Karistianis [1], Dr. Louise A Corben [2], Prof. Eric F. Lock [3], Dr. Helena Bujalka [4], Dr. Isaac Adanyeguh [5], Dr. Jonathan J. Cherry [6], Dr. Manuela Corti [7], Prof. Martin Delatycki [8], Dr. Imis Dogan [9], Ms. Jennifer Farmer [10], Prof. Marcondes França [11], Dr. Anthony S. Gabay [12], Prof. William Gaetz [13], Prof. Ian Harding [14], Dr. James Joers [5], Ms. Michelle A. Lax [15], Mr. Jiakun Li [3], Prof. David Lynch [16], Prof. Thomas Mareci [17], Prof. Alberto Martinez [11], Prof. Massimo Pandolfo [18], Dr. Marina Papoutsi [12], Dr. Richard Parker [12], Dr. Myriam Rai [10], Prof. Kathrin Reetz [9], Dr. Thiago Rezende [19], Prof. Timothy P. Roberts [13], Dr. Sandro Romanzetti [20], Dr. David A. Rudko [21], Dr. Susmita Saha [22], Prof. Jörg B. Schulz [23], Prof. S. H. Subramony [7], Dr. Veena G. Supramaniam [12], Prof. Christophe Lenglet [24], Prof. Pierre-Gilles Henry [25]

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Background

TRACK-FA [1] is the largest neuroimaging natural history study in Friedreich Ataxia (FRDA) to date, with a total enrollment of 169 patients and 95 controls over 7 sites worldwide. Baseline enrollment ended in Aug 2023. As of May 2024, about 60% of 12-month neuroimaging data have been acquired and analyzed. Here we report preliminary 12-month longitudinal results.

Methods

Primary outcome measures [1] were obtained using TRACK-FA pipelines, including FastSurfer (brain morphometry), ACAPULCO and SUIT (cerebellum morphometry) and SCT (spinal cord morphometry). For each primary outcome measure (POM), the standardized response mean (SRM) between controls and patients was calculated as: (mean slope controls - mean slope FRDA) / (pooled SD of slopes).

Results

In FRDA participants, compared with controls, the largest absolute longitudinal effect size was found for spinal cord cross-sectional area (CSA) at C1-C2 (SRM -0.47) followed by cerebellum volume (-0.31).

When stratifying by age, higher absolute effect size was found for CSA at C1-C2 in the 11-17 age group (-0.92) and the 5-10 age group (-0.79) compared to adults (-0.26), and for cerebellum volume in the 5-10 age group (-0.99) compared to adults (-0.28). In children, the size of those two structures increased in controls over time (reflecting development) but decreased in FRDA.

Discussion and conclusion

Because of hypoplasia, higher longitudinal effect size can be achieved in children than in adults for key imaging metrics such as spinal cord CSA and cerebellum volume. Contrary to clinical scales such as mFARS or SARA (not designed to measure development in healthy controls), neuroimaging can measure differential development between controls and FRDA. This presents an opportunity for the design of therapeutic clinical trials specifically focusing on children with smaller groups of patients and/or shorter follow-up.

Reference

[1] Georgiou-Karistianis et al. PLoS One 17(11):e0269649 (2022).

Funding: FARA and TRACK-FA consortium.

Longitudinal evaluation brain structural changes in RFC1-related disorder

Wednesday, 13th November - 16:15: (Trinity & Goodmans Suite) - Oral Presentation

Dr. Camila Lobo [1], ***Dr. Thiago Rezende*** [1], ***Mr. Gustavo Jarola*** [1], ***Dr. Gabriel Schmitt*** [1], ***Dr. Paula Matos*** [2], ***Dr. Fabrício Lima*** [1], ***Prof. Alberto Martinez*** [3], ***Dr. Orlando Barsottini*** [2], ***Dr. José Luiz Pedroso*** [2], ***Dr. Wilson Marques Jr*** [4], ***Prof. Marcondes França Jr*** [1]

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Background and Objective: The structural characteristics of the brain in RFC1/CANVAS are well-documented, but the progression of brain damage remains unclear. This study aims to delineate the natural history of brain changes in RFC1/CANVAS. **Methods:** A cohort of ten patients with RFC1/CANVAS and ten age- and sex-matched healthy controls underwent 3T MRI scans at a two-year interval. Gray matter was assessed using FreeSurfer, cerebellar structures were analyzed with CerebNet, white matter integrity was evaluated using DTI multiatlas, and the spinal cord was examined with the Spinal Cord Toolbox. Covariance analysis was employed to investigate longitudinal changes between the groups, and effect sizes (ES) were calculated for significant findings. **Results:** Compared to controls, the RFC1/CANVAS group exhibited progressive atrophy in the brainstem (ES=2.18), left hippocampus (ES=1.21), and right thalamus (ES=1.24), a reduction in the spinal cord cross-sectional area at C1 (ES=1.09), and an increase in middle cerebellar peduncle axial diffusivity (ES=1.44). Over the same period, mean SARA scores increased from 15.2±5.1 to 19.4±7.9 (ES=0.62). **Discussion and Conclusions:** Quantitative neuroimaging is capable of capturing the progression of RFC1/CANVAS and proves to be more sensitive than clinical evaluation.

Dorsal root ganglia and spinal cord imaging in genetic and acquired sensory neuronopathies

Wednesday, 13th November - 16:30: (Trinity & Goodmans Suite) - Oral Presentation

Mrs. Rafaella Tacla [1], ***Dr. Thiago Rezende*** [2], ***Paulo Wolmer*** [3], ***Mr. Gustavo Jarola*** [4], ***Prof. Alberto Martinez*** [5], ***Prof. Marcondes França Jr*** [4]

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Objectives: The etiology of sensory neuronopathies (SN) can be divided into two key groups: acquired and genetic. We designed an MRI-based study to compare volumetry of the dorsal root ganglia (DRG) and structural imaging of the spinal cord (SC) across healthy controls, patients with acquired SN and patients with Friedreich's Ataxia (FRDA), the prototypical genetic SN. We also assessed clinical correlates of these imaging parameters.

Methods: Sixty-two subjects (21 healthy controls, 20 acquired SN patients, and 21 FRDA patients) underwent 3T MRI acquisitions including: Diffusion-weighted imaging and T2WI of the cervical spine as well as PROSET isotropic T2WI of the lumbosacral spine to assess the DRG. Analyses correlating MRI metrics and clinical parameters were performed. Sex, age, and body-mass index were used as covariates in between-group comparisons.

Results: Patients with FRDA, but not acquired SN had widespread (C1-T1) SC atrophy relative to controls. Fractional anisotropy reduction in the dorsal columns was observed in both groups (effect size 1.42 SN group versus 1.58 FRDA group), but spinocerebellar and corticospinal tracts were affected only in FRDA patients. DRG volumes at L3-L5 were smaller in both groups compared to controls. Dorsal column fractional anisotropy at cervical SC correlated with disease severity in both groups (R: -0.6-0.7).

Discussion: A dedicated MRI protocol can capture SC and DRG abnormalities in both genetic and acquired SN. SC damage seems to be more widespread in genetic SN, which may be useful from a diagnostic perspective. The existing correlation between disease severity and SC diffusivity changes suggests MRI may be also relevant to track disease progression.

Conclusion: Combined MRI-based evaluation of SC and DRG may be a potential (diagnostic and status) biomarker for sensory neuronopathies.

Reduced Mitochondrial Complex 1 density in the brain and heart of Friedreich's ataxia patients revealed using [18F]BCPP-EF PET imaging

Wednesday, 13th November - 16:45: (Trinity & Goodmans Suite) - Oral Presentation

Prof. Richard Festenstein [1], ***Dr. Yoann Petibon*** [2], ***Dr. Cristian Salinas*** [2], ***Dr. Gaia Rizzo*** [3], ***Dr. Yvonne Lewis*** [3], ***Dr. Paul Wilkens*** [2], ***Mr. Julius Labao*** [4], ***Dr. Alexander Whittington*** [3], ***Dr. Leon Fonville*** [3], ***Dr. Frans Van Den Berg*** [3], ***Dr. Ozlem Yardibi*** [2], ***Prof. Paola Giunti*** [5], ***Dr. Caterina Rua*** [3], ***Dr. James Davies*** [3], ***Dr. Adam Schwarz*** [2], ***Dr. Ilan Rabiner*** [3]

1. *Imperial College London,* ***2.*** *Takeda Pharmaceuticals, Cambridge MA,* ***3.*** *Invicro, London,* ***4.*** *Imperial College Healthcare Trust,* ***5.*** *University College London*

Background: Friedreich's ataxia (FA) is mainly due to a guanine-adenine-adenine (GAA) trinucleotide repeat expansion in intron 1, leading to neurodegeneration and premature death from cardiac dysfunction. Loss of the frataxin protein affects mitochondrial function including mitochondrial complex 1 (MC1) activity. Novel biomarkers evaluated by non-invasive techniques are needed to monitor disease progression and treatment effects in people with FA.

Methods: Positron emission tomography (PET) with [^{18}F]BCPP-EF, a ligand with high binding specificity for MC1, was used to measure cardiac and brain MC1 density in 10 participants with FA and 10 age-matched controls. Patients had clinical assessment (SARA, SCAFI motion-capture analysis) at 2 visits separated by 1 year.

Results: PET scanning followed a previously developed imaging protocol, consisting of a 70-minute brain scan immediately post-[^{18}F]BCPP-EF administration followed by a 30-minute cardiac scan 255 minutes post-[^{18}F]BCPP-EF administration. Cardiac [^{18}F]BCPP-EF binding was significantly lower in participants with FA compared with healthy volunteers. Brain uptake showed a correlation with age (in both HV and FA). FA subjects exhibited lower DVR than HV in nearly all surveyed brain regions. When adjusted for age using ANCOVA, significant cross-sectional differences were observed in several brain regions, including thalamus, brain stem, and frontal cortex.

Discussion: An objective *in vivo* biomarker for FA progression will have a significant impact in assessing novel therapies for FA.

Conclusions: MC1 density in the heart and brain, as measured using [^{18}F]BCPP-EF PET, is a promising biomarker of mitochondrial deficit in people with FA. An ongoing 1-year longitudinal study in patients cross-correlating with SARA/SCAFI, FXN levels and motion capture analysis is underway and the initial results will be presented.

Plenary session: Cerebellar Neurodevelopment and Cognitive Disorders

Human cerebellar development: from cells to disease

Thursday, 14th November - 09:00: (Trinity & Goodmans Suite) - Invited Speaker

Kimberly Aldinger [1]

1. *Seattle Children's Research Institute*

The cerebellum undergoes an extended developmental process, resulting in a finely structured brain region that houses more than half of the neurons in the entire brain, densely packed within a highly organized framework. At birth, the human cerebellum is only a quarter of its adult size, yet it already contains the blueprint essential for the development of motor, cognitive, and emotional skills that continue to unfold into adulthood. Although the mature neuroanatomy of the cerebellum is well understood, our knowledge of its developmental origins and relationship to disease is still limited. To bridge this critical knowledge gap, we profiled the molecular, cellular, and spatial composition of the human cerebellum during early and mid-gestation to provide its first developmental characterization. These emerging profiles offer invaluable resources for investigating the molecular basis of cerebellar development, validating ex vivo models, and comparing them to diseased tissues.

Cognitive impairment in SCA3: a multi-center cohort study with demographic and biomarker correlates

Thursday, 14th November - 09:30: (Trinity & Goodmans Suite) - Oral Presentation

Dr. Roderick Maas [1], ***Dr. Jennifer Faber*** [2], ***Prof. Paola Giunti*** [3], ***Prof. Ludger Schöls*** [4], ***Dr. Jeroen de Vries*** [5], ***Dr. Khalaf Bushara*** [6], ***Prof. Kathrin Reetz*** [7], ***Dr. Chiadi Onyike*** [8], ***Dr. Heike Jacobi*** [9], ***Prof. Dagmar Timmann*** [10], ***Prof. Jon Infante*** [11], ***Dr. Magda Santana*** [12], ***Dr. Jeannette Hübener-Schmid*** [13], ***Prof. Luis Pereira de Almeida*** [14], ***Prof. Manuela Lima*** [15], ***Prof. Thomas Klockgether*** [16], ***Prof. Bart van de Warrenburg*** [17]

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Objective: To evaluate cognitive impairment in a large international cohort of ataxic and pre-ataxic SCA3 mutation carriers and explore associations with posterior cerebellar lobule volumes, basal ganglia volumes, and plasma neurofilament light chain (NfL) concentration.

Methods: In a prospective, multinational cohort study involving 11 European and 2 US sites, the Montreal Cognitive Assessment (MoCA) was used as a screening tool for cognitive impairment. Brain MRI scans were acquired in part of the participants. Cerebellar subsegmentation was performed using CerebNet, while basal ganglia volumes were obtained using FreeSurfer software. All volumes were divided by estimated total intracranial volume (eTIV) in order to yield relative values. Clinical and MRI characteristics and plasma NfL concentrations were compared between SCA3 patients with a MoCA score ≥ 26 and those with a score ≤ 25.

Results: Baseline MoCA data were collected from 61 pre-ataxic and 231 ataxic SCA3 mutation carriers, as well as from 111 healthy controls. MRI and plasma NfL data were available from 72/231 (31.2%) and 149/231 patients (64.5%), respectively. After correction for educational level and age, there were significant differences in MoCA total score and visuospatial/executive, attention, language, and abstraction subscores between healthy controls and ataxic, but not pre-ataxic individuals. SCA3 patients with a MoCA score ≤ 25 (n = 80) had a significantly lower educational level, higher SARA score, lower pallidum volume, and higher plasma NfL concentration than those with a score ≥ 26 (n = 141). There were no significant differences in age, repeat length, and volumes of posterior cerebellar lobules between both groups.

Conclusion: The MoCA differentiates ataxic but not pre-ataxic SCA3 mutation carriers from healthy controls. Our findings underscore the important role of education in cognitive assessments, but also seem to suggest that the basal ganglia are involved in cognitive impairment in SCA3.

Cognitive performance and its correlates in spinocerebellar ataxia types 1, 2, 3, and 6

Thursday, 14th November - 09:45: (Trinity & Goodmans Suite) - Oral Presentation

Dr. Louisa P. Selvadurai [1], ***Ms. Sarah Wallis*** [1], ***Ms. Chiara Lo Giudice*** [1], ***Ms. Tabitha Krishnan*** [1], ***Ms. Yasmin Yassin*** [1], ***Mr. James Morgan*** [1], ***Ms. Sheryl Gullia*** [1], ***Prof. Adam P. Vogel*** [2], ***Dr. Kishore R. Kumar*** [3], ***Dr. David J. Szmulewicz*** [4], ***Dr. Ian Harding*** [5]

1. *Monash University, Melbourne,* ***2.*** *The University of Melbourne and Redenlab Inc.,* ***3.*** *Garvan Institute of Medical Research, Sydney and UNSW Sydney; Concord Repatriation General Hospital and University of Sydney,* ***4.*** *Balance Disorders & Ataxia Service, Royal Victorian Eye and Ear Hospital,* ***5.*** *Monash University, Melbourne and QIMR Berghofer Medical Research Institute, Brisbane*

Background and objectives: There is growing evidence of cognitive deficits in spinocerebellar ataxias, with the Cerebellar Cognitive Affective Syndrome Scale (CCAS-S) an increasingly common measure of this dysfunction. There remain ongoing questions as to how scale performance relates to day-to-day cognitive function and other features of SCAs. We aimed to evaluate CCAS-S performance amongst individuals with SCA1, SCA2, SCA3, and SCA6 relative to matched controls, and to investigate relationships between performance and demographic factors, ataxia severity, psychomotor performance, and non-motor functions.

Methods: Using data collected remotely via an online and teleconference-based approach, we evaluated performance on the CCAS-S amongst individuals with SCA1 (n=14), SCA2 (n=16), SCA3 (n=18) and SCA6 (n=26) relative to control groups matched for age, sex, and education level. We examined associations between CCAS-S performance and a) age and education, b) measures of ataxia symptom severity, c) performance on computerised finger-tapping and reaction time tasks d) self-rated cognition, depression, emotional regulation, psychosocial function, and fatigue.

Results: CCAS-S performance was significantly reduced in SCA2, SCA3, and SCA6 compared to controls. Better CCAS-S performance was associated with more years of education and lower ataxia symptom severity. Better CCAS-S performance was also associated with both average psychomotor task speed (motor tapping speed, simple reaction time, decision reaction time) and variability in psychomotor performance across task trials (motor tapping speed, motor timing, decision time). Finally, CCAS-S performance was significantly associated with self-reported day-to-day cognitive function.

Discussion and conclusion: The CCAS-S is a useful cognitive measure in SCAs that maps on to self-reported cognitive function as well as motor function. This study motivates further investigation into the manifestation of cognitive dysfunction in these conditions and the role ataxia severity motor symptoms and psychomotor variability play in the development of these deficits.
Funding contribution by the National Ataxia Foundation.

Cerebellar contribution to cognitive deficits and prefrontal cortex dysfunction in SCA1

Thursday, 14th November - 10:00: (Trinity & Goodmans Suite) - Oral Presentation

Mrs. Kaelin Sbrocco [1], Mrs. Ella Borgenheimer [2], Prof. Harry Orr [1], Dr. Marija Cvetanovic [1]

1. *University of Minnesota,* ***2.*** *Baylor University*

Background and objectives:

SCA1 patients suffer from motor and cognitive deficits, especially in executive function. While dysfunction of cerebellar Purkinje cells (PCs) is thought to underly motor deficits, etiology of SCA1 cognitive deficits is unknown. Originally known for its role in movement, cerebellum's role in cognition is increasingly recognized. We found that transgenic *ATXN1[82Q]* mice expressing mutant ATXN1(mATXN1) only in PCs, exhibit cognitive deficits, indicating that cerebellar dysfunction can contribute to SCA1 cognitive deficits. Yet how cerebellum impacts cognition remains unclear.

Executive function is associated with prefrontal cortex (PFC) function. We investigated how mATXN1 expression in PCs impacts PFC and whether mATXN1 expression in PCs contributes to cognitive deficits and PFC dysfunction in SCA1.

Methods:

Immunohistochemistry and confocal imaging were used to quantify numbers (NeuN+), and neuronal activity (c-Fos+), and synapses (excitatory (VGLUT2/PSD95) and inhibitory (VGAT/Gephryn)). Conditional SCA1 knock-in model, *f-ATXN1*^{146Q} and *Pcp2-Cre* mice were used to delete mutant ATXN1 only in PCs. RNA sequencing was used to evaluate transcriptional changes. Fear conditioning, Barnes maze, and pairwise discrimination were used to evaluate cognition.

Results:

We have found no neuronal loss, increase in the cFOS+ neurons and a decrease in the number of inhibitory synapses in PFC of *ATXN1[82Q]* mice. We also identified 341 differentially expressed genes (DEGs) impacting sugar transport and IL17 signaling in the PFC of *ATXN1[82Q]* mice.

We found 1274 DEG in PFC of *f-ATXN1*^{146Q} mice impacting multiple signaling pathways. Surprisingly, numbers of DEGs and impacted pathways, decrease in inhibitory synapses, and cognitive performance were exacerbated in *f-ATXN1*^{146Q}*;Pcp2-Cre* mice.

Discussion and conclusion:

Our results indicate that selective expression of mATXN1 in PCs is sufficient to cause PFC dysfunction.

Surprisingly, selectively deleting mATXN1 in PCs worsens PFC dysfunction and cognitive performance in SCA1 knock-in mice. These results suggest a complex, important cerebellum-PFC interaction in SCA1.

Cognition in spinocerebellar degenerations measured by CCAS scale

Thursday, 14th November - 10:15: (Trinity & Goodmans Suite) - Oral Presentation

Dr. Daniel Lopez Dominguez [1], Dr. Cecilia Marelli [2], Mr. Emilien Petit [3], Mrs. Sabrina Sayah [1], Prof. Stefan Pulst [4], Dr. Thomas Klockgether [5], Dr. Gulin Oz [6], Dr. Henry Paulson [7], Dr. Tetsuo Ashizawa [8], Dr. Sophie Tezenas du Montcel [9], Prof. Alexandra Durr [10], Dr. Giulia Coarelli [10]

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Background and objectives: Cerebellar Cognitive-Affective Syndrome (CCAS) arises from cerebellar injury. However, its prevalence and severity in spinocerebellar degenerations have not been extensively investigated. In this study, we aimed to compare cognitive impairment among genotypes and explore its longitudinal progression along with motor worsening.

Methods: We enrolled 328 participants from READISCA and SPATAX networks including 66 SCA1, 28 SCA2, 158 SCA3, 24 SCA7, 35 SPG7, and 17 SCA27B individuals. Cognitive function was assessed using the CCAS scale, and ataxia severity by the SARA scale and CCFS. We correlated CCAS at baseline with disease severity, brain MRI findings, and plasma neurofilament light chain (NfL) levels. In polyQ carriers, we explored CCAS longitudinal progression over one year. Subtests comparisons among genotypes were adjusted for age, education level, and SARA scores.

Results: Definitive CCAS (failure in three or more subtests of the CCAS scale) was observed at baseline in 88% SCA27B, 68% of SPG7 patients followed by 57% SCA2, 41% SCA7, 33%SCA1, and 31% of SCA3 patients. None of the 89 polyQ preataxic carriers had cognitive impairment. Adjusted subtests comparisons revealed different impairments across groups: SPG7 showed lower scores in executive function and affect subtest but performed better in visuospatial test. CCAS score correlated with SARA scores ($p<0.001$) and plasma NfL levels ($p=0.008$). The affect subtest correlated with cerebellar volume ($p=0.001$). Longitudinal follow-up showed improvement in CCAS score after one year, especially for verbal recall and phonemic fluency ($p=0.021$ and $p=0.020$, respectively).

Discussion and conclusion: We observed a high frequency of definitive CCAS in ataxic patients, especially for SCA27B and SPG7. Improvement over one year suggests a learning effect, potentially limiting the scale's effectiveness for longitudinal assessment. Interestingly, the significant correlations observed between CCAS and clinical scores, NfL levels, and cerebellum volume indicate that cognitive dysfunction is integral to the neurodegenerative process.

Parallel session: Disease Mechanism II

A PRKN missense polymorphism modifies the age at onset in Spinocerebellar Ataxia Type 3 (SCA3) and impacts protein-protein interaction as well as mitophagy

Thursday, 14th November - 11:00: (Trinity & Goodmans Suite) - Oral Presentation

Dr. Jonasz Jeremiasz Weber [1], Ms. Leah Czisch [2], Ms. Priscila Pereira Sena [2], Ms. Natasa Schwarz [2], Ms. Rana Dilara Incebacak Eltemur [1], Mr. Florian Fath [2], Ms. Chrisovalantou Huridou [1], Ms. Anna Würth [2], Dr. Daniel Weishäupl [2], Ms. Miriam Döcker [2], Dr. Sandra Martins [3], Prof. Jorge Sequeiros [4], Prof. Guy A. Rouleau [5], Prof. Laura Bannach Jardim [6], Prof. Maria Luiza Saraiva-Pereira [6], Prof. Marcondes França [7], Prof. Carlos Gordon [8], Prof. Mario Cornejo-Olivas [9], Prof. Bart van de Warrenburg [10], Prof. Alexandra Durr [11], Prof. Alexis Brice [12], Prof. Peter Bauer [13], Prof. Thomas Klockgether [14], Prof. Ludger Schöls [15], Prof. Olaf Rieß [2], Dr. Thorsten Schmidt [2]

1. *Institute of Medical Genetics and Applied Genomics, Eberhard Karls University, Tübingen & Department of Human Genetics, Ruhr University, Bochum,* ***2.*** *Institute of Medical Genetics and Applied Genomics, Eberhard Karls University, Tübingen,* ***3.*** *i3S - Instituto de Investigação e Inovação em Saúde & IPATIMUP - Institute of Molecular Pathology and Immunology, University of Porto,* ***4.*** *i3S-Instituto de Investigação e Inovação em Saúde; ICBAS-Instituto Ciências Biomédicas Abel Salazar; CGPP-Centro de Genética Preditiva e Preventiva; IBMC-Institute for Molecular and Cell Biology,* ***5.*** *Department of Neurology and Neurosurgery & The Neuro (Montreal Neurological Institute□Hospital), McGill University, Montréal,* ***6.*** *Universidade Federal do Rio Grande do Sul,* ***7.*** *Department of Neurology, University of Campinas, Campinas, Sao Paulo,* ***8.*** *Department of Neurology, Tel Aviv University, Tel Aviv,* ***9.*** *Neurogenetics Working Group, Universidad Cientifica del Sur, Lima, Peru,* ***10.*** *Radboud university medical center,* ***11.*** *Sorbonne University, Paris Brain Institute, Pitié-Salpêtrière Hospital,* ***12.*** *Sorbonne Université, Institut du Cerveau - Paris Brain Institute- ICM, Inserm, CNRS, APHP, University Hospital Pitié-Salpêtrière, Paris, France,* ***13.*** *Centogene GmbH, Rostock,* ***14.*** *German Center for Neurodegnerative Diseases (DZNE),* ***15.*** *Department of Neurology, University of Tübingen*

Background and objectives: Spinocerebellar ataxia type 3 (SCA3), also known as Machado-Joseph disease (MJD), is the most common autosomal dominant spinocerebellar ataxia worldwide and caused by a CAG repeat expansion in the *ATXN3* gene resulting in a polyglutamine expansion in the encoded Ataxin-3 protein. Statistically, a correlation between the number of CAG repeats and the age at onset of SCA3 patients exists: Patients with more CAG repeats have an earlier onset of symptoms. However, this statistical correlation is not perfect and the number of CAG repeats contributes only about 50% to the age at onset. Therefore, the remaining 50% are influenced by other factors, which we aim to identify in this study.

Methods: We analyzed missense single nucleotide polymorphism in the *PRKN* gene encoding the Parkinson's disease-associated protein Parkin. Parkin is an interaction partner of ataxin-3. While Parkin is a ubiquitin ligase, Ataxin-3 is a deubiquitinase. In a combined European, Canadian, and South American approach, we genotyped more than 900 SCA3 patients, to-date the largest cohort, for polymorphisms within *PRKN*.

Results: We identified the V380L variant within Parkin as genetic modifier of SCA3, decreasing the age at onset by three years in homozygous carriers. Subsequent functional analysis in SCA3 cell models demonstrated the functional relevance of the identified SNP for the pathophysiology of the disease: The Parkin V380L variant impacts the protein-protein interaction of Parkin and Ataxin-3. Moreover, Parkin V380L interfered with the execution of mitophagy, the autophagic removal of surplus or damaged mitochondria, thereby compromising cell viability.

Discussion and Conclusion: We identified the V380L variant in Parkin as a genetic modifier of SCA3, with negative repercussions on its molecular pathogenesis and the age at onset. Our results will improve the prediction of clinical

symptoms and contribute to the understanding of pathogenic processes in SCA3.

Purkinje-Enriched snRNA-seq in SCA7 Cerebellum Reveals Zebrin-II Stripe Loss as a Shared Feature of Polyglutamine Ataxias

Thursday, 14th November - 11:15: (Trinity & Goodmans Suite) - Oral Presentation

Prof. Albert La Spada [1]

1. *University of California Irvine*

Background: Spinocerebellar ataxia type 7 (SCA7) is an inherited neurodegenerative disorder caused by a CAG-polyglutamine repeat expansion. To determine the relative contribution of cerebellar cell types to SCA7 pathogenesis, we performed single-nucleus RNA-seq on SCA7 266Q knock-in mice.

Methods: Although single-nucleus sequencing offers the ability to isolate transcriptional signals of individual cell types in complex tissues, it is difficult to study Purkinje cell (PC) neurons, because they are a rare cell type. To overcome this, we developed a simple, robust PC nuclear enrichment protocol, which increases the relative proportion of PCs from 1% to >40% when applied to the adult mouse cerebellum.

Results: Our snRNA-seq analysis of SCA7 266Q knock-in mouse cerebellum revealed significant expression alterations involving synapse organization genes. We verified and further investigated this signal by directly examining synapse distribution and electrical circuit function in SCA7 mice. In addition, we detected a striking reduction of SCA7 PC zebrin-II subtype specification at the level of gene expression, and upon anatomical analysis, we discovered an ablation of zebrin-II parasagittal striping. To understand if this loss of cell subtype specification is specific to SCA7, or has the potential to be a unifying disease-causing mechanism, we evaluated zebrin-II parasagittal striping patterns in related polyglutamine SCAs. We discovered that this reduction of PC zebrin-II striping extends to other polyglutamine disease mouse models, showing that breakdown of zebrin-II subtypes is a shared feature of polyglutamine ataxias. To assess the relevance of these findings to human patients, we obtained post-mortem cerebellum from SCA7 patients and controls, and documented reduced expression of zebrin-II subtype genes. We also uncovered evidence for disrupted subtype regulation in Molecular Layer Interneurons in SCA7 patients, thereby extending this finding to another important cerebellar cell type.

Conclusions: Our findings reveal an unexpected role for altered developmental cell-type specification in polyglutamine cerebellar degeneration.

Repeat associated non-AUG translation as a common mechanism for the polyGln ataxias

Thursday, 14th November - 11:30: (Trinity & Goodmans Suite) - Oral Presentation

Dr. Monica Banez Coronel [1], ***Dr. Tao Zu*** [2], ***Ms. Madeline Denton*** [1], ***Dr. Shu Guo*** [1], ***Mr. Ramadan Ajredini*** [1], ***Ms. Deborah Morrison*** [1], ***Lisa Duvick*** [3], ***Mrs. Alexis Tays*** [1], ***Dr. Olga Pletnikova*** [4], ***Dr. Tony Yachnis*** [5], ***Dr. Juan Troncoso*** [6], ***Prof. Henry Paulson*** [7], ***Dr. Hayley McLoughlin*** [7], ***Prof. Harry Orr*** [3], ***Dr. Tetsuo Ashizawa*** [8], ***Prof. S. H. Subramony*** [9], ***Dr. Laura Ranum*** [10]

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[Background] Repeat associated non-AUG (RAN) proteins have been reported in eleven repeat expansion disorders. In *C9orf72*-ALS/FTD, RAN proteins have been shown to be important drivers of disease. In spinocerebellar ataxia type 8 and Huntington disease, which are both caused by CAG·CTG repeat expansions, RAN protein aggregates increase with disease severity. Determining if RAN protein pathology occurs across the CAG•CTG polyglutamine spinocerebellar ataxias (CAG-SCAs) is critical for understanding the mechanisms of these diseases.

[Methods] To examine sense and antisense RAN protein accumulation across the CAG-SCAs, we developed novel α-polySer, α-polyLeu and locus-specific antibodies against unique C-terminal regions of each polySer and polyLeu RAN protein expressed from SCA1, SCA2, SCA3, SCA6 or SCA7 expansions mutations. Using these tools, we performed immunostaining in SCA1,2,3,6 & 7 human postmortem tissue and SCA1 and SCA3 mouse brains. RAN proteotoxicity was determined using alternative-codon minigenes to individually express each locus specific polySer and polyLeu RAN protein.

[Results] Sense polySer and antisense polyLeu RAN protein aggregates show robust accumulation in SCA1, SCA2, SCA3, SCA6 and SCA7 throughout the most affected cerebellum and brainstem regions. In the less affected frontal cortex, RAN protein aggregates are rare. In cell culture experiments: polySer and polyLeu RAN proteins are toxic to neural cells; RAN protein aggregates are ubiquitin-positive; cause autophagic dysfunction; and decreasing RAN proteins levels with metformin reduces cytotoxicity.

In SCA3-YAC-84Q mice, cerebellar and pontine RAN protein aggregates increase with age. Pcp2-ATXN1^{82Q} SCA1 mice designed to express polyGln-expanded ATXN1 in Purkinje cells, also show prominent RAN protein aggregates throughout the cerebellum. Mice with disrupted ATXN1^{82Q}:capicua binding show both improved phenotypes and markedly reduced RAN protein aggregates.

[Discussion/Conclusion] These data support a pathogenic role of RAN proteins across the CAG-SCAs, and highlight the need to understand their role in these diseases and evaluate therapeutic strategies targeting both sense and antisense transcripts.

Identification of genetic modifiers of somatic GAA repeat instability in Friedreich's ataxia by in vivo CRISPR-Cas9 genome editing

Thursday, 14th November - 11:45: (Trinity & Goodmans Suite) - Oral Presentation

Mr. Maheswaran Kesavan [1], ***Dr. Antonia G Vitalo*** [2], ***Ms. Anh Nhu*** [2], ***Mr. Muzhou Wu*** [2], ***Mr. Neil Doherty*** [1], ***Dr. Ali Shahryari*** [1], ***Dr. Shota Shibata*** [2], ***Dr. Ricardo Mouro Pinto*** [1]

1. *Center for Genomic Medicine, Massachusetts General Hospital, Boston, USA.*, ***2.*** *Center for Genomic Medicine, Massachusetts General Hospital, Boston, USA*

Background and objectives: Friedreich ataxia (FRDA) is primarily caused by a GAA expansion in the frataxin gene (*FXN*), which leads to transcriptional silencing and low levels of FXN. Intergenerational and somatic instability (SI) of the GAA repeat tract has been previously documented in FRDA patients and recapitulated in cellular and animal models. SI has recently been implicated as a strong driver of disease onset and progression in Huntington's disease. As in other repeat expansion disorders, the role of mismatch repair (MMR) genes has been previously implicated in GAA repeat instability. However, a complete understanding of the contribution of individual MMR genes is still lacking. We will present a systematic and complete characterization of the role of MMR genes on GAA SI in a FRDA mouse model.

Methods and Results: First, we developed a novel method to quantify SI of *FXN* GAAs which we used to characterize its age- and tissue-specificity in YG8s.300 mice. We observe a remarkable degree of expansion-prone SI by 24weeks in multiple CNS tissues and liver. Second, we established a novel *in vivo* CRISPR/Cas9-based system for identifying *FXN* GAA SI modifier genes. This consists of systemic delivery of AAVs expressing sgRNAs targeting genes of interest in Cas9-expressing YG8s.300/800 mice. Taking advantage of efficient AAV8 and PhP.eB delivery to liver and brain, respectively, we confirmed involvement of previously described MMR genes. We also discovered additional MMR genes as novel genetic modifiers of GAA SI (both suppressors and promoters of expansions) in neuronal and peripheral tissues.

Conclusion: The YG8s.300 mouse model exhibits expansion-prone age- and tissue-specific GAA SI. We used this model to obtain a comprehensive understanding of how the MMR machinery is involved in this mechanism, which is critical for rational therapeutic development for FRDA targeting somatic GAA expansions.

Funding sources: NIH; Pfizer Inc.; NAF.

Development of a cellular ataxin-3 protein-protein interaction assay for high-throughput screening of PPI modifiers

Thursday, 14th November - 12:00: (Trinity & Goodmans Suite) - Flash Talk

Ms. Ana Rita Fernandes [1], Ms. Liliana Meireles-Costa [2], Dr. Bruno Almeida [1], Dr. Fernando Rodrigues [1], Dr. Andreia Teixeira-Castro [1], Dr. Sara Duarte-Silva [1], Dr. Stephanie Cabantous [3], Prof. Patrícia Maciel [1]

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Machado-Joseph disease (MJD) is a neurodegenerative disorder caused by expansion of a polyglutamine tract within the protein ataxin-3 (ATXN3). Evidence supports the nuclear presence of ATXN3 as a key event in pathogenesis. However, the mechanisms through which it promotes toxicity in the neuronal cell nucleus remain mostly unknown. We hypothesized that mutant ATXN3 interacts abnormally with different proteins in the nucleus, and that those interactions contribute to neuronal toxicity. This reinforces the interest in studying relevant protein-protein interactions (PPIs) of the wild-type and mutant ATXN3.

Therefore, we developed a new cellular platform to detect nuclear ATXN3 PPIs, based on the previously described tripartite split-GFP (triSFP) system. In this system, the proteins of interest are fused to GFP10 and GFP11 subunits, and upon interaction, those tags get tethered and spontaneously assemble with the co-expressed GFP1-9 subunit, resulting in full GFP reconstitution and fluorescence emission.

Having generated and transfected the relevant constructs and validated the triSFP system in a human cell line, we confirmed the interaction of ATXN3 with the glucocorticoid receptor (GR) in the nucleus and cytoplasm. Upon treatment with TUDCA, a therapeutic agent with beneficial effect in animal models of MJD, this interaction shifted to the nucleus, preventing UPS-mediated degradation of GR in the cytoplasm, that is accelerated in these models. Using the triSFP system, we also validated the interaction of ATXN3 with the SRSF7, a key regulator of MAPT (tau) exon 10 splicing.

In summary, this novel system constitutes a valuable tool to detect ATXN3 PPIs, study their subcellular localization at the basal level and in response to different stimuli, and can be used to identify novel therapeutic targets and promising modulators of disease-relevant interactions, that might constitute lead molecules for MJD therapy.

Dysregulated Lipid Profiles in Cerebellar Tissues of SCA3 Mice and Human Patients

Thursday, 14th November - 12:07: (Trinity & Goodmans Suite) - Flash Talk

Ms. Alexa Putka [1], ***Dr. Varshasnata Mohanty*** [2], ***Mr. Vikram Sundararajan*** [1], ***Dr. Stephanie Cologna*** [2], ***Dr. Hayley McLoughlin*** [1]

1. *University of Michigan,* ***2.*** *University of Illinois Chicago*

Background and Objective: While RNA-sequencing and proteomic studies abound in the neurodegenerative disease literature, one class of macromolecules remains understudied: lipids. Our lab studies Spinocerebellar ataxia type 3 (SCA3), the most common dominantly inherited ataxia. We recently identified cholesterol/sterol biosynthetic processes as the top gene ontology biological pathways dysregulated in SCA3 transgenic mice compared to WT controls. Whether transcriptional dysregulation translated to altered lipid levels, however, remained unknown. Here, we investigated dysregulated lipid profiles in SCA3 disease.

Methods: Cerebella from post-mortem SCA3 patients and healthy controls were obtained from the Michigan Brain Bank. Cerebella from YACQ84 transgenic and *Atxn3*-Knockout mice were collected at 16 weeks of age. Lipids were isolated from all samples using the Folch extraction method and quantified by positive and negative ion mode liquid chromatography-mass spectrometry. Lipid identifications were obtained via Lipid Annotator Software and differential analysis was carried out. Lipids with a fold-change < -1.5 or > 1.5 and a p-value < 0.05 (unpaired Student's two-tailed t-test) were considered differentially expressed.

Results: SCA3 patients and YACQ84 transgenic mice share reduced cerebellar lipid content, with a majority of differentially expressed lipids downregulated. We identified seven lipids reduced in both SCA3 patients and YACQ84 mice, representing potential lipid biomarkers of disease. In contrast, *Atxn3*-Knockout mice displayed increased cerebellar lipid content, with most differentially expressed lipids upregulated. No lipid changes were uniformly observed across SCA3 patients, YACQ84 mice, and *Atxn3*-Knockouts, suggesting ATXN3 loss-of-function is likely not the primary driver of lipidomic alterations in SCA3.

Conclusion: We uncovered broad lipid dysregulation in SCA3 patients and mice likely due to mutant ATXN3 toxic gain-of-function. This work establishes lipid perturbations as an uncharacterized feature of SCA3. We are particularly interested in determining how lipid alterations affect disease pathogenesis and how these lipids change with mutant ATXN3 silencing therapeutic approaches.

What is required for GAA repeat expansion at the endogenous Friedreich's ataxia locus?

Thursday, 14th November - 12:14: (Trinity & Goodmans Suite) - Flash Talk

Dr. Jixue Li [1], Dr. Yanjie Li [1], Dr. Hongjun Wang [2], Dr. Gabriel Matos-Rodrigues [3], Dr. Andre Nussenzweig [3], Dr. Karen Usdin [4], Dr. Jill Napierala [5], Dr. Marek Napierala [5]

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Introduction: Instability expressed as the propensity to expand or contract is a major feature of long, pathogenic tandem repeat sequences. Expansion of these sequences is a cause of more than 50 human diseases, including Friedreich's ataxia (FRDA). Large, biallelic expansion of intronic GAA repeats results in decreased transcription of the frataxin (*FXN*) gene leading to deficiency of frataxin protein and progressive multi-organ deterioration. While length of the shorter of the two expanded GAA tracts (GAA1) corelates well with disease onset and progression, the high degree of variability observed between FRDA patients cannot be solely explained by the size of GAAs established at diagnosis. We postulate that tissue-specific somatic instability affects clinical presentation.

Methods: Using CRISPR-Cas9, we generated several FRDA induced pluripotent stem cell (iPSC) lines to determine the role of active transcription, proximity of DNA replication origins, level of frataxin as well as length, chromosomal context and repeat orientation on the propensity of GAA sequences to expand. GAA repeat expansion assays were utilized to monitor size changes over an extended period of time.

Results: Transcription, tract length and orientation of the repeats relative to transcription direction are the major factors affecting GAA repeat expansion.

Conclusions: Considering potential long-term treatment of FRDA patients, the effect of therapy, especially those affecting processes occurring at the endogenous *FXN* gene, on GAA expansion should be incorporated in clinical trial design and patient monitoring.

Nano narratives: unraveling spinocerebellar ataxia type 2 pathogenesis through exosomes

Thursday, 14th November - 12:21: (Trinity & Goodmans Suite) - Flash Talk

Mr. Rafael Costa [1], ***Prof. Carlos Matos*** [2], ***Prof. Tiago Outeiro*** [3], ***Prof. Clévio Nóbrega*** [4]

1. *Algarve Biomedical Center Research Institute, University of Algarve, Faro, Portugal,* ***2.*** *Universidade do algarve, UAlg; Algarve Biomedical Center Research Institute, University of Algarve, Faro, Portugal,* ***3.*** *Department of Experimental Neurodegeneration, Göttingen, Germany,* ***4.*** *Algarve Biomedical Center – Research Institute*

Objectives: The main goal of this study is to understand and characterize the role of exosomes in spinocerebellar ataxia type 2 (SCA2) pathogenesis. Specifically, we aim to elucidate whether *ATXN2* mRNA, deriving from the causative gene for SCA2, is present in exosomes and if they can be internalized by recipient cells, promote ATXN2 translation, and induce aggregation akin to SCA2 neuropathology.

Methods: Exosomes were obtained from distinct cellular and animal models of disease and characterized based on their content, size, and morphology using molecular techniques and various microscopy-based approaches. Exosome internalization was assessed using fluorescence microscopy and flow cytometry. Aggregation of ATXN2 was analyzed *in vitro* and *in vivo* using fluorescence microscopy.

Results: Our study reveals the presence of *ATXN2* mRNA in exosomes. Furthermore, we demonstrate that *ATXN2*-containing exosomes can be internalized by recipient cells, leading to ATXN2 translation and aggregation, mimicking some features of SCA2 neuropathology.

Conclusions: These findings suggest exosome-mediated spreading of mutant *ATXN2* mRNA as a contributing mechanism to SCA2 pathogenesis. Further research into the molecular mechanisms involved in this process is needed, as it could lead to the identification of novel therapeutic targets to delay SCA2 progression.

Parallel session: Emerging and Existing Therapeutics - Pre-Clinical Research

CRISPR-Cas9-mediated ATXN3 gene inactivation as a potential therapeutic approach for Machado-Joseph disease

Thursday, 14th November - 14:00: (Trinity & Goodmans Suite) - Oral Presentation

Dr. Sara Lopes [1], ***Mr. Miguel Lopes*** [1], ***Dr. Rui Jorge Nobre*** [2], ***Dr. Magda Santana*** [1], ***Dr. Ana Vasconcelos-Ferreira*** [3], ***Dr. Dina Pereira*** [1], ***Ms. Ana Rita Fernandes*** [1], ***Ms. Inês Barros*** [1], ***Prof. Clévio Nóbrega*** [4], ***Prof. Carlos Matos*** [4], ***Prof. Neville Sanjana*** [5], ***Prof. Patrick Hsu*** [5], ***Dr. F Ann Ran*** [5], ***Dr. Lukasz Swiech*** [5], ***Dr. Le Cong*** [5], ***Prof. Feng Zhang*** [5], ***Prof. Luís Pereira de Almeida*** [6]

1. *CNC-UC, Univ.Coimbra; CIBB, Univ.Coimbra; GeneT, Gene Therapy Center of Excellence, Coimbra; Institute for Interdisciplinary Research, Univ.Coimbra,* ***2.*** *CNC-UC, Univ.Coimbra; CIBB, Univ.Coimbra; GeneT, Gene Therapy Center of Excellence, Coimbra; Institute for Interdisciplinary Research, Univ.Coimbra; ViraVector, Univ.Coimbra,* ***3.*** *CNC-UC, Univ.Coimbra; CIBB, Univ.Coimbra; Faculty of Pharmacy, Univ.Coimbra,* ***4.*** *CNC-UC, Univ.Coimbra; CBMR, Univ.Algarve,* ***5.*** *Broad Institute of MIT and Harvard,* ***6.*** *CNC-UC, Univ.Coimbra; CIBB, Univ.Coimbra; GeneT, Gene Therapy Center of Excellence, Coimbra; ViraVector, Univ.Coimbra; Faculty of Pharmacy, Univ.Coimbra*

Introduction: Machado-Joseph disease (MJD) is an autosomal dominantly-inherited neurodegenerative disorder, caused by an over-repetition of the polyglutamine-codifying region in the ataxin-3 (*ATXN3*) gene. Expanded ATXN3 is prone to aggregate, ultimately leading to cell dysfunction and death. Strategies based on the suppression of the deleterious gene products have demonstrated promising results in pre-clinical studies. Nonetheless, these strategies do not target the root cause of the disease, producing incomplete and/or transient therapeutic effects. CRISPR-Cas9 systems have been successfully used to permanently inactivate disease-related genes, holding promise for the development of definitive cures for inherited diseases.

Objectives: In order to prevent the downstream toxic pathways, our goal was to develop a CRISPR-Cas9-based strategy to efficiently inactivate the human *ATXN3* gene.

Methods: A panel of sequences were designed and constructed to target an early exon of the human *ATXN3* gene. Functional characterization was performed in HEK293T cells, through the surveyor mutation-detection assay and the top-ranked sequence was selected for *in vivo* experiments.

Results: Surveyor assay revealed the ability of the designed sequences to bind and edit the target region. Consequently, a significant decrease in protein levels were observed *in vitro*. Adeno-associated viruses (AAV1/2) encoding CRISPR-Cas9 sequences were delivered in the striatum of a lentiviral-based mouse model of MJD by intracranial injection. Here, we observed a drastic reduction of aggregates and the preservation of neuronal function. Additionally, upon cerebellar injection of YAC-MJD84.2/84.2 transgenic mice, the CRISPR-Cas9 system efficiently reduced the nuclear accumulation of mutant ATXN3 in deep cerebellar nuclei. Importantly, neonatal administration of the engineered system in YAC-MJD84.2/84.2 mice mediated a delay in disease progression, when compared with non-treated littermates.

Discussion/Conclusion: This work provides evidence of the efficacy of a CRISPR-Cas9-based approach to permanently inactivate the *ATXN3* gene in two mouse models, supporting its potential as a new therapeutic avenue in the context of MJD.

Base editing of pathogenic GAA repeats reduces somatic repeat expansions in Friedreich's ataxia

Thursday, 14th November - 14:15: (Trinity & Goodmans Suite) - Oral Presentation

Dr. Zaneta Matuszek [1], ***Mandana Arbab*** [2], ***Mr. Maheswaran Kesavan*** [3], ***Alvin Hsu*** [4], ***Jennie Roy*** [5], ***Jing Zhao*** [6], ***Tian Yu*** [7], ***Ben Weisburd*** [8], ***Gregory Newby*** [9], ***Mr. Neil Doherty*** [3], ***Ana Christian*** [4], ***Allen Tao*** [4], ***Heidi Rehm*** [10], ***Jun Xie*** [11], ***Guangping Gao*** [12], ***Dr. Ricardo Mouro Pinto*** [13], ***David Liu*** [4]

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Friedreich's ataxia (FRDA), the most prevalent hereditary ataxia in humans, is caused by expansion of GAA repeats at the *FXN* locus. Interruptions in the GAA repeat region of *FXN* intron 1 with GGA and GAG triplets are associated with either the absence of FRDA disease phenotypes or later disease onset and mild phenotypes compared to patients with similar sized uninterrupted GAA allele.

First, to characterize the protective role of interruptions in individuals with pathogenic GAA repeat expansions, we analyzed the *FXN* locus for all genomes of the UK Biobank using ExpansionHunter. We found that 10 of the 31 individuals (32%) with pathogenic *FXN* loci carried two completely pure or near-completely pure GAA repeat tracts, including all four individuals with a G11 hereditary ataxia diagnosis. The remaining 21 individuals had clear evidence of GAG interruptions in one or both *FXN* alleles with expanded GAA repeats. These findings further support the hypothesis that repeat interruptions reduce the penetrance of pathogenic GAA expansions in FRDA.

Next, we explored genome editing approaches to introduce A·T>G·C interruptions at GAA repeats to reduce the repetitiveness of these sequences. We utilized base editing to target pathogenic GAA repeats and reproduce the natural genetic variation of *FXN* alleles associated with reduced disease severity. We identified base editing strategies that efficiently introduce codon interruptions at repeat expansions in vitro and in patient cells, with proclivity for editing longer repeat alleles. AAV9 delivery of optimized base editors in YG8s mouse models of FRDA resulted in efficient repeat interruption in transduced tissues *in vivo*, and significantly reduced somatic expansions in the CNS. These findings establish for the first time that introducing naturally occurring interruptions in pathogenic trinucleotide repeats can mitigate a key disease-associated neurological feature of these repeat tracts in animal models of FRDA.

Allele-specific silencing of mutant ataxin-3 via single administration of AAV9 vectors mitigates neuropathology and motor deficits in Spinocerebellar Ataxia Type 3

Thursday, 14th November - 14:30: (Trinity & Goodmans Suite) - Oral Presentation

Ms. Ana Carolina Silva [1], ***Ms. Carina Henriques*** [2], ***Ms. Diana Lobo*** [3], ***Ms. Ana Rita Fernandes*** [3], ***Mr. Miguel Monteiro Lopes*** [3], ***Mr. Kevin Leandro*** [4], ***Dr. Dina Pereira*** [5], ***Dr. Sónia Duarte*** [5], ***Mr. Rafael Baganha*** [6], ***Dr. Sara Lopes*** [5], ***Dr. Magda Santana*** [5], ***Dr. Rui Jorge Nobre*** [7], ***Prof. Luís Pereira de Almeida*** [8]

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Spinocerebellar ataxia type 3 (SCA3) is an autosomal dominantly inherited neurodegenerative disorder characterized by expansion of CAG trinucleotide repeats located in the *ATXN3* gene. This mutation induces a toxic gain-of-function in the encoded ATXN3 protein, leading to neurodegeneration, particularly in the cerebellum. Despite ongoing investigations, no treatment currently exists to alter disease progression in SCA3 patients.

In this study, we explored the therapeutic potential of adeno-associated virus (AAV9) encoding polycistronic artificial microRNAs (miATXN3) targeting the mutant *ATXN3* allele to induce gene silencing.

First, silencing capacity and selectivity for the mutant allele of different silencing constructs was evaluated in HEK293T cell lines transiently expressing human mutant or wild-type ATXN3. Our *in vitro* results identified a lead construct displaying significant and selective gene silencing targeted for the mutant *ATXN3* allele. Next, AAV9 encoding the lead silencing construct was delivered via intra-cisterna magna (ICM) injection, on post-natal day 1 in severely impaired transgenic SCA3 mice. Significant and robust improvements in motor behavior were observed at 5-, 8-, and 11-weeks post-injection, assessed by rotarod performance, beam-walking testing and catwalk XT analysis. Importantly, histological analysis indicated a reduction in the number of ATXN3 aggregates and a trend toward preventing layer thickness shrinkage within the cerebellar lobules in AAV9 miATXN3-injected mice. These findings were supported by a dose-dependent reduction in mutant ATXN3 mRNA levels and decreased expression of neuroinflammatory markers typically elevated in the cerebellum of SCA3 mice. Notably, a significant increase of neuronal marker NeuN at the protein level was also observed. Finally, widespread detection of AAV genome copies and miATXN3 levels in disease-relevant brain regions was observed 13 weeks post-ICM injection. In summary, our results support allele-specific AAV-based therapy in SCA3, representing a step toward closer clinical translation for SCA3 patients.

Funding: This work was funded by an industrial partner (identity non-disclosed).

Effects of the novel therapeutic SBT-589 across models of Friedreich's ataxia

Thursday, 14th November - 14:45: (Trinity & Goodmans Suite) - Oral Presentation

Mrs. Alyssa Handler [1], Dr. Hatim Zariwala [1], Ms. Yunmi Park [1], Dr. Kristy Vardy [1], Dr. John Ciallella [1], Dr. Jim Wakefield [1], Dr. Jim Carr [1], Dr. David Brown [1], Dr. Laura Kropp [1]

1. *Stealth BioTherapeutics*

Mitochondrial dysfunction is a central contributor to pathology in Friedreich's ataxia (FA), a disease in which heart failure and sudden cardiac events are among the leading causes of mortality. Novel therapeutics that directly target the impaired mitochondrial bioenergetics and cardiac manifestations of FA are desperately needed to facilitate improved patient outcomes. In this study, we evaluated the protective effects of the novel small molecule SBT-589 across cell and mouse models of FA. SBT-589 has been shown to bypass dysfunctional complex I and lower redox stress, leading to the hypothesis that this compound may provide beneficial effects across models of FA. In cell-based studies, FA patient-derived fibroblasts (GM03665) were used to determine the mechanistic effects of SBT-589 on various aspects of mitochondrial function. Using high-resolution respirometry, SBT-589 restored oxygen consumption rates to near baseline levels after chemical inhibition of complex I, and decreased mitochondrial reactive oxygen species (ROS) emission. SBT-589 also improved cell viability and lowered markers of lipid peroxidation following treatment with redox stressors. In parallel studies, we utilized the cardiac MCK-CreFXN conditional knockout mouse, a model with prominent cardiac hypertrophy and an aggressive mortality rate. SBT-589 was dosed daily by subcutaneous injection (60 mg/kg) starting at 4 weeks of age (WOA) until the animals succumbed to disease. Echocardiogram was performed at 4 (baseline) and 7 WOA. SBT-589 significantly reduced various parameters of cardiac hypertrophy after 3 weeks of dosing and delayed the onset of mortality by 15 days compared to vehicle control. This observation was particularly notable in that this model typically does not live past 80 days. Additionally, SBT-589 delayed the onset of mortality by 15 days compared to omaveloxolone. The cardioprotective effects in a mouse model of aggressive FA cardiomyopathy, and restoration of bioenergetics in FA patient-derived fibroblasts support further development of SBT-589 as a disease-modifying therapy.

Pharmacological potentiation of mitochondria-mediated integrated stress response is beneficial for Spastic Ataxia type 5 preclinical models

Thursday, 14th November - 15:00: (Trinity & Goodmans Suite) - Flash Talk

Dr. Alessandra Rocco [1], Dr. Camilla Franchino [1], Dr. Daniele De Ritis [1], Dr. Camilo Toro [2], Dr. Emmanuel Scalais [3], Dr. Angelo Quattrini [4], Dr. Francesca Maltecca [1]

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Background and Objectives: Spastic Ataxia type 5 (SPAX5) is an ultrarare, severe, childhood-onset condition characterized by cerebellar ataxia with spasticity and neuropathy. SPAX5 is caused by biallelic mutations in the *AFG3L2* gene, encoding a key mitochondrial protease exerting protein quality control in the inner mitochondrial membrane (IMM). Currently, there is no cure for SPAX5.

We previously reported that dysfunctional AFG3L2 causes mitochondrial proteotoxicity, leading to the overactivation of the stress-sensitive protease OMA1. In experimental cell models, OMA1 was shown to be the key player communicating mitochondrial stress to the cytosol, via a pathway involving the IMM protein DELE1 and the cytosolic kinase HRI, thus eliciting the integrated stress response (ISR). Our objective was to test ISR potentiation as potential therapeutic approach for SPAX5.

Methods: We studied SPAX5 patients' skin fibroblasts and the *Afg3l2*$^{-/-}$ mouse model, which recapitulates SPAX5 features. We performed RT-qPCR and Western blot to assess ISR activation and a pharmacological treatment with Sephin-1 to modulate it.

Results: We demonstrated the activation of the OMA1-DELE1-HRI pathway eliciting the ISR in both skin fibroblasts from SPAX5 patients and in the cerebellum of *Afg3l2*$^{-/-}$ mice. We indeed detected increased phosphorylation of eIF2α, increased levels of ATF4 and upregulation of its downstream targets. In agreement, pharmacological potentiation of ISR via Sephin-1, a drug that selectively inhibits the stress-induced eIF2alpha phosphatase GADD34 (encoded by Ppp1r15a), improved cell growth of SPAX5 fibroblasts, and cell survival and dendritic arborization *ex vivo* in primary *Afg3l2*$^{-/-}$ Purkinje neurons (PNs). Notably, Sephin-1 treatment *in vivo* extended the life span of *Afg3l2*$^{-/-}$ mice, improved PN morphology, mitochondrial ultrastructure and respiratory capacity.

Discussion and Conclusions. Sephin-1 has desirable safety and pharmacologic profile to act in the CNS and PNS, making it a promising candidate for the pharmacologic treatment of SPAX5 and potentially other ataxias with altered mitochondrial proteostasis.

Screening approach for the discovery of molecules replacing Frataxin in Fe-S cluster biosynthesis for the treatment of Friedreich's ataxia

Thursday, 14th November - 15:07: (Trinity & Goodmans Suite) - Flash Talk

Dr. Benoit D'Autreaux [1]***, Dr. Hubert Gorny*** [1]***, Mr. Kristian Want*** [1]

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Background and objectives. Friedreich's ataxia (FA) is caused by defective expression of the frataxin protein (FXN), with FXN level decreased by 70 to 95 % in FA patient. FXN is a mitochondrial protein accelerating the production of iron-sulfur (Fe-S) cluster that are protein cofactors essential for a multitude of cellular functions. Our strategy to cure or stopped disease progression is to find small molecules replacing FXN in this process. We developed a high throughput screening (HTS) assay to discover molecules specifically replacing FXN in Fe-S cluster biosynthesis using an *in vitro* reconstituted human Fe-S cluster assembly system.

Methods. We performed virtual screening to narrow down the number of molecules to be tested. Using the structure of the NFS1-ISCU-FXN complex (PDB 6NZU) we screened for molecules binding at the FXN binding site in the complex. The molecules with the highest predicted affinity were tested in HTS assays. FXN stimulates Fe-S cluster biosynthesis by accelerating the transfer of persulfide from the NFS1 to ISCU, but persulfide transfer is not amenable to HTS assays. Instead, we relied on the kinetics of Fe-S cluster formation by detection of the Fe-S cluster in ISCU at 456 nm by UV-visible as a read-out. The hits and available structural analogues were validated in dose response assays and by circular dichroism (CD).

Results. We virtually screened 58,848 molecules from the French National Chemical Library and performed HTS assays on 420 compounds. We identified 8 hits and validated 10 hits and analogues in dose response assays and CD, with 7 of them being analogues.

Discussion and Conclusion. This screening approach led to the identification of molecules accelerating Fe-S cluster biosynthesis in place of FXN. The identification of a family of compounds encompassing 7 structural analogues is encouraging for the development of drug candidates.

Funding sources: FARA and Ataxia UK

CAG repeat-selective compounds reduce abundance of expanded CAG RNAs in patient cell and murine models of SCAs

Thursday, 14th November - 15:14: (Trinity & Goodmans Suite) - Flash Talk

Dr. Hannah Shorrock [1], ***Ms. Asmer Aliyeva*** [1], ***Dr. Jesus Frias*** [1], ***Ms. Victoria DeMeo*** [1], ***Dr. Subodh Mishra*** [1], ***Ms. Claudia Lennon*** [1], ***Ms. Cristina DeMeo*** [1], ***Ms. Amy Mascorro*** [1], ***Dr. Ashif Bhuiyan*** [1], ***Ms. Sharon Shaughnessy*** [1], ***Mr. Hormoz Mazdiyasni*** [1], ***Dr. Jia Sheng*** [1], ***Dr. John Cleary*** [1], ***Dr. Kaalak Reddy*** [1], ***Dr. Sweta Vangaveti*** [1], ***Dr. Damian Shin*** [2], ***Dr. J. Andrew Berglund*** [1]

1. *University at Albany,* ***2.*** *Albany Medical College*

Background

Spinocerebellar ataxias (SCAs) are a genetically heterogenous group of devastating neurodegenerative conditions for which clinical care currently focuses on managing symptoms. Across these diseases there is an unmet need for therapies that address underlying disease mechanisms. We utilised the shared CAG repeat expansion mutation causative for a large subgroup of SCAs, to develop a novel disease-gene independent and mechanism agnostic small molecule screening approach to identify compounds with therapeutic potential across multiple SCAs.

Method

We generated a reporter cell line that expresses both a tagged (CAG)60 expansion and a no-repeat control and used this cell line to perform a chemical screen of 1584 compounds and selected candidate small molecules that selectively reduce levels of CAG-expansion transcripts and associated polyglutamine expansion proteins.

Results

Using this approach, we identified the FDA approved microtubule inhibitor Colchicine and a novel CAG-repeat binding compound that reduce expression of disease associated transcripts across SCA1, 3 and 7 patient derived fibroblast lines and the *Atxn1*$^{154Q/2Q}$ SCA1 mouse model in a repeat selective manner. Furthermore, we demonstrated that our lead candidate compound is safe and well tolerated *in vivo*, can be quantified in the brain of treated mice using HPLC and rescues dysregulated alternative splicing in *Atxn1*$^{154Q/2Q}$ mice with minimal off-target transcriptional effects.

Conclusion

This work provides the first example of small molecules capable of targeting the underlying mechanism of disease across multiple CAG SCAs and provides proof of concept that our disease-gene independent, mechanism agnostic small molecule repeat selective screening system can identify compounds that regulate CAG expansion transcript abundance in a repeat dependent manner across multiple CAG expansion diseases. Together this study lays a strong foundation for the potential for repeat selective small molecules as shared therapeutics across CAG microsatellite expansion diseases.

NMDAR-TRPM4 coupling drives neurotoxicity and disease progression in models of spinocerebellar ataxias

Thursday, 14th November - 15:21: (Trinity & Goodmans Suite) - Flash Talk

Mr. David Brito [1], ***Ms. Inês Afonso*** [1], ***Prof. Hilmar Bading*** [2], ***Prof. Clévio Nóbrega*** [1]

1. *Algarve Biomedical Center – Research Institute,* ***2.*** *Interdisciplinary Center for Neurosciences*

Methods: Until now, nearly all attempts to use traditional N-methyl-d-aspartate receptor (NMDAR) antagonists to treat neurodegenerative diseases have failed. This is because NMDARs are not only promoters of neuronal death, but are also essential for high brain function. Recently, it was discovered that the interaction between NMDAR and an ion channel (TRPM4) underlies several types of neurodegenerative disorders. We hypothesized that this neurodegenerative trigger might occur in SCA. To evaluate the therapeutic potential of blockade of NMDAR/TRPM4 coupling in mitigating neuropathology, we expressed recombinant interface inhibitors that compete with endogenous NMDAR/TRPM4 binding in SCA2 and SCA3 lentiviral and transgenic mouse models. As a complementary therapeutic strategy, we evaluated the neuroprotective effects of small molecules identified in a structure-based computational compound screening from over 1.13 million candidates that block NMDAR/TRPM4 interaction. We analysed by immunohistochemistry the levels of neuroinflammation markers, aggregate deposition, and neuronal integrity. Motor behavior was also performed on transgenic SCA3 and SCA2 mice.

Results

We found that both therapeutic strategies resulted in a pronounced reduction of neuronal damage, protein aggregation and neuroinflammation in these disease models. Moreover, we found that this treatment drastically improved performance of transgenic mice in motor coordination.

Discussion

These findings underscore a previously unknow biochemical dysregulation on glutamate-signaling which seems to be conserved in the cerebellum and in other brain regions in SCA2 and SCA3 and can potentially be transversal to several polyglutamine SCAs. In the next stage of this project, we aim to evaluate whether this therapy can reverse gait ataxia patterns specific to patients.

Conclusion

These results indicate that NMDAR/TRPM4 interaction interface inhibitors are an effective therapeutic strategy to treat SCAs.

Parallel session: Emerging and Existing Therapeutics - Clinical Research

Longitudinal progression, SARA metrics, and a sustained modifying effect of 4-aminopyridine treatment in SCA27B: a multicenter study in 219 patients

Thursday, 14th November - 16:00: (Trinity & Goodmans Suite) - Oral Presentation

Dr. Andreas Traschütz [1], ***Prof. Ralf-Dieter Hilgers*** [2], ***Dr. Friedrich Erdlenbruch*** [3], ***Prof. Christel Depienne*** [4], ***Dr. Thomas Wirth*** [5], ***Dr. Clarisse Delvallée*** [5], ***Dr. Astrid Nümann*** [6], ***Dr. Catherine Ashton*** [7], ***Dr. David Pellerin*** [8], ***Dr. Elisabetta Indelicato*** [9], ***Prof. Michael Strupp*** [10], ***Dr. Mathilde Renaud*** [11], ***Dr. Max Borsche*** [12], ***Dr. Marcus Grobe-Einsler*** [13], ***Dr. Jennifer Faber*** [13], ***Prof. Ludger Schöls*** [14], ***Dr. Bernard C. Brais*** [7], ***Prof. Mathieu Anheim*** [5], ***Prof. Dagmar Timmann*** [15], ***Prof. Matthis Synofzik*** [1]

1. *Division of Translational Genomics of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research and Center of Neurology, University of Tübingen, Tübingen, Germany,* ***2.*** *RWTH Aachen University, Institute of Medical Statistics, Aachen, Germany,* ***3.*** *UK Essen,* ***4.*** *) Institute of Human Genetics, University Hospital Essen, University of Duisburg-Essen, Essen, Germany,* ***5.*** *Department of Neurology, Hautepierre University Hospital, Strasbourg, France,* ***6.*** *Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Neurology, Berlin, Germany,* ***7.*** *Department of Neurology and Neurosurgery, Montreal Neurological Hospital and Institute, McGill University, Montreal, QC, Canada,* ***8.*** *Department of Neurology and Neurosurgery, Montreal Neurological Hospital and Institute, McGill University, Montreal, QC, Canada.,* ***9.*** *Center for Rare Movement Disorders Innsbruck, Department of Neurology, Medical University Innsbruck, Innsbruck, Austria,* ***10.*** *Department of Neurology and German Center for Vertigo and Balance Disorders, University Hospital, Ludwig-Maximilians University, Munich, Germany,* ***11.*** *Laboratoire de Génétique, CHRU de Nancy, France,* ***12.*** *Institute of Neurogenetics, University of Lübeck, Lübeck, Germany,* ***13.*** *German Center for Neurodegnerative Diseases (DZNE),* ***14.*** *Department of Neurology, University of Tübingen,* ***15.*** *Department of Neurology, University of Essen*

Background and Methods: Disease progression, underlying modifiers and SARA metrics, and impact of 4-aminopyridine (4-AP) treatment have not yet been well understood in SCA27B. This multicenter study compiled 661 assessments in 219 patients (age: 68±10 years; SARA: 9±6) to characterize the longitudinal progression of SCA27B, assess its demographic/genetic progression modifiers and SARA metrics, and test the 4-AP impact thereon. Progression was estimated by mixed-effect modelling of the SARA and its items, including factors age, sex, baseline severity, GAA repeat size on the longer (range: 251-937) and shorter allele (7-348), and treatment with 4-AP (117 assessments, 21 patients). Distribution-based analysis was performed mapping the item-level evolution of the SARA relative to normative data of 390 healthy elderly controls (age: 19-79 years).

Results: Progression of the SARA was 0.68 points/year [95%CI: 0.46-0.91]. Faster progression was associated with repeat size on the shorter allele (+0.005 points/year per additional repeat) above an estimated threshold of 264 repeats, but not the longer allele. 4-AP reduced progression by 0.55 SARA points/year [0.26-0.83], with marginal residual progression on treatment (0.24 points/year [-0.05-0.51]). The distribution of SARA items gait and stance exceeded age norms by a 1-point difference between the medians of SCA27B and controls, while the respective medians of all other items overlapped across the age range.

Discussion: This first larger multicenter study provides a longitudinal progression rate for SCA27B, identifies the shorter allele as genetic progression modifier, and suggests a sustained effect of 4-AP on progression. Item-level comparisons to healthy aged controls reveal that responsiveness of the SARA in SCA27B primarily depends on balance items as key effector domain. Yet small differences to healthy elderly populations indicate limited effect sizes of this scale for future treatments.

Conclusion: 4-AP might modify disease progression in SCA27B. Future trials should complement the SARA with balance-centric endpoints to improve sensitivity.

Home- and Clinic-Based Rehabilitation Programs for People Living with ARSACS

Thursday, 14th November - 16:15: (Trinity & Goodmans Suite) - Oral Presentation

Prof. Elise Duchesne [1], ***Mrs. Isabelle Lessard*** [2], ***Prof. Luc J. Hébert*** [1], ***Dr. Bernard C. Brais*** [3], ***Dr. Xavier Rodrigue*** [4], ***Dr. Jean-Denis Brisson*** [5], ***Prof. Mylène Aubertin-Leheudre*** [6], ***Mrs. Isabelle Côté*** [7], ***Prof. Cynthia Gagnon*** [8]

1. *École des sciences de la réadaptation, Faculté de médecine, Université Laval, Québec, Canada,* ***2.*** *Centre ÉCOBES-Recherche et Transfert, Cégep de Jonquière, Québec, Canada,* ***3.*** *Department of Neurology and Neurosurgery, Montreal Neurological Hospital and Institute, McGill University, Montreal, QC, Canada,* ***4.*** *Centre intégré universitaire de santé et de services sociaux de la Capitale-Nationale, Québec, Canada,* ***5.*** *Centre intégré universitaire de santé et de services sociaux du Saguenay–Lac-St-Jean, Quebec, Canada,* ***6.*** *Département des Sciences de l'activité physique, Faculté des sciences, Université du Québec à Montréal, Montréal, Canada,* ***7.*** *Groupe de recherche interdisciplinaire sur les maladies neuromusculaires (GRIMN), Centre intégré universitaire de santé et de services sociaux du Saguenay–Lac-St-Jean, Québec, Canada,* ***8.*** *Faculté de médecine et des sciences de la santé, Université de Sherbrooke, Québec, Canada*

Background. Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is manifested by ataxia, incoordination and impairments of motor control, strength, and balance. A few studies have shown that participation in a rehabilitation program improves functional abilities, mobility and coordination in ARSACS or comparable populations. We developed two rehabilitation programs targeting motor impairments and specifically adapted for people living with ARSACS: 1) PACE-ARSACS, a home-based exercise program, and 2) IMPACT, a supervised rehabilitation program.

Objective. To determine whether those adapted exercise programs can improve the physical capacity of adults living with ARSACS.

Methods. PACE-ARSACS: 20 participants were recruited to complete a control phase (usual activities) followed by an experimental phase (exercise program to be carried out three times a week), lasting 12 weeks each. Physical assessments were performed at the beginning of the control phase, at the end of control phase/start of intervention, and at the end of intervention. IMPACT: 38 participants were recruited on 3 sites, and they are currently following the program (n=16) or usual activities (n=22). For both programs, feasibility and acceptability were evaluated as well as balance (Berg balance scale [BBS], Ottawa sitting scale [OSS]), mobility (10-meter walk test), social participation (LIFE-H), and ataxia severity (SARA).

Results. PACE-ARSACS: 14 participants completed the study. Our results showed no disease progression during the control phase, but a significant improvement in social participation (LIFE-H) and a reduction in disease severity (SARA) following the intervention phase. Also, the individual results showed at least 43% of the participants improved with scores above the standard error of measurement for standing (BBS) and sitting (OSS) balance. IMPACT: preliminary results will be presented at the conference.

Conclusion. Those studies provide preliminary results supporting the use of tailored rehabilitation interventions specifically adapted for the ARSCAS population.

Funding: Fondation de l'Ataxie Charlevoix-Saguenay and Canadian Institutes of Health Research.

Home Balance Verse Aerobic Training: A Randomized Controlled Trial

Thursday, 14th November - 16:30: (Trinity & Goodmans Suite) - Oral Presentation

Dr. Scott Barbuto [1], ***Mr. Michael Spinner*** [1], ***Dr. Sheng-Han Kuo*** [1], ***Dr. Joel Stein*** [1], ***Dr. Lori Quinn*** [1], ***Dr. Seonjoo Lee*** [1], ***Dr. Yaakov Stern*** [1]

1. *Columbia University Medical Center*

Background and Objective: Balance and aerobic training have shown benefits in cerebellar ataxias, but it is unclear if one training is better than the other.

Methods: We conducted a single center, assessor-blinded, randomized controlled trial. Individuals with cerebellar ataxia were assigned (1:1) to either home balance or aerobic training. Aerobic training consisted of 30-minute cycling sessions, 5x per week at greater than 80% maximum heart rate. Balance training consisted of the same frequency and duration. During the first 6-months, individuals had study support comprised of biweekly phone calls. After 6-months this study support was withdrawn, and individuals were expected to train independently with up to 1-year follow-up. The primary outcome was improvement in ataxia severity as measured by the Scale for the Assessment and Rating of Ataxia (SARA).

Results: Thirty-one individuals were randomized to each group with 6 dropouts in the balance group and 5 in the aerobic group after the one-year program. There were no serious adverse events caused by training, and adherence to exercise protocols was over 80% at 6-months. Adherence in both groups, however, dropped to less than 40% after study support was withdrawn. There was a mean improvement in ataxia severity of 2.5 SARA points (SD 1.92) in the aerobic group compared to an improvement of 1.0 points (SD 1.87) in the balance group at 6-months. Individuals in the aerobic group who halted or limited training had improvements revert back to baseline. However, individuals who continued to train regularly had an average improvement of almost 4.0 SARA points (SD 1.84) at one-year.

Conclusions: There was a statistically and clinically significant improvement in ataxia severity with aerobic training compared to balance training. Individuals who continuously trained maintained and improved benefits whereas individuals who stopped training reverted back to baseline.

Safety and Efficacy of Vatiquinone Treatment in Friedreich Ataxia Patients from MOVE-FA: a Phase 3, Double-blind, Placebo-controlled Trial

Thursday, 14th November - 16:45: (Trinity & Goodmans Suite) - Flash Talk

Prof. David Lynch [1], ***Dr. Antoine Duquette*** [2], ***Prof. Marcondes França*** [3], ***Dr. Susan Perlman*** [4], ***Prof. Alexandra Durr*** [5], ***Dr. Enrico Bertini*** [6], ***Dr. Alejandra Darling*** [7], ***Dr. Katherine Mathews*** [8], ***Prof. Ludger Schöls*** [9], ***Dr. Anne Fournier*** [10], ***Prof. Martin Delatycki*** [11], ***Prof. S. H. Subramony*** [12], ***Prof. Richard Roxburgh*** [13], ***Dr. Olivia Zhang*** [14], ***Dr. Christian Rummey*** [15], ***Dr. Alana Salvucci*** [16], ***Dr. Bert Yao*** [16], ***Dr. Jonathan J. Cherry*** [14], ***Dr. Lee Golden*** [14], ***Dr. Theresa Zesiewicz*** [17]

1. *Children's Hospital of Philadelphia,* ***2.*** *Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Montreal, QC, Canada.,* ***3.*** *Department of Neurology, University of Campinas, Campinas, Sao Paulo,* ***4.*** *University of California at Los Angeles,* ***5.*** *Sorbonne University, Paris Brain Institute, Pitié-Salpêtrière Hospital,* ***6.*** *Bambino Gesù Children's Hospital, IRCCS, Unit of Muscular and Neurodegenerative Disorders,* ***7.*** *Sant Joan de Déu Children´s Hospital,* ***8.*** *University of Iowa,* ***9.*** *Department of Neurology, University of Tübingen,* ***10.*** *CHU Sainte-Justine, Montreal,* ***11.*** *Monash University; Murdoch Children's Research Institute; University of Melbourne; Victorian Clinical Genetics Service,* ***12.*** *Department of Neurology and Fixel Center for Neurological Disorders, Gainesville, FL,* ***13.*** *Auckland City Hospital,* ***14.*** *PTC Therapeutics Inc, Warren, NJ,* ***15.*** *Clinical Data Science GmbH,* ***16.*** *Formally PTC Therapeutics,* ***17.*** *University of South Florida*

Background and Objective: Friedreich ataxia (FA), the most common inherited ataxia, is characterized by progressive neurological damage and loss of ambulation. Vatiquinone is an oral, first-in-class inhibitor of 15-lipoxygenase. 15-Lipoxygenase inhibition prevents accumulation of lipid peroxides, proinflammatory signaling, and ferroptosis, which are underlying pathologies of FA.

MOVE-FA (NCT04577352) is a global phase 3 trial, evaluated the safety and efficacy of vatiquinone in patients with FA. Here, we describe the results from the 72-week placebo-controlled phase.

Methods: The study enrolled 143 subjects with FA aged ≥7 years, modified FA Rating Scale (mFARS) score of 20–70, and the ability to ambulate ≥10 feet in 1 minute +/- assistance. The primary endpoint was placebo corrected change from baseline in mFARS at 72-weeks. The Intent-to-Treat (ITT) population had a mean age of 18.7 years and the primary analysis population (modified ITT; mITT) included 123 subjects 7-21 years (mean 14.6).

Results: In the mITT population, there was a -1.61 (p=0.144) change in mFARS at 72-weeks relative to placebo. A consistent vatiquinone treatment benefit was observed across the primary, secondary, and exploratory endpoints. Notably, there were nominally significant benefits recorded in the Upright Stability subscale (USS) of mFARS (-1.26 [p=0.021]), a relevant metric of disease progression in younger, ambulatory FA patients, and the Modified Fatigue Impact Scale (MFIS), -5.05 (p=0.025).

Vatiquinone was well tolerated and there was no difference in treatment related AEs between treatment and placebo groups.

Discussion and Conclusion: MOVE-FA demonstrated clinically relevant benefits across the primary, secondary, and exploratory endpoints. Vatiquinone treatment resulted in a clinically meaningful and statistically significant treatment effect on the USS, a sensitive and predictive endpoint for risk of loss of ambulation, prevention of which is a key goal for therapy in ambulatory FA patients.

A clinical update from a first-in-human, phase 1/2a trial of the CAG repeat-targeting ASO VO659 in patients with Spinocerebellar ataxia types 1 and 3 and Huntington's disease

Thursday, 14th November - 17:00: (Trinity & Goodmans Suite) - Flash Talk

Dr. Scott Schobel [1], ***Dr. Katja Obieglo*** [1], ***Dr. Nicole Datson*** [1]

1. *VICO Therapeutics BV*

Methods

Spinocerebellar ataxia 1 and 3 (SCA1 and SCA3) and Huntington's disease (HD) belong to a group of polyglutamine (polyQ) diseases caused by CAG repeat expansion, leading to the expression of mutant proteins. VO659 is a CAG repeat-targeting and allele preferential antisense oligonucleotide (ASO) that inhibits protein translation via steric hindrance in the case of HD and SCA1, and induces exon skipping in the case of SCA3. Non-clinical data demonstrate that VO659 reduces levels of mutant polyQ proteins in SCA1, SCA3, and HD models.

A first-in-human, phase 1/2a trial of VO659 (NCT05822908, EudraCT 2022-001314-19) is ongoing in HD, SCA1 and SCA3. This open-label multiple ascending dose trial evaluates the safety and tolerability of four-doses of VO659 administered every 4 weeks over a 13 week dosing period with a six month post-dose recovery period. Secondary objectives include VO659 pharmacokinetics in cerebrospinal fluid (CSF) and blood, and exploratory objectives include pharmacodynamic and clinical endpoints. Dose-escalation is planned in up to five dose levels. Dose levels one and two will include participants with SCA3 only, and dose level three onwards participants with SCA1, SCA3, and HD.

Results

To date, VO659 has been safe and well tolerated in participants through the 10mg dose level (n=4 SCA3), 20mg dose level (n=4 SCA3), and 40mg dose level (n=6 SCA3, n=3 SCA1, and n=6 HD, dosing ongoing). Further accrued safety and tolerability data will be shared from the study at this meeting.

Discussion and Conclusion

VO659 is safe and well tolerated in the ongoing Phase 1/2a trial. VO659 is the first investigational treatment designed for all polyQ diseases. We hypothesise that VO659's CAG repeat targeting mechanism will enable a broader therapeutic window than non-allele preferential approaches in SCA1 and HD, and provide a unique advantage via the splice mechanism in SCA3 patients relative to non-allele selective approaches.

Post hoc subgroup analysis: age of Friedreich ataxia onset in MOXIe trial of omaveloxolone

Thursday, 14th November - 17:15: (Trinity & Goodmans Suite) - Flash Talk

Dr. Suneeta Chimalapati [1]***, Dr. Whitney Costello*** [1]***, Dr. Angie Goldsberry*** [1]***, Dr. Shannon Rich*** [1]***, Dr. Cody Ruhl*** [1]***, Prof. David Lynch*** [2]

1. *Biogen, Inc.,* ***2.*** *Children's Hospital of Philadelphia*

Background and objectives: Age of onset is considered the most important predictor of clinical progression in patients with Friedreich ataxia (FA). This post hoc analysis assessed the change in the modified Friedreich Ataxia Rating Scale (mFARS) scores by age of onset subgroups using data from MOXIe Part 2 full analysis set (NCT02255435; EudraCT2015-002762-23) and propensity-matched data from the MOXIe open-label extension (OLE) and the FACOMS natural history study (NCT03090789).

Methods: MOXIe Part 2 randomized patients 1:1 to placebo or omaveloxolone 150 mg once daily (primary endpoint: change from baseline in mFARS at Week 48). MOXIe OLE (ongoing study to assess long-term safety and tolerability of omaveloxolone) patients were propensity score-matched 1:1 to FACOMS patients. Changes in mFARS scores by age of onset subgroups were analyzed using mixed models repeated measures.

Results: In MOXIe Part 2 (median age of FA onset: 15 years), patients with age of onset >15 years had lower mean (SD) baseline mFARS scores (omaveloxolone: 37.64 [9.559]; placebo: 36.52 [12.683]) versus patients with age of onset ≤15 years (omaveloxolone: 43.40 [10.501]; placebo: 40.84 [9.080]). Placebo-treated patients with age of onset ≤15 years versus >15 years had more progression by Week 48 (least squares mean [95% CI]: 2.02 [0.23, 3.81] versus −0.40 [−2.26, 1.47]), whereas omaveloxolone-treated patients demonstrated a similar change from baseline regardless of age of onset (−1.59 [−3.50, 0.32] versus −1.46 [−3.53, 0.61]). In both age of onset subgroups, progression directionally favored omaveloxolone versus placebo-treated patients at Week 48 and versus propensity-matched FACOMS controls after 3 years.

Discussion and conclusion: Although MOXIe Part 2 was not powered to detect efficacy by age of onset subgroups, results directionally favored omaveloxolone versus placebo regardless of age of FA onset. Similar trends were observed after 3 years in MOXIe OLE relative to propensity-matched FACOMS patients.

Funding: Biogen

Effect of nomlabofusp administration on tissue frataxin levels, plasma lipid profiles, and gene expression in patients with Friedreich's ataxia

Thursday, 14th November - 17:22: (Trinity & Goodmans Suite) - Flash Talk

Ms. Noreen Scherer [1], Prof. Adrienne Clements-Egan [1], Mr. Mohamed Hamdani [1], Dr. Magdy Shenouda [2], Ms. Anik Forest [3], Prof. Christine Des Rosiers [4], Prof. Gopi Shankar [1], Dr. Russell Clayton [1]

1. *Larimar Therapeutics, Inc.,* ***2.*** *Clinilabs, Inc.,* ***3.*** *Montreal Heart Instititute,* ***4.*** *Montreal Heart Institute*

Methods: In a Phase 2 placebo-controlled double-blind dose exploration study, adults with Friedreich's ataxia (FRDA) were enrolled into 2 cohorts and randomized 2:1 to receive nomlabofusp 25 mg (Cohort 1) and 50 mg (Cohort 2) or placebo via subcutaneous injection daily for 14 days followed by alternate day administration for 14 days. Tissue frataxin concentrations were measured using a hybrid LC-MS/MS assay in buccal and skin cells before, during, and after treatment. Buccal cells were collected before, during, and after treatment for gene expression profiling using the NanoString nCounter system. Blood samples were collected before, during, and after treatment for lipid profiling using high-resolution liquid chromatography quadrupole time-of-flight.

Results: Thirteen (9 nomlabofusp, 4 placebo) and 15 adults with FRDA (10 nomlabofusp, 5 placebo) participated in Cohorts 1 and 2, respectively. In general, nomlabofusp appeared to be well tolerated. Compared with baseline, median frataxin concentration increased by 0.56 pg/mcg and 0.72 pg/mcg in buccal cells and 2.81 pg/mcg and 5.57 pg/mcg in skin cells in Cohorts 1 and 2, respectively, after 14 days of daily administration of nomlabofusp, with no change from baseline observed in subjects receiving placebo. Abnormal lipid profiles were identified in adults with FRDA at baseline with directional dose-dependent normalization observed post nomlabofusp treatment. Preliminary analyses indicated similar observations with abnormal gene expression identified in adults with FRDA.

Discussion: Frataxin deficiency results in metabolic dysfunction in patients with FRDA. Increased tissue frataxin concentrations observed after nomlabofusp administration appear to affect metabolic function as evidenced by normalization of gene expression and lipid profiles.

Conclusion: In patients with FRDA, daily administration of 25 mg and 50 mg nomlabofusp was well tolerated and resulted in increased tissue frataxin concentrations, and directional dose-dependent normalization of lipid profiles and gene expression was observed following treatment with nomlabofusp.

Plenary: Emerging and Existing Therapeutics

Molecular and synaptic adaptations promote resilience in posterior SCA6 cerebellum

Friday, 15th November - 09:00: (Trinity & Goodmans Suite) - Invited Speaker

Dr. Alanna Watt [1]

1. *Department of Biology, McGill University, Montreal*

Regional heterogeneity is important for normal function in the cerebellum and can be disturbed in disease. Atrophy in spinocerebellar ataxia type 6 (SCA6) has been reported to be restricted to the anterior cerebellum while the posterior is spared. We showed that in a mouse model of SCA6, degeneration occurs in a similar pattern to that reported in patients: anterior zone Purkinje cell degenerated while nodular zone Purkinje cells survived. Long before degeneration takes place, anterior Purkinje cells in SCA6 mice fired with reduced frequency and regularity compared to litter-matched WT mice, while nodular Purkinje cells firing was unaffected. Does this mean that the posterior cerebellum is untouched by disease in SCA6? We used RNA-sequencing to determine molecular changes in anterior and posterior vermis. To our surprise, we found that the majority of differentially expressed genes in SCA6 were in the posterior cerebellum that does not degenerate. Our results suggest that unexpected molecular and synaptic adaptations occur in posterior cerebellum in SCA6 that may act to protect posterior Purkinje cells from degeneration.

Atrophin-1 Antisense Oligonucleotides Provide Robust Protection from Pathology in a Novel Humanized DRPLA Model

Friday, 15th November - 09:30: (Trinity & Goodmans Suite) - Oral Presentation

Mx. Velvet Smith [1], Ms. Bereket Gidi [1], Mr. Jiaxu Feng [1], Ms. Veronica Guerra [1], Mr. Robert Bragg [1], Ms. Aliza Ben-Varon [2], Dr. Silvia Prades [3], Dr. Holly Kordasiewicz [4], Ms. Briana Buscemi [4], Dr. Hien Zhao [4], Dr. Jeff Carroll [1]

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Methods: We have developed a novel, fully-humanized, model of Dentatorubral-pallidoluysian atrophy (DRLA), in which one mouse allele of *Atrophin-1* (*Atn1*) is replaced with human sequence, from the 3' to the 5'-UTR. This human allele includes 112 pure CAG repeats, to model DRPLA, which is caused by a CAG expansion in *ATN1*. We then evaluated antisense oligonucleotides (ASOs) that selectively target either mouse or human *ATN1*, to test the hypothesis that ASO treatment targeting human *ATN1* might provide protection from any emergent DRPLA-like symptoms. Due to severe phenotypes in the mice, we delivered ASOs two days postnatally, and again at five weeks of age.

Results: Our mouse- and human-targeted ASOs robustly reduce levels of the targeted transcript, but not the non-targeted one[HZ1] . Treatment with human ASO, but not mouse ASO, provides very robust protection from DRPLA-relevant changes in the mice, including improving many behavioral changes including: rotarod, balance beam performance, grip strength, tremors, and gait. It also reduces brain mass reduction by 66% (2.9% brain weight loss in Hu ASO treated mice versus 8.4% loss in saline). Detailed neuropathological assessments are underway, and will be discussed. Finally, we conducted bulk RNA sequencing in the cerebellum, and have observed that human- but not mouse-, ASO treatment robustly rescues a number of the transcriptional changes observed in our mice.

Discussion: We have generated a novel, fully-humanized, mouse model of DRPLA. This model provides an excellent resource for sequence-specific interventions such as oligonucleotides. These mice have very pronounced DRPLA-relevant symptoms, for which we have provided significant rescue using ASO treatment.

Conclusion: We believe these results support ATN1-lowering treatment as a therapeutic intervention in DRPLA patients, as well as providing an exciting proof of concept of a novel means of using fully humanized mice to test hypotheses about repeat expansion disorder pathogenesis.

Synergy in stimulating FXN expression by co-treatment with Synthetic Genome Regulators (SynGR1) and molecules that stabilize active chromatin marks

Friday, 15th November - 09:45: (Trinity & Goodmans Suite) - Oral Presentation

Prof. Aseem Ansari [1]

1. *St. Jude Children's Research Hospital*

Background and Objectives: In Friedreich's ataxia (FRDA/FA), expression of frataxin (*FXN*) is downregulated by expansion of GAA trinucleotide repeats within the gene. We previously reported the development of a novel synthetic genome regulator SynGR1/SynTEF1 to restore *FXN* expression in patient-derived cells. SynGR1 traffics to the nucleus, enriches at the disease-causing GAA repeat expansion, and licenses gene transcription through the repressive GAA repeats. To investigate the role repressive chromatin on SynGR1 function, we now co-treat cells with pharmacological agents that rewire repressive marks on chromatin. We observe that a selective histone deacetylase inhibitor HDACi-109, stabilizes active chromatin marks and enhances SynGR1-mediated *FXN* expression in FRDA/FA cells.
Methods: Using RNA-seq, we examined whether the HDACi-109-mediated inhibition of class I histone deacetylases (HDACs) impacts SynTEF1-responsive FXN expression. Additionally, quantitative chromatin immunoprecipitation (ChIP) assays were used to measure HDACi-109 mediated enrichment of histone H3 lysine 9 acetylation (H3K9Ac) at the *FXN* locus.
Results: Upon co-treatment with SynGR1 and HDACi-109, *FXN* mRNA levels greatly exceeded the maximal activation achieved by either small molecule alone. Remarkably, HDACi-109 enabled SynGR1 to override chromatin-based attenuation at *FXN* but it did not permit SynGR1 to function at other non-targeted genomic loci.
Discussion: We demonstrate that HDACi-109 reduced the barrier to transcription initiation while SynGR1 licensed transcription elongation past the repressive repeats. Together, these molecules act on two consecutive mechanistic steps in transcription and result in dramatically increased levels of *FXN* in patient-derived cells. From a therapeutic perspective, these data indicate that modulating flux between transcription initiation and elongation will greatly expand the therapeutic window for treatment.

Preliminary Results from SUNRISE-FA: A Phase1/2 Study of Investigational Gene Therapy, LX2006, for Cardiomyopathy of Friedreich Ataxia

Friday, 15th November - 10:00: (Trinity & Goodmans Suite) - Oral Presentation

Dr. Theresa Zesiewicz [1], ***Dr. Aarti Patel*** [1], ***Dr. Teerapat rojsajjakul*** [2], ***Prof. Ian Blair*** [2], ***Dr. Aashir Khan*** [3], ***Dr. Nithya Selvan*** [3], ***Dr. Richie Khanna*** [3], ***Dr. Franca Angeli*** [3], ***Dr. Eric Adler*** [3]

1. *University of South Florida,* ***2.*** *University of Pennsylvania, Perelman School of Medicine,* ***3.*** *Lexeo Therapeutics*

Objectives. Friedreich ataxia (FA) is a serious autosomal recessive disorder that manifests as neurologic and cardiac disease. While the neurologic disease is disabling, the cardiac disease, characterized by concentric remodeling/hypertrophic cardiomyopathy (CM), is the cause of premature death in most affected individuals. The objective of this study (SUNRISE-FA) is to assess the safety and preliminary efficacy of AAVrh.10hFXN (LX-2006), a cardiotropic adeno-associated gene transfer vector coding for human frataxin (FXN), in treating FA-CM.

Methods. SUNRISE-FA is an ongoing first-in-human, dose-escalating, open label study (NCT05445323) assessing the safety and efficacy of LX-2006. At the time of the submission, 4 participants were dosed intravenously (n=1, $1.8x10^{11}$ gc/kg; n=3, $5.6x10^{11}$ gc/kg). Cardiac structure and function were monitored by cardiac MRI and echocardiograms, cardio-pulmonary hand crank exercise testing, cardiac biomarkers, Holter monitoring and cardiac biopsies to assess FXN levels in the target organ before and 3 months post-therapy.

Results. At the time of this submission, there were no reported serious adverse events related to the LX-2006. In the 3 subjects who underwent cardiac biopsies, there was a 22% to 187% dose-dependent increase in cardiac FXN levels assessed by liquid chromatography–mass spectrometry (LCMS) 12 weeks after therapy.

Conclusions. This first-in-human study of gene therapy for FA-CM has demonstrated that administration of LX-2006 results in a dose-dependent increase of FXN in the heart. Additional data on preliminary safety and protein expression will be presented at the conference.

The efficacy of a 30-week goal-directed rehabilitation program for individuals with hereditary cerebellar ataxia, a randomised controlled trial.

Friday, 15th November - 10:15: (Trinity & Goodmans Suite) - Oral Presentation

Dr. Sarah Milne [1], Ms. Melissa Roberts [2], Mrs. Shannon Williams [3], Mrs. Jillian Chua [4], Ms. Alison Grootendorst [5], Ms. Genevieve Agostinelli [5], Dr. Anneke Grobler [6], Ms. Hannah Ross [2], Ms. Amy Robinson [7], Ms. Kristen Grove [8], Ms. Gabrielle Modderman [9], Ms. Annabel Price [7], Ms. Megan Thomson [2], Ms. Libby Massey [5], Dr. Christina Liang [10], Dr. Kishore Kumar [11], Prof. Kim Dalziel [12], Prof. Joshua Burns [13], Prof. Carolyn Sue [14], Prof. Pubudu Pathirana [15], Prof. Malcolm Horne [16], Ms. Nikki Gelfard [17], Ms. Helen Curd [17], Dr. David J. Szmulewicz [18], Dr. Louise A Corben [19], Prof. Martin Delatycki [20]

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Background and objectives: Rehabilitation is recommended for individuals with hereditary cerebellar ataxia (HCA), yet questions remain about its efficacy. The aim of this multicentre randomised controlled trial was to examine the efficacy of a 30-week goal-directed rehabilitation program on improving functioning, balance, and ataxia severity for individuals with HCA, compared to standard care.

Methods: Individuals with autosomal dominant or recessive ataxia were randomised to receive rehabilitation (six weeks of outpatient physiotherapy followed by a 24-week home exercise program) or continued their usual care. The primary outcome was the motor domain of the Functional Independence Measure (mFIM). Secondary outcomes included the Scale for the Rating and Assessment of Ataxia (SARA), Berg Balance Scale (BBS) and SF-36v2 Health Survey. Outcomes were administered by blinded assessors at baseline, seven, 18 and 30 weeks.

Results: Seventy-six individuals, mean (SD) age 45.1 (16.5) years, were recruited. Compared with standard care, mFIM scores improved after six-weeks of outpatient physiotherapy (mean difference 2.26, 95%CI:0.30-4.21, p=0.025). Similar improvements were demonstrated in the SARA (mean difference -1.18, 95%CI:-2.27 to -0.09, p=0.035), BBS (mean difference 4.08, 95%CI:2.30-5.85, p<0.001) and SF-36v2 Role Limitations due to Physical Health domain (mean difference 4.78 95%CI:0.56-9.01, p=0.027). Compared with standard care, rehabilitation maintained improvement in the SARA at 30-weeks (mean difference -1·51, 95%CI -2·76 to -0·27, p=0·017), but not mFIM (1·74, 95%CI -0·32-3·81, p=0·098).

Discussion and Conclusion: Outpatient rehabilitation reduces ataxia severity and improves functioning, balance, and health related quality of life (HRQOL) in individuals with HCA, beyond what occurs with usual care. Improvements were maintained with the 24-week home exercise program, with the exception of functioning, a critical goal

of rehabilitation. Further research is required to understand the optimal approach and rehabilitation dosage. This study provides justification for equitable access to rehabilitation for individuals with HCA.
Funding: Medical Research Future Fund, Rebecca L Cooper

Parallel session: Biomarkers and Clinical Outcomes II

Assessing Progression in Ataxias - The Rating Scale Dilemma

Friday, 15th November - 11:00: (Trinity & Goodmans Suite) - Oral Presentation

Dr. Christian Rummey [1]

1. *Clinical Data Science GmbH*

Background

Despite outstanding technological developments in ataxia research, rating scales have remained the hallmark of progression assessment for more than 2 decades. The SARA, and 8-item assessment of gait, balance, speech and appendicular function is the currently most widely used tool, known for its brevity and simplicity. However, the compartmentalized nature of such scales often fuels concerns, mostly related to individual item relevance and within-scale weighting of individual functions. As a result, recent Phase III trials, typically after regulatory guidance, have focused on axial function, employed by various SARA and ICARS derivatives. The detailed rationales are often unclear, and do not seem to rely on natural history data.

This leads to less effective studies and endangers the development of promising therapies, while alternatives are available.

Methods

Results from several studies and analyses were synthesized: 1) a correlation analysis of SARA/mFARS/ICARS, 2) a breakdown of placebo effects in RCTs, 3) findings in pediatric- and 4) elderly populations, and 5) on the clinical meaningfulness of ataxia rating scales. Findings will be put into regulatory context.

Results and Discussion

Appendicular function assessments show prominent placebo effects, high variability in young children, and age-related scoring in elderly patients. In addition, direct patient relevance is often questioned. A solution should be applicable to most ataxias: Focus on the most prominent and unifying symptom of all ataxia types: Gait and balance, by means of the Upright Stability Score (Section E of the mFARS). This construct is more objective (timed tasks), more sensitive in mild disease stages, and was shown to predict loss of ambulation in Friedreich's ataxia. Its use is currently expanded into Spinocerebellar Ataxias.

Conclusion

The USS Score constitutes the most objective rating scale, and it's use follows the logic of recent SARA and ICARS derivatives: Focus on axial function.

A Longitudinal Analysis of One-Year Spinocerebellar Ataxia (SCA) Progression using the Patient-Reported Outcome Measure of Ataxia (PROM-Ataxia)

Friday, 15th November - 11:15: (Trinity & Goodmans Suite) - Oral Presentation

Ms. Anna L. Burt [1], ***Dr. Gilbert L'Italien*** [2], ***Dr. Susan Perlman*** [3], ***Dr. Liana S. Rosenthal*** [4], ***Dr. Sheng-Han Kuo*** [5], ***Dr. Tetsuo Ashizawa*** [6], ***Dr. Theresa Zesiewicz*** [7], ***Dr. Cameron Dietiker*** [3], ***Dr. Puneet Opal*** [8], ***Dr. Antoine Duquette*** [9], ***Dr. George Wilmot*** [10], ***Dr. Vikram G. Shakkottai*** [11], ***Dr. Christopher M. Gomez*** [12], ***Dr. Sharan Srinivasan*** [13], ***Prof. Henry Paulson*** [13], ***Dr. Michael D. Geschwind*** [3], ***Dr. Sandie Worley*** [5], ***Dr. Chiadi Onyike*** [14], ***Dr. Andrew Billnitzer*** [15], ***Dr. Amy Ferng*** [13], ***Ms. Kristen Matulis*** [5], ***Dr. Marie Y. Davis*** [16], ***Prof. S. H. Subramony*** [17], ***Dr. Anoopum Gupta*** [1], ***Dr. Christopher D. Stephen*** [1], ***Prof. Jeremy D. Schmahmann*** [1]

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Background: The PROM-Ataxia has been cross-sectionally validated in patients with ataxia (Schmahmann et al., 2021) and is being considered for use in clinical trials. It remains to be validated longitudinally to evaluate its responsiveness to change in disease and patient experience over time, and to explore the overall trajectory of progression of cerebellar ataxia and the trajectory of progression of its different features.

Methods: We performed psychometric analysis of PROM-Ataxia data from 177 individuals with SCA types 1, 2, 3, 6, 7, 8 or 10, obtained from the CRC-SCA natural history study collected at baseline and one year later. Patients were classified as *mild*, *moderate*, and *severe* according to baseline Friedreich's Ataxia Rating Scale functional stage. Analyses included assessment of internal consistency, sensitivity to disease severity, predictive modelling of score changes over time, correlations with clinical outcome assessments (COAs) - Brief Ataxia Rating Scale, Scale for the Assessment and Rating of Ataxia, Fatigue Severity Scale, Cerebellar Cognitive Affective Syndrome scale, EuroQol 5-Dimension, 25-foot timed walk), and responsiveness to progression.

Results: We confirmed PROM-Ataxia's high internal consistency and correlation with other COAs. We demonstrate sigmoidal progression in relationship to PROM-Ataxia scores, the change most notable in patients of *moderate* severity. Significant variation in PROM-Ataxia progression was not observed between different SCA genotypes. Compared to other COAs, PROM-Ataxia captured the greatest degree of change over one year. Analysis of responsiveness by severity revealed variation in domain-specific progression: MENTAL changed most significantly in the *mild*, early stage; PHYSICAL in the *moderate*, middle stage subgroup; and Activities of Daily Living in the later, *severe* stages.

Discussion: PROM-Ataxia is sensitive to change, reliably capturing disease evolution over a one-year time frame. Insights into domain-specific progression and patient experience over longer periods in larger cohorts will be critical for informing and enhancing clinical care and research.

Longitudinal progression of digital gait measures in patients with spastic paraplegia type 7 (SPG7): an international multi-center study (PROSPAX)

Friday, 15th November - 11:30: (Trinity & Goodmans Suite) - Oral Presentation

Dr. Lukas Beichert [1], ***Mr. Jens Seemann*** [2], ***Dr. Christoph Keßler*** [3], ***Dr. Andreas Traschütz*** [4], ***Dr. Ivana Ricca*** [5], ***Dr. Sara Satolli*** [6], ***Prof. Nazli Basak*** [7], ***Dr. Giulia Coarelli*** [8], ***Prof. Dagmar Timmann*** [9], ***Prof. Cynthia Gagnon*** [10], ***Prof. Bart van de Warrenburg*** [11], ***Dr. Winfried Ilg*** [2], ***Prof. Matthis Synofzik*** [4], ***Prof. Rebecca Schüle*** [12]

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Background and Objective: With treatment trials on the horizon, sensitive outcome measures are highly needed for the >100 spastic ataxias. Digital-motor gait measures, assessed by wearable sensors, are considered prime outcome candidates for spastic ataxias and have shown favourable cross-sectional properties in spastic paraplegia type 7 (SPG7). However, their longitudinal sensitivity to change is yet unknown. This study aimed to assess 1-year progression of digital gait measures in patients with SPG7.

Methods: Longitudinal multi-center study (7 centers, 6 countries), assessments at baseline and after 1 year. Gait was analysed in 49 SPG7 patients (baseline, median [min-max]: age=52 [22-69], SARA=9.0 [3.5-17.0], SPRS=14 [3-28]) using 3 wearable motion sensors (Opal APDM) during laboratory-based walking and 'supervised free walking', resembling real-life walking. Assessments included rating of the Scale for the assessment and rating of ataxia (SARA) and the Spastic paraplegia rating scale (SPRS). Effect size and significance of 1-year changes were assessed using non-parametric matched-pairs rank biserial correlation (r_{prb}) and Wilcoxon signed-rank test, respectively.

Results: In laboratory-based walking, 1-year progression was observed for measures of trunk range of motion variability (CoronalRoM_CV: r_{prb}=0.46, p=0.0051), of gait smoothness (harmonicRatioML: r_{prb}=-0.40, p=0.015) and of spatiotemporal stride variability (e.g. DoubleSupport_MADN: r_{prb}=0.31-0.37). In the trial-relevant subcohort of mildly affected patients (SPRS items 1-6≤9; n=34), CoronalRoM_CV (r_{prb}=0.59, p=0.0027) exhibited larger effect size than clinician-reported outcomes like SARA (r_{prb}=0.53, p=0.0055) or SPRS (r_{prb}=0.30, p=0.087). In supervised free walking, progression was observed for measures of gait smoothness and temporal variability (e.g. harmonicRatioML, DoubleSupport_MADN: $|r_{prb}|$=0.28-0.44).

Discussion and Conclusion: In this first longitudinal multi-center study of digital gait measures in SPG7, 1-year progression was captured for several gait measures, with effect sizes partly exceeding those of key clinician-reported outcomes (SARA, SPRS). These gait measures could thus improve sensitivity to treatment effects in future clinical trials in SPG7 and possibly also other spastic ataxias.

Longitudinal MRI reveals early structural changes in pre-symptomatic SCA3: A one- and two-year follow-up study

Friday, 15th November - 11:45: (Trinity & Goodmans Suite) - Oral Presentation

Ms. Mónica Ferreira [1], ***Mr. Philipp Wegner*** [2], ***Prof. Thomas Klockgether*** [3], ***Dr. Jennifer Faber*** [4], ***Prof. Bart van de Warrenburg*** [5], ***Dr. Heike Jacobi*** [6], ***Prof. Dagmar Timmann*** [7], ***Dr. Gulin Oz*** [8], ***Dr. Peter Barker*** [9]

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Background and Objectives

Spinocerebellar ataxia type 3 (SCA3) is characterized by cerebellar and brainstem atrophy. First gene therapy trials have been initiated in SCA3. Thus, there is an urgent need for non-invasive biomarkers, particularly for pre-ataxic SCA3 (preSCA3), to pave the way towards preventive trials. This study analyzes MRI data over one- and two-year follow-up periods to identify volumetric and diffusion changes in SCA3.

Methods

In a multicenter, longitudinal study, we acquired T1-weighted, and diffusion MRI of 162 participants. For volumetric analysis, brain region volumes were obtained with FastSurfer and CerebNet, except for the deep cerebellar nuclei, which were manually segmented. Diffusion data was preprocessed with FSL, followed by tractography using MRtrix. We applied ComBat harmonization and calculated mean fractional anisotropy, mean diffusivity, axial diffusivity, and radial diffusivity values for each tract. Linear mixed-effect models were used to assess changes over time, controlling for age and sex.

Results

Significant volumetric changes were observed over time in both preSCA3 and SCA3. The dentate nucleus, brainstem, medulla, pons, midbrain, cerebellum cortex and cerebellum white matter volumes showed significant differences ($p < 0.01$) over time. However, no significant changes were found in the diffusion mean tract values over the same periods.

Discussion and Conclusion

These longitudinal findings highlight early structural changes in preSCA3, confirming that brain changes start before clinical ataxia onset. Significant volumetric reductions in SCA3 patients, particularly in cerebellar and brainstem regions, align with the disease's known pathology. The lack of significant longitudinal diffusion changes suggests volumetry is more sensitive for detecting early neurodegenerative changes in SCA3. Volume measurements are potential candidates for outcome parameters in clinical trials, but larger cohorts are needed to better assess the accuracy of the different imaging parameters.

Unusual age-dependent behaviour of Leukocyte Telomere Length in Friedreich's ataxia

Friday, 15th November - 12:00: (Trinity & Goodmans Suite) - Flash Talk

Dr. Suran Nethisinghe [1], ***Dr. Daniela Scarabino*** [2], ***Dr. Liana Veneziano*** [3], ***Dr. Elide Mantuano*** [3], ***Dr. Nita Solanky*** [1], ***Dr. Alessia Fiore*** [4], ***Dr. Giulia Granata*** [4], ***Dr. Ginevra Zanni*** [5], ***Dr. Francesca Cavalcanti*** [6], ***Dr. Rosa Maria Corbo*** [4], ***Prof. Paola Giunti*** [1]

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Background: Friedreich's ataxia (FRDA) is an autosomal recessive neurodegenerative disorder caused by an expanded GAA repeat in the first intron of the *FXN* gene. We aim to identify a novel biomarker.

Methods: We investigated Leukocyte Telomere Length (LTL) in a cohort of FRDA homozygous patients (n=61) and heterozygous carriers (n=29), comparing them with age-matched healthy subjects and 12 compound heterozygous patients.

Results: The results showed that prior to 35 years of age, leukocyte telomeres were longer in patients than in controls, while the reverse applies in patients above 36 years of age. Interestingly, LTL was greater than controls at any age in heterozygous carriers. This picture mirrors what has been previously observed *in vitro* in FRDA cultured fibroblasts, showing significantly longer telomeres at early passages due to activation of an Alternative Lengthening of Telomeres (ALT)-like mechanism but showing accelerated telomere shortening as population doubling increases. The relationship of LTL with clinical parameters (cardiomyopathy, diabetes, dependence on a wheelchair) was also analyzed. Significantly shorter leukocyte telomeres were associated with the presence of cardiomyopathy but not with diabetes and the dependence on a wheelchair.

Conclusion: Overall, the present study indicates that telomere length analysis in FRDA may be a relevant biomarker to follow the stages of the disease and its clinical evolution.

Cerebellum as geometrical object: Investigating SCA disease patterns in cerebellar shape analysis using graph neural networks and explainable AI

Friday, 15th November - 12:07: (Trinity & Goodmans Suite) - Flash Talk

Mr. Philipp Wegner [1], ***Mr. Jahn Theisen*** [1], ***Ms. Mónica Ferreira*** [1], ***Prof. Thomas Klockgether*** [2], ***Dr. Jennifer Faber*** [2]

1. *German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany,* ***2.*** *German Center for Neurodegnerative Diseases (DZNE)*

Objectives

Spinocerebellar Ataxia Type 3 (SCA3) features brainstem and cerebellar atrophy. Cortical surface alterations may serve as imaging biomarkers. Inspired by cortical shape analysis in the whole brain, this study used graph neural networks to analyze cerebellar surface features, aiming to classify healthy, pre-symptomatic, and symptomatic SCA3.

Methods

In a cohort of 167 participants of ESMI we obtained 368 T1-weighted scans of 111 healthy controls (HC), 73 pre-ataxic (preSCA3), and 184 ataxic SCA3 (SCA3) mutation carriers. Using CerebNet, we reconstructed cerebellar gray matter and triangulated the surface. The resulting mesh was fed into a graph neural network (GNN) trained to distinguish HC, preSCA3, and SCA3. Subsequently, we used an explainable AI approach to highlight the crucial areas for classification.

Results

The GNN could distinguish between HC and preSCA with a ROC-AUC of 62.7%, between preSCA and SCA with 96.9%, and between HC and SCA with 97.2%. The crucial areas identified by the explainable AI efforts were the fissures in motor function-related areas, mainly within the anterior lobe.

Discussion

We used GNNs to capture geometric alterations from cerebellar gray matter atrophy in SCA3. The model showed promising but not yet satisfying classification for HC vs. preSCA. However, it almost perfectly classified preSCA vs. SCA, and HC vs. SCA, aligning with known cerebellar atrophy in later disease stages. The explainable AI approach allowed to gain insight into the most important surface areas, that were overlapping with the known somatotopology of motor functions in the anterior lobe.

Conclusion

Geometric alterations in the cerebellar cortex surface may serve as imaging biomarkers in advanced SCA3. Successful preSCA vs. SCA classification encourages further exploration of the cerebellum using shape analysis methods.

Responsiveness and predictive value of biomarkers in SCA1: Insights from a two-year multimodal longitudinal study

Friday, 15th November - 12:14: (Trinity & Goodmans Suite) - Flash Talk

Mr. Teije van Prooije [1]***, Ms. Kirsten Kapteijns*** [1]***, Dr. Sjaak van Asten*** [2]***, Prof. Tom Scheenen*** [2]***, Prof. Bart van de Warrenburg*** [3]

1. *Radboud University Medical Center; Donders Institute for Brain, Cognition and Behaviour; Department of Neurology; Nijmegen, The Netherlands,* ***2.*** *Department of Medical Imaging, Radboud University Medical Center,* ***3.*** *Department of Neurology, Donders Institute for Brain, Cognition, and Behavior, Radboud University Medical Center*

Objective To identify sensitive progression markers in SCA1 and explore the relationship between MR changes and subsequent changes in Clinical reported outcome measures (ClinRO) and patient reported outcomes (PROMs)
Methods We conducted a two-year longitudinal study with 33 symptomatic SCA1 patients and 20 controls. ClinRO, PROMs, neuro-imaging (MRI/MR spectroscopy), and biofluids (plasma/CSF) were collected at baseline, year 1 and year 2. Standardized response means (SRMs) were calculated for all outcomes and compared after one and two years. Sample size estimates for hypothetical trials were computed for promising markers. We also explored the relationship between MR changes after one year and ClinRO/PROM changes at two years.
Results Several MR markers were more responsive than SARA after one and two years, with pontine volume being the most sensitive for capturing disease progression. Sample size calculations showed that a one-year trial, powered to detect a 50% reduction in pontine volume change, would need 19 SCA1 patients per arm, compared to 116 per arm to detect a 50% reduction in SARA change. Estimates further decreased for a two-year trial: 5 patients per arm for pontine volume change and 34 patients per arm for SARA change. Strong correlations were found between changes in multiple MR markers after one year and SARA or ADL changes after two years, including a strong correlation between delta pontine volume after one year and delta SARA after two years ($\rho = -0.5$) and a strong correlation ($\rho = 0.5$) between delta cerebral cortical volume after one year and declining ADL scores after two years.
Discussion and conclusion This study highlights pontine volume as a highly sensitive progression marker and aids in selecting sensitive outcome markers and trial duration in interventional trials. It also provides the first anchoring of short-term MR changes to longer-term ClinRO and PROMs changes.

How to improve statistical power in a trial with SCA2 patients

Friday, 15th November - 12:21: (Trinity & Goodmans Suite) - Flash Talk

Ms. Maylis Tran [1], ***Mr. Pierre-Emmanuel Poulet*** [1], ***Ms. Cécile Di Folco*** [1], ***Dr. Giulia Coarelli*** [2], ***Prof. Alexandra Durr*** [2], ***Prof. Thomas Klockgether*** [3], ***Dr. Heike Jacobi*** [4], ***Dr. Tetsuo Ashizawa*** [5], ***Dr. Sophie Tezenas du Montcel*** [1]

1. *ARAMIS, Sorbonne Université, Institut du Cerveau-Paris Brain Institute-ICM, CNRS, Inria, Inserm, AP-HP, Groupe Hospitalier Sorbonne Université, Paris, France,* ***2.*** *Sorbonne Université, Institut du Cerveau - Paris Brain Institute- ICM, Inserm, Inria, CNRS, APHP, University Hospital Pitié-Salpêtrière,* ***3.*** *German Center for Neurodegnerative Diseases (DZNE),* ***4.*** *Department of Neurology, University of Heidelberg,* ***5.*** *The Houston Methodist Research Institute, Houston, TX 77030, USA*

Background and objectives: ATRIL study showed that riluzole did not improve clinical outcomes (Coarelli et al., 2022). SARA scores showed indeed a median increase (ie, worsening) of 0.5 points (IQR –1.5;1.5) in the riluzole group versus 0.3 points (–1.0;2.5) in the placebo group (p=0.70). Our objective was to use recent advancements in statistical methodologies to improve the trial analysis.

Methods: SCA2 patients from different cohorts of the US and European centers were selected in a longitudinal dataset (EUROSCA, SPATAX, CRC-SCA, RISCA), to train a progression model capturing the natural disease development in untreated patients (Disease Course Mapping using Leaspy python library). Using the prognostic score predicted by this model, we applied prognostic covariate adjustment and prediction-powered inference (PPI++) to enhance the power of the ATRIL clinical trial, involving both treated and placebo SCA2 patients. In contrast, matching-adjusted indirect comparison (MAIC) enhances analysis by adjusting baseline characteristics between treatment groups rather than leveraging prognostic scores.

Results: Our progression model accurately forecasted prognostic scores for SCA2 patients, providing insights into disease progression trajectories. The variance of the average treatment effect decreased more with PPI++ than with prognostic covariate adjustment, in comparison to the classical method, allowing a gain in power for the treatment comparison. Incorporating the prognostic score among the covariates allowed to reduce the sample size, while maintaining the results validity.

Discussion and Conclusion: Our study addresses the dual challenge of identifying the optimal longitudinal model for predicting disease progression and improving the conclusions of the ATRIL trial through statistical methods incorporating natural disease change predictions. The methods tested aim to reduce confidence intervals, improve power and decrease the required sample size for the clinical trial while maintaining the results validity. This approach holds promise for refining treatment efficacy assessments and facilitating informed clinical decisions in ataxia management.

Late-breaking research

Frataxin loss in animals is rescued by intra-complex mutations in the mitochondrial iron sulfur cluster biosynthesis machinery

Friday, 15th November - 12:30: (Trinity & Goodmans Suite) - Oral Presentation

Joshua Meisel [1], ***Pallavi Joshi*** [1], ***Amy Spelbring*** [2], ***Hong Wang*** [1], ***Sandra Wellner*** [1], ***Presli Wiesenthal*** [1], ***Maria Miranda*** [1], ***David Barondeau*** [2], ***Gary Ruvkun*** [1], ***Dr. Vamsi Mootha*** [1]

1. *Harvard Medical School and Massachusetts General Hospital,* ***2.*** *Texas A&M University*

Objectives Frataxin is an essential component of mitochondrial iron sulfur cluster (ISC) biosynthesis, allosterically activating the cysteine desulfurase NFS1. Reduced levels of Frataxin underly the human disorder Friedreich's ataxia (FA). Here, we aimed to identify genetic suppressors that bypass the need for Frataxin in animals.

Methods We conducted a forward genetic screen in *C. elegans* for mutations that allow Frataxin null animals to survive at a high, non-permissive oxygen tension. We identified causal mutations through whole genome sequencing, and validated our findings using *C. elegans* genetics, *in vitro* biochemistry, mammalian cell culture, and a mouse model of FA.

Results We found that specific amino acid substitutions at the interface of the ferredoxin FDX-2 and the cysteine desulfurase NFS-1 dominantly rescue the growth and development of Frataxin null *C. elegans*. The suppressor mutations boost ISC levels in the absence of Frataxin, likely through weakening the NFS1-FDX2 interaction. Conversely, over-expression of FDX2 inhibits NFS1 activity *in vitro* and blocks ISC synthesis in mammalian cell culture. Lowering levels of wild type FDX2 was also sufficient to rescue the growth of Frataxin null *C. elegans* and we are currently testing whether lowering FDX2 levels will rescue the ataxia of a mouse model of FA.

Discussion and Conclusion Recent work has shown that Frataxin and FDX2 use overlapping binding sites on NFS1 and are competitive inhibitors of each other. Our results reveal that in the absence of Frataxin, when FDX2 is constitutively bound to NFS1, ISC synthesis can be restored by lowering the amount of wild type FDX2 or by genetically perturbing the NFS1-FDX2 interaction. These results motivate knockdown of FDX2 as a potential therapy for Friedreich's ataxia.

Myeloid CRISPR/Cas-9 repeat editing as a therapeutic approach for cerebellar neurodegeneration in Friedreich's ataxia.

Friday, 15th November - 12:40: (Trinity & Goodmans Suite) - Oral Presentation

Carla Pernaci [1], ***Sydney Gillette*** [1], ***Ms. Avalon Johnson*** [2], ***Ms. Sammy Weiser Novak*** [3], ***Priyanka Mishra*** [1], ***Dr. Anusha Sivakumar*** [1], ***Prof. Stephanie Cherqui*** [1], ***Nicole G Coufal*** [4]

1. *University of California, San Diego,* ***2.*** *University of Barcelona,* ***3.*** *Salk Institute for Biological Studies, La Jolla, CA,* ***4.*** *Univeristy of California, San Diego*

Friedreich's ataxia is a devasting neurodegenerative disease caused by an aberrant GAA trinucleotide repeat expansion within the FXN gene. This leads to halted transcription and reduced levels of frataxin; a mitochondrial protein involved in iron-sulfur cluster and iron homeostasis. Its absence causes progressive muscle weakness, ataxia, and cardiomyopathy with no cure available. Neuroinflammation has been noted in FRDA, but whether dysfunction in microglia is a primary disease mediator or secondary to neurodegeneration has not been investigated. An enhanced understanding of cell type-specific contributions to FRDA could aid in identifying novel therapeutical targets. To this aim, we generated a unique cohort of human induced pluripotent stem cell (hiPSC) derived microglia and neurons from FRDA patients, carriers and healthy donors. To test the therapeutic potential of a gene editing approach, we applied a dual-guide CRISPR approach to correct the GAA repeat expansion in FRDA patient lines. We find that FRDA GAA repeats in microglia cause iron overload, excessive lipid peroxidation, elevated mitochondrial ROS, and reduced mitochondrial function eventually leading to a hyperinflammatory microglial profile *in vitro*. To untangle the microglial contribution to FRDA pathology, we used a 3D model wherein healthy neurons were co-cultured with diseased and gene-edited microglia and find that diseased microglia perturb healthy neuronal viability *in vitro* leading to neuronal blebbing and increased Caspase-3^{+} apoptotic neurons. Finally, we extended these findings *in vivo* wherein hiPSC-derived microglial progenitors are transplanted into neonatal humanized mice genetically depleted for endogenous murine microglia. We find that, FRDA microglia in the cerebellum are hyperinflammatory, accumulate in the Purkinje cell and reduce PC survival. All the above alterations were strongly attenuated by the GAA gene editing approach. Altogether, these findings identify a critical role for microglia in the pathogenesis of neurodegeneration in FRDA and suggest the therapeutic potential for a dual-guide gene editing strategy.

Comparative Effectiveness of Troriluzole versus Untreated Natural History Cohorts in Spinocerebellar Ataxia Leveraging Propensity Score Matching Methods

Friday, 15th November - 12:50: (Trinity & Goodmans Suite) - Oral Presentation

Dr. Gilbert L'Italien [1], Dr. Michele Potashman [1], Dr. Melissa Wolfe-Beiner [1], Ms. Basia Rogula [2], Ms. Lauren Powell [2], Ms. Victoria Wirtz [1], Dr. David Stock [1], Dr. Irfan Qureshi [1], Dr. Sheng-Han Kuo [3], Dr. Liana S. Rosenthal [4], Dr. Susan Perlman [5], Dr. Vlad Coric [1], Prof. Jeremy D. Schmahmann [6]

1. *Biohaven Pharmaceuticals, Inc.,* ***2.*** *Broadstreet HEOR,* ***3.*** *Columbia University Medical Center,* ***4.*** *Johns Hopkins University,* ***5.*** *University of California at Los Angeles,* ***6.*** *Department of Neurology, Massachusetts General Hospital and Harvard Medical School*

Objective: Examine the treatment benefits of troriluzole over 3 years in patients with Spinocerebellar Ataxia (SCA) by conducting a matched comparison of troriluzole-treated subjects vs untreated external controls.

Background: The SCAs are rare autosomal-dominant neurodegenerative diseases characterized by atrophy of the cerebellum and associated with severe disability and premature death. Study BHV4157-206-RWE (NCT06529146) examines troriluzole effectiveness slowing or delaying progression compared with untreated natural history subjects.

Design/Methods: Data on troriluzole treatment was obtained from BHV4157-206 (NCT03701399), a 48-week double blinded study with 3-year open-label extension. Three natural history cohorts were leveraged as comparators: CRC-SCA, EUROSCA, and a combined cohort selected from CRC-SCA/EUROSCA. Propensity score matching (PSM) between patient-level natural history data with troriluzole-treated subjects created equipoise, matching on baseline characteristics of: modified-functional Scale for the Assessment and Rating of Ataxia (f-SARA), genotype, CAG length, sex, age, and age of symptom onset. The between-group least squares (LS) mean change from baseline differences on f-SARA were derived at years 1, 2, and 3.

Results: Comparison of 101 troriluzole-treated subjects and 202 CRC-SCA subjects showed LS mean change differences in f-SARA of -0.45, -0.67, and -0.79 at years 1, 2, and 3, favoring troriluzole (all $p<0.005$). When compared with subjects from the EUROSCA dataset (N=85 troriluzole vs N=170 EUROSCA; SCA genotypes 1/2/3), LS mean change differences in f-SARA of -0.88, -1.39, and -1.75 were observed at Years 1, 2, and 3, favoring troriluzole (all $p<0.0001$). Results with the combined cohort were comparable. These results correspond to 50-70% slowing of disease progression (i.e., 1.5 to 2.2 years delay) for troriluzole-treated subjects, compared to the untreated external controls.

Conclusions: Compelling and sustained treatment effects were observed out to 3 years when troriluzole-treated subjects were compared to 3 different matched untreated natural history cohorts, supporting that long-term daily dosing of troriluzole attenuates the progression of disease among SCA subjects.

Poster session I: Biomarkers and clinical outcomes

Novel biomarker candidates for Fragile X-associated Tremor/Ataxia Syndrome

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Amy Krans [1], Dr. Bryan Killinger [2], Dr. Deborah Hall [2], Dr. Peter Todd [3]

1. *University of Michgan,* ***2.*** *Rush University Medical Center,* ***3.*** *University of Michigan*

Background and Objective:
Fragile X-associated tremor ataxia syndrome (FXTAS) is caused by a transcribed trinucleotide CGG repeat expansion in the 5' UTR of FMR1. CGG repeats drive neurodegeneration through formation of aberrant repeat RNA - RNA binding protein complexes and by triggering repeat-associated non-AUG initiated ("RAN") translation of toxic proteins, with the most abundant being a polyglycine protein known as FMRpolyG. FMRpolyG triggers inclusion formation within neurons and non-neuronal tissues. Despite recent advances, no effective therapies exist for this progressive fatal condition and no biomarkers exist that predict disease development or track disease progression.
Methods:
We used skin biopsies to assess for the abundance and molecular characteristics of inclusions in dermal cells as a potential biomarker of FXTAS. We also developed a meso-scale delivery (MSD) assay system to measure FMRpolyG quantitatively in model systems and patient derived samples.
Results:
We observed P62 and FMRPolyG positive intranuclear inclusions in skin biopsies taken from cases with FXTAS. These inclusions were present in multiple cell types (sweat glands and dermal fibroblasts), with ultrastructural features that align with inclusions reported in FXTAS brain samples. Using custom designed antibodies to FMRpolyG, we established an MSD assay with linear quantification into the femtomolar range with recombinant protein. This assay reliably measures FMRpolyG in brain samples from CGG repeat expressing transgenic mice and FXTAS patient derived brain samples with little or no signal in controls. Ongoing assays are being conducted in patient derived iNeurons, fibroblasts, and blood samples will discern the sensitivity and specificity of these measures as predictive and progressive biomarkers.
Discussion and Conclusion:
Both skin-biopsy based assays for FMRpolyG inclusions and MSD based measurement of FMRpolyG hold promise as biomarkers for disease prediction and progression, with relevance to future therapy development and clinical trials in FXTAS.

Foundation-collected Patient Data Provide Epidemiological Insights for Ataxias

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Lauren Moore [1], ***Dr. Kelsey Trace*** [1], ***Laura Crespo*** [1], ***Andrew Rosen*** [1]

1. *National Ataxia Foundation*

Objectives: Here we aimed to glean information about ataxia prevalence, demographics, and distribution through patient-level data collected by the National Ataxia Foundation (NAF), one of the largest ataxia non-profit patient organizations.
Methods: Patient data was collated from four foundation-affiliated datasets.

1. NAF Member Database (n≥7500 individuals who self-report as having ataxia).
2. The CoRDS Ataxia Patient Registry (n>2000).
3. CRC-SCA Natural History Study (n≥1000).
4. Required annual patient reporting data collected from 26 NAF Ataxia Centers of Excellence.

Results: Data will be presented on the number of unique patients and the regional population densities of a variety of the most prevalent ataxia disorders across these datasets. Derived distribution and incidence will be compared to published epidemiological reports on several of the most common forms of ataxia.
Discussion: Epidemiological research on ataxias faces many obstacles, such as difficulty and delay in diagnoses, limited longitudinal data sets and the lack of defined ICD codes for most ataxias. To date, the estimated prevalence of genetic ataxias has largely been defined through small, region-restricted genetic studies. Here, we improve upon our understanding of ataxia epidemiology through real-world, patient organization collected data that represents more than 7500 ataxia patients.
Conclusion: Patient organizations and the programs they sponsor are an important source for patient-level data that can provide critical information about rare disease epidemiology.

Modeling disease progression in spinocerebellar ataxias

Tuesday, 12th November - 18:10: (Minories) - Poster

Elisabeth Georgii [1], Prof. Thomas Klockgether [2], Dr. Heike Jacobi [3], Dr. Tanja Schmitz-Hübsch [4], Sheng Han Kuo [5], Dr. Tetsuo Ashizawa [6], Tim Elter [2], Marie Piraud [1], Dr. Jennifer Faber [2]

1. *Helmholtz AI, Helmholtz Munich, Germany,* ***2.*** *German Center for Neurodegnerative Diseases (DZNE),* ***3.*** *Department of Neurology, University of Heidelberg,* ***4.*** *NCRC-Neuroscience Clinical Research Center, Charité–Universitätsmedizin Berlin, corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, 10117 Berlin,* ***5.*** *Houston Methodist Research Institute, Houston, TX, US,* ***6.*** *The Houston Methodist Research Institute, Houston, TX 77030, USA*

Background and objectives: The disease course of the most common PolyQ spinocerebellar ataxias (SCA), SCA1, SCA2, SCA3 and SCA6 is characterized by progressive ataxia and additional neurological signs. To comparatively investigate determinants of disease progression, we analyzed clinical data of three-year time courses. The aim was to provide tailored marker candidates and prediction models to support type-specific clinical monitoring and trial design.

Methods: We examined co-occurrence patterns of deterioration events. Predicting disease progression was treated as a survival analysis problem.

Results: The data set contained 1538 subjects from five longitudinal cohorts (EUROSCA; RISCA; ESMI; SCA Registry; CRC-SCA) and 3802 visits. The pattern of progressive neurological symptoms varied with the SCA type. Mining of the progression data revealed the Scale for the Assessment and Rating of Ataxia (SARA) sum score to be the most representative descriptor of disease progression. We trained predictive models for the progression of each neurological symptom for each SCA type. The most universal predictors included the besides SARA sum score, gait and the CAG repeat length of the expanded allele. Beyond the data-driven characterization of relationships between observable features and symptom progression, deterioration in disease staging was studied: For the meaningful milestone of deterioration, (i) the need to use walking aids and finally (ii) the requirement to use a wheelchair, we discovered common as well as changing markers. For ease of interpretation, a decision tree was built including the main contributing clinical features and the resulting probability of progression within 3 years.

Discussion: Data-driven approaches are potent tools for identification of the main contributing features of progression prediction. Progression events for the disease stage were predictable from the baseline neurological status. Remarkably, a limited number of features had predictive importance, and only few were shared among all four SCA types, including gait and the SARA sum score.

Otolith organ function in CANVAS

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. David J. Szmulewicz [1]

1. *Balance Disorders & Ataxia Service, Royal Victorian Eye and Ear Hospital*

Purpose:
CANVAS is a disease which results in imbalance and incoordination due to the triad of cerebellar, vestibular and somatosensory impairment. Our previous research has found that the primary site of vestibular pathology in CANVAS is Scarpa's (vestibular) ganglia, noting that the end organs (both semicircular canals and otolith organs) remain unaffected. Ours and other groups have previously shown that in CANVAS the semicircular canal function is invariably reduced. This is putatively in keeping with the severe Scarpa's ganglionopathy. Here, we sought to understand whether otolith function was similarly affected to semicircular canal function in people with CANVAS.
Methods:
A multi-centre analysis of objective otolith organ function in 35 individuals with CANVAS was performed.
Results:
Analysis of Vestibular Evoked Myogenic Potentials (VEMP) in 35 people with CANVAS found that approximately half had normal results.
Conclusion:
This finding is of particular interest given afferent nerve fibres from both the otolith organs and the semicircular canals route via Scarpa's (vestibular) ganglia. Potential explanations include the different sensitivities of the objective modalities utilised to measure end organ function, and potential anatomical distinctions between afferent vestibular nerve fibres. Additionally, existing literature indicates that vestibular function parameters show promise as disease severity biomarkers.

Enhancing Ataxia Care in Non-Dominant Cultures: Reflections on patient care and research models

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Libby Massey [1], ***Ms. Janine Ryan*** [1], ***Ms. Alison Grootendorst*** [1], ***Dr. Rebecca Amery*** [2], ***Dr. David J. Szmulewicz*** [3]

1. *MJD Foundation,* ***2.*** *Charles Darwin University,* ***3.*** *Balance Disorders & Ataxia Service, Royal Victorian Eye and Ear Hospital*

Methods: Ethnographic, Descriptive.

Discussion The MJD Foundation provides comprehensive case management and support to Australian Aboriginal people living with SCA3/MJD and SCA7 across remote north Australia. These families are a minority culture in Australia, who face challenges accessing quality health care, resulting in well-documented health disparity. The additional burden of long-term, genetic degenerative disease confers an even higher risk of poor health outcomes. We have developed a model grounded in cultural competency, acknowledging the importance of understanding diverse worldviews and practices and addressing the social determinants of health to foster effective engagement and overcome barriers to access. The MJDF has implemented an integrated therapy program and recently commenced place-based multidisciplinary clinics led by a neurologist in remote communities.

Research is an important part of the MJDF integrated model, and we have conducted, partnered, and collaborated on research that considers sleep, rehabilitation, physical activity, strategies to maximise engagement, the collaborative development of aided augmentative and alternative (AAC) system, and the utility of assisted reproductive technology.

Conclusion/Results: This presentation will outline the model's clinical and research activities and outcomes, which serve as a blueprint for culturally responsive care and emphasises collaboration, empowerment, and inclusivity.

Australian Aboriginal Families living with MJD -Acceptability, Access and Equity: Issues associated with using Assisted Reproductive technology

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Libby Massey [1]

1. *MJD Foundation*

Qualitative methodologies including *Indigenous Standpoint* and *Grounded theory*,
designed to give primacy to the knowledge and lived experiences of Australian Aboriginal families living with Machado-Joseph Disease (MJD) in remote north Australia, are utilised to explore their understanding and perspectives of family planning and the use of assisted reproductive technology to manage genetic disease. The research explores the importance of worldview and health literacy on reproductive decision making.
Australian Aboriginal Families living with MJD report limited exposure to modern assisted reproductive technology. Women describe attitudes and understanding stratified by age. Older women recount traditional cultural concepts of family creation, including adherence to traditional marriage patterns along moiety lines. Middle-aged women also adhere to traditional relationship structures but describe awareness of mainstream Western/biomedical concepts learnt at school. Younger women have had lower school attendance and describe less strict adherence to traditional relationship structures and understanding of conception and pregnancy, reflecting both traditional and biomedical perspectives. All groups report limited working knowledge of invitro fertilisation and assisted reproductive technology.

Discussion and Conclusion

A biomedical Western worldview/framework dominates Australian approaches to the use of medically assisted reproduction to minimise disability. The fit with these families' worldviews requires careful consideration and the examination of standpoints from other cultural and sociological perspectives. This presentation will outline perspectives about family, marriage patterns, health literacy and the utility of the concept of disability for Aboriginal MJD families, which raise ethical, legal, strategic and public health issues related to the use or avoidance of technology and access to advice and support, which, although specific to this situation, will provide insights to guide the management of genetic disease in other culturally-diverse contexts.

Perspectives about Disease and Research Participation of Aboriginal Australians living with Machado-Joseph Disease in remote North Australia

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Libby Massey [1], ***Ms. Janine Ryan*** [1], ***Ms. Alison Grootendorst*** [1]

1. *MJD Foundation*

Objectives: This study explores the perspectives and knowledge of Australian Aboriginal individuals living with Machado-Joseph Disease (Spinocerebellar Ataxia Type 3) in isolated remote communities. Specifically, it sought to understand their views on participation in a rehabilitation trial and identify barriers and facilitators to engagement.
Methods: Using constructivist grounded theory principles, in-depth interviews and focus groups were conducted with thirty individuals from seven remote communities with lived experience of the disease. Data were thematically analysed to uncover insights into participants' understanding of their condition, motivations for research participation, and perceptions of the study design.
Results: Participants exhibited varied understandings of Machado-Joseph Disease, reflecting non-western/biomedical worldviews. While genetic inheritance was generally well understood, beliefs about onset and causation often aligned with traditional perspectives, attributing the disease to magical origins. Motivations for research participation included positive experiences with rehabilitation and a desire to contribute to disease management. The randomised research design was well accepted, with recommendations for maximising engagement emphasising culturally safe practices and flexible implementation protocols.
Discussion: The findings highlight the importance of considering worldview and contextual factors in research design and implementation, particularly in populations with diverse cultural backgrounds. Collaboration with participants and understanding underlying beliefs can enhance engagement and ensure the relevance of interventions.
Conclusion: Understanding the perspectives and motivations of Australian Aboriginal individuals living with Machado-Joseph Disease is crucial for designing culturally sensitive and effective research interventions. Incorporating culturally safe practices and fostering relationships with participants can maximise engagement and improve the translation of research findings into meaningful outcomes for affected communities.

Characterization of clinical serum cardiac biomarker levels in individuals with Friedreich ataxia

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Sonal Sharma [1], ***Prof. David Lynch*** [2], ***Ms. Kimberly Schadt*** [2], ***Ms. Medina Keita*** [2], ***Ms. Katherine Gunther*** [2], ***Ms. Courtney Park*** [1], ***Dr. Kimberly Lin*** [2], ***Ms. Cassandra Strawser*** [3], ***Ms. Lauren Hauser*** [3], ***Mr. Nathaniel Greeley*** [3], ***Mr. Patrick Hearle*** [3]

1. *Department of Pediatrics and Neurology, The Children's Hospital of Philadelphia,* ***2.*** *Children's Hospital of Philadelphia,* ***3.*** *Childrens Hospital of Philadelphia*

BACKGROUND AND OBJECTIVES

To characterize troponin values in Friedreich ataxia (FA) and establish correlation with phenotypic features. Friedreich Ataxia, a progressive neurodegenerative disorder, caused by homozygous Guanine-Adenine-Adenine (GAA) trinucleotide expansion in the frataxin gene is the most common inherited ataxia. Hypertrophic cardiomyopathy is common with premature mortality occurring due to cardiac failure or supraventricular arrhythmia. While troponin can be a marker of acute myocardial infarction, the significance of its elevation in FA is incompletely understood.

METHODS

We identified 950 troponin values collected between 9/28/2010 to 10/05/2023 in FA patients. Data included age, age of onset, sex, cTnI value, GAA1 repeat length, provoked (symptoms at time of draw) vs unprovoked status of values, cardiac status including echocardiography and electrocardiograms. Statistical analysis was performed using STATA SE version 11.1.

RESULTS

Mean age was 22.4 ± 13.7 yrs, representing a younger cohort with early age of onset (11.7 ± 8.5 yrs). Mean GAA1 length (666 ± 225) was longer than typical for FA. Mean cTnI value was 0.37 ng/ml. 36% of unprovoked and 70% of provoked cTnI samples were abnormal. Numerical troponin values were higher among provoked values. Age was predictive ($p < 0.001$), sex was marginally predictive ($p=0.025$) and GAA1 repeat length was not predictive ($p=0.876$) of abnormal troponin value at first visit. Absolute level of cTnI (analyzed as a log) significantly predicted future low ejection fraction and marginally predicted future cardiac death.

DISCUSSION AND CONCLUSION

Large number of FRDA patients carry elevations of cTnI without cardiac symptomatology. Unprovoked levels are substantially lower than provoked levels that occur in the context of a cardiac insult. Elevated cTnI levels are associated with younger age, female sex, and to some degree GAA1 length. Further studies to understand the molecular mechanisms relating to cTnI levels in FRDA are required.

FUNDING SOURCES

Friedreich Ataxia Research Alliance

Impact of foundation-sponsored Ataxia Clinical Training (ACT) program on health care professional (HCP) competence and confidence in ataxia care and diagnosis.

Tuesday, 12th November - 18:10: (Minories) - Poster

Mrs. Aimee Alcott [1], ***Dr. Kelsey Trace*** [1], ***Dr. Lauren Moore*** [1]

1. *National Ataxia Foundation*

Objective:

To assess the efficacy of completion of the Ataxia Clinical Training (ACT) program, a foundation-sponsored ataxia clinical course on proficiency and perceived competency of healthcare professionals (HCPs) in the clinical care of patients with ataxic disorders.

Background:

Diagnosing and treating ataxia-related disorders is challenging due to the high number and heterogeneity of ataxic disorders, rarity of individual cases, and lack of approved treatments. Despite these complexities, clinical training for ataxias is highly variable, and often minimal, amongst today's medical training programs.

Methods:

To address educational gaps in ataxia clinical training, the National Ataxia Foundation developed an in-person ataxia-focused educational program for HCPs that included interactive clinical training with ataxia patients and a series of didactic lectures by ataxia clinical experts on the diagnosis and comprehensive care of ataxic disorders. A pre- and post-participation ataxia comprehension exam and attendee survey were completed by attendees to assess prior experience, learnings, and perceived course efficacy.

Results:

A total of 85 HCPs completed the ACT in 2023-2024 (72 movement/neuromuscular/genetics fellows, 7 neurologists, 4 residents, 2 allied HCPs). Over 70% of fellows reported exposure to ≥20 ataxia patients during their medical training prior to ACT. Significant improvements in average and within subject ataxia comprehensive exam scores, as well as self-reported increases in attendee confidence, were achieved across matriculated HCPs.

Conclusions:

In its first two years, the ACT program achieved its goal of improving comprehension and confidence of HCPs in the treatment of ataxic disorders, suggesting that this course may fill critical gaps in the comprehensive training of movement disorder professionals.

Social network analysis in ataxia patients: exploring correlations with quality of life and functional outcomes.

Tuesday, 12th November - 18:10: (Minories) - Poster

Mr. James Concepcion [1]***, Dr. Amar Dhand*** [2]***, Prof. Jeremy D. Schmahmann*** [3]

1. *Massachusetts General Hospital Department of Neurology,* ***2.*** *Neurosciences Center, Department of Neurology, Brigham and Women's Hospital and Harvard Medical School,* ***3.*** *Department of Neurology, Massachusetts General Hospital and Harvard Medical School*

Background

Patients with inherited and acquired cerebellar disorders often experience fatigue, depression, impaired executive function, emotional processing difficulties, and psychosocial deficits. As the disease evolves, patients encounter increasing challenges maintaining functional independence, performing activities of daily living, and remaining socially engaged.

Social interactions and dynamics impact mental and physical health. An individual's personal social network is the web of relationships with others in everyday life. Analysis of social networks of an individual makes it possible to quantify how social context including network size (number of contacts), density (degree of interconnectedness), and composition (percentage of members in the network) influence a patient's wellbeing. There are no data regarding the role of social networks in the ataxia population. We hypothesized that social network metrics correlate with quality of life and functional ability in patients with neurodegenerative ataxias. We tested this for the first time in patients with cerebellar disorders.

Methods

We employed a cross-sectional five-survey design, using the Personal Network Survey for Clinical Research, World Health Organization Quality of Life-BREF, Functional Staging Scale for Ataxia, Functional Assessment Staging-ADL, and the Patient-Reported Outcome Measure of Ataxia. We conducted visits through Microsoft Teams with data entered into REDCap. We are now testing the correlations between social network size, density, composition and other metrics with the patient-reported outcomes.

Results

We have recruited 105 patients with 30 ataxia diagnoses, most commonly spinocerebellar ataxia types 1, 2, 3, 6, 7, 8, and 27B, ARSACS, CANVAS, MSA-C and Friedreich's Ataxia, and 17 other unique causes of ataxia.

Conclusion

This is the first investigation of relationships between social network dynamics and quality of life and disease severity in patients with cerebellar ataxia. This work has the potential to advance understanding of support systems that impact health outcomes, opening the way to novel interventions to improve patient health and well-being.

Coproducing a family-centred resource to support communication about children and young people with ataxia and the management of their associated health and care needs

Tuesday, 12th November - 18:10: (Minories) - Poster

Prof. Lisa Bunn [1], ***Ms. Munira Khan*** [1], ***Ms. Lisa Bunn*** [2]

1. *University of Plymouth,* ***2.*** *Various*

Objectives: Ataxia diagnosed in childhood presents evolving therapeutic need and requires carefully coordinated care across health services, education, third sector services and family life. Patient and public involvement and engagement (PPIE) revealed that families can feel burdened coordination of complex care and the amount of information necessary to repetitively share with professionals. This research project aimed to design a family-owned resource to promote person-centred care whist reducing the burden of self-management.

Methods: A qualitative research approach involving focus groups, with two children with complex ataxia and eight parents (predominantly ataxia telangiectasia), acted to determine key considerations for the pack. This study explored the views of families about the utility, acceptability, design, and content of this pack. Feedback cycles utilising PPIE served to refine two first edition resources: My A-T Pack, for those specifically with ataxia telangiectasia, and My A+ Pack, for those with a wider range of ataxia diagnoses. Data was analysed using the framework method, with themes and sub-themes inferred by connecting conceptually related ideas and categories.

Results: Three themes (and 21 sub-themes) were generated: 1) accessing, managing, organising, and sharing information with others, 2) pack content, and 3) design features.

Discussion: Participants shared important insights and views, which acted to direct the remit, design and functionality of the packs designed. A dedicated artist engaged in focus group sessions ensured that children with ataxia and their views were inclusively represented in attractive design features whilst the pack remained accessible and low cost to produce.

Conclusion: Free to download first edition packs now provide children and families with a new resource for effective record keeping, symptomatic management, and information sharing. Pack users have the option to share feedback to inform subsequent editions.

The neural mechanisms underlying cortical control of gait and cognition in Fragile X-Associated Tremor/Ataxia Syndrome (FXTAS)

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Emily Timm [1], ***Mr. Jack Paras*** [1], ***Dr. Deborah Hall*** [1], ***Dr. Joan O'Keefe*** [1]

1. *Rush University Medical Center*

Background: Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) is a neurodegenerative disorder characterized by cerebellar ataxia, tremor, and executive dysfunction that negatively impacts balance and gait, and increases fall risk. Previous studies have investigated the relationship between gait and cognition impairments in FXTAS; however, the neural mechanisms underlying these cognitive-motor relationships remain largely undescribed.
Objectives: Examine the cortical control of gait and cognition in FXTAS during simple and complex gait tasks and executive functioning assessments.
Methods: Six participants with FXTAS (69.9±6.5 years) and six healthy controls (66.8±7.4 years) performed gait testing under single-task, fast-paced, dual-task, and obstacle navigation conditions, then completed an extensive battery of neurocognitive tests. Simultaneous functional near-infrared spectroscopy (fNIRS) data was collected from the dorsolateral prefrontal cortex (DLPFC), premotor cortex (PMC), and supplementary motor area (SMA) during all assessments.
Results: A trend of increased activation in the DLPFC was observed in FXTAS participants versus controls under the single-task gait condition (p=0.073). FXTAS participants scored significantly lower on executive functioning assessments than controls, including the SDMT, Stroop, COWAT, and Serial-3 Subtraction scores were significantly lower in FXTAS versus controls (p=0.005–0.029), and nearly reached significance for the Auditory N-Back test (p=0.098); however, there were no significant differences in DLPFC activation during these neurocognitive assessments in FXTAS participants compared to controls.
Discussion: These preliminary data may indicate that more DLPFC resources are required in FXTAS during simple gait due to decreased automaticity. With an increased sample size, we anticipate discovering significantly reduced activation during executive functioning tests in FXTAS, which may indicate reduced maximum recruitment due to ceiling effects in the DLPFC.
Conclusion: Individuals with FXTAS may inefficiently recruit the DLPFC during simple gait, making it a potential target for rehabilitative therapeutics to improve mobility.

The Visually enhanced VOR (VVOR) in Spinocerebellar Ataxia Type 27B (SCA27B).

Tuesday, 12th November - 18:10: (Minories) - Poster

Prof. David Szmulewicz [1], ***Dr. Lewis Tang*** [2]

1. *3. Balance Disorders and Ataxia Service, Royal Victorian Eye and Ear Hospital, Bionics Institute/University of Melbourne, Victoria Australia.,* ***2.*** *Balance Disorders & Ataxia Service, Royal Victorian Eye and Ear Hospital*

Purpose:
SCA27B is a recently discovered autosomal dominant spinocerebellar ataxia. It is estimated to account for around 20% of inherited cerebellar disease and as such, is the most common inherited cause of ataxia. Since its discovery in late 2022, thousands of cases have been diagnosed globally. Whilst the range of clinical phenotypes are progressively elucidated, we note that a key oculomotor parameter, the visually enhanced vestibular ocular reflex (VVOR), has yet to be examined in this patient group.
Methods:
Video Head Impulse Test equipment (vHIT) was employed to quantitatively assess the VVOR in patients with a genetic diagnosis of SCA27B. Data was then analyzed to assess vestibular function including VOR gain and VVOR metrics across three frequencies,
which was then correlated with a number of key parameters including patient age, age at disease onset, disease duration and FGF14 GAA repeat expansion number.
Results:
Quantitative VOR and VVOR gain and saccades analysis revealed substantial abnormalities in both.
Conclusion:
We found prevalent abnormalities in VVOR in a cohort of people with SCA27B. This finding reflects a significant phenotypic combination, that of bilateral vestibular hypofunction and cerebellar impairment. This test may be carried out at the bedside, is non-invasive and requires only modest skill in its interpretation. The value in this discovery is manifold, an abnormal VVOR narrows the number of differential diagnoses significantly, it offers non-invasive insight into the underlying pathology, is a potential quantitative metric of disease progress and biomarker in treatment trials.

Retinal Nerve Fiber Layer Metrics as Biomarkers for Functional Outcomes in Friedreich Ataxia: Insights from a Czech Cohort

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Lucie Stovickova [1], ***Dr. Libor Eichenmann*** [2], ***Ms. Katerina Lebedova*** [2], ***Mr. Filip Kolman*** [3], ***Mr. David Nemecek*** [3], ***Ms. Alexandra Mizikova*** [3], ***Mr. Martin Simcik*** [3], ***Ms. Katerina Stanzelova*** [3], ***Dr. Alena Zumrova*** [4]

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Methods: We conducted a comprehensive analysis of retinal nerve fiber layer (RNFL) metrics in a cohort of Friedreich ataxia (FA) patients from the Czech Republic. Using optical coherence tomography (OCT), we measured average RNFL thickness and correlated these findings with various functional outcomes, including activities of daily living (ADL), the Friedreich Ataxia Rating Scale (FARS), the modified Friedreich Ataxia Rating Scale (mFARS), mobility measured by the Spinocerebellar Ataxia Functional Index (SCAFI), Montreal Cognitive Assessment (MoCA), Scale for the Assessment and Rating of Ataxia (SARA), disease duration, and other relevant clinical metrics.
Results: Significant correlations included:

- mFARS (max. 93): A negative correlation ($r = 0.351$, $p < 0.05$, $n = 16$), greater RNFL thinning is associated with higher disability.
- SARA (max. 40): A positive correlation ($r = 0.278$, $p < 0.0001$, $n = 49$), RNFL thinning is linked to more severe ataxia symptoms.
- Timed 8-meter walking test: A positive correlation ($r = 0.214$, $p = 0.0008$, $n = 49$), better-preserved RNFL thickness is associated with greater mobility.
- Muscle atrophy in lower limbs: A positive correlation ($r = 0.373$, $p < 0.0001$, $n = 49$), RNFL thinning correlates with increased muscle atrophy.
- Permanent wheelchair use: A positive correlation ($r = 0.545$, $p < 0.0001$, $n = 32$), patients with thinner RNFL tend to require wheelchair use earlier.

Discussion: The findings from our Czech cohort align with the recent study by Rodden et al. (2023), which reported retinal hypoplasia and degeneration as contributing factors to vision loss in FA. Our data extend these observations by linking RNFL metrics to broader functional outcomes. The significant correlations between average RNFL and clinical scales underscore the potential of RNFL measurements as non-invasive biomarkers for disease severity and progression in FA.
Conclusion: Further research should explore longitudinal changes in RNFL and their relationship with disease-modifying therapies.

Artificial intelligence applied to motion-capture data in Friedreich's ataxia outperforms clinical scales in monitoring disease progression

Tuesday, 12th November - 18:10: (Minories) - Poster

Prof. Richard Festenstein [1], ***Dr. Balasundaram Kadirvelu*** [1], ***Dr. Constantinos Gavriel*** [1], ***Dr. Sathiji K. Nageshwaran*** [2], ***Dr. Jackson (Ping Kei) Chan*** [1], ***Dr. Suran Nethisinghe*** [3], ***Dr. Stavros Athanasopoulos*** [1], ***Dr. Valeria Ricotti*** [4], ***Dr. Thomas Voit,*** [4], ***Prof. Paola Giunti*** [5], ***Prof. Aldo Faisal*** [1]

1. *Imperial College London,* ***2.*** *Neurogenetics Program, Department of Neurology, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA, USA.,* ***3.*** *UCL Queen Square Institute of Neurology,* ***4.*** *UCL,* ***5.*** *University College London*

Background

Friedreich's ataxia (FA) is caused by a mutated *Frataxin* (*FXN*) gene, leading to its downregulation and progressively impaired cardiac and neurological function. Current gold-standard clinical scales (SARA, FARS) use behavioural assessments, which require 12- to 24-month-long trials to measure disease progression. There is a pressing need for more patient-relevant and accurate methods to reduce the prohibitively large number of patients required for efficacy trials of novel treatments.

Methods

We captured full-body movement kinematics from patients with wearable sensors, enabling us to define digital behavioural features based on the data from nine FA patients (six females and three males) and nine age- and sex-matched controls, who performed the 8-m walk (8-MW) test and 9-hole peg test (9 HPT) as well as activities of daily living in a mock-up of a one bedroom flat.

Results

We used artificial intelligence to combine these features to predict the clinical scores of the FA patients, and compared with standard clinical assessments, the Spinocerebellar Ataxia Functional Index (SCAFI) and the Scale for the Assessment and Rating of Ataxia (SARA). The digital behavioural features enabled longitudinal predictions of personal SARA and SCAFI scores 9 months into the future and were 1.7 and 4 times more precise than longitudinal predictions using only SARA and SCAFI scores.

Discussion and Conclusion

Our work demonstrates how data-derived wearable biomarkers can track personal disease trajectories and indicates the potential of such biomarkers for substantially reducing the duration or size of clinical trials testing disease-modifying therapies. The development of a digital biomarker of activities of daily living is ongoing - and promises to provide a reliable assessment of changes in movement more relevant to the patients' normal activities outside the hospital environment.

Evaluating outcome measures of gait and balance in adults with Primary Mitochondrial Disease and Spinocerebellar ataxia type 6.

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Lisa Alcock [1], ***Dr. Aye Moe*** [2], ***Dr. Jane Newman*** [2], ***Dr. Silvia Del Din*** [1], ***Prof. Grainne Gorman*** [2], ***Dr. Yi Shiau Ng*** [2]

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INTRODUCTION: Two-thirds of adults with primary mitochondrial disease (PMD) demonstrate cerebellar dysfunction (unsteady gait, lack of co-ordination). Laboratory gait and balance assessments are emerging as promising tools to characterise and track gait changes in cerebellar ataxia. Understanding how gait and balance differ due to pathology will enable the development of targeted therapeutics.

METHODS: 67 participants were recruited to the Ataxia in Mito study (Controls n=21, PMD n=34, Spinocerebellar ataxia type 6; SCA6 n=12).

The following clinical outcomes were obtained: Newcastle Mitochondrial disease Assessment Scale, Scale for the Assessment and Rating of Ataxia, International Cooperative Ataxia Rating Scale, Functional Gait Assessment, Falls efficacy scale, Activities Balance Confidence Scale.

6x 10-metre intermittent walks were completed (self-selected pace). Temporal-spatial and gait variability outcomes were extracted (instrumented walkway; n=12).

Two 60-second stands were completed with feet 10-cm apart, eyes open. Balance outcomes were extracted (force platform; n=8).

Statistical between-group differences (Kruskal-Wallis test, Bonferroni adjusted p-value) and discriminative capacity (ROC curve, Area under the curve; AUC) were evaluated.

RESULTS: All clinical scores were significantly different between controls and patients (PMD and SCA6) but not between patients (PMD vs. SCA6).

For gait, 10/12 outcomes were significantly different for controls vs. PMD compared to 9/12 outcomes which were significantly different for controls vs. SCA6. Only step width variability was significantly higher in PMD vs. SCA6 (AUC=75%).

For standing balance, 5/8 outcomes were significantly different for controls vs. PMD compared to 4/8 outcomes which were significantly different for controls vs. SCA6. Sway area (centre of pressure) and force range in the medio-lateral and anterior-posterior planes of motion were significantly higher in PMD vs. SCA6 (AUC>81%).

CONCLUSION: Discrete gait and balance outcomes are selective to the presence of ataxia in PMD and SCA6 and were able to discriminate pathology. These findings will inform outcome selection for future clinical trials.

Cardiac MRI in Friedreich's ataxia young adults: exploring new biomarkers

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Vito Luigi Colona [1], Dr. Nicoletta Cantarutti [2], Dr. Susanna Summa [1], Dr. Marianna Cicenia [2], Dr. Enrico Bertini [3], Dr. Sara Di Marzio [2], Dr. Martina Favetta [1], Dr. Rachele Adorisio [2], Dr. Aurelio Secinaro [4], Dr. Gessica Vasco [1]

1. *Department of Neurorehabilitation and Robotics, Bambino Gesù Children's Hospital, IRCCS, Rome,* ***2.*** *Pediatric Cardiology and Cardiac Arrhythmias Complex Unit, Neonatal and Cardiological Area, Bambino Gesù Children's Hospital, IRCCS, Rome,* ***3.*** *Bambino Gesù Children's Hospital, IRCCS, Unit of Muscular and Neurodegenerative Disorders,* ***4.*** *Advanced Cardiothoracic Imaging Unit, Department of Imaging, Bambino Gesù Children's Hospital, IRCCS, Rome*

Objectives: Friedreich's ataxia (FRDA) is a progressive mitochondrial disorder. It primarily affects the neural pathways, and also the heart muscle. Cardiac involvement occurs in 60% of cases, leading to cardiac impairment. In 30% of cases, it progresses to heart failure (HF), which is the leading cause of death among affected patients. The heart involvement is a multistep process. It begins as non-obstructive concentric cardiomyopathy of the left ventricle (LV). Over time, there is continuous fibrotic replacement of the tissue. This eventually results in dilated dysfunctional cardiomyopathy in the end stage. Echocardiography (Echo) is the gold standard for follow-up in patients. However, cardiac MRI (cMRI) can offer additional valuable information for their management.

Methods: A cMRI with contrast was conducted on a cohort of 22 FRDA patients (10 males and 12 females), with an average age at diagnosis of 8.8±3.3 years (range 4-16 years), and a disease duration of 11.6±7.4 years (GAA1 799.5±403.1). All these patients were diagnosed with hypertrophic cardiomyopathy (HCM). Subsequently, correlations were established between neurological metrics with serum parameters, Echo and cMRI indices (volume, masses, tissue remodeling and functionality).

Results: No correlation with serum parameters was found. Regarding Echo, correlation between disease burden (DB) and LVDs was significant (p=0.003). Finally, LVEF% correlated with DB (p=0.001) and SARA (p=0.03), ECV% with GAA1 (p=0.02), and all LGE parameters with DB (p=0.02), in cMRI.

Discussion: The diagnostic refinement through cardiac MRI could thus become a crucial tool in providing a more detailed characterization of cardiac tissue throughout the disease progression. Furthermore, it might identify specific endpoints for the various stages of cardiac dysfunction, potentially serving as valuable biomarkers for evaluating therapeutic efficacy in the future.

Conclusions: cMRI provide parameters of tissue remodeling correlated with disease progression and severity.

Multiomics approach leads to the identification of SERPINB1 as a candidate progression biomarker for Spinocerebellar Ataxia type 2

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Luis Enrique Almaguer-Mederos [1], ***Dr. Jana Key*** [1], ***Dr. Nesli-Ece Sen*** [1], ***Dr. Julia Canet-Pons*** [1], ***Dr. Claudia Döring*** [1], ***Dr. David Meierhofer*** [2], ***Dr. Suzana Gispert-Sánchez*** [1], ***Mr. Dany Cuello-Almarales*** [3], ***Mr. Dennis Almaguer-Gotay*** [3], ***Dr. Georg Auburger*** [1]

1. *Faculty of Medicine, Goethe University,* ***2.*** *Max Planck Institute for Molecular Genetics,* ***3.*** *Center for the Investigation and Rehabilitation of Hereditary Ataxias*

Background. Spinocerebellar ataxia type 2 (SCA2) is a polyglutamine disorder, and variants in its disease protein Ataxin-2 act as modifiers in the progression of Amyotrophic Lateral Sclerosis. There are no reliable molecular progression biomarkers for SCA2. **Methods.** Using cerebellar and cervicothoracic spinal cord RNA from *Atxn2*-CAG100-KnockIn and wildtype mice, global transcriptome studies were conducted. Extracted proteins were analyzed by LC-MS/MS for global proteomics, and Immobilized Metal Affinity Chromatography for phosphoproteomics. Validation documented expression by RT-qPCR, and protein abundance by quantitative immunoblots and ELISA (LS Bio). **Results.** Venn diagram comparisons across all OMICS datasets indicated that only *Serpinb1a*-transcript, SERPINB1A-protein and -phosphopeptides were consistently downregulated at terminal stage in 14-month-old KnockIn mice. Expression studies in cerebellum and spinal cord from 10 weeks (pre-manifest), 6-month-old (early ataxic), and 14-month-old (late ataxic stage) mice confirmed a progressive decrease of *Serpinb1a* and SERPINB1A, from 67% initially to 15% at end-stage. Very preliminary results obtained in ten SCA2 patients *versus* 14 age- and sex-matched controls showed patients' SERPINB1 plasma levels to be lowered without significance, but to display a trend towards association with CAG repeat length at expanded *ATXN2* alleles. Studies of additional patients and controls are underway. **Discussion.** SERPINB1 shows anti-protease and endonuclease activities, through the inhibition of secreted neutrophil elastase and proteinase-3, but also of lysosomal cathepsin G and granzyme H. It has been implicated in the modulation of innate immunity, apoptosis, vesicular trafficking, and autophagy. Previous reports established that these biological processes are relevant to SCA2 pathogenesis. **Conclusion.** The progressive downregulation of *Serpinb1a*/SERPINB1A in the nervous system identifies a dysregulated upstream proteostasis dysregulation mechanism, which appears prior to aggregate formation, macroautophagy, locomotor deficits, and apoptosis. Its specificity for SCA2 suggests a potential use as molecular biomarker in therapy studies of patient groups. Further studies are needed to clarify its translational value in humans.

Patient perception of walking ability and balance confidence in relation to symptoms of ataxia

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Tanja Schmitz-Hübsch [1], ***Mr. Andreas Rösch*** [2], ***Dr. Pia Sophia Sperber*** [2], ***Dr. Lina Carlotta Anderhalten*** [2], ***Dr. Eva-Maria Dorsch*** [2], ***Dr. Rebekka Rust*** [2], ***Mr. Adam Berlijn*** [3], ***Dr. Andreas Thieme*** [4], ***Prof. Jutta Peterburs*** [5], ***Prof. Andrea Kühn*** [6], ***Prof. Friedemann Paul*** [7], ***Prof. Dagmar Timmann*** [4], ***Dr. Astrid Nümann*** [6], ***Dr. Martina Minnerop*** [8]

1. *NCRC-Neuroscience Clinical Research Center, Charité–Universitätsmedizin Berlin, corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, 10117 Berlin,* ***2.*** *Experimental and Clinical Research Center, a cooperation of Max Delbrueck Center of Molecular Medicine and Charité, Berlin,* ***3.*** *Institute of Experimental Psychology, Heinrich-Heine-University Dusseldorf,* ***4.*** *Department of Neurology and Center for Translational Neuro- and Behavioral Sciences (C-TNBS), University Hospital Essen, University of Duisburg-Essen, Duisburg, Germany,* ***5.*** *Institute of Systems Medicine and Department of Human Medicine, MSH Medical School Hamburg, Hamburg, Germany,* ***6.*** *Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Neurology, Berlin, Germany,* ***7.*** *Experimental and Clinical Research Center, a cooperation of Max Delbrueck Center of Molecular Medicine and Charité - Universitätsmedizin Berlin, Berlin,* ***8.*** *Institute of Neuroscience and Medicine (INM-1), Research Centre Jülich, Jülich, Germany*

Background and objectives: Patient-based assessment has been established as a mandatory component in clinical efficacy trials. Patient-reported outcomes (PROs) can be easily applied remotely and may allow for cost-effective monitoring of disease course. However, criteria for selecting appropriate PROs for a given condition and context are not well described. Methods: Here, we explore the utility of PROs in patients with symptoms of ataxia. This included PROs specific to walking ability (MSWS-12) and balance self-confidence (ABC) – both constructs of known relevance in ataxia disorders – and a generic PRO of general health perception (PROMIS global health) as well as maximum gait speed as a commonly used performance outcome in ataxias. Analysis contrasted patient groups with ataxia symptoms (multiple sclerosis (MS-ATX n=31) and neurodegenerative ataxias (ATX n=41)) with a group of MS patients without ataxia symptoms (MS-NATX n=131) as well as 45 healthy controls (HC, no data on ABC). Validity was explored by correlation to clinical and performance measures of ataxia severity (SARA-score) and diagnostic precision by receiver operator curve (ROC) analysis. Results: In patients with mild to moderate ataxia (SARA 0-24), both symptom-specific PROs showed high variance with no single individual scoring unimpaired, while MS patients without ataxia clustered at values indicating no difficulty with walking or balance confidence. Correlations with SARA and maximum speed were remarkable for MSWS-12 (r=.709 and -.647) and moderate for ABC (r=-.560 and .468). ROC-analysis indicated that all PROs except for PROMIS global mental health were able to discern the presence of ataxia among patients with MS (AUC .811 to .866) with highest discriminatory ability for MSWS-12. Conclusion: Though not developed for ataxia cohorts, this first exploration of PROs on walking ability and balance confidence indicates their potential to define manifestation and severity of ataxia symptoms.

Speech Therapy for People with Progressive Ataxia: A Process Evaluation of Patient and Clinician Expectations and Experiences

Tuesday, 12th November - 18:10: (Minories) - Poster

Prof. Anja Lowit [1], ***Ms. Phoebe Ashworth*** [1], ***Prof. Marios Hadjivasssiliou*** [2]

1. *Strathclyde University,* ***2.*** *Sheffield Ataxia Centre*

Introduction: Clinical interventions frequently encounter substantial barriers when seeking adoption into healthcare provision despite their well-documented effectiveness (Kirchner et al., 2020). This has prompted increased research aimed at understanding the factors that facilitate the uptake of evidence-based practises (Moullin et al., 2015). Conducting a process evaluation can ensure all stakeholder needs are considered and addressed during intervention development, that the mechanisms by which the intervention works are clearly understood, and that relevant outcomes are considered.

This poster presents the outcomes of a process evaluation of a novel mixed individual / group therapy approach (ClearSpeechTogether (CST), Lowit et al. 2022). CST is a six week programme that focuses on two strategies - Loud and Clear. It involves four individual therapist led sessions over two weeks, followed by four weeks of intensive, daily group practice which is patient led. This study aimed to explore the requirements and expectations of both patients and clinicians in relation to an intervention that can be successfully integrated into existing healthcare systems and addresses patient needs.

Methods: We conducted semi-structured interviews with 17 patients immediately post-treatment. Inductive thematic analysis was employed to capture the participants' experiences and perspectives of the intervention. A logic model was then developed based on these findings. The model was subsequently refined by conducting follow-up focus groups, consisting of eight participants from the CST trials and two NHS speech and language therapists (SLTs).

Results: There was wide agreement between participants that speech treatment was important in progressive ataxias, and that CST was successful in improving speech performance, and more importantly, communication participation and confidence.

Discussion: The process evaluation highlighted which aspects of the intervention were a priority for patients as well as SLTs. The information will now be used as the basis for designing a larger RCT to demonstrate effectiveness of the approach.

Understanding the impact of Friedreich ataxia on French patients' daily life

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Juliette Dieusaert [1], Dr. Cecilia Estrella [1], Mrs. Caroline Gaboriau [1], Mr. Jean-François Joguet [1], Mrs. Sandra Lechene [1], Mr. Stéphan Rouillon [1]

1. *AFAF French Friedreich Ataxia Patients Association*

Background and Objectives

Friedreich ataxia (FA) is the most common inherited ataxia in Europe. About 1,500 people have been diagnosed with FA in France. The French Friedreich Ataxia Patients Association (AFAF) conducted a survey with the aim of understanding the FA impact on French patients' everyday life, their needs, priorities and expectations.

Methods

FA patients, families and caregivers, were invited to answer an online survey between December 2022 and January 2023. Close and open-ended questions were included.

Results

A total of 209 responses were received, including 103 from FA patients aged 10 to 76 years. Most responses were from patients aged of 30 to 40 years (22%) who were diagnosed in their teens. The age of onset was between 8 and 14 years in 35% of patients, although 16% showed an early onset (<7 years). More than 50% of patients were diagnosed after 2 years, but almost 25% in 1 year or less.

Results showed a geographical distribution throughout France, with 20% of FA patients living in the Ile-de-France region (Paris and its surrounding area). Most patients (73%) live on their own or with a partner, 29% with their parents; 60% use a wheelchair and 15% walk without assistance.

Overall, 60% of patients benefit from regular multidisciplinary medical care, but 20% are not interested or do not have access to a FA competent service close to their home. Additionally, 44% of patients are treated with Idebenone and only 16% report not to take any medication.

Discussion and Conclusion

Problems faced by FA patients and their impact on daily life were highlighted. FA still takes too long to be diagnosed. External caregivers need to be better trained to handle their needs. This work has enabled us to identify paths to improve AFAF services for the FA community.

Work funded by AFAF

Longer, less frequent video-based training units seem more effective than shorter but more frequent units in patients with cerebellar ataxia

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Clara Rentz [1], ***Ms. Alisha Reinhardt*** [1], ***Ms. Naomi Jung*** [1], ***Prof. Berwin Turlach*** [2], ***Dr. Mehran Sahandi Far*** [3], ***Prof. Jutta Peterburs*** [4], ***Dr. Maik Boltes*** [5], ***Dr. Heike Jacobi*** [6], ***Prof. Dagmar Timmann*** [7], ***Dr. Andreas Thieme*** [7], ***Mrs. Doris Brötz*** [8], ***Prof. Jürgen Dukart*** [3], ***Prof. Alfons Schnitzler*** [9], ***Prof. Katrin Amunts*** [1], ***Dr. Martina Minnerop*** [1]

1. *Institute of Neuroscience and Medicine (INM-1), Research Centre Jülich, Jülich, Germany,* ***2.*** *Centre for Applied Statistics, The University of Western Australia, Perth, Australia,* ***3.*** *Institute of Neuroscience and Medicine, Brain & Behaviour (INM-7), Research Centre Jülich, Jülich, Germany,* ***4.*** *Institute of Systems Medicine and Department of Human Medicine, MSH Medical School Hamburg, Hamburg, Germany,* ***5.*** *Institute for Advanced Simulation (IAS-7), Research Centre Jülich, Jülich, Germany,* ***6.*** *Department of Neurology, University of Heidelberg,* ***7.*** *Department of Neurology and Center for Translational Neuro- and Behavioral Sciences (C-TNBS), University Hospital Essen, University of Duisburg-Essen, Duisburg, Germany,* ***8.*** *Institute of Medical Psychology and Behavioral Neurobiology, University of Tübingen, Tübingen, Germany,* ***9.*** *Institute of Clinical Neuroscience and Medical Psychology, Medical Faculty & University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany*

Introduction:
Progressive gait and balance impairment is a prominent and disabling clinical feature in patients with degenerative cerebellar ataxia. Intensive coordinative training has short- and long-term effects (Ilg et al., 2009, Milne et al., 2017). We performed a rater-blinded, parallel 3-arm RCT with delayed-start-design for the control group to assess if patients benefit from additional video-based training, and if frequency and duration of training units have an impact on the outcome.
Methods:
Clinical (ataxia rating: SARA, balance confidence: ABC-D, Timed Up and Go: TuG) and digital (force plate) gait and balance measures were assessed in patient groups with degenerative hereditary cerebellar ataxias before and after a three-week video-based gait and balance training: 4x20min/week training was performed in GroupA (n=15, age 55±11 years, 8 female, 9 pure cerebellar, baseline SARA score of 8.9±3.3) and 2x40min/week in GroupB (n=16, 57±12 years, 6 female, 10 pure cerebellar, SARA 8.3±3.9). 9/31 patients belonged to the control group. Training groups did not differ in age, gender, number of pure cerebellar ataxias, SARA score, amount of physiotherapy, and sports/week ($p>0.155$).
Results:
Compared to controls, only GroupB showed significantly increased gait velocity (GV) in normal gait and a mean reduction in SARA score ($p<0.023$) by 1.3 points (≡16%; vs. 1% in GroupA, $p=0.890$). Worse initial gait and stance performance strongly correlated with a higher training effect ($r=0.414$-0.992, $p\leq0.05$). Patients with initially higher SARA score (>8), low balance confidence (ABC-D<69%) and longer TuG (>12s) had improved GV in backward and normal gait ($p<0.05$).
Discussion/Conclusion:
In summary, ataxia patients have a short-term benefit from video-based training, particularly longer and less frequent units seem to be more effective. Patients with initially higher SARA score and increased fall risk according to established cut-off values on lower balance confidence and slower TuG times have a larger benefit from the

video-based training.

Clinical features on Friedreich´s Ataxia patients in São Paulo, Brazil

Tuesday, 12th November - 18:10: (Minories) - Poster

Mrs. Daiana Machado [1]***, Prof. Marcondes França*** [2]

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Background and Objective

There are few reports characterizing Friedreich´s Ataxia (FRDA) phenotype beyond Europe and USA. Herein, we describe the clinical profile of a representative FRDA cohort from São Paulo, Brazil.

Methods

Patients were invited to answer an online survey comprising 24 to 32 questions, which was sent by physicians and the Brazilian Association of Acquired and Hereditary Ataxias via email and releases. We collected data on sex, current age, age at diagnosis, affected relatives, parental consanguinity, ability to walk, the need of ambulatory assistive device or wheelchair and presence of diabetes, cardiomyopathy, and scoliosis. Descriptive statistics was used to report the results - means, proportions, and Standard Deviations (SD). Age at loss of ambulation (LOA) was assessed both for classical and late-onset (LOFA) groups using Kaplan-Meier curves.

Results

We gathered 104 answers from FRDA patients or legal guardians, being 79 (75.9%) patients with classical FRDA and 25 (24%) with late onset (LOFA). There were 64 (61.5%) women and 40 (38.5%) men. Mean age and age at diagnosis were 37.3± 13.8 y and 24.1± 11.5 years, respectively. Parental consanguinity and familial disease recurrence were seen in 24 (23%) and 50 (48.7%) of the patients, respectively. Fifty five out of 104 patients lost ambulation at the mean age of 29.4±11.5 years. Those who are still ambulatory but need assistive devices, first required them at a mean age of 27.1±13.1 years. As expected, patients with classical FRDA had LOA earlier than LOFA (25.5±7.7 vs 47.8±10.2 years). The frequency of diabetes, cardiomyopathy, hearing impairment and scoliosis in this cohort was 36±10.9, 23.6±14.2, 32.5±8.8 and 18.5±9.1, respectively.

Discussion and Conclusion

The overall phenotype of FRDA in Brazil is similar to previous European and US reports, but age at LOA was slightly higher. Further studies are needed to better understand such difference.

Understanding the relationship of static and dynamic balance measures in ataxic stance and gait

Tuesday, 12th November - 18:10: (Minories) - Poster

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Impairments in gait– with a key component of dynamic balance– and stance– with a key component of static balance- represent the key hallmarks of ataxia; not only in clinical assessments and clinician-reported outcomes; but also in patients' voice burden of disease severity and patient-reported outcomes. While it is obvious that both features are not independent from each other, their interplay in ataxia – in terms of underlying control mechanisms- remains unknown. Here we aimed to assess the interaction between dynamic balance (gait) and static balance (stance) in response to longitudinal changes in cerebellar ataxia using wearable sensors;

We assessed cross-sectional and longitudinal balance of subjects with degenerative cerebellar disease (SARA:7.5±5.14) at baseline and 1-year follow-up (n=60) by 3 body-worn inertial sensors in two conditions: (1) stance with feet together (30 seconds), (2) straight walking (2 minutes). Based on the hip sensor, sway path length was calculated as a measure of static balance during stance using both directions of sway (PLtotal), as well as exclusively anterior-posterior (PLap) and medial-lateral (PLml) direction. Gait analysis focussed on ataxic-sensitive measures of spatio-temporal variability: stride length variability (SLCV) in gait direction and lateral step deviation (LSD) as well as upper body range of motion during gait in respective directions (ROMap, ROMml).

Cross-sectional analyses revealed significant correlations between PLtotal and LSD as well as ROMml (r >0.6), and between PLtotal and SLCV and ROMap (r>0.4). Matching directions of sway showed a mildly increased effect (e.g. LSD\PLap:r_total=0.61,r_ap=0.63). Corresponding stance and gait measures showed similar correlations to patient-reported balance confidence (ABC-score;PLtotal:0.65,LSD:0.69).

Longitudinal changes in static balance were correlated with changes in dynamic balance specifically in the corresponding direction (e.g. deltaLSD\deltaPLml:r=0.40).

We were able to identify specific influences of the static balance mechanism on gait, demonstrating the patient's relevance of static stance testing and related balance exercises in rehabilitation.

Gait event detection in cerebellar ataxia: A single vs. multiple device approach

Tuesday, 12th November - 18:10: (Minories) - Poster

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INTRODUCTION: Monitoring gait with wearable sensors provides an opportunity for improving clinical management and evaluating therapeutic interventions in patients with degenerative cerebellar ataxia (DCA). While multi-sensor configurations are recommended for robust gait evaluation, using a single sensor offers several advantages including reduced data footprint, minimized patient burden, and extended battery life.
METHODS: 96 participants (control: n=42; preclinical DCA: n=19; clinical DCA: n=35) completed two 25m straight walks at their self-selected preferred pace in a laboratory setting. A wearable sensor (APDM, Opal 128Hz) was affixed to the lower back and the dorsum of both feet. Gait events (initial contact-IC, final contact-FC) were detected using a single sensor and multiple sensors (reference system). Agreement between the single and multi-sensor configurations (bias, limits of agreement, intraclass correlation coefficient) and accuracy (Positive predictive value; PPV, median absolute error; MAE) were quantified. Relationships between event detection accuracy and gait outcomes derived by the reference system were explored.
RESULTS: A total of 8473 steps were included in the analyses. Accuracy was high for identification of IC in controls (PPV=97%), preclinical DCA (PPV=96%) and clinical DCA (PPV=86%). Accuracy was lower for FC compared to IC for controls (PPV=88%), preclinical DCA (PPV=90%) and clinical DCA (PPV=82%). The MAE was low for all groups (<0.12s). Significant correlations were observed indicating that gait events were detected less accurately for individuals walking with a reduced cadence, longer stride duration, and increased gait variability (gait speed, stride length and duration).
DISCUSSION AND CONCLUSION: Accuracy for the single sensor approach was high and exceeded the threshold of 80% indicating that this approach may be used with confidence. Noticeable differences were observed in FC identification for clinical DCA, which may impact the calculation of gait outcomes. Additional refinements to optimize the algorithm should be considered to improve gait event detection accuracy.

Morphofunctional evaluation of visual pathways in pediatric and young adult patients with Friedreich's ataxia: a possible role for bipolar cells?

Tuesday, 12th November - 18:10: (Minories) - Poster

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Objectives: Abnormalities in retinal structure and function have been described in Friedreich's ataxia (FA). Approximately 73% of patients with FA experience visual system involvement (VSI), which includes optic nerve sub-atrophy or atrophy and abnormal bioelectrical responses in the visual cortex. This study aims to assess the extent of VSI and identify early subclinical manifestations of FA by evaluating morphofunctional retinal alterations in children and young adults' patients.

Methods: Forty-eight eyes of twenty-four patients (11 females and 13 males; mean age 18.9±7.9 years; range 10-38) with FA were evaluated using Visual Evoked Potentials (VEP), Pattern Electroretinogram (PERG), Multifocal Electroretinogram (MfERG), Optical Coherence Tomography (OCT). An age-matched group of subjects served as controls.

Results: VEP showed a statistically significant reduction and delay in the P100 implicit time response (p=0.01). PERG demonstrated a statistically significant reduction in response (p=0.02). MfERG revealed a statistically significant reduction in response amplitude densities (p=0.04), indicating additional dysfunction in the inner retinal layers. OCT analysis showed a reduction in the Retinal Nerve Fiber Layer (RNFL) (p=0.002) and retinal thickness (RT) (p=0.040).

Discussion: Stratigraphic analysis of subretinal layers revealed a significant reduction in the Ganglion Cell Layer (GCL) (p=0.033) and a non-statistically significant reduction in the inner nuclear layer (INL), confirming the functional alteration of the inner retinal layers as indicated by the PERG and MfERG responses. Indicators of the severity stage of FA (e.g., SARA) were positively correlated with the PERG and more significantly with the MfERG responses.

Conclusions: Morphofunctional evaluation of visual pathways in FA patients showed significant alterations in the inner retinal layers. The selective alteration of the MfERG may indicate bipolar cells as a possible additional biomarker of FA.

Genomic variants in the insulin pathway as potential modifiers of age at onset in patients with spinocerebellar ataxia type 3 and type 2

Tuesday, 12th November - 18:10: (Minories) - Poster

Prof. Maria Luiza Saraiva-Pereira [1], ***Ms. Aurora Ubatuba de Melo*** [1], ***Ms. Grasiely Moreira Machado*** [1], ***Ms. Amanda de Souza*** [1], ***Dr. Gabriel Vasata Furtado*** [2], ***Prof. Laura Bannach Jardim*** [1]

1. *Universidade Federal do Rio Grande do Sul,* ***2.*** *Hospital de Clinicas de Porto Alegre*

Background: Spinocerebellar ataxia type 3 (SCA3) and type 2 (SCA2) are hereditary progressive neurodegenerative diseases due to CAG repeats expansions in ATXN3 e ATXN2, respectively. These disorders are by far the most prevalent types of SCA worldwide, and some clinical features are similar. Although an inverse correlation between CAG expansion length and age at onset (AO) of symptoms is observed, an unexplained variability of symptoms remains. Therefore, additional factors may play a role in this variability, and previously published studies indicate that variants near FOXO1 gene could be good candidates.

Objectives: To investigate rs4509910 and rs9532809 variants, both associated with the insulin pathway, as modifiers of the AO in SCA3 patients and in SCA2 patients.

Methods: We have included samples from the biorepository of the Neurogenetics Translational Laboratory at Hospital de Clinicas de Porto Alegre, Brazil. Samples were selected based on the availability of AO data and CAG expansion length. Variants described above were genotyped, and additional data was correlated to these results. Statistical analyzes were performed using the SPSS18 program.

Results: rs4509910 genotype distribution were determined as follows: 0.192 (GG), 0.411 (GT), and 0.397 (TT) among SCA3/MJD patients, and 0.156 (GG), 0.414 (GT), and 0.430 (TT) among SCA2 patients. rs9532809 genotype distribution was 0.040 (CC), 0.291 (CT), and 0.669 (TT) for SCA3/MJD patients, and 0.023 (CC), 0.250 (CT), and 0.727 (TT) for SCA2 patients. Mean AO(y) of SCA3 patients was 38.66 (GG), 30.87 (GT) and 34.53 (TT).

Discussion: The GG genotype (rs4509910) showed an association with AO of SCA3 patients and a later onset of approximately 7.78 years (compared to the GT genotype). No difference was seen in the group of SCA2 patients with neither variants.

Conclusion: Data presented here indicate that those variants can play a role as additional factors to modulate AO in SCA3.

Diagnosis Journey of Brazilian Friedreich´s Ataxia patients

Tuesday, 12th November - 18:10: (Minories) - Poster

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Background and Objective

Friedreich´s Ataxia (FRDA) is the most common recessive ataxia worldwide, but its diagnosis is often delayed for multiple reasons. This impacts the timely introduction of therapeutic interventions and therefore, prognosis. Herein, we sought to characterize the diagnostic journey of FRDA in São Paulo, the most populous state in Brazil.

Methods

Patients were invited to answer an online survey comprising 24 to 32 questions, which was sent by physicians and the Brazilian Association of Acquired and Hereditary Ataxias via email and releases.

We recorded information on age at first symptoms, age at diagnosis, first symptom observed, time since first symptoms to diagnosis, medical specialty first sought, number of physicians visited up to the diagnosis and misdiagnoses. Descriptive statistics was used to report the results - means, proportions, and Standard Deviations (SD). Comparison between classical (onset<25 yo) and late-onset - LOFA (onset>25yo) diagnostic journey was accomplished using parametric tests.

Results

One-hundred four FRDA patients or legal guardians answered the survey. There were 79 (75.9%) classical and 25 (24%) LOFA FRDA. Mean age at first symptoms was 17.6± 9.2 years, whereas mean age at diagnosis was 24.1±11.5 years. Overall, diagnostic delay was around 7.8±6.7 years, for the whole group. There was no difference between classical and LOFA subgroups (7.6±6.8 vs 7.6±6.4 years, p=0.978). Unsteadiness was the most frequent heralding symptom (50%). Neurologists (35.8%) closely followed by orthopedical surgeons (30.8%) were the most frequent medical specialties first sought. On average, 6 different physicians were visited until diagnosis was reached. Misdiagnoses were reported by 38 (46.3%) of patients.

Discussion and conclusion

In Brazil, FRDA is characterized by impressive diagnostic delay and frequent misdiagnoses, with no relevant differences between classical and LOFA patients.

Evaluating Outcome Measures of Ataxia in Adult Patients with Primary Mitochondrial Disease and Spinocerebellar Ataxia Type 6

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Jane Newman [1], ***Dr. Aye Moe*** [1], ***Dr. Sylvia Del Din*** [2], ***Prof. Grainne Gorman*** [1], ***Dr. Lisa Alcock*** [2], ***Dr. Yi Shiau Ng*** [1]

1. *Wellcome Centre for Mitochondrial Research, Translational and Clinical Research Institute, Newcastle University, UK,* ***2.*** *National Institute for Health and Care Research (NIHR) Newcastle Biomedical Research Centre (BRC), Newcastle University and The Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK*

Objectives

There are limited clinical studies that systematically characterised ataxic features and their severity in patients with primary mitochondrial disease (PMD). This project aims to investigate the validity of existing clinician- and patient-reported measures on capturing ataxic symptoms experienced by patients with PMD compared to patients with Spinocerebellar Ataxia type 6 (SCA6).

Methods

In this cross-sectional study, adults with genetically-confirmed PMD and SCA6, and healthy controls were recruited. The following assessments were performed:

- Clinician-reported measures: SARA (Scale for the Assessment and Rating of Ataxia), ICARS (International Cooperative Ataxia Rating Scale), Inventory of Non-Ataxia Signs;

Performance assessments: Functional Gait Assessment, nine-hole peg test and keyboard-tapping test;

- Patient-reported measures: Activities-Specific Balance Confidence Scale, Falls Efficacy Scale-International, Fatigue Severity Scale, and Neuro-QoL.

Results

Sixty-seven participants (PMD, n=34; SCA6, n=12; healthy controls, n=21) were recruited; pathogenic *POLG* and m.3243A>G (*MT-TL1*) variants accounted for 56% of PMD cases. The mean age of PMD patients was younger than SCA6 patients (46 vs 60, p=0.016). The severity of ataxia, measured by SARA (9 vs 8.8) and ICARS (17.3 vs 15.2), was similar between two patient groups (p>0.05); however, a higher mean ICAR oculomotor score was observed in SCA6 than in PMD patients (1.7 vs 0.8, p=0.003). Patients with PMD had more non-ataxia signs than SCA6 (3 vs 1, p<0.001). Significant correlations were identified between clinician- and patient-reported measures in both patient groups. Patients with PMD and SCA6 had similar profiles in Neuro-QoL, except patients with PMD had a higher Fatigue T score (mean difference 6.5, p=0.03).

Conclusion

All outcome measures could differentiate patients with PMD from controls. Our findings show that the overall ataxic severity in PMD is comparable to SCA6. However, the impairment of balance and mobility in patients with PMD is complicated by other additional neurological features such as neuropathy, myopathy, ophthalmoplegia and vestibular dysfunction.

R-PROMS: A Feasibility Study of Remote Patient Reported Outcome Measures in Patients with Ataxia

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Aye Moe [1], ***Dr. Louisa P. Selvadurai*** [2], ***Ms. Sarah Wallis*** [3], ***Dr. Jane Newman*** [1], ***Prof. Ian Harding*** [4], ***Dr. Yi Shiau Ng*** [1]

1. *Wellcome Centre for Mitochondrial Research, Translational and Clinical Research Institute, Newcastle University, UK,* ***2.*** *Monash University,* ***3.*** *Monash University, Melbourne,* ***4.*** *QIMR Berghofer Medical Research Institute, Brisbane, Queensland*

Objectives – To investigate the feasibility of remote assessments of patient-reported outcomes (PRO) and explore the digital biomarkers of speech, upper limb, and cognitive function in patients with spinocerebellar ataxia (SCA) and ataxia in patients with primary mitochondrial disease (PMD).

Method – The initial stage of the pilot study involves recruiting adult patients with confirmed genetic diagnoses of PMD (n=20) and SCA (n=10). The participants undergo the following assessments at the baseline face-to-face visit:

- Clinician-reported outcome measures (CRO): 9-hole Peg Test, SARA (Scale for Assessment and Rating of Ataxia, INAS (Inventory of Non-Ataxia Signs), Mini-Best (Balance Evaluation Systems Test), and Newcastle Mitochondrial Disease Adult Scale
- Baseline PRO: ABC Scale (Activities-specific Balance), HADS (Hospital Anxiety and Depression Scale)
- Digital Assessments: Patient-reported outcome measure of Ataxia (PROM-Ataxia), Activities of Daily Living (ADLs), Components of the Quality of Life in Neurological Disorders (Neuro-QoL), Cognitive-Motor Tasks and Speech Assessment tasks. These digital platforms are supported by the Neurosciences team at Monash University, Australia (led by Prof I Harding).

The participants are asked to complete digital assessments remotely every two months for up to a year.

Results – The study has recruited nine SCA patients, a mean age of 42 (range 33 -75), and two PMD patients by the end of May 2024. Datasets of three remote assessments and their correlations with clinician reported measure will be presented at the conference.

Discussion – This project offers the research opportunity to patients who have a more advanced stage of ataxia (loss of ambulation) or with rarer genetic aetiologies that often preclude them for participating in other outcome measure studies. The utilisation of remote digital assessments for repeated measures of different symptoms will potentially reduce participant burden in future clinical trials.

Disease characteristics and tissue frataxin concentrations in adults with Friedreich's ataxia participating in nomlabofusp interventional studies

Tuesday, 12th November - 18:10: (Minories) - Poster

Mr. Mohamed Hamdani [1], ***Ms. Noreen Scherer*** [1], ***Dr. Magdy Shenouda*** [2], ***Dr. Russell Clayton*** [1]

1. *Larimar Therapeutics, Inc.,* ***2.*** *Clinilabs, Inc.*

Methods: Disease characteristics (e.g. age at onset, guanine adenine adenine [GAA] repeat length) of adults with Friedreich's ataxia (FRDA) participating in Phase 1 and 2 nomlabofusp interventional studies were summarized and evaluated relative to baseline buccal and skin cell frataxin concentrations.

Results: Sixty-one subjects participated in at least one study; 18 participated in more than one study. Mean age was 31.9 years (range 19- 69). Mean age of onset was 15.9 years (range 5- 60). Mean (range) shorter and longer GAA repeat lengths were 555.8 (99- 1000) and 890.2 (265- 1300). Mean baseline modified Friedreich's ataxia Rating scale neurologic score was 49.5 (13.2- 74.5). Mean (range) baseline buccal and skin cell frataxin concentrations were 1.90 (0.70- 4.95) and 3.25 (1.40- 8.10) pcg/mcg, respectively. There is a relationship between frataxin concentrations and age of onset and GAA repeat length. There is also a relationship between skin and buccal cell frataxin concentrations.

Discussion: Early age of onset is associated with more rapid disease progression. Data from the nomlabofusp interventional studies are consistent with previously published data that suggest that lower tissue frataxin concentrations are associated with more rapid disease progression. Increasing frataxin concentrations in patients with FRDA may decrease the rate of disease progression.

Conclusion: The study population in the nomlabofusp interventional studies is representative of the FRDA population, and tissue frataxin concentration data from these studies are consistent with prior studies demonstrating that lower frataxin concentrations are associated with earlier onset of disease. Buccal and skin cell frataxin levels correlate with each other.

Metabolic Cerebellar Ataxias-Single Centre Experience

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Shpresa Pula [1], ***Dr. Amanda Lam*** [2], ***Ms. Katarina Manso*** [1], ***Ms. Suzanne Booth*** [1], ***Prof. Simon Heales*** [2], ***Prof. Paola Giunti*** [1]

1. *Ataxia Centre, Clinical and Movement Neurosciences Department, UCL, Queen Square Institute of Neurology, London,* ***2.*** *Neurometabolic Unit, UCL, Queen Square, Institute of Neurology, London*

Background and Objectives: Metabolic causes of cerebellar ataxias encompass all categories of inherited metabolic disorders (IEM). The prevalence and phenotype in adults with IEMs are unknown. The study aims to identify the causes of metabolic cerebellar ataxias, the clinical phenotype, outline the diagnostic approach, and identify delays.

Methods: We conducted a retrospective study at the Ataxia Centre, National Hospital for Neurology and Neurosurgery in London. We recruited 450 patients with unexplained ataxias. The study took place from 2007 to 2024 and involved reviewing patient records for clinical features, neuroimaging, neurophysiology, biomarkers, and genetic investigations.

Results: Twelve patients with pathogenic variants in seven genes (*PMM2, NPC-1, POLG, MT-TK, MT-ATP6, CoQ8A, TTPA*) were identified, including five females. The median age was 33 years (range: 17-76), with a median diagnosis age of 30.5 years (range: 17-66), despite early presentation in most cases. Nerve conduction studies showed axonal sensory-motor neuropathy in one patient and axonal sensory neuropathy in two. Neuroimaging was available in 7/12 patients and showed cerebellar atrophy in all patients. The median diagnostic delay from presentation to diagnosis was 16 years (range: 2-30).

Conclusion and discussion: At the Ataxia Centre of Excellence, out of 450 unexplained ataxias, 2.6 % were attributed to IEMs, with the majority being related to an energy deficit disorder. This underscores the importance of investigating IEMs as potential causes of “undetermined” ataxias in adulthood. Most cases were diagnosed using an expanded genetic approach; biomarkers were crucial for prompt intervention in cases involving deficiencies in Vitamin E, CoQ10, and 3β,5α,6β-trihydroxy-cholanoyl glycine for Niemann Pick type C. Recognizing potentially treatable IEM related ataxias is critical not only to implement treatments, but also to prevent further neurological deterioration.

Elevated Bile Acid 3β,5α,6β-Trihydroxycholanoyl Glycine in a Subset of Adult Ataxias Including Niemann–Pick Type C

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Nazgol Motamed-Gorji [1], Dr. Youssef Khalil [2], Dr. Cristina Gonzalez-Robles [1], Mr. Shamsher Khan [1], Dr. Philippa Mills [2], Dr. Hector Garcia-Moreno [1], Dr. Heather Ging [1], Ms. Ambreen Tariq [1], Prof. Peter Clayton [2], Prof. Paola Giunti [1]

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Background: Ataxia is a common neurological feature of Niemann–Pick disease type C (NPC). In this disease, unesterified cholesterol accumulates in lysosomes of the central nervous system and hepatic cells. Oxidation by reactive oxygen species produces oxysterols that can be metabolised to specific bile acids. These bile acids have been suggested as useful biomarkers for detecting NPC.

Methods: Concentrations of 3β,5α,6β-trihydroxycholanoyl glycine (3β,5α,6β-triOH-Gly) and 3β,7β-dihydroxy-5-cholenyl glycine (3β,7β-diOH-Δ5-Gly) were measured in plasma of 184 adults with idiopathic ataxia. All patients were tested with whole genome sequencing containing hereditary ataxia panels, which include *NPC1*and *NPC2* mutations and other genetic causes of ataxia.

Results: Plasma 3β,5α,6β-triOH-Gly was above normal (>90 nM) was in 8 out of 184 patients. One patient was homozygous for the p.(Val1165Met) mutation in the *NPC1* gene. The remaining seven included one patient with Friedreich's ataxia and three patients with autoimmune diseases. Since oxidative stress is known to be increased in Friedreich's ataxia and autoimmune diseases, this subset of patients possibly shares a common mechanism that determines the increase of this bile acid.

Discussion and Conclusion: In a large cohort of adults with ataxia, plasma 3β,5α,6β triOH-Gly was able to detect the one patient in the cohort with NPC1 disease, but also detected oxidation of cholesterol by ROS in other disorders. Plasma 3β,7β-diOH-Δ5-Gly is not a potential biomarker for NPC1.

Progression of Non-Ataxia Signs in Spinocerebellar Ataxia Types 1, 2, 3, and 6

Tuesday, 12th November - 18:10: (Minories) - Poster

Mr. William Legendre [1], Dr. Sophie Tezenas du Montcel [2], Prof. Thomas Klockgether [3], Dr. Heike Jacobi [4], Prof. Alexandra Durr [5], Mr. Emilien Petit [1]

1. *Sorbonne Université, Paris Brain Institute, Inserm, INRIA, CNRS, APHP, 75013 Paris, France,* ***2.*** *ARAMIS, Sorbonne Université, Institut du Cerveau-Paris Brain Institute-ICM, CNRS, Inria, Inserm, AP-HP, Groupe Hospitalier Sorbonne Université, Paris, France,* ***3.*** *German Center for Neurodegnerative Diseases (DZNE),* ***4.*** *Department of Neurology, University of Heidelberg,* ***5.*** *Sorbonne University, Paris Brain Institute, Pitié-Salpêtrière Hospital*

Background and Objectives: Global scores assessments often mask the heterogeneous progression of symptoms in spinocerebellar ataxias (SCAs). To address this, we used Disease Course Mapping, a Bayesian model in LEASPY framework, to model individual Inventory of Non-Ataxia Signs (INAS) trajectories in SCA types 1, 2, 3, and 6.
Methods: Longitudinal data from 546 genetically confirmed SCA1, 2, 3, and 6 participants (170 SCA2, 145 SCA1, 131 SCA3, 100 SCA6) from the EUROSCA and RISCA cohorts were analyzed. Bayesian models estimated population-and individual-level trajectories, accounting for onset age (τ), progression speed (ξ), feature specific time shift compared to the mean progression in years (ωi), and time transitions between severity levels ($\Delta 1 \rightarrow 2$, $\Delta 2 \rightarrow 3$).
Results: SCA6 (75.70 ± 11.84) had significantly later disease onset than SCA3 (56.31 ± 11.78), SCA2 (54.43 ± 13.49), and SCA1 (47.85 ± 13.44), all $p < 0.0001$, as shown by higher τ. Of the 11 ordinal sub-items, 10 showed steady progression with $\Delta 1$ close to $\Delta 2$ (e.g., Gait Spasticity: $\Delta 1$ = 16.97 years, $\Delta 2$ = 18.09 years), except for Fasciculation Face/Tongue ($\Delta 1$ = 30.56 years, $\Delta 2$ = 79.56 years). Significant differences in ω values were found among different SCA. For example, Muscle Atrophy UL Distal showed later onset in SCA1 compared to SCA3 and SCA6 (ω = 2.49±3.76 vs. -1.06±5.24 and -4.06±3.54, p=0.007 and p<0.0001 respectively), while Impaired Vibration Sense Right Foot appeared earlier compared to SCA2 (ω=-5.51±9.70 vs 4.07±9.52 p = 0.0047). For SCA3, Cognitive Impairment appeared earlier than SCA1 (ω=-0.67±3.51 vs ω = 1.67±2.52, p = 0.0082). In SCA6, Dysphagia appeared later than SCA2 (ω = 4.72±4.16 vs ω = -3.15±4.18, p < 0.0001).
Discussions/Conclusion: These rankings underscore the heterogeneity in disease progression across SCAs and highlight the need for personalized monitoring and treatment strategies.

Context matters: Gait analysis in real-life -but not in-lab or SARA- reveal disease progression in spinocerebellar ataxias already after 1 year

Tuesday, 12th November - 18:10: (Minories) - Poster

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OBJECTIVES: In this observational study, we aim to unravel performance markers of ataxic gait for upcoming therapy trials using wearable sensors. We hypothesize that in short, trial-like time-frames gait measures captured in complex real-life settings of patients are more sensitive to natural disease progression compared to lab-based gait assessments and clinical rating scales.

METHODS: We assessed longitudinal gait changes of 24 subjects with spinocerebellar ataxia (SCA types: 1, 2, 3, 6) at baseline (SARA:9.4±4.1), 1-year and 2-years follow-up assessment by three body-worn inertial sensors in two conditions: (1) laboratory-based walking; (2) real-life walking in everyday environment. In the real-life walking condition, a context-sensitive analysis was performed by selecting comparable walking bouts according to bout length and number of performed turns. Movement analysis focussed on measures of spatio-temporal variability, in particular lateral step deviation (LSD) and a compound measure of spatial variability (SPcmp).

RESULTS: Cross-sectional analyses revealed high correlation to ataxia severity (SARA) and patients subjective balance confidence (ABC-Scale) in both conditions ($r>0.7$). While clinical ataxia score and gait measure in lab-based gait assessments identified changes after two years only (SARA: rprb=0.71; LSD: rprb=0.67), real life assessment of lateral step deviation and a compound measure of spatial step variability identified changes already after one year, with high effect sizes (LSD: rprb=0.66; SPcmp: rprb=0.68) and additionally increased effect sizes after two years (LSD: rprb=0.77; SPcmp: rprb=0.82).

DISCUSSION: Utilizing a context-sensitive matching procedure with high robustness to disease-independent changes of environment, real-life gait measures capture longitudinal change within one year with high effect size. In contrast, clinical scores like the SARA or lab-based gait measures show longitudinal change only after two years.

CONCLUSIONS: Features of real-life gait constitute promising performance markers for upcoming therapy trials, yielding ecologically validity, earlier sensitivity and increased effect sizes in comparison with clinical scores and lab-based gait assessment.

Exploring Dysarthria Profiles Across Diverse Ataxia Types

Tuesday, 12th November - 18:10: (Minories) - Poster

Mr. Suleyman Kahraman [1], Prof. Anja Lowit [1], Prof. Mario Parra Rodriguez [1], Prof. Marios Hadjivasssiliou [2]

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Introduction: Dysarthria profiles in progressive ataxia exhibit significant heterogeneity among ataxia types, suggesting the presence of subgroups within ataxic dysarthria. However, speech phenotyping studies are largely limited to Friedreich's Ataxia and Spinocerebellar Ataxia. Therefore, we aimed to extend this understanding by investigating dysarthria manifestation across other, less frequently investigated ataxia types.
Methods: We will report data on ten individuals with Spastic Paraplegia-7, ten with CANVAS, and ten with Gluten Ataxia. The participants engaged in online speech assessment meetings via Zoom and the RedenLab® platforms. Speech data of sustained phonation, alternating and sequential motion tasks (AMR/SMR), producing the days of the week, a standard passage reading, and a monologue were collected. Moreover, participants' age of onset, disease durations, and SARA scores were obtained from their neurologists.
Results: Data analysis is currently ongoing. We will report on the parameters of maximum phonation time, fundamental frequency variability, diadochokinetic rate, as well as articulation rate and intelligibility ratings from connected speech tasks. The dysarthria features will be examined in relation to ataxia severity, age of onset, and disease duration. Within and across-group comparisons will be performed, in addition, our data will be compared to other, more widely reported ataxia types in order to establish to what degree subtypes are distinct in their dysarthria presentation.
Discussion: Knowledge about the distinct dysarthria profiles across ataxia subtypes is important to design effective treatment approaches. Speech symptoms might also have a role to play in differential diagnosis. Furthermore, a more profound understanding of dysarthria symptoms associated with different neuropathological involvements in ataxia may enhance our knowledge of the neurological mechanisms underlying speech production. Our study is the first step towards larger investigations by providing information on which speech aspects should be explored further to establish distinctive profiles of dysarthria across progressive ataxias.

French patients' experiences following treatment with Skyclarys (omaveloxolone): a qualitative approach.

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Juliette Dieusaert [1], ***Dr. Cecilia Estrella*** [1], ***Dr. Patricia Etienne*** [1], ***Mrs. Sandra Lechene*** [1], ***Mr. Stéphan Rouillon*** [1]

1. *AFAF French Friedreich Ataxia Patients Association*

Background and Objectives

Omaveloxolone (Skyclarys®) received FDA approval to treat Friedreich Ataxia (FA) in adults and adolescents aged 16 and older in February 2023. In France, the Early Access to Skyclarys (before EU approval) was granted by the French National Health Authority by December 2023. Since then, French patients have been able to benefit from this treatment. In order to support the application to the French Health Authorities for a potential reimbursement of the costs of Skyclarys, the French Friedreich Ataxia Patients Association (AFAF) collected patients' experiences following treatment prescriptions.

Methods

A comprehensive qualitative approach was used consisting in semi-structured interviews. First, the AFAF launched an invitation to its ~500 FA patients member base, to collect testimonials from those who have received Omaveloxolone (OMA) treatment for at least 4 weeks. Non-directive interviews were carried out by AFAF administrators using non-invasive techniques to facilitate the patient's discourse. Themes included FA symptoms and psychological perceptions before and after the treatment. Interviews were recorded, transcribed and analysed according to a defined interview guide.

Results

Female (n=7) and male (n=2) patients aged from 21 to 65 years, living with FA for 6 to 27 years were included. Among those who experienced effects after the treatment with OMA, 2/3 reported reduced fatigability, improved coordination, less clumsiness, and improved speech. No changes after the treatment were also reported.

Discussion and Conclusion

Qualitative interviews offered patients the opportunity to describe their own experiences with OMA. Part of testimonies put emphasis on the improvement of motor aspects as well as speech troubles and fatigability, whilst confirming that not all patients experience the same response to treatment.

Our approach to representing patients' voices may complement scientific analyses currently conducted by French physicians of quantitative data collected as part of the Early Access program in France.

Work funded by AFAF

SARA Progression Characteristics of SCA 1, 2, 3, and 6 in the CRC-SCA Natural History Study

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Christian Rummey [1], ***Dr. Tetsuo Ashizawa*** [2], ***Dr. Andrew Billnitzer*** [2], ***Dr. Marie Y. Davis*** [3], ***Dr. Cameron Dietiker*** [4], ***Dr. Antoine Duquette*** [5], ***Dr. Michael Geschwind*** [4], ***Dr. Anoopum Gupta*** [6], ***Dr. Christopher M. Gomez*** [7], ***Dr. Ali Hamedani*** [8], ***Dr. Chiadi Onyike*** [9], ***Dr. Puneet Opal*** [10], ***Prof. Henry Paulson*** [11], ***Dr. Susan Perlman*** [12], ***Prof. Jeremy D. Schmahmann*** [6], ***Prof. S. H. Subramony*** [13], ***Dr. Sharan Srinivasan*** [11], ***Dr. Christopher D. Stephen*** [6], ***Dr. George Wilmot*** [14], ***Dr. Theresa Zesiewicz*** [15], ***Dr. Sheng-Han Kuo*** [16], ***Dr. Liana S. Rosenthal*** [9], ***Dr. Vikram G. Shakkottai*** [17], ***Dr. Lauren Moore*** [18]

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Introduction

As knowledge about the natural history of Spinocerebellar Ataxia (SCA) grows, we must bridge gaps in translating these findings into clinical trial designs. Natural history studies are crucial for enhancing the understanding of disease progression and the effectiveness of assessments like the SARA score, and the CRC-SCA study offers vital into both. These results need to be put in the context of the EUROSCA data that was reported several years ago.

Methods

Progression rates in SARA scores for the four most prevalent SCA types (SCA1, SCA2, SCA3, and SCA6) were reported. The study analyzed total and sub-scores, with item-level data where appropriate, using linear mixed-effect modeling. The analysis included the complete population and relevant subgroups.

Results

The longitudinal analysis included 863 patients: 152 SCA1 (17%), 198 SCA2 (23%), 334 SCA3 (39%) and 179 SCA6 (21%). Follow-up data was available for 79 SCA1 (52%), 119 SCA2 (60%), 182 SCA3 (55%), and 103 SC6 (58%) patients. Progression rates in SARA scores did not significantly differ between genotypes: Rates of decline in points per year were, for SCA1 1.2 (95%CI 0.6, 1.7), SCA2 by 0.98 (95%CI 0.5, 1.4), SCA3 by 0.88 (95%CI 0.5, 1.2), and SCA6 0.62 (95%CI 0.2, 1.1). Decline was driven by axial function, particularly gait and balance, with no significant decline in appendicular function.

Discussion

The CRC-SCA study found substantially lower progression rates compared to EUROSCA, and more variability within the genotypes. Since CRC-SCA is continuously enrolling, results also depend on the amount of follow-up considered. Reasons for the discrepancy with European results may involve different enrollment strategies, healthcare systems and incentives for patients to participate. The findings highlight the importance of considering these factors in clinical trial design. The direct comparison of natural history data acquired in the US (CRC-SCA) vs Europe EUROSCA) is not straightforward.

Instrumented balance assessment for evaluating response to rehabilitation for individuals with hereditary cerebellar ataxia

Tuesday, 12th November - 18:10: (Minories) - Poster

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BACKGROUND/OBJECTIVES

Improving balance is a key rehabilitation goal for individuals with hereditary cerebellar ataxia (HCA) hence assessment is critical. We aimed to determine if Biokin Inertial Measurement Unit (IMU) sensors worn during sitting and standing balance tests were responsive to change after rehabilitation in individuals with HCA.

METHODS

Seventy individuals with HCA, mean (SD) age 46.4 (18.6) years, were randomly allocated to receive a 30-week rehabilitation program or standard care underwent assessment at baseline, week-7, week-18, and week-30. Participants completed standing tests: feet apart and together, eyes closed (EC) and eyes open (EO), and sitting EC and EO, while wearing IMU sensors on their back and chest. The Berg Balance Scale (BBS) and Function in Sitting Test (FIST) were administered. Regression models analysed balance impairment. IMU sensor ability to distinguish between the groups was validated using the gradients of progression between assessments. A regression model to generate a balance score that differentiated between the groups was also developed.

RESULTS

Supervised machine learning models identified a significant correlation between the back IMU during sitting with EC and the FIST (r=0.882), between the back IMU during feet together EO and the BBS (r=0.892) and classified participants as receiving rehabilitation or standard care with an accuracy of 71.7%. Regression models trained on BBS clinical scores produced severity scores which correlated with the BBS (r=0.410) and distinguished the two cohorts ($p<0.013$, Cohen's d=0.496).

DISCUSSION/CONCLUSION

The Biokin IMU sensors worn during sitting and standing were able to detect change after rehabilitation. However, features were different to those strongly correlated with raw BBS and FIST scores, suggesting IMU sensors capture distinct aspects of motor function and balance not reflected by these clinical outcomes. These IMU-derived features, potentially related to movement quality, could provide a more comprehensive picture of neurological rehabilitation progress.

FUNDING

Medical Research Future Fund

Sensitivity of advanced MR imaging to progression in early SCA1 and SCA3: READISCA findings

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Thiago Rezende [1], ***Mr. Emilien Petit*** [2], ***Dr. Young Woo Park*** [3], ***Dr. Sophie Tezenas du Montcel*** [4], ***Dr. James Joers*** [5], ***Dr. Jonathan DuBois*** [6], ***Dr. H. Moore Arnold*** [6], ***Dr. Michal Povazan*** [7], ***Dr. Ipek Özdemir*** [7], ***Dr. Guita Banan*** [8], ***Dr. Romain Valabregue*** [2], ***Dr. Philipp Ehses*** [9], ***Dr. Jennifer Faber*** [10], ***Dr. Pierrick Coupé*** [11], ***Dr. Chiadi Onyike*** [12], ***Dr. Peter Barker*** [7], ***Prof. Jeremy D. Schmahmann*** [13], ***Dr. Eva-Maria Ratai*** [14], ***Prof. S. H. Subramony*** [15], ***Prof. Thomas Mareci*** [16], ***Dr. Khalaf Bushara*** [17], ***Dr. Henry Paulson*** [18], ***Prof. Thomas Klockgether*** [10], ***Prof. Alexandra Durr*** [19], ***Dr. Tetsuo Ashizawa*** [20], ***Prof. Christophe Lenglet*** [21], ***Dr. Gulin Oz*** [17]

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Background/Objective: Emerging therapies are in the pipeline for SCAs, however clinical outcomes are not sensitive to early disease progression when therapeutics will be most effective. Baseline MRI data from READISCA, a trans-Atlantic consortium, demonstrated morphometric, microstructural and neurochemical alterations in preataxic and early SCA1 and SCA3. Here we present the first longitudinal MRI findings from READISCA.

Materials and Methods: 112 participants (n(SCA1)=26, n(SCA3)=69, n(control)=17; including 42% of SCA at preataxic stage) were scanned at 3T at baseline. A subset were followed up at 6 months and then annually during the next 48 months. Structural, diffusion MRI (dMRI) and MR spectroscopy data were analyzed blinded to diagnosis. Ataxia severity was assessed with SARA. Annual change of MR measures and SARA was compared between groups using nonparametric testing.

Results: We are first reporting short-term follow-up findings at median (IQR) of 6.2 (5.9-6.7) months. Forty-four participants (n(SCA1)=10, n(SCA3)=25, n(control)=9; including 44% pre-ataxic gene carriers) returned for 6-month follow up from 2019-2021, primarily during the COVID-19 pandemic. Rate of change in microstructural integrity (decrease in fractional anisotropy (FA), increase in diffusivities) in the middle cerebellar peduncle (MCP), corona radiata and superior longitudinal fasciculus significantly differed in SCAs from controls (P<0.005) with high effect sizes (Cohen's d=1-2) and moderate-high responsiveness (|SRM|=0.6-0.9) in SCAs. SARA scores did not change, and their rate of change did not differ between groups. We estimate a 5-fold reduction in sample sizes with dMRI relative to SARA as endpoint for 6-month long trials. The analysis of the full longitudinal dataset is underway.

Discussion/Conclusion: Diffusion MRI is sensitive to disease progression in a trial-relevant follow-up period at very early stage SCA1 and SCA3, and may substantially reduce necessary trial cohort sizes. The analysis of the full time course of the larger cohort will provide more reliable effect sizes for clinical trial design.

Development of SARA-Q: An Automated Assessment System Based on SARA for Enhanced Precision in Evaluating Motor Coordination

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Chuyeon Lee [1]***, Mr. Mincheol Song*** [2]***, Mr. Joey Kim*** [2]***, Mr. Hyunmyung Jang*** [3]***, Mr. Alex Lee*** [4]***, Mr. Eric Lee*** [5]***, Mr. Jaeshin Kim*** [1]

1. *Voice of Calling NPO Life and Research Center,* ***2.*** *University of California, Los Angeles,* ***3.*** *Gyeongnam International Foreign School (GIFS),* ***4.*** *Harvard-Westlake School,* ***5.*** *Harvard Westlake School*

The Scale for the Assessment and Rating of Ataxia (SARA) is frequently employed in clinical evaluations. However, challenges arise when assessments must be carried out outside of a clinical setting or without an examiner present. These limitations present challenges in tracking daily variations and conducting precise analyses of outcomes. Additionally, the subjective nature of the examiner's assessment could impact objectivity and quantitative evaluation. In response to these challenges, we designed and implemented SARA-Q, an automated assessment platform for ataxia based on the SARA assessment tool.

SARA-Q employs a method where patients draw on a computer touchscreen, capturing x and y coordinates for analysis, thereby enabling a more precise evaluation of motor coordination. This app focuses on two upper limb assessment items: finger tracking and the finger-to-nose test. SARA-Q enhances the accuracy and reliability of assessments through various quantitative metrics, including deviation area analysis, reverse movement analysis, discontinuity analysis, test area overshoot analysis, tremor frequency analysis, maximum and average rotational amplitude analysis, and modulation speed analysis. Furthermore, SARA-Q allows assessments to be conducted by patients alone or with minimal caregiver assistance, facilitating data collection in various settings within a short time frame. In a beta test conducted on 10 brain injury patients at Chungbuk National University Hospital, it was confirmed that SARA-Q could more precisely differentiate between patients who were classified with the same score on the traditional SARA test. This was accomplished by employing precise data measured in increments of one-thousandth of a second and one-thousandth of a centimeter.

Additionally, the integration of AI technology is in progress to provide a personalized testing environment and to expand the system from simple assessments to rehabilitation exercises.

RE Pathways: Advocating and Advancing Genetic Testing for Cerebellar Ataxia in Australia

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Penny Snell [1], ***Dr. Haloom Rafehi*** [2], ***Ms. Kayli Davies*** [1], ***Ms. Tess Field*** [1], ***Mrs. Greta Gillies*** [1], ***Dr. Justin Read*** [3], ***Ms. Genevieve Thompson*** [3], ***Prof. Lauren Sanders*** [4], ***Dr. David J. Szmulewicz*** [5], ***Prof. Martin Delatycki*** [6], ***Prof. Melanie Bahlo*** [7], ***Prof. Paul Lockhart*** [8]

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Background and Objectives: For many individuals with a suspected genetic cerebellar ataxia (CA) the disease-causing variant remains unknown. Genetic testing for CA in diagnostic laboratories in Australia is limited, especially for repeat expansions. In Victoria, testing typically involves single gene tests for SCA1, 2, 3, 6 & 7 and FRDA, with a diagnostic yield of ~5%. Short-read genome sequencing (GS) can identify small variants, copy number variants, and repeat expansions. Unfortunately, GS is rarely accessible for individuals with CA. Our program, RE Pathways, aims to better understand monogenic causes of ataxia and improve availability of genomic testing for this condition.

Methods: RE pathways will: offer genomic testing to 800 individuals with CA, work with a diagnostic laboratory to offer further clinical repeat expansion testing, and support medical specialists by provision of ataxia specific genomic knowledge. Individuals with ataxia will be contacted, with permission from their specialist doctor, or can express their interest directly.

Results: More than 90% of those referred by their doctor have consented to our research. We have completed GS for 127 individuals with CA, with pathogenic or likely pathogenic variants identified in 48 (38%). Biallelic *RFC1* repeat expansions have been identified by PCR testing for an additional 43 individuals. Finding causal variants has provided diagnostic certainty and facilitated recurrence risk counselling and cascade testing for families.

Discussion and Conclusion: There is considerable utility in genomic testing in individuals with CA, including ending the diagnostic odyssey for individuals who otherwise might not have access to appropriate testing. Individuals with CA and their families are enthusiastic to be part of furthering knowledge in this area and receive important information from the results. RE Pathways is a program to progress understanding of genetic CA and to demonstrate the value of GS for this condition.

Deriving composite biomarkers of disease progression in Friedreich Ataxia using machine learning

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Susmita Saha [1], ***Dr. Paul H Harrison*** [2], ***Dr. Louise A Corben*** [3], ***Dr. Ian Harding*** [4], ***Prof. Nellie Georgiou-Karistianis*** [5]

1. *School of Psychological Sciences, The Turner Institute for Brain and Mental Health, Monash University, Clayton, Victoria, Australia; Department of Neuroscience, School of Translational Medicine, Monash University, Australia,* ***2.*** *Monash Genomics and Bioinformatics Platform, Biomedical Discovery Institute, Monash University, Australia,* ***3.*** *Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia,* ***4.*** *Monash University, Melbourne and QIMR Berghofer Medical Research Institute, Brisbane,* ***5.*** *Monash University*

Background

A major challenge in advancing drug development and clinical trials for Friedreich Ataxia (FRDA) is the lack of objective, sensitive and comprehensive biomarkers to track disease progression. There is a critical requirement to validate improvements in traditional clinical-endpoints with alternative, clinically significant measures for more conclusive outcomes. This study explores the potential of machine learning (ML) approaches to derive fully objective composite biomarkers for monitoring FRDA progression, surpassing traditional clinical scores in effectiveness.

Methods

We developed a ML model using elastic net regression to predict Friedreich's Ataxia Rating Scale (FARS) scores using different combinations of neuroimaging (structural, diffusion, and quantitative susceptibility imaging) and demographic data (sex, age-at-onset, GAA1/GAA2 repeat length, medications, age-at-scan, disease-duration, and years-of-education). The model was trained and validated with repeated 10-fold cross-validation, combining visit1 and visit2 (2-years-follow-up) data from 15 individuals with FRDA, from the IMAGE-FRDA study. Standardised coefficients measured each input's contribution to prediction. Sensitivity to disease progression was assessed for the top-performing combinations, using Cohen's d effect sizes of the differences between visit 2 and visit1(d-score).

Results

The highest FARS prediction was achieved with a weighted combination of all neuroimaging and demographic variables (R^2 = 0.663, d = 1.13), outperforming only neuroimaging (R^2 = 0.527, d = 0.516) or only demographic combinations (R^2 = 0.606, d = 0.924). The highest-weighted individual predictors were age-at-onset, GAA1/GAA2 repeat lengths, disease-duration, volume of cerebellar lobules IV, crus II, and X, and integrity (fractional-anisotropy and radial-diffusivity) of the inferior cerebellar peduncles.

Discussion and Conclusion

A weighted composite of neuroimaging and demographic variables was more sensitive to short-term disease progression than the FARS (d = 0.855) and individual imaging measures. We suggest this objective composite as a stronger biomarker of FRDA progression than individual clinical or imaging scales and a possible alternative or complement to conventional clinician-rated outcome measures.

Prevalence Estimation of the Main Spinocerebellar Ataxias in Latin America: A Meta-analysis Approach

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Milagros Galecio Castillo [1], ***Dr. Maryenela Illanes-Manrique*** [1], ***Dr. Elison Sarapura-Castro*** [1], ***Prof. Mario Cornejo-Olivas*** [1]

1. *Neurogenetics Working Group, Universidad Científica del Sur, Lima, Perú. Neurogenetics Research Center, Instituto Nacional de Ciencias Neurológicas, Lima, Peru*

BACKGROUND AND OBJECTIVES

Spinocerebellar ataxias (SCAs) are autosomal dominant neurodegenerative disorders characterized by cerebellar ataxia with variable extracerebellar symptoms. Currently, more than 40 SCAs have been reported, and their prevalence varies significantly across different regions. We aim to estimate the prevalence of the main SCAs in Latin America (LA).

METHODS

We conducted a systematic search in PubMed, EMBASE, Scopus, WOS, Lilacs, and Scielo, combining the concepts "autosomal dominant cerebellar ataxia", "prevalence", "Latin America" and SCA genes, from inception to 04/2024. We included observational studies where the prevalence of one or more SCA types was reported independently by country. We conducted a meta-analysis of proportions to calculate pooled rates using a random effect method.

RESULTS

Of 601 records, 15 were included in our study, comprising 4 404 individuals with clinical diagnosis of SCA. 7 studies were conducted in Brazil, 2 in Peru, 2 in Mexico, 1 in Argentina, 1 in Cuba, 1 in Venezuela, and 1 in Chile.

We found that MJD/SCA3 was the most prevalent type in LA (21%, 95%CI 9–42%), followed by SCA2 (17%, 95% CI 7-36%), SCA7 (11%, 95%CI 4-26%), and SCA10 (9%, 95%CI 3–24%). Brazil had the highest prevalence of SCA3, Mexico and Cuba of SCA2, Mexico of SCA7, and Peru of SCA10. Other reported types were SCA1, SCA6, and DRPLA (5%, 2%, and 2%, respectively). Of note, cases of SCA28, SCA36, and SCA48 were reported in Argentina; SCA5, SCA8, and SCA27b in Brazil; SCA17 and SCA19 in Mexico, SCA19 in Chile, and an important proportion of SCA with undetermined genotype (17%).

DISCUSSION AND CONCLUSION

Among individuals with genetic confirmation, MJD/SCA3, SCA2, SCA7, and SCA10 are the most prevalent types in Latin America, with variable geographic differences across countries. Importantly, the accuracy of our results is affected by the significant lack of prevalence studies in most countries.

The North American Ataxia Natural History Study: Focus on biomarkers and other outcome measures

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Liana S. Rosenthal [1], ***Dr. Christian Rummey*** [2], ***Dr. Tetsuo Ashizawa*** [3], ***Dr. Andrew Billnitzer*** [3], ***Dr. Marie Y. Davis*** [4], ***Dr. Cameron Dietiker*** [5], ***Dr. Antoine Duquette*** [6], ***Dr. Michael Geschwind*** [5], ***Dr. Anoopum Gupta*** [7], ***Dr. Christopher M. Gomez*** [8], ***Dr. Ali Hamedani*** [9], ***Dr. Chiadi Onyike*** [1], ***Dr. Puneet Opal*** [10], ***Dr. Henry Paulson*** [11], ***Dr. Susan Perlman*** [12], ***Prof. Jeremy D. Schmahmann*** [7], ***Dr. Christopher D. Stephen*** [7], ***Prof. S. H. Subramony*** [13], ***Dr. Sharan Srinivasan*** [11], ***Dr. George Wilmot*** [14], ***Dr. Theresa Zesiewicz*** [15], ***Dr. Lauren Moore*** [16], ***Dr. Sheng-Han Kuo*** [17], ***Dr. Vikram G. Shakkottai*** [18]

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Background

The Clinical Research Consortium for the Study of Cerebellar Ataxia (CRC-SCA) is the largest SCA natural history study in North America. It provides crucial data on disease progression and identification of key outcome measures to guide the design of effective clinical trials. The study spans three phases: the initial grant of 12 sites focused on SCA 1, 2, 3, and 6 (2010-2011), the intermediate phase (2011-2020), and the current phase (2020-present). To adapt to the changing clinical and research landscape, the CRC-SCA has expanded to include additional assessments, more genetic ataxias and biofluid acquisition.

Methods

The CRC-SCA currently enrolls individuals with SCA 1, 2, 3, 6, 7, 8, 10. SCA 27b and RFC1-ataxia were added in mid-2024. Participants are seen annually at 15 U.S. and 1 Canadian site. Demographic data, cognitive testing, psychiatric symptomatology, motor examination, and patient reported outcome measures are assessed. All participants undergo venipuncture for serum and plasma-based biomarker investigations. Participants are encouraged to undergo a lumbar puncture for cerebrospinal fluid collection and storage.

Results

De-identified clinical data is entered into and available to approved researchers and pharmaceutical companies through the University of South Florida's Health Informatics Institute. Biospecimens are stored at BioSEND and obtained through application to NINDS's SCA-Biospecimen Resource Access Committee. Baseline data is available for 929 individuals (152 SCA1, 198 SCA2, 334 SCA3, 179 SCA6, 24 SCA7, 41 SCA8, 9 SCA10). Follow up data is available for approximately half the participants. Serum and plasma is available for approximately 40% of the cohort and CSF is available from approximately 67 individuals.

Discussion

The CRC-SCA study's broad scope and evolving methodologies underscore its role in providing comprehensive data on spinocerebellar ataxias. The dynamic adaptation of outcome measures ensures the study remains relevant and

useful for future clinical trials and regulatory discussions.

Funding

National Ataxia Foundation

Informing clinical trial readiness in ARSACS

Tuesday, 12th November - 18:10: (Minories) - Poster

Prof. Cynthia Gagnon [1]***, Mrs. Isabelle Lessard*** [2]***, Prof. Elise Duchesne*** [3]***, Prof. Luc J. Hébert*** [4]***, Dr. Bernard C. Brais*** [5]***, Dr. Xavier Rodrigue*** [6]***, Prof. Francois Routhier*** [3]***, Prof. Krista Lynn Best*** [3]***, Dr. Jean-Denis Brisson*** [7]***, Mrs. Isabelle Côté*** [8]

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Objectives. Since therapy development in some ataxias is accelerating, this study aimed to improve trial readiness by documenting over a trial-relevant time frame of two years the disease progression in adults with autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) according to their disease stage, and the responsiveness to change of the related clinical outcome assessments (COAs).

Methods. Sixty participants from two neuromuscular clinics (Saguenay and Quebec City, Canada) were included. The COAs were the Scale for the Assessment and Rating of Ataxia (SARA), Disease Severity Index for ARSACS (DSI-ARSACS), 10-meter Walk Test (10mWT), Timed Up & Go test (TUG), 30-second Chair Stand test (30s-CST), Berg Balance Scale (BBS), Activities-specific Balance Confidence-simplified (ABC-S) scale, Lower Extremity Motor Coordination Test (LEMOCOT), co-contraction, Swallowing Disturbance Questionnaire, grip and pinch strength, Standardized Finger-to-Nose test, TEMPA, Barthel Index, and Assessment of Life Habits questionnaire. Self-perception of last year's progression of related COA outcomes was also documented.

Results. A significant progression was observed at the SARA (+1.6 points), DSI-ARSACS (-1.5 points), 10mWT (-0.111 to - 0.165 m/s), TUG (+7.8 seconds), BBS (-4.3 points), ABC-S (-4.3 points), LEMOCOT (-2.3 repetitions) grip (-2.3kg) and pinch (-0.25kg) strength, and Barthel Index (-7.4 points). Differences between disease stages were observed for some COAs.

Discussion. The 10mWT, TUG, BBS, and grip strength are the COAs most sensitive to change; they detected changes specifically in participants who reported getting worse. However, a high proportion of participants reported not having some impairments, which limited the statistical power of some responsiveness analyses, particularly for COAs assessing upper limb functions.

Conclusion. The results of the present study take us one step further toward clinical trial readiness. The progression rate of impairments and identification of the most sensitive to change COAs are of the utmost importance for designing future trials in ARSACS.

Adaptability index of Prism Adaptability Test stably evaluates disease conditions of cerebellar degenerations in clinical environment

Tuesday, 12th November - 18:10: (Minories) - Poster

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[Objectives]
Prism adaptation test is able to be used to quantify human motor-learning capability of the cerebellum. The subjects wearing the prism, which considerably shifts the visual field, are instructed to touch with their finger the target presented in front of them. Healthy subjects can quickly learn how to precisely touch the target with their fingers, even when their gaze is artificially shifted rightward or leftward by the prism lens.
In our previous study, we quantified cerebellar motor-learning capability by calculating the adaptability index (*AI*) from the results of the prism adaptation task. Whereas the *AI*s of healthy subjects showed typically value of around one, the *AI*s of the patients with cerebellar degeneration showed value of around zero. Furthermore, by comparing *AI*s with SARA scores, we found a negative correlation between them. However, it has not been clarified whether *AI*s be able to stably detect a cerebellar disease by measurement in many hospitals. Here, we aim to clarify about stability of *AI*s measured in many hospitals.
[Methods]
We analyzed *AI*s of 74 patients with cerebellar degeneration (21 patients with SCA6, 20 patients with SCA31, 15 patients with SCA3, nine patients with MSA-C, five patients with IDCA, one patient with DRPLA, one patient with left inferior olivary degeneration, and two patients with other type of SCD) measured in seven hospitals.
[Results]
Comparing *AI*s with SARA scores, it was indicated that a negative correlation between them (p=0.001, C=-0.364). This result is the same as that of measurement at a hospital in our previous study. Moreover, we found a negative correlation between *AI*s and SARA scores of patients with either SCA6 or SCA31(p=0.001, C=-0.504).
[Discussion/Conclusion]
Our results show that *AI*s are able to stably measured in any hospital and evaluate motor-learning capability.

Risk factors for the development of anxiety and depression in patients with Friedreich's Ataxia

Tuesday, 12th November - 18:10: (Minories) - Poster

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Background and objectives

Anxiety and depression are increasingly prevalent in the general population. Living with a chronic neurodegenerative condition such as in patients with Friedreich's Ataxia (FRDA) confers a significant risk, which can considerably impact their quality of life. The aim of this study was to investigate the risk factors predisposing FRDA patients to neuropsychiatric disorders.

Methods

Data from 34 CHUM patients (10 males) with a genetically confirmed diagnosis of FRDA was collected as part of the large observational Friedreich's Ataxia Clinical Outcomes Measures Study (FACOMS). Disease severity was assessed using the Friedreich's Ataxia Rating Scale (FARSn) score. The Hospital Anxiety and Depression Scale (HADS) was used to quantify anxiety (HADS-A) and depression (HADS-D).

Results

The prevalence of anxiety and depression was 5.88% and 2.94% respectively, with whole-cohort average scores of 4.18±2.24(mean±SD)(HADS-A) and 3.21±2.1(HADS-D).

HADS-D correlated with FARSn upper limb (UL) function sub-scores(r=0.34,P=0.05), but not overall FARSn, with a stronger positive correlation among females(r=0.48,P=0.018) compared to males(r=-0.01,P=0.98). Higher BMI moderately correlated with depression scores(r=0.47,P=0.02), but did not vary with ambulation status(P=0.07).

HADS-A scores were higher in patients with an FRDA-related heart conditions(P=0.0032), and varied with mobility impairment(one-way ANOVA F(2,32)=3.43,P=0.045), with higher scores in severely impaired patients(P=0.039). HADS scores did not correlate with age at diagnosis or symptom onset.

Discussion and conclusions

UL motor function, mobility impairment and increased BMI represent potential risk factors for the development of neuropsychiatric symptoms and could constitute helpful targets for intervention. Heterogeneous trajectories between males and females are observed in relation to UL function, suggesting a disproportionally increased risk in females. In accordance with previous studies, HADS scores were not contingent on global disease severity as measured by the FARSn scores. Younger age, and hence more severe disease did not prove to be a risk factor.

Funding: Friedreich's Ataxia Research Alliance (FARA), Ataxia Canada

Submovements from wearable sensors are predictive of ataxia severity and differ across motor tasks and directions of motion

Tuesday, 12th November - 18:10: (Minories) - Poster

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Background and Objective: Movement patterns extracted from wearable sensors during natural behavior capture ataxia severity and progression. Improved understanding of how submovement kinematics vary based on behavior could inform more sensitive assessments.

Method: Seventy individuals with ataxia (4 SCA1, 2 SCA2, 9 SCA3, 6 SCA6, 7 SCA-Other, 5 MSA-C, 3 HSP, 1 A-T, 1 FA, 1 ARCA-1, 1 ARCA-3, 1 CANVAS) and 27 controls performed finger-to-nose, finger-chase, alternating hand movements, heel-to-shin, and foot-tapping tasks. Sixteen submovement kinematic features were extracted from wrist and ankle accelerometers from each task. t-Distributed Stochastic Neighbor Embedding (t-SNE) was used to visualize the submovement feature space. Two severity modeling approaches were compared: training a single regression model where data is combined across all tasks (task-pooled), versus training separate regression models for each task and combining model outputs into a single score (task-separated). Both modeling approaches were trained to predict the Brief Ataxia Rating Scale (BARS).

Results: Submovement kinematic variance was predominantly explained by the task and direction of motion. The task-separated approach (r=0.82 [95% CI: 0.77 to 0.86]) correlated more strongly with BARS than the task-pooled approach (r=0.75 [0.68-0.80]). All tasks contributed positively but disproportionately to the task-separated measure, with finger-chase and heel-to-shin contributing the most.

Discussion: Submovement properties are influenced not just by ataxia severity, but also by the type and direction of movement. Modeling each task separately produces modest improvements in severity estimation.

Conclusion: Submovements are a useful construct for assessing ataxia across a broad range of motor tasks. For submovement-based analysis of natural behavior, parcellation of different motor behavior categories may produce improved estimates of motor impairment.

Funding: NIH R01 NS117826

Speech, Language, and Swallowing Research in Children with Ataxia (SLICA)

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Caroline Spencer [1], ***Prof. Adam P. Vogel*** [2]

1. *Indiana University,* ***2.*** *The University of Melbourne*

Ataxia affects communication and swallowing function, leading to dysarthria, impaired language processing, and dysphagia. Much of our current knowledge comes from research on adults, often many years after their ataxia onset. Capturing only later-in-life symptomatology leaves early trajectories relatively unaddressed. Clinically, interventions designed primarily for adults are unlikely to be suitable for children. Here we outline the launch of an inclusive global initiative to investigate the speech, language, and swallowing functions in children with ataxia.
We have established a clinical-research collective focused on assessing speech, language, and swallowing abilities of children with ataxia throughout their development. Utilizing a network of clinical sites specializing in ataxia care, we will collect data from children (under age 18) with Friedreich's ataxia, spinocerebellar ataxia, and ataxia telangiectasia. We plan to collect clinical, behavioral and acoustic data from patients, as well as patient reported outcomes and questionnaires from caregivers. Initial research questions include:

- Are speech, language, and swallowing functions in children with ataxia age-dependent, and severity-dependent?
    - Pediatric patients may have a symptom trajectory that differs from adult patients
- Do symptoms differ by specific etiology? Are there subgroups within specific diagnostic categories?
- Since language and cognitive skills continue to develop throughout childhood, are there different symptoms of the language and cognitive abilities in children ataxia, compared to adults with ataxia?
- How can we use this information to inform future clinical trials in child and adolescent populations?

Our long-term goal is to improve early identification of dysarthria, language, and dysphagia in children with ataxias. This information will ultimately assist us in improving clinical outcomes by developing treatment paradigms tailored to children.

Excellent concordance in plasma and serum concentrations of NfL and GFAP in SCA3 mutation carriers enrolled in the ESMI study

Tuesday, 12th November - 18:10: (Minories) - Poster

Mr. Jérémy Manry [1], Dr. Hector Garcia-Moreno [2], Dr. Magda Santana [3], Dr. Jennifer Faber [4], Ms. Aurore SORS [5], Ms. Natacha MOULHARAT [5], Prof. Manuela Lima [6], Prof. Ludger Schöls [7], Dr. Jon Infante [8], Prof. Bart van de Warrenburg [9], Ms. Cécilia GABRIEL GRACIA [5], Prof. Luís Pereira de Almeida [3], Prof. Thomas Klockgether [4], Prof. Paola Giunti [2]

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Introduction. Neurofilament light chain (NfL), glial fibrillary acidic protein (GFAP), total tau (t-tau) and ubiquitin carboxy-terminal hydrolase L1 (UCHL1) have been proposed as biomarkers in neurological diseases, and some of them are currently being evaluated in several natural history studies and clinical trials (using CSF and different blood matrices). We evaluated the concordance between plasma and serum concentrations of these four biomarkers using samples from SCA3 mutation carriers.

Methods. We selected 120 samples from 40 SCA3 mutations carriers enrolled in different ESMI sites. Participants were selected based on SARA scores (covering different disease stages) and previous blood NfL measurements (covering a broad range of concentrations). Samples were processed following a common protocol. Per each participant, we analysed two types of plasma samples (Cell Preparation Tubes -CPT- and Plasma Preparation Tubes -PPT) and one serum sample (Serum Separator Tubes -SST). Samples were analysed with a single lot of the Neurology 4-Plex "A" assay, in the HD-X Simoa platform. To assess concordance between plasma CPT, plasma PPT and serum SST, intraclass correlation coefficient (ICC) and pairwise concordance correlation coefficients (CCC) were calculated. Then, limits of agreement were evaluated by Bland-Altman plots, and Passing-Bablok regression analysis was applied to assess proportional differences between matrices.

Results. We found excellent concordance across matrices for NfL and GFAP [ICC_{NfL}=0.92 (95%CI: 0.87–0.95); ICC_{GFAP}=0.92 (95%CI: 0.88–0.96)], and most differences between paired measurements fell within limits of agreement. Proportional and systematic differences between matrices for NfL and GFAP were defined through regression equations. Poor and moderate concordance were observed for t-tau [ICC=0.17 (95%CI: -0.02–0.38)] and UCHL1 [ICC=0.61 (95%CI: 0.44–0.75)].

Discussion and Conclusions. Our findings support the use of corrective factors for NfL and GFAP measurements performed in different blood matrices. This will contribute to the combination of biomarker data obtained in SCA3 samples from different cohorts.

Funding. Servier.

Natural history and neurological progression in xeroderma pigmentosum: a cohort study in the UK

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Hector Garcia-Moreno [1], ***Prof. Douglas R Langbehn*** [2], ***Dr. Adesoji Abiona*** [3], ***Dr. Isabel Garrood*** [3], ***Dr. Zofia Fleszar*** [1], ***Dr. Marta Manes*** [4], ***Ms. Ana M S Morley*** [5], ***Ms. Emma Craythorne*** [3], ***Dr. Shehla Mohammed*** [6], ***Ms. Tanya Henshaw*** [3], ***Ms. Sally Turner*** [3], ***Ms. Harsha Naik*** [3], ***Dr. Istvan Bodi*** [7], ***Dr. Robert P E Sarkany*** [3], ***Dr. Hiva Fassihi*** [3], ***Prof. Alan R Lehmann*** [8], ***Prof. Paola Giunti*** [1]

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Xeroderma pigmentosum (XP) results from biallelic mutations in any of eight genes involved in DNA repair systems (defining eight genotypes, from XPA to XPG, and XP variant or XPV). In addition to cutaneous and ophthalmological features, some patients present with XP neurological disease. We aim to characterise the XP neurological disease and its evolution in the heterogeneous UK XP cohort.

XP patients were followed in the UK National XP Service, from 2009 to 2021. Patients' mutations received scores based on their predicted effects.

Ninety-three XP patients were recruited. Thirty-six (38.7%) reported neurological symptoms, especially in the XPA, XPD and XPG groups, with early-onset and late-onset forms, and typically appearing after cutaneous and ophthalmological symptoms. XPA, XPD and XPG patients showed higher SARA scores compared to XPC, XPE and XPV. SARA total scores significantly increased over time in XPD (0.91 points/year) and XPA (0.63 points/year). Hyporeflexia, hypopallesthaesia, upper motor neuron signs, chorea, dystonia, oculomotor signs and cognitive impairment were frequent in XPA, XPD and XPG. Cerebellar and global brain atrophy, axonal sensory and sensorimotor neuropathies, and sensorineural hearing loss were common findings in patients. Some XPC, XPE and XPV cases presented with abnormalities on examination and/or ancillary tests, suggesting an asymptomatic XP neurological disease. More severe mutations were associated with a faster progression in SARA in XPA (increasing 0.40 points/year per 1-unit change in severity score) and XPD (0.60 points/year per 1-unit change), and in ADL in XPA (0.35 points/year per 1-unit change).

Neurological disease is frequent in XP, being typically preceded by cutaneous and ophthalmological features, and these symptoms should be actively searched in patients with idiopathic late-onset neurological syndromes. Scales assessing cerebellar function and disability can show progression in some of the groups. Mutation severity can be used as a prognostic biomarker in clinical trials.

Plasma neurofilament light chain, total tau, glial fibrillary acidic protein and ubiquitin carboxy-terminal hydrolase L1 in SCA1, SCA2, SCA6 and SCA7

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Hector Garcia-Moreno [1], ***Prof. Douglas R Langbehn*** [2], ***Dr. Amanda Heslegrave*** [3], ***Prof. Henrik Zetterberg*** [4], ***Prof. Paola Giunti*** [5]

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Background and objectives. Monitoring biomarkers tracking disease progression are an unmet need in spinocerebellar ataxias. Neurofilament light chain (NfL), total tau (t-tau), glial fibrillary acidic protein (GFAP) and ubiquitin carboxy-terminal hydrolase L1 (UCHL1) have shown potential as fluid biomarkers in neurological conditions. We aim to study plasma concentrations of these biomarkers in a UK cohort of SCA1, SCA2, SCA6 and SCA7 carriers.
Methods. Plasma NfL, t-tau, GFAP and UCHL1 were measured with the Neurology 4-Plex "A" kit in the Simoa HD-X analyser.
Results. We recruited SCA1 (n=14), SCA2 (n=11), SCA6 (n=36), and SCA7 (n=5) carriers, as well as healthy controls (n=33). Carriers were classified into preataxic (SARA<3) or ataxic participants (SARA>=3). Preliminary results showed that unadjusted NfL concentrations were higher in ataxic SCA1 (3.4 logpg/mL; 95% CI: 3.1, 3.7), SCA2 (3.2 logpg/mL; 95% CI: 2.9, 3.5), SCA6 (2.7 logpg/mL; 95% CI: 2.5, 2.8) and SCA7 participants (3.4 logpg/mL; 95% CI: 2.9, 3.9) compared to controls (2.2 logpg/mL; 95% CI: 2.0, 2.4; all contrasts, p<0.001). Ataxic SCA6 carriers showed lower plasma NfL compared to SCA1, SCA2 and SCA7 (all contrasts, p<=0.002). In SCA6, plasma NfL was associated with age (0.019; p=0.017). Unadjusted GFAP concentrations were higher in ataxic SCA1, SCA2 and SCA6 participants compared to controls (all contrasts, p<=0.005). In SCA2 and SCA6, GFAP was associated with disease duration (both, p<=0.009).
T-tau concentrations did not differ between groups. Plasma UCLH1 measurements were unreliable, as a high proportion of values showed CV above the acceptable threshold (20%).
Discussion and Conclusion. Plasma NfL is increased in ataxic patients in all the SCA groups. Studies in larger cohorts, with longitudinal sampling, are required to clarify its potential in tracking disease progression. Plasma GFAP was increased in ataxic patients in some of the groups, but further analyses are required.
Funding. Medical Research Council, CureSCA3.

Dentatorubral-pallidoluysian Atrophy (DRPLA) Clinical and genetic findings in a Chinese cohort

Tuesday, 12th November - 18:10: (Minories) - Poster

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Background

Dentatorubral-pallidoluysian atrophy (DRPLA) is an autosomal dominant cerebellar ataxia caused by cytosine-adenine-guanine (CAG) repeat expansion (>48 tandem copies) in *ATN1*. The clinical manifestations of DRPLA vary depending on the age of onset, including epilepsy, myoclonus, ataxia, choreoathetosis, and dementia. It is a group of rare and difficult to identify diseases.

Objective

Here we report cross-sectional baseline data to describe DRPLA profile and deepen the understanding of the disease from clinical, biomarker, genetic and other multi-dimensions.

Methods

This prospective analysis included the clinical, molecular data, biomarker, and imaging of patients diagnosed with DRPLA from December 4, 2021, through May 12, 2024. Analyses were done on the baseline cross-sectional data from patients.

Results

We enrolled 111 patients from 45 families were diagnosed with DRPLA (69 males [62%],42 females [38%]), including 91 patients (72 adult-onset patients [86%]), 19 juvenile-onset patients [14%]) and 20 premanifest patients. A shared haplotype of 286.0kb in length containing DRPLA CAG expansion was identified in 8 families. The degree of cognitive impairment is related to course of disease and the severity of the disease, with extensive cognitive domains involved. The level of Plasma neurofilament light (NfL) were significantly higher in manifest DRPLA than in controls(p<0.001). DRPLA patients had widespread gray-matter volume reductions. White matter lesions were present in 86% of the manifest DRPLA patients.

Discussion and Conclusion

The clinical manifestations of DRPLA patients in our cohort were consistent with those reported in the previous literature. Founder effect was also observed in Chinese DRPLA patients. Five cognitive domains are affected in DRPLA patients. Plasma NfL is a potential biomarker of disease progression and treatment evaluation in DRPLA. White matter lesions are important points of differentiation from other SCAs.

Funding

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SCA27B in a Czech late-onset cerebellar ataxia cohort: phenotypic spectrum, episodic symptoms and 4-aminopyridine treatment response

Tuesday, 12th November - 18:10: (Minories) - Poster

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Background and objectives: SCA27B is a late-onset cerebellar ataxia caused by repeat expansion in the *FGF14* gene. Studies from different cohorts suggest that it accounts for a large number of late-onset ataxia cases.
Methods: Seventy-nine patients with unresolved late-onset cerebellar ataxia or downbeat nystagmus followed at the Center of Hereditary Ataxias, Motol University Hospital, Prague, underwent genetic analysis for the presence of GAA repeat expansion in the intronic part of the *FGF14* gene.
Results: Twenty-eight patients (35%) had expansion above the pathogenic threshold (³ 250 GAA repeat units); 8 of them had expansion in the reduced penetrance range (250–300 GAA repeat units). The disease occurred sporadically in 58 % of the patients. The average age at disease onset was 55,8 years. All patients had progressive cerebellar syndrome. Downbeat nystagmus and significant fluctuations were present in 64% and 46% of patients, respectively. Treatment with 4-aminopyridine was initiated in 18 patients, with a favorable effect in 15 of them.
Discussion and conclusion: SCA27B is a common cause of late-onset cerebellar ataxia in the Czech Republic. We confirm a significant number of patients with episodic features and downbeat nystagmus. Due to its relatively high prevalence, SCA27B should be included in a standard testing algorithm for patients with adult-onset chronic ataxia.
Funding: National Institute for Neurological Research Project No. LX22NPO5107

Clinical outcomes predict patient-relevant disease milestones in SCA3: a large SARA-based multicenter study

Tuesday, 12th November - 18:10: (Minories) - Poster

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Background and Objective

It's key for natural history studies and treatment trials to predict future patient-relevant milestones - ideally with readily usable clinical outcome assessments (COAs), *e.g.* SARA. In turn, to yield patient meaningfulness of COAs, as mandated by the FDA, it is urgently warranted to charge COAs with patient-relevant meaningfulness. Both goals might be achieved by time-to-event predictions (events reflecting patient-relevant milestones) based on prognostic variables of the COA. We hypothesized that predictive patterns for loss of patient-relevant functions could be identified for SCA3 based on SARA score.

Methods

We analysed the ability of SARA, $SARA_{gait}$ (gait item), and $SARA_{g\&p}$ (gait&posture items 1-3) to predict patient-relevant milestones in SCA3 progression, leveraging the ESMI cohort undergoing annual assessments, including SARA, FARS-ADL with 1213 annual follow-up visits of 385 patients. Critical patient-relevant milestones were defined as disease stages 2 (permanent walking aid dependence) and 3 (permanent wheelchair dependence) (Klockgether1998), and different levels of the FARS-ADL items.

Results

We determined $SARA_{gait}$ and $SARA_{g\&p}$ levels that longitudinally predict the risk of reaching disease stages 2 and 3, respectively. For example, the risk of reaching stage 2 for participants with $SARA_{g\&p}$=6 was 24% at baseline, 62% after two years, and 71% after three years. The risk of reaching stage 3 for participants with $SARA_{g\&p}$=11 was 22% at baseline and 77% after three years; for participants with $SARA_{gait}$=5 it was 2.9% at baseline and 87% after three years. The risk of reaching $FARS\text{-}ADL_{walk}$=3 for participants with $SARA_{g\&p}$=6 was 26% at baseline, 61% after two, and 74% after three years.

Discussion

$SARA_{g\&p}$ items are effective predictors of patient-relevant milestones in SCA3. This predictive anchoring can be utilized in clinical trial settings to justify the selection of SARA levels that may not directly correspond to a patient-relevant endpoint, yet reliably predict the occurrence of a patient-relevant milestone.

Development of clinically objective parameters for spinocerebellar ataxia type 31

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Hanako Aoki [1], Dr. Akiko Amano [1], Dr. Takeru Honda [1], Dr. Miwa Higashi [1], Prof. Tetsuya Nagata [1], Prof. Takanori Yokota [1], Prof. Kinya Ishikawa [1]

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Background and Objective

Spinocerebellar ataxia type 31 (SCA31), caused by a mutation of complex penta-nucleotide repeats in *BEAN1* and *TK2*, is characterized by a late-onset and relatively pure cerebellar symptom. Longitudinal natural history study showed that the annual progression of Scale for the Assessment and Rating of Ataxia (SARA) score was 0.8 points/year (Cerebellum. 2017;16:518-24). We need further clinical evaluation to assess the efficacy of emerging disease modifying therapies.

Methods

We designed a prospective-and cross-sectional study of patients with SCA31. All patients were clinically evaluated using SARA, and International Cooperative Ataxia Rating Scale (ICARS). Participants also underwent assessments using 9 hole-peg test (9HPT), 6 minutes walking test (6MWT), Timed 25-foot walk test (T25W) and Kinect recordings (Front Neurol. 2020;11:179). Additionally, we collected cerebrospinal fluid and blood samples, and conducted brain MRI evaluations.

Results

A total of 18 patients with genetically confirmed diagnosis of SCA31 were enrolled. The mean age of onset was 68.3± 7.2 (standard deviation) years, and the mean disease duration was 12.3± 4.1 years. The participants had an average SARA score of 12.6± 3.1 and ICARS score of 35.2± 9.2. SARA and ICARS showed a strong correlation (Pearson's r= 0.777, p <0.05), and the disease duration also correlated with SARA and with ICARS (r= 0.522, p <0.05). SARA also correlated with 9HPT. For the ataxia indices of gait, T25W and 6MWT significantly correlated with gait scores in SARA and ICARS. The distance between both knees at Kinect v2 during 6MWT was also correlated with SARA and with ICARS. However, disease duration did not correlate with T25W, nor with 6MWT.

Discussion and Conclusion

We found that walking performance is reliable and objective clinical parameters in SCA31 patients, making us include these walking tests in future clinical trial employing disease-modifying therapy. Investigations are ongoing to find fluid and radiological markers.

Plasma glial fibrillary acidic protein correlates with disease severity in spinocerebellar degenerations

Tuesday, 12th November - 18:10: (Minories) - Poster

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Background and objectives: The search for blood biomarkers is a primary need for future clinical trials in spinocerebellar degenerations. Our study aimed to explore Glial fibrillary acidic protein (GFAP) as a potential biomarker in these diseases.

Methods: We measured plasma GFAP and Neurofilament light chain (NfL) levels in inherited spinocerebellar ataxias (SCAs) and spastic paraplegias (SPGs) carriers using Simoa assay (Quanterix). Clinical assessments included SARA and CCAS scales. We examined correlations between plasma GFAP and NfL with disease severity.

Results: We included 138 individuals: 12 SCA1, 24 SCA2, 37 SCA3, 11 SCA7, 18 SCA27B, 20 SPG7, and 16 SPG4. SCA27B patients were the oldest (mean age: 73.8±7.2 years), followed by SPG7 (55.8± 9.9), with other groups ranging from 43.1 to 51.9 years. SCA3 carriers had the lowest SARA scores (8.9±6.1) compared to the other groups (13.0 to 18.4). Mean plasma GFAP levels were higher in SCA27B (219±154 pg/ml) compared to the other groups (104 to 130 pg/ml, p=0.003), which were all significantly elevated compared to healthy controls (30-70 pg/ml). Plasma Nfl levels were higher in SCAs than SPGs (21.5±15.2 vs 11.8±7.2 pg/ml, p<0.001). For the pooled cohort, plasma GFAP levels positively correlated with NfL (r=0.48, p<0.001), and this correlation was consistent across genotypes taken separately. GFAP and Nfl levels correlated with disease severity scored by SARA (r=0.39, p<0.001 and r=0.26, p=0.005, respectively) and GFAP also with cognitive impairment assessed by CCAS (r=-0.38 p=0.002). Adjusting for age, SARA, and NfL, GFAP levels did not differ significantly between genotypes (p=0.07).

Discussion and conclusion: Plasma GFAP levels are mainly linked to age, SARA score, and NfL levels in spinocerebellar degenerations. Interestingly, GFAP showed stronger correlations with disease severity than NfL, indicating astrocytes involvement in these diseases. GFAP could be a promising biomarker for spinocerebellar degenerations, warranting further research, including studies on presymptomatic carriers.

Examining free-living motor activity measurements and task-based diadochokinetic rate in ataxia-telangiectasia

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Anna Luddy [1], ***Ms. Faye Yang*** [2], ***Dr. Nancy Soja*** [1], ***Dr. Kathryn Connaghan*** [3], ***Ms. Jennifer Thornton*** [4], ***Ms. Sara Reiling*** [4], ***Dr. Anoopum Gupta*** [2]

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Background: As disease therapies for ataxias are being investigated, it is necessary to develop improved biomarkers to evaluate disease severity and progression. We tested the hypothesis that movement metrics derived from a wrist-worn accelerometer and speech syllable rate can provide accurate, interpretable, and reliable information about disease severity and progression.

Methods: Data were analyzed from 25 participants with ataxia-telangiectasia (A-T) and 26 controls ranging from six to twenty-six years of age. 9 participants with A-T and 10 controls completed the study at two-time points separated by one year. Participants continuously wore a GENEActiv accelerometer device on their dominant wrist and ankle for one week. An overall motor severity score was generated based on wrist movement patterns. Participants completed a speech survey three times over a week, including repeating "pah-tah-kah" and "tah-kah-pah" continuously for 10 seconds. The number of syllables per second was calculated for each recording and averaged across the two tasks per participant per time point to determine the overall diadochokinetic (DDK) rate. Caregivers also completed outcome measures, including the CPCHILD and Dysarthria Impact Scale.

Results: DDK rate was highly informative for distinguishing individuals with A-T from controls. Rates for participants with A-T ranged from 1.1 syllables/second to 4.1 syllables/second, with 92% of the samples falling at or below 3.3 syllables/second. Controls spanned 1.8 syllables/second to 6.5 syllables/second, and 62% of the samples were above 3.3 syllables/second. For controls, variance was higher for DDK rate compared to the sensor-based motor score. Conversely, in the A-T population, the sensor-based score had higher variance than DDK rate.

Discussion/Conclusion: These results demonstrate that data from the wrist sensor and DDK speech task produce informative measures but have different statistical properties that inform about the context of use and future development.

Funding: A-TCP, NIH NS134597

The two faces of pediatric SCA2

Tuesday, 12th November - 18:10: (Minories) - Poster

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Background and objectives: Spinocerebellar ataxia type 2 (SCA2) is an autosomal dominant neurological disease largely described in adults. Although first signs usually appear between 30 and 40 years, the phenotype may worsen through generations due to the CAG expansion instability in the *ATXN2* gene, leading to a pediatric onset. This study aims to describe the natural history of SCA2 in children.

Methods: Pediatric neurologists, neurologists, and geneticists from 17 institutions pooled resources to identify pediatric SCA2. We analysed clinical and genetic data and compared them to those of 20 children previously reported in the literature and to paediatric SCA7 patients.

Results: We included 22 SCA2 patients with a mean age at onset of 6.5±5.8 years (range 0.25-15). Unlike pediatric SCA7, which exhibits a continuum within the phenotypic spectrum, the phenotype of pediatric SCA2 can be divided into two distinct groups based on CAG repeat size and age, independent of the parental origin. In the infantile group,

the disease onset was in the first months of life and all children carried more than 100 CAG repeats. Developmental delay and seizures were prominent features, along with pontocerebellar atrophy; all died before 3 years of age. In the juvenile group, the mean age at onset was 10.7 ± 3.5, with CAG repeats ranging from 43 to 69. Cerebellar ataxia was the major feature with a mean age at loss of walking at 23.8±8.9 years. Four individuals developed chorea or dystonia, and three had limbs and trunk myoclonus. Epilepsy was not reported in any juvenile patients.

Discussion and Conclusion: We identified two phenotypic groups of pediatric SCA2 related to CAG repeat sizes, with 88-90 CAG being the threshold separating the two groups. Our series provides the first comprehensive description of pediatric SCA2, which will help to improve early clinical diagnosis of this rare condition.

The daily life and psychosocial impacts of Friedreich ataxia: a qualitative study of patient lived experiences

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Andrea Bever [1], Ms. Shelagh Szabo [1], Dr. Julie Vallortigara [2], Prof. Paola Giunti [2], Prof. David Lynch [3], Dr. Gessica Vasco [4], Dr. Ioannis Tomazos [5]

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Background and Objectives: Friedreich ataxia (FA) is a progressive and systemic neurologic movement disorder characterized by impaired motor function and speech. Although impacts of FA on patient health-related-quality of life (HRQoL) have been documented, generic HRQoL measures can miss key disease-specific impacts, particularly in rare conditions. Qualitative methods can provide unique insights into patient lived experiences, contextualizing the profound impact of clinical manifestations. This study sought to understand patient perspectives on the impact of FA symptoms on daily life and psychosocial wellbeing.

Methods: Qualitative interviews were conducted among individuals with FA, or caregivers as proxies, in the US, UK and Germany. Participants responded to open-ended questions about the impact of FA symptoms on day-to-day life, including key challenges and adaptations. Interviews were held virtually, audio-recorded and transcribed. Patient demographic and clinical characteristics were summarized, and conventional content analysis was used to explore patterns in the data.

Results: Sixteen patients and 13 caregivers were interviewed. Mean (standard deviation, SD) patient age was 33 (12.1) years and 62.1% were female. Among 14 (48.3%) non-ambulatory patients, mean (SD) age at loss of ambulation was 24.0 (8.8 years). All 29 participants described impaired motor skills, which leads to doing everyday tasks slowly and with greater effort. Twenty-two participants reported challenges with managing fatigue, creating obstacles to participation. Emotional impacts, including frustration, sadness or worry, were common (n=27). These impacts were often provoked by the progression of symptoms and consequent loss of independence. Despite these challenges, individuals adapt to life with FA by finding ways to maintain participation and social connection, developing coping strategies, and identifying sources of optimism.

Discussion and Conclusion: This study augments scarce data to describe the lived experiences of individuals with FA. Findings highlight psychosocial challenges that permeate everyday life, and sources of strength and adaptation.

Age at loss of ambulation among patients with Friedreich ataxia using health administrative claims data in the United States: A retrospective study

Tuesday, 12th November - 18:10: (Minories) - Poster

Prof. David Lynch [1]***, Ms. Christina Qian*** [2]***, Ms. Lauren Powell*** [2]***, Dr. Gessica Vasco*** [3]***, Dr. Alana Salvucci*** [4]***, Dr. Karissa Johnston*** [2]***, Dr. Ioannis Tomazos*** [5]

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Background and Objectives: Friedreich Ataxia (FA) is a progressive and systemic neurologic movement disorder, characterized by worsening ataxia, scoliosis, and loss of ambulation (LOA). This study aimed to characterize the age at LOA among a cohort of patients with FA, using retrospective United States (US) claims data.

Methods: The US Merative MarketScan Commercial database from Aug 2010 to Sept 2020 was used. All incident patients ≤24 years of age were identified. Incidence was defined with an 18-month washout period. Included patients were followed up for 12 months. This 30-month period was used to observe LOA by age at FA diagnosis, through the presence of ≥1 LOA diagnosis or claim for mobility aid.

Results: Seventy-seven patients with FA were diagnosed before 24 years of age (43 [56%] were diagnosed <16 years and 34 [44%] between 16-24 years). Of these, 42 (55%) had LOA captured within this 30-month period, and most patients were identified through their use of manual wheelchairs (83%). Among these 42 patients, the majority (57%) had LOA <16 years, with an overall mean (standard deviation [SD]) age at LOA of 15.0 (5.6) years. When stratified by age, patients who were diagnosed with FA <16 years (10.9 [4.0] years), and were observed to have LOA (n=19), had LOA at 10.3 (4.3) years; whereas those diagnosed later (16-24 years; n=23; 20.2 [2.7] years at the time of FA diagnosis) had LOA at 18.9 (3.0) years on average.

Discussion and Conclusion: In this cross-sectional analysis, most patients who were diagnosed with FA before the ages of 24 years experienced LOA and wheelchair use before the age of 16 years. While limitations exist in ascertainment of LOA using claims data, findings suggest that those who had earlier onset of FA also had earlier LOA.

‘We have conversations that other couples don’t have to have’: A qualitative study of the impact of caregiving in Friedreich ataxia (FA)

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Shelagh Szabo [1], Ms. Andrea Bever [1], Prof. David Lynch [2], Dr. Gessica Vasco [3], Prof. Paola Giunti [4], Dr. Julie Vallortigara [4], Dr. Ioannis Tomazos [5]

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Background and Objectives: In FA, a rare neuromuscular disease characterized by impaired motor function and speech, caregiving is typically required. A recent review of caregivers in rare inherited diseases identified gaps in understanding mediators of burden and how impacts differ between parental and spousal caregivers. We sought to understand FA caregiver experiences, and how impacts vary by caregiver type.
Methods: Qualitative interviews were conducted among FA caregivers from the US, UK and Germany, recruited through patient advocacy groups. Participants responded to open-ended questions about caregiving impact on daily activities and emotional outlook, including challenges and strategies to mitigate these. Interviews were virtual, audio-recorded and transcribed. Transcript data were analyzed using conventional content analysis.
Results: Mean (standard deviation) caregiver age (n=13) was 43.2 (5.9) years; 76.9% were female and 69.2% cared for patients with symptom onset at <16 years of age. All 13 participants described ‘making space’ for caregiving, which impacts ability to work, socialize, and maintain a healthy lifestyle. Emotional stressors were substantial and demands ever-present; frustration and worry were common. Six caregivers highlighted sources of positivity like increased empathy and strengthened relationships. Nine caregivers described coping strategies including making time for themselves, mindfulness, and research participation. Perceived caregiving burden varied based on effectiveness of coping strategies, intensity of demands, availability of external support, and other personal factors. Spousal caregivers (n=7) discussed unique challenges around family planning and intimacy, needing to shoulder more household tasks, or having to consider the impact of premature mortality.
Discussion and Conclusion: While caregiving impact on emotional health, daily activities, and relationships can be substantial, these can be balanced by sometimes surprising positive aspects. Perceptions of caregiving intensity, which are multifactorial, can vary substantially, and differ between spousal and parental caregivers. These findings augment scarce data on caregiving impact in FA.

Fiberoptic Endoscopic Evaluation of Swallowing is Feasible and Shows High Prevalence of Dysphagia in Patients with Friedreich's Ataxia

Tuesday, 12th November - 18:10: (Minories) - Poster

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Background:
At least a third of patients with Friedreich's ataxia (FRDA) show unsafe swallowing on video-fluoroscopy. Fiberoptic endoscopic evaluation of swallowing (FEES) is a procedure that can be completed at bedside, provides information regarding swallowing safety and efficiency without radiation exposure, and allows for simultaneous noninvasive respiratory testing.

Methods: Pilot study of patients aged ≥18 years with FRDA. Patients underwent FEES, pulmonary and respiratory muscle testing, diaphragm ultrasound, modified Friedreich's Ataxia Rating Scale (mFARS), Swallowing Quality of life questionnaire (SWAL-QOL), Eating Assessment Tool (EAT-10), and Functional oral intake scale (FOIS). Demographics and medication history were extracted from the health records. Worst Penetration Aspiration Scale (PAS) (range 1-7, abnormal ≥3) and dynamic imaging grade of swallowing toxicity (DIGEST) (range 0-4, abnormal ≥1,) scores were obtained from FEES.

Results: Five subjects (four male) were enrolled. Median age was 33 years (range: 18-42), median mFARS was 66 (range: 36-84.5), three were non-ambulant.

EAT-10 scores were abnormal in 4 subjects (≥3), FOIS was abnormal in two subjects. DIGEST scores were 1 in 4 subjects and 2 in one subject. PAS score ranged from 3 to 5.

Forced Vital Capacity was 33 to 106% predicted, cough peak flow ranged from 107 to 573 L/min (<270L/min in two). Paradoxical diaphragmatic movement was observed in one patient. SWAL-QOL provided relevant insight related to dysphagia in all subjects.

Discussion: Dysphagia was present in all subjects. EAT-10 was able to identify 4 out of 5 patients with abnormal FEES results. However, the results did not correspond with other clinical metrics or patient reported outcomes. Our study is limited by the small sample.

Conclusion: FEES is feasible in patients with FRDA and provides a comprehensive assessment of swallowing. Dysphagia is common in patients with FRDA, even in those with reassuring self-reported symptoms and respiratory testing.

Towards digital-motor outcomes for limb movements in spastic ataxia: a comparative validation of Q-Motor in ataxia and hereditary spastic paraplegia

Tuesday, 12th November - 18:10: (Minories) - Poster

Mrs. Malin Schulze [1], ***Mr. Dominik Hermle*** [1], ***Mr. Robin Schubert*** [2], ***Mr. Pascal Barallon*** [2], ***Dr. Winfried Ilg*** [3], ***Prof. Rebecca Schüle*** [4], ***Dr. Ralf Reilmann*** [2], ***Prof. Matthis Synofzik*** [5], ***Dr. Andreas Traschütz*** [5]

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Background and Methods: Quantitative motor (Q-Motor) assessment captures upper limb ataxia with digital-motor outcomes that are patient-meaningful and sensitive to change. Whether Q-Motor also captures damage to the pyramidal tract – an ideal prerequisite for trial outcomes in >100 spastic ataxias –, and how this would interfere with digital measures for cerebellar ataxia is unknown. This cross-sectional single-center study validated digital upper limb measures of finger tapping, diadochokinesia, grip-lift, spiral drawing and target reaching, together with lower limb measures of foot tapping, in 41 patients with hereditary spastic paraplegia (HSP; age: 48±14 ; Spastic Paraplegia Rating Scale [SPRS]: 19±9) as compared to 46 cross-genotype ataxia patients (age: 50±18; SARA: 12±6). Validation criteria comprised discrimination from 48 matched controls, and significant correlations to each disease severity (SPRS or SARA), activities of daily living (FARS-ADL), and 9-hole peg-test (9HPT).

Results: Speed measures of finger tapping and diadochokinesia, but no other movement feature or task, captured HSP motor impairment in the upper limbs. These measures better reflected impaired fine motor function in HSP than in ataxia ($|rho_{9HPT}|$=0.7-0.8 vs. 0.5-0.6), comparable to smoothness of target reaching as most sensitive measure for upper limb ataxia ($|rho_{9HPT}|$=0.8). Correlations of finger tapping to disease severity were limited to the dominant hand in HSP (($|rho_{SPRS}|$=0.3-0.4), while upper limb ataxia was better captured in the non-dominant hand. In the lower limb, speed of foot tapping captured both ataxia ($|rho_{SARA}|$=0.4-0.7) and spastic paraplegia ($|rho_{SPRS}|$= 0.4-0-6), with almost perfect discrimination of HSP from controls (AUC=0.96), while variability measures of tap duration were specific for ataxia.

Discussion: Digital tapping speed measures capture cerebellar ataxia and pyramidal tract involvement, while variability measures appear specific for ataxia. Sensitivity for the interaction of both systems in spastic ataxias is being validated in ARSACS and SPG7.

Conclusion: Q-Motor presents a promising digital-motor outcome for spastic ataxias.

Physical activity levels and acceptability of eccentric exercise in inherited ataxia: a thematic analysis of co-production workshops

Tuesday, 12th November - 18:10: (Minories) - Poster

Mr. Jude Offe Krampah [1], ***Prof. Karen Anthony*** [1], ***Prof. Anthony Kay*** [1]

1. *University of Northampton*

Background and objectives

Our research on frail populations has confirmed the superior efficacy of eccentric exercise to improve balance, muscle strength and mobility but this has not been trialed in ataxia populations. Our objective was to conduct co-production workshops with people with ataxia to identify barriers and facilitators to physical activity and to determine the acceptability and feasibility of home- and laboratory-based eccentric-dominant exercises.

Methods

Five online workshops were held with 21 adults with an inherited ataxia (mean age in years ± SD = 59 ± 17 [range = 26 - 77]) to discuss physical activity and introduce eccentric home- and laboratory-based exercises. Eight participants attended a follow-up workshop at the University of Northampton where they trialled different eccentric-dominant exercises and co-produced a feasible and acceptable programme. Thematic analysis was used to identify emerging themes.

Results

Barriers to physical activity included poor motivation, balance, fear of falling, and fatigue, but needing to attend a local facility to access specialist equipment was not a barrier. In fact, a preference to exercise away from home was revealed, with reduced motivation for, and adherence to, home-based activities. There was a high level of acceptability for our proposed eccentric-dominant modifications to familiar home-based exercises. Laboratory-based gamified seated eccentric strength training machines were well received whilst gamified balance training exercises on an unsteady platform was met with reluctance, despite the presence of handles to prevent falls.

Discussion and conclusion

These findings are consistent with literature reporting limited enjoyment and poor adherence to home-based exercise programmes. We provide reassurance regarding the feasibility and acceptability of eccentric-dominant exercises to develop and pilot an eccentric exercise regime for people with ataxia.

Funding sources: University of Northampton Public, Community Engagement and Participatory Research Fund.

Longitudinal outcomes measures in preataxic and early ataxic spinocerebellar ataxia type 2 and 7 carriers

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Giulia Coarelli [1], ***Mx. Charlotte Dubec-Fleury*** [2], ***Mr. Emilien Petit*** [3], ***Mrs. Sabrina Sayah*** [1], ***Mrs. Clara Fischer*** [4], ***Dr. Marco Nassisi*** [5], ***Mrs. Peggy Gatignol*** [6], ***Mr. Karim Dorgham*** [7], ***Mrs. Lina Daghsen*** [8], ***Mr. Pierre Daye*** [9], ***Mrs. Rania Hilab*** [10], ***Mrs. Hortense Hurmic*** [1], ***Dr. Antonin Lamaziere*** [11], ***Mr. Jean-Charles Lamy*** [1], ***Dr. Marie-Laure Welter*** [1], ***Mrs. Marie Chupin*** [4], ***Mr. Jean-François Mangin*** [4], ***Dr. Roger Lane*** [12], ***Dr. Bertrand Gaymard*** [13], ***Mr. Pierre Pouget*** [1], ***Dr. Isabelle Audo*** [5], ***Prof. Alexis Brice*** [14], ***Dr. Sophie Tezenas du Montcel*** [2], ***Prof. Alexandra Durr*** [15]

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Background and Objective: Identifying biomarkers is crucial for future clinical trials in spinocerebellar ataxias (SCAs). Our study aimed to identify longitudinal changes in biological, clinical, and/or imaging biomarkers in SCA2 and SCA7 carriers over one year.

Methods: We conducted a one-year single-centre study (NCT04288128) including neurological examination, quality of life, oro-facial motor, neuropsychological and ophthalmological assessments, gait and oculomotor recordings, brain MRI, CSF and blood sampling. Inclusion criteria included SARA (Scale for the Assessment and Rating of Ataxia) scores between 0 and 15. The primary outcome was the longitudinal change in these assessments over one year.

Results: We included 15 SCA2 carriers, 15 SCA7 carriers, and 10 controls. SARA scores were low but different [4 (1.25, 6.5) in SCA2, 2 (0, 11.5) in SCA7, 0 in controls, $p<0.01$]. Pons and medulla volumes were smaller in SCAs ($p<0.05$), cerebellum volume only in SCA2 ($p=0.01$). Increased NfL levels were apparent more than 10 years before the estimated age at onset, and the levels were higher in SCA participants than in controls ($p<0.001$). After a one-year follow-up, in SCA2, there was significant pons (-144±60 mm^3) and cerebellum (-1508±580 mm^3) volume loss, and a worsening of gait assessment; in SCA7, SARA score significantly increased (+1.3±0.4) and outer retinal nuclear layer thickness decreased (-15.4±1.6 µm). For preataxic and early ataxic carriers, the strongest longitudinal deterioration on outcome measures was oro-facial motility in SCA2 and retinal thickness in SCA7.

Discussion and conclusion: Changes over one year can be detected even in preataxic SCA individuals. Increased NfL levels best-indexed disease activity, followed by atrophy of the brainstem and cerebellum in all, and loss of retinal thickness in SCA7. This insight allows us to foresee therapeutic strategies for preataxic individuals, leveraging

the utility of these endpoints that are reasonably likely to predict clinical outcomes.

Cross-sectional validation of a smartphone app for remote monitoring in spastic ataxias.

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Ilse Willemse [1], ***Dr. Sabato Mellone*** [2], ***Dr. Carlo Tacconi*** [3], ***Dr. Winfried Ilg*** [4], ***Prof. Rebecca Schüle*** [5], ***Prof. Matthis Synofzik*** [6], ***Dr. Jorik Nonnekes*** [7], ***Prof. Bart van de Warrenburg*** [8]

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Objectives: To develop a smartphone application for spastic ataxia (SPAX-app) to obtain reliable and valid digital outcome measures for use in clinical trials.

Methods: The app contains four motor tasks assessing gait, standing balance, and finger and hand movements. We carried out a lab-based validation study in 21 spastic ataxia patients and 10 controls. Subjects performed the app-based tasks three times during one visit, along with APDM sensors, Q-motor assessment, and the SARA.

Results: Outcome measures of the SPAX-app were correlated with APDM sensors or Q-motor in terms of means, but not with variability measures. Significant correlations were found between the SARA and step time ($r = 0.70$, $p \leq 0.01$), finger tapping frequency ($r = -0.59$, $p \leq 0.05$) and hand turning frequency ($r = -0.78$, $p \leq 0.01$). The stance task did not show significant correlations with SARA. Furthermore, test-retest measurements yielded consistent results for all outcomes of the gait (ICC ≥ 0.75) and the finger movements tasks (ICC ≥ 0.86), but not for all outcomes of the stance (ICC ≤ 0.20) and hand movements task (ICC between 0.54 and 0.79).

Discussion: Significant cross-sectional correlations between SARA and various measures of the SPAX-app support the clinical concurrent validity of the app. However, the lack of correlation for variability measures implies limitations in the SPAX-app to capture certain aspects of abnormal motor performance relevant and highly sensitive to change in this group. Moreover, the current stance task seems unsuitable to serve as a digital outcome measure for SPAX.

Conclusion: With the SPAX-app, we present a set of promising digital outcome measures, including step time, tapping frequency, and hand turning frequency, for use in clinical trials. Nevertheless, longitudinal studies are needed to evaluate whether these measures can track disease progression.

This work is funded by ZonMw(grant number: 463002002)

Stride Width Haptic Feedback for Gait Stability in Spinocerebellar Ataxia: Development and Preliminary Results

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Hong Wang [1], Mr. Zakir Ullah [1], Mr. Eran Gazit [2], Ms. Marina Brozgol [2], Prof. Jeffrey M. Hausdorff [2], Prof. Peter Shull [1], Dr. Penina Ponger [3]

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Background: Spinocerebellar ataxia (SCA) often results in balance and gait impairments, leading to irregular, unsteady gait, and falls. Wider stride width and lower variability have been associated with gait stability and reduced fall risk in other cohorts, however, it is not yet clear patients with SCA can learn to adjust these aspects of gait.
Objectives: This study examined the feasibility of using a novel wearable, real-time stride width haptic biofeedback approach to enhance gait stability and reduce fall risk in individuals with SCA.
Methods: Thirteen participants with Spinocerebellar ataxia type 3 (SCA3) performed stride width modification training using real-time haptic feedback. Stride width and its variability, crossover steps, and gait adaptation were evaluated before and after training; short-term retention was also assessed.
Results: Participants effectively adopted new gait pattern and demonstrated wider stride width during post-training (19.3cm, interquartile range IQR 16.3-20.2cm) and retention (16.6cm, IQR 16.2-21.1cm) compared to baseline (11.0cm, IQR 5.2-15.2cm; $p<0.001$) and reduced stride width variability during post-training (19.7%, IQR 17.4-26.2%) and retention (22.3%, IQR 18.6-30.2%) compared to baseline (44.5%, IQR 28.5-71.2%; $p<0.001$). Crossover steps occurrence decreased after training ($p<0.031$).
Conclusions: This novel, biofeedback approach improved stride width, decreased stride width variability, and reduced crossover steps. These initial findings support the feasibility and effectiveness of biofeedback training and lay the groundwork for the use of portable rehabilitation tools in daily training to reduce fall risk in people with SCA.

Further Development and Validation of the Cerebellar Neuropsychiatric Rating Scale (CNRS)

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Louisa P. Selvadurai [1], Ms. Anna L. Burt [2], Dr. Maureen Daly [3], Dr. Janet C. Sherman [4], Prof. Jeremy D. Schmahmann [5]

1. *Monash University,* ***2.*** *Department of Neurology, Massachusetts General Hospital,* ***3.*** *Mesulam Center for Cognitive Neurology & Alzheimer's Disease, Northwestern University Feinberg School of Medicine,* ***4.*** *Psychology Assessment Center, Department of Psychiatry, Massachusetts General Hospital and Harvard Medical School,* ***5.*** *Department of Neurology, Massachusetts General Hospital and Harvard Medical School*

Background

The neuropsychiatric / affective component of the cerebellar cognitive affective / Schmahmann syndrome (CCAS) comprises five domains: attentional control, emotional control, autism spectrum, psychosis spectrum, and social skill set, each with positive (exaggerated) and negative (diminished) symptoms (Schmahmann et al., 2007). Our beta version of a Cerebellar Neuropsychiatric Rating Scale (CNRS; Daly et al., 2015) detects and quantifies these neuropsychiatric features. Here we refine the CNRS and explore its validity.

Methods / Preliminary Results

Phase 1: Conceptual Framework. We generated (2015) the 35-item beta version from clinical experience spanning >20 years. We disambiguated items based on preliminary use and feedback, resulting in a revised 45-item CNRS.

Phase 2: Cognitive Debrief. In focus group sessions with five individuals with cerebellar conditions and their informants (family member, friend, caregiver), we gathered feedback on the scale to evaluate readability, relevance, and importance of each item. We clarified and added items to form 50-item self-report and informant-report versions.

Phase 3. Psychometric Validation. We will enroll 40 cerebellar patients and 40 healthy controls. Each will identify an informant, for a total of 80 participant-informant pairs. They will complete online questionnaires including the 50-item CNRS, the Behavior Rating Inventory of Executive Function (BRIEF-A), ASEBA Behavior scales, Social Responsiveness Scale (SRS-2), and Neuropsychiatric Inventory Questionnaire (NPI-Q). We will test CNRS internal consistency, floor and ceiling effects, concordance of ratings within the participant-informant pairs, and responsiveness to disease severity. We will investigate construct validity of the CNRS by analyzing correlations between domain scores and relevant subscales of external measures, and the differences in scores between patients and controls.

Phase 4: Test Re-test reliability. Patients, controls, and their informants will complete the CNRS 2 weeks later.

Conclusion

We predict that the CNRS has potential to advance the diagnosis and care of mental health in patients with cerebellar disorders.

Sensitive SMCxPRO immunoassays for assessing ATXN3 levels in clinical trial samples

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Chantal Beekman [1]***, Dr. Cira Dansokho*** [2]***, Mr. Ioannis Lingos*** [2]***, Ms. Nadine Ruske*** [2]***, Ms. Svenja Hüser*** [2]***, Dr. Jean-Philippe Castaing*** [2]***, Dr. Katja Obieglo*** [1]***, Dr. Scott Schobel*** [1]***, Dr. Alexander Weiss*** [2]***, Dr. Nicole Datson*** [1]

1. *VICO Therapeutics BV,* ***2.*** *Evotec SE*

Background & objective. Spinocerebellar ataxia type 3 (SCA3) is a neurodegenerative disorder caused by a CAG repeat expansion in exon 10 of the ATXN3 gene. The expanded poly-glutamine (polyQ) tract in the mutant ATXN3 protein leads to toxicity and degeneration of neurons in the cerebellum, brainstem and spinal cord. VICO Therapeutics has developed an antisense oligonucleotide (ASO) to lower the levels of toxic polyQ proteins by directly targeting the CAG repeat on the mRNA. Pre-clinical studies revealed an exon skip mechanism of action for VO659 in SCA3, resulting in a reduction of full-length unexpanded wild type and expanded mutant ATXN3, with concurrent formation of a truncated functional ATXN3 isoform that lacks exon 10 and 11. To demonstrate pharmacodynamic target engagement of VO659 in clinical trials, VICO needed specific assays to measure ATXN3 levels in the cerebrospinal fluid (CSF) of treated SCA3 patients.

Methods. The Translational Biomarker department at Evotec was enlisted to develop high-sensitivity immunoassays on the SMCxPRO platform for the detection of mutant, total and full-length ATXN3 in human CSF and plasma. Recombinant ATXN3 proteins were used for antibody screening, before further testing in biosamples.

Results. The mutant and total assays are specific and sensitive and were validated to support clinical sample measurements in a regulated environment. The full-length ATNX3 assay detects mutant and wild-type ATXN3 protein, but not the truncated delta-polyQ isoform.

Conclusion and discussion. Our results show that the SMCxPRO assays can detect mutant and total ATXN3 levels with high sensitivity and specificity and are suitable for quantitating ATXN3 levels in clinical samples.

Funding. This development work was partly funded by VICO Therapeutics and partly by Evotec SE.

Objective and quantified instrumented measurement of ataxia in children with Friedreich ataxia.

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Louise A Corben [1], ***Prof. Malcolm Horne*** [2], ***Prof. Martin Delatycki*** [3], ***Dr. Sarah Milne*** [4], ***Dr. Thang Ngo*** [5], ***Prof. Pubudu Pathirana*** [6]

__1.__ Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute; University of Melbourne; Monash University., __2.__ Bionics Institute; Department of Medicine, St Vincents Hospital, University of Melbourne., __3.__ Monash University; Murdoch Children's Research Institute; University of Melbourne; Victorian Clinical Genetics Service, __4.__ Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute; University of Melbourne; The Turner Institute for Brain and Mental Health, Monash University; Physiotherapy Department, Monash Health, __5.__ School of Engineering, Deakin University., __6.__ School of Engineering, Deakin University

Background

Concurrent maturation of the cerebellum (peaking around 12 years) confounds the use of clinical rating scales in pre-teen children with Friedreich ataxia (FRDA). We utilized the Ataxia Instrumented Measure (AIM), previously validated in adults with FRDA, to develop an objective, developmentally appropriate measure of ataxia in children with FRDA.

Methods

The AIM system comprises a data logger (in the form of a spoon, cup or pendant) with inertial sensors and machine learning (ML) based algorithms using carefully extracted features from kinetic and kinematic measurements, providing an ataxia severity score. The AIMs system was administered to all participants in conjunction with the modified Friedreich Ataxia Rating Scale (mFARS), Activities of Daily Living (ADL) scale and Pediatric Berg Balance Scale. An Adaptive Filtering (AF) based approach facilitated dissociation of motor deficits due to FRDA from developmental related effects.

Results

The AIM ataxia score was obtained from 11 children with FRDA ($\bar{x}$ age =9.0, SD=2.2) and 13 matched controls ($\bar{x}$ age=8.2, SD=1.6). Scores on the AIMs and the mFARS in controls decreased according to developmental age. Application of AF to movement kinematic data made it possible to disentangle the contribution of age-related development of coordination and, signs of FRDA. The ataxia scores of children with FRDA were significantly different ($p<0.001$) from controls. In children with FRDA the AIM ataxia score was strongly correlated with the mFARS ($r(10) = 0.80$, $p=0.005$) and the Upright Stability subscore ($r(10) = 0.81$, $p=0.0043$) of the mFARS (reported to be the most reliable subscore of the mFARS in children).

Conclusion

We demonstrated the ability to accommodate age-related development of coordination in the measurement of ataxia in children with FRDA via the AIM devices. Specifically, we provide a personalized severity score that accommodates typical variability in the development of coordination in pre-teen children with FRDA.

Funding: FARA Grant.

Developing an instrumented measure of movement disorder in Dentatorubral-pallidoluysian atrophy (DRPLA): DRPLA Natural History and Biomarkers Study.

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Louise A Corben [1], ***Prof. Pubudu Pathirana*** [2], ***Prof. Malcolm Horne*** [3], ***Mr. Sahan Dissanayake*** [2], ***Prof. Henry Houlden*** [4], ***Dr. Hector Garcia-Moreno*** [5], ***Dr. Silvia Prades*** [6], ***Ms. Ola Volhin*** [5], ***Dr. Claire Miller*** [7], ***Ms. Danika Anganoo-khan*** [7], ***Dr. Yael Shiloh-Malawsky*** [8], ***Ms. Yulissa Gonzalez*** [8], ***Prof. Paola Giunti*** [9]

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Background

Dentatorubral-pallidoluysian atrophy (DRPLA) is a rare neurodegenerative disease caused by an expanded CAG repeat in the ATN1 gene. Future clinical trials depend on natural history studies, valid biomarkers and sensitive outcome measures. We have previously validated the Ataxia Instrumented Measure – Spoon (AIM-S) as a sensitive and reliable measure of movement in Friedreich ataxia (FRDA). As a component of the international, longitudinal, multisite *DRPLA Natural History and Biomarker Study* (DRPLA-NHBS), we aim to evaluate the efficacy of the AIM-S in quantifying the movement disorder manifest in DRPLA.

Method

The AIM-S system, a simulated feeding task, comprises a data logger (in the form of a spoon) with inertial sensors and machine learning (ML) based algorithms using carefully extracted features from kinetic and kinematic measurements, providing a score of ataxia severity. The AIM-S was administered to DRPLA-NHBS participants (adult/pediatric carriers, controls) in conjunction with clinical scales. The applicability of the algorithm developed to quantify the movement disorder related to FRDA was determined and optimized to develop a DRPLA-specific ML based algorithm.

Results

Nineteen participants recruited from 3 sites (UK, USA) have undergone baseline assessment. The AIM-S captured significant features ($p<0.05$, $d\geq0.95$) that quantified task difficulty, especially when bringing the device down in a controlled descent. These features seemed to measure the translational and rotational jerk and shake in the participant's motion, with jerk over the entire movement being the most noteworthy ($p<0.005$, $d=2.49$). Preliminary 10-fold cross-validated classification (training accuracy: 100%, testing accuracy: 93.3%) and regression (training R2:0.77, testing R2:0.89) models were trained using these features.

Discussion/Conclusion

We have identified features characterizing DRPLA-specific movement and, the capacity of the AIM-S to provide a sensitive measure of such disorder. Ongoing longitudinal data collection will validate the use of the AIM-S in natural history and clinical trials for DRPLA.

Funding: Ataxia UK, Cure DRPLA.

Movement Patterns from At-Home Wearables Differentiate Patients with Ataxia-Telangiectasia (A-T) and Friedreich's Ataxia (FA) from Healthy Controls

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Divya Kulkarni [1], ***Ms. Anna Luddy*** [2], ***Ms. Faye Yang*** [1], ***Ms. Sara Reiling*** [3], ***Ms. Victoria Profeta*** [4], ***Ms. Jennifer Thornton*** [3], ***Prof. David Lynch*** [4], ***Dr. Anoopum Gupta*** [1]

1. *Department of Neurology, Massachusetts General Hospital and Harvard Medical School,* ***2.*** *Department of Neurology, Massachusetts General Hospital,* ***3.*** *A-T Children's Project,* ***4.*** *Children's Hospital of Philadelphia*

Background and Objectives: There is a need to develop tools to reliably and sensitively assess motor function in support of clinical trials. We investigate the ability of movement patterns derived from at-home wearable data for capturing motor impairment in individuals with Ataxia-Telangiectasia (A-T) and Friedreich's Ataxia (FA).

Method: One week of continuous wrist and ankle accelerometer data was collected from two disease populations, A-T and FA. The A-T population (12.4 ± 6.2 years old) had 54 individuals with A-T and 47 controls; the FA population (30.5 ± 15.6 years old) had 30 FA and 30 controls. Activity Bouts (4 and 18 seconds of continuous periods of activity) were extracted from the velocity time series, and movement features were obtained from each bout. Weekly-bout vectors were created for each participant's ankle and wrist data, summarizing the average and standard deviation of these features. Logistic regression models were trained to differentiate disease and control groups based on these vectors.

Results: For A-T versus controls, the area under the receiver operating characteristic curve (AUROC) score was 0.93 for both ankle and wrist data analyzed independently and 0.94 when the data was combined. The FA group had AUROC scores of 0.85 and 0.84 for independent analyses of ankle and wrist data, respectively, and 0.83 when the data from both limbs were combined.

Discussion: A single weekly-bout vector strongly differentiated between ataxia and control populations, even with simple logistic regression models. We expect substantially enhanced performance through the filtering of relevant bouts and the use of additional machine learning modeling approaches.

Conclusion: Movement patterns derived from natural behavior at-home capture information about the disease state. Future work with advanced models and longitudinal analysis could lead to improved digital biomarkers for diagnosis, severity, and disease progression.

Funding: NIH R01 NS134597, A-T Children's Project, FARA

Progression of Early Clinical, Imaging, and Gait Measures of SCA2: A Longitudinal Study

Tuesday, 12th November - 18:10: (Minories) - Poster

Prof. Luis Velázquez-Pérez [1], Prof. Roberto Rodriguez Labrada [2], Mr. Yasmany González-Garcés [3], Mr. Reidenis Torres Vega [4], Dr. Jacqueline Medrano Montero [3], Prof. Yaimee Vazquez [2], Prof. Evelio Gonzalez [2], Dr. Georg Auburger [5], Dr. Fay Horak [6], Dr. Imis Dogan [7], Dr. Sandro Romanzetti [8], Prof. Kathrin Reetz [7], Prof. Ulf Ziemann [9], Dr. Christopher M. Gomez [10]

1. *Cuban Academy of Sciences and Medical University of Havana,* ***2.*** *Cuban Center of Neurosciences,* ***3.*** *Center for Research and Rehabilitation of Hereditary Ataxias.,* ***4.*** *Center for Reserach and rehabilitation of Hereditary Ataxias,* ***5.*** *Faculty of Medicine, Goethe University,* ***6.*** *Oregon Health and Science University, OR,* ***7.*** *Department of Neurology, University of Aachen,* ***8.*** *Department of Neurology, RWTH Aachen University, Aachen; JARA-BRAIN Institute Molecular Neuroscience and Neuroimaging, Forschungszentrum Jülich GmbH and RWTH Aachen University, Aachen,* ***9.*** *University of Tübingen,* ***10.*** *University of Chicago*

Background and Objectives. The identification of valid progression biomarkers for spinocerebellar ataxias represents a key research objective for the forthcoming era of early interventional approaches in hereditary ataxias. Objective. to assess the progression of digitally measured gait ataxia and imaging features in preclinical and symptomatic SCA2. Methods. A total of 47 clinically-manifest SCA2 patients and 27 preclinical subjects were followed up by four times over a period of four years. At each visit, all participants underwent a two-minute natural walking test while wearing a set of six body-worn inertial measurement units. The stride-to-stride mean and standard deviation of the gait speed, double support time, foot strike angle, and toe-off angle were analyzed. In addition, the Scale for the Assessment and Rating of Ataxia (SARA) and the Inventory of Non-Ataxia Symptoms (INAS) were obtained in each visit. Also, the SCA2 subjects underwent to imaging evaluation. Results. Digital measures captured the progression of gait impairment in clinically manifest SCA2, demonstrating sensitivity to identify subtle longitudinal motor changes in preclinical SCA2. The primary factor influencing the significant longitudinal changes observed in the digital measures of gait impairment was the total SARA score at baseline. In clinically manifest SCA2, the total SARA score demonstrated the most pronounced effect size for disease progression, while in preclinical SCA2, it was the digital double-support time. Longitudinal imaging study showed a significant decrease of volumetric measures of cerebellum and brainstem in SCA2 patients and preclinical Subjects. Discussion and Conclusions This study confirmed the progressive nature of SCA2 and demonstrated the utility of digitally measured metrics and imaging analysis to assess longitudinal changes in SCAs, even at a preclinical stage of the disease. Findings suggest that these measures may serve as biomarkers of disease progression in future clinical trials.

Foundation-Sponsored Genetic Counseling and Testing Program for SCA types 1, 2, & 3 Facilitates Patient Access, Understanding, and Decision Making

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Kelsey Trace [1], ***Ms. Michelle Hearn*** [2], ***Dr. Celeste Suart*** [1], ***Ms. Natalie Beck*** [2], ***Dr. Lauren Moore*** [1]

1. *National Ataxia Foundation,* ***2.*** *Genome Medical*

Objective: Describe the usage, genetic results, and participant experience of a foundation-sponsored genetic counseling and testing program for SCA 1, 2, and 3.

Background: Individuals at-risk for hereditary ataxias benefit from genetic testing (GT) to aid in clinical care and lifestyle decisions. Obstacles to GT include cost, fear of discrimination, and limited access to specialized healthcare. Foundation-sponsored GT programs have shown promise in Parkinson's disease. The National Ataxia Foundation (NAF) launched a sponsored genetic counseling (GC) and GT program in February 2022 for SCA 1, 2 & 3. Eligibility criteria require participants to be ≥18 years old, reside in the U.S., and have at least one relative with a diagnosis of SCA types 1, 2, or 3. Required pre-test and optional post-test GC visits are provided via telehealth by a certified genetic counselor. A CLIA-approved clinical lab processes the tests.

Methods: Individuals referred to the program receive an optional, anonymous survey (questions on demographics, attitudes towards GC/GT, and participant satisfaction) from NAF 6-10 weeks later. Data compiled represents deidentified metrics from virtual GC provider, Genome Medical, and testing laboratories.

Results: In the first 24 months of this program, 319 patients had pre-test GC, 219 opted for GT including the following molecular diagnoses: SCA1 n=39, SCA2 n=60, SCA3 n=12. The most common reasons for not seeking GT previously were cost (27/54) and not knowing how (26/54). Of participants completing the survey, 86% had not previously had GC, 87% indicated GC helped them prepare to make a decision about GT, and 97% would recommend this program to family members

Discussion and Conclusion: Overall, utilization and survey responses indicate high satisfaction rates demonstrating that this foundation-initiated framework can improve access to GT in rare disease. Data collection is ongoing and will be updated to reflect a 2.5 year period.

Cross-sectional Analysis of International Cooperative Ataxia Rating Scale (ICARS) Subcomponent Scores in 152 Children with Ataxia-Telangiectasia (A-T)

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Dirk Thye [1], ***Prof. Biljana Horn*** [1], ***Mrs. Maureen Roden*** [1]

1. *Quince Therapeutics*

Objectives:

Reliable measures of neurodegenerative disease progression over time are important for anticipatory guidance, and assessment of treatment efficacy. The ICARS, developed for quantification of symptoms in cerebellar ataxia, was adopted in clinical trials for children with ataxia-telangiectasia as a research efficacy endpoint. Modified ICARS (mICARS), used in the ATTeST study, and Rescored mICARS (RmICARS) with condensed kinetic and oculomotor measures were also introduced as efficacy endpoints. The aim of this analysis is to describe baseline ICARS subcomponent scores by age, in a cross-sectional analysis of treatment-naïve patients from ATTeST dataset, and to identify ICARS subcomponents that best reflect progression of disease by age.

Methods

Mean baseline ICARS scores ± SD were calculated for each of 7 age-groups (age 6-12) for walking, standing, sitting, knee-tibia test, finger-nose test, pronation-supination, drawing, speech, and oculomotor subcomponents.

Results

The subcomponents of ICARS that showed disease progression with age and increased >25% between 6 and 10 years of age were: walking (3.3±1.58 to 6.6±2.55; 100% increase), standing capacities (8.7±3.35 to 12.2±2.77; 40% increase), and sitting (1.1±0.93 to 1.4±0.56, 27% increase). Scores in the kinetic function domain including drawing, speech, and oculomotor did not show age-related trends. No trends were identified in 11- and 12-year-olds in any of ICARS subcomponents, but the numbers of participants were small in these age groups.

Discussion

In this cross-sectional analysis, the posture and gait disturbance category of ICARS showed progression with age in untreated 6-10-year-old A-T children. Scores in the kinetic function category, comprising 52% of ICARS, showed no trends in progression over time, regardless of age. Scales with reduced kinetic function domain may be more sensitive than the full ICARS scores when assessing disease progression in younger children.

Conclusion

Additional data and new measures that correlate better with disease progression, particularly in older children with A-T, are needed.

At-home Wearables and Machine Learning Models Reliably Capture Motor Impairment and Disease Progression in Ataxias

Tuesday, 12th November - 18:10: (Minories) - Poster

Mr. Rohin Manohar [1], ***Ms. Faye Yang*** [2], ***Dr. Christopher D. Stephen*** [2], ***Prof. Jeremy D. Schmahmann*** [2], ***Ms. Nicole Eklund*** [3], ***Dr. Anoopum Gupta*** [2]

1. *Department of Neurology, Massachusetts General Hospital,* ***2.*** *Department of Neurology, Massachusetts General Hospital and Harvard Medical School,* ***3.*** *Behavioral Neuroscience PhD, Boston University School of Medicine*

Background and Objectives: A major barrier to developing novel ataxia therapies is the scarcity of tools to sensitively measure disease progression in clinical trials. Quantitative analysis of movement data during natural behavior could support precise assessments of motor function over time.

Methods: 51 individuals with ataxia (SCAs 1, 2, 3, and 6, MSA-C), including 13 preataxic individuals, and 25 age-matched controls participated in fully remote studies. Longitudinal data were collected from 34 individuals (6 controls). Participants wore a GENEActiv tri-axial accelerometer on their dominant ankle and wrist for one week during natural behavior at home. Data were analyzed to characterize submovements and create composite severity scores.

Results: A machine-learned composite outcome measure, previously trained on a large and longitudinal amyotrophic lateral sclerosis (ALS) dataset, demonstrated strong properties when applied to data from individuals with ataxia. The composite outcome from wrist data separated ataxia from controls (Cohen's d = 1.1), had high within-week reliability (ICC = 0.95), correlated with SARA (r = -0.72) and PROM-Ataxia (r = -0.63), and captured disease progression ($p < 0.001$). Similarly, when applied to ankle data, the composite outcome separated ataxia from controls (Cohen's d = 1.1), had high within-week reliability (ICC = 0.95), correlated with SARA (r = -0.75) and PROM-Ataxia (r = -0.62), and captured disease progression ($p < 0.005$).

Discussion: Passively collected limb movement data obtained via low-cost accelerometers can produce highly reliable and sensitive measures that reflect the ataxia phenotype, and may be appropriate for use in future clinical studies. We expect an ataxia-specific longitudinal model to be more sensitive for capturing change than the current ALS model.

Conclusion: Accelerometers worn at home during natural behavior can sensitively measure disease progression and may be used in research or clinical settings with relatively low burden to individuals.

Funding: NIH NS117826, Biogen, FARA

Cardiac Features of Friedreich Ataxia as Predictors of Mortality

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Medina Keita [1], ***Ms. Kimberly Schadt*** [1], ***Ms. Katherine Gunther*** [1], ***Ms. Courtney Park*** [2], ***Dr. Kimberly Lin*** [1], ***Prof. David Lynch*** [1]

1. *Children's Hospital of Philadelphia,* ***2.*** *Department of Pediatrics and Neurology, The Children's Hospital of Philadelphia*

Cardiomyopathy represents the main cause of death in FRDA. Patients develop cardiac hypertrophy with later fibrosis, loss of systolic function, arrhythmias, and heart failure. Echocardiographic results from a large cohort of FRDA patients are used to investigate the relationships between genetic severity of cardiac features, and clinical outcomes of death, loss of systolic function, and development of arrhythmias.

Methods: Data was collected from all echocardiographs. Analysis focused on the most commonly available variables: mortality, age at death, age of onset, GAA1, sex, age, age at echo, duration, wall thicknesses (IVST), and ejection fraction (EF).

Results:

Demographics: The overall cohort had 534 subjects; 2969 echocardiographs were extracted. 7.1% carried point mutations/deletions, 52% were female. The mean GAA1 repeat (691) was slightly longer than the average FRDA patient.

Cohort features: At analysis 81.5% of the cohort was alive, 11.1% deceased and 7.5% unknown. Mean age of death was 33 + 15.5. 80.1% had neither an arrhythmia nor low EF, 7.1% had a low EF but no arrhythmia, 6.1% had an arrhythmia but normal EF, and 6.6% had both.

Predictors of age at death: Longer GAA1 predicted earlier age of death. The age of death differed above and below a GAA1 length of 500. The maximal ejection fraction (EFmax) significantly predicted age at death in subjects whose initial EFmax was > 50%, such that a higher EFmax was associated with an earlier age of death. EFmax also marginally predicted the likelihood of death. Additionally, the maximal septal diameter (IVSTmax) predicted age at death ($P<0.005$).

Conclusion: In this cohort the age of death in FRDA matches previous studies, however two markers of hypertrophic cardiomyopathy (increased EF, thicker IVST) are linked to earlier age of death. This study thus supports the proposed pathophysiology that eventual systolic dysfunction is a direct association of earlier cardiac hypertrophy.

Clinical outcomes of omaveloxolone in patients with Friedreich Ataxia: a ten-month review

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Katherine Gunther [1], ***Ms. Victoria Profeta*** [1], ***Ms. Kimberly Schadt*** [1], ***Ms. Gina Coll*** [1], ***Prof. David Lynch*** [1]

1. *Children's Hospital of Philadelphia*

Omaveloxolone, the only approved medication for Friedreich Ataxia (FRDA) is an Nrf2 activator available since July 2023. We examined the course of response in individuals with FRDA over the first 12 months of administration.

Methods

We recorded baseline and follow-up transaminases, albumin, bilirubin, and other lab values, adverse events, and features of the access to omaveloxolone.

Results

95.4% of 244 patients obtained omaveloxolone through payors with 5.3% of those approved utilizing the patient assistance program. Although all patients were provided orders for baseline lab values, only 57.3% completed baseline labs (Lipid panel, BNP, CMP) prior to starting omaveloxolone. 30.6% had elevated total cholesterol levels, 3.4% had elevated BNP, and 9.4% had elevated ALT at baseline. At one month, 58.2% of patients had elevated ALT values above 1x ULN with 11.3% above 3x ULN. At 2 months, 53.1% were elevated with 4.9% above 3x ULN. At 3 months, 39.2% were elevated with 1.4% above 3x ULN. At 6 months 33.0% were elevated with 3.7% above 3x ULN. Patients with elevated LFT levels paused dosing with 90.9% resolution of abnormalities and 48.5% eventually returned to initial dosing. 1.9% had abnormal albumin and 2.3% had abnormal total bilirubin protein. Common side effects included gastrointestinal upset, headache, and fatigue. 7.0% withdrew from drug and 2.9% restarted drug after stopping. Reasons for discontinuing included gastrointestinal upset, rash, lack of immediate benefit, death unrelated to omaveloxolone (two patients), and lack of communication between patient and pharmacy.

Discussion

While access to omaveloxolone was difficult, the vast majority eventually had access to the agent. Abnormalities in liver function were limited to transaminases and resolved with dose pausing or reduction. Side effects were modest and overall, the vast majority remained on drug. Thus, the features of omaveloxolone after administration largely resemble those noted during clinical trials.

The abundance of the expanded ATXN3 mRNA allele in blood samples of Machado-Joseph disease subjects.

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Mafalda Raposo [1], ***Mrs. Sara Pavão*** [2], ***Dr. Ana Rosa Vieira Melo*** [3], ***Dr. Luís Teves*** [4], ***Dr. Ana F. Ferreira*** [5], ***Prof. Jorge Sequeiros*** [6], ***Dr. Sandra Martins*** [7], ***Prof. Manuela Lima*** [8]

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Machado-Joseph disease (MJD), the most prevalent hereditary spinocerebellar ataxia worldwide remains untreatable, 30 years after the discovery of its causative mutation, an expanded CAG repeat in the *ATXN3* gene. MJD pathogenesis is mainly driven by its mutation, but the *cis*-regulatory effect of the CAG repeat in the expression of mRNA *ATXN3* alleles has not yet been elucidated. Our aim was to determine the expression levels of the expanded *ATXN3* allele in blood samples of MJD subjects by distinguishing allelic mRNA species based on the heterozygous status of single nucleotide variants (SNVs) at the *ATXN3* gene. We developed and tested a novel protocol comprising: (1) the identification of MJD mutation carriers, heterozygous for rs1048755; (2) the calculation of the relative expression of mRNA levels of normal/expanded alleles, using TaqMan SNP Genotyping Assays; and (3) the haplotyping of rs1048755-(CAG)n by PCR and Sanger Sequencing. We identified 51 MJD carriers, heterozygous for rs1048755 (discrimination power of 44%) and retrieved the relative abundance of *ATXN3* mRNA alleles. Next, in a subset of 24 samples, we assessed the allelic phase of rs1048755 and the CAG repeat, and detected expression levels of the expanded allele 1.23 (±0.11 SD) higher on average than its normal counterpart. Additionally, to extend our protocol to other informative SNVs and raise the potential of discriminating normal *vs* expanded alleles, we genotyped 12 additional SNVs in coding and 3'UTR regions of *ATXN3*. Using genotype information of six SNVs a discrimination power of 79% was achieved, a value which might be variable in cohorts of different ethnic backgrounds. Further studies in a large cohort of MJD carriers and comparisons between relative and absolute quantification methods to calculate the number of expanded *ATXN3* mRNA copies will be of upmost importance to better elucidate the role of the CAG expansion in the *ATXN3* transcription.

Sensor-free motion registration and automated movement evaluation: Leveraging machine learning for clinical gait analysis in ataxia disorder

Tuesday, 12th November - 18:10: (Minories) - Poster

Mr. Philipp Wegner [1], ***Dr. Marcus Grobe-Einsler*** [2], ***Dr. Lara Reimer*** [3], ***Mr. Fabian Kahl*** [4], ***Dr. Berkan Serdal Can Koyak*** [1], ***Mr. Tim Elters*** [1], ***Mr. Alexander Lange*** [1], ***Dr. Okka Kimmich*** [1], ***Dr. Daniel Soub*** [1], ***Dr. Felix Hufschmidt*** [1], ***Dr. Sarah bernsen*** [1], ***Ms. Mónica Ferreira*** [1], ***Prof. Thomas Klockgether*** [2], ***Dr. Jennifer Faber*** [1]

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Objectives: Gait disturbances are the clinical hallmark of ataxia disorders, fundamentally impairing the mobility of ataxia patients. In clinical routine and research the severity of the gait disturbances is assessed within a well-established clinical scale (SARA) and graded into categorial levels. Sensor-free motion registration and subsequent movement analysis allowed reconstructing that clinical rating solely from videotaped assessments and could overcome lack of sensetivity of the clinical scale.

Methods: 119 participants from neurodegenerative ataxias studies at DZNE in Bonn, Germany, were assessed using the SARA scale, including a 10m walk. This task was videotaped and rated by a certified rater. The videos were processed with a deep learning-based motion-capturing model, tracking 17 body positions. The data was analyzed with two time series machine learning models to reconstruct the on-site rating from video data and model disease progression. For comparison, 44 videos were rated by 3 human raters.

Results: The proposed model successfully reproduced categorical scaling, achieving an F1-score of 80.28%, compared to 44.88% by human effort. The model's time series analysis extracted features that significantly outperformed SARA-based ratings in longitudinal modeling. The SARA gait score had a non-significant Pearson's correlation coefficient of -0.060 with the time since the first visit. In contrast, a specific time series feature had a significant correlation with a coefficient of -0.626. Stratifying the cohort by initial SARA gait score yielded even greater absolute correlation coefficients.

Discussion: Using videotaped assessments and a markerless motion-capturing model, we employed time series machine learning models to reconstruct the on-site rating, significantly outperforming human efforts. Additionally, the models captured longitudinal changes more effectively than the clinical score

Conclusion: This work successfully demonstrated the capabilities of markerless motion capturing in combination with time series modeling to model ataxia diseases. We see great potential for this in clinical practice and for home assessments.

Impact of specialist ataxia centres on health service resource utilisation and costs across Europe: cross-sectional survey

Tuesday, 12th November - 18:10: (Minories) - Poster

Prof. Stephen Morris [1], Dr. Julie Vallortigara [2], Dr. Julie Greenfield [3], Prof. Barry Hunt [3], Ms. Deborah Hoffman [4], Dr. Carola Reinhard [5], Dr. Holm Graessner [6], Prof. Antonio Federico [7], Ms. Vinciane Quoidbach [8], Prof. Paola Giunti [2]

1. *Primary Care Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK,* ***2.*** *Ataxia Centre, UCL Queen Square Institute of Neurology, London, UK.,* ***3.*** *Ataxia UK,* ***4.*** *Takeda Pharmaceuticals, Cambridge MA,* ***5.*** *Centre for Rare Diseases and Institute of Medical Genetics and Applied Genomics, University Hospital Tübingen,* ***6.*** *Ataxia Global Initiative, University Hospital Tübingen, 7. Department of Medicine, Surgery and Neurosciences, Medical School, University of Siena and European Academy of Neurology,* ***8.*** *European Brain Council, Brussels*

Background

Little is known about the costs of treating ataxia and whether treatment at a specialist ataxia centre affects the cost of care. We have explored the resource use and health service costs of patients affected by ataxia in the United Kingdom, Italy and Germany over a 12-month period.

Aim

The aim of this study was to investigate whether patients who attended SACs in three European countries reported differences in their health care utilisation and costs compared with patients who did not attend a SAC.

Methods

Data were obtained from a survey distributed to people with ataxia in the three countries. We compared mean resource use for each contact type and costs per patient, stratifying patients by whether they were currently attending a specialist ataxia centre or had never attended one.

Results

Responses were received from 181 patients from the United Kingdom, 96 from Italy and 43 from Germany. Differences in the numbers of contacts for most types of health service use between the specialist ataxia centre and non-specialist ataxia centre groups for each country were non-significant. In the United Kingdom the mean total cost per patient was €2209 for non-specialist ataxia centre patients and €1813 for specialist ataxia centre patients (P=0.59). In Italy these figures were €2126 and €1971, respectively (P=0.84). In Germany they were €2431 and €4087, respectively (P=0.19). Inpatient stays made the largest contribution to total costs. For the SAC group, there were significant differences in mean costs between countries with mean costs per patients highest in Germany and similar lower costs in Italy and the UK (P<0.01).

Conclusion: Within each country, resource use and costs were broadly similar for specialist ataxia centre and non-specialist ataxia centre groups. There were differences between countries in terms of health care contacts and costs.

Patient pathways for rare diseases in Europe: ataxia as an example

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Julie Vallortigara [1], ***Dr. Julie Greenfield*** [2], ***Prof. Barry Hunt*** [2], ***Ms. Deborah Hoffman*** [3], ***Dr. Carola Reinhard*** [4], ***Dr. Holm Graessner*** [5], ***Prof. Antonio Federico*** [6], ***Ms. Vinciane Quoidbach*** [7], ***Prof. Paola Giunti*** [1]

1. *Ataxia Centre, UCL Queen Square Institute of Neurology, London, UK.,* ***2.*** *Ataxia UK,* ***3.*** *Takeda Pharmaceuticals, Cambridge MA,* ***4.*** *Centre for Rare Diseases and Institute of Medical Genetics and Applied Genomics, University Hospital Tübingen,* ***5.*** *Ataxia Global Initiative, University Hospital Tübingen,* ***6.*** *Department of Medicine, Surgery and Neurosciences, Medical School, University of Siena and European Academy of Neurology,* ***7.*** *European Brain Council, Brussels*

Background

Progressive ataxias are rare complex neurological disorders that represent a challenge for the clinicians to diagnose and manage. This study explored the patient pathways of individuals attending specialist ataxia centres (SAC) compared with non–specialist settings. We investigated how diagnosis was reached, the access to healthcare services, treatments, and care satisfaction. The focus of this study was on early intervention, coordination of treatment to understand the care provision in different countries.

Aim

The aim was to have a landscape of the rare diseases using ataxia as an example, in different health systems in Europe that will lead to a white paper to improve policy for patients with rare diseases.

Methods

A patient survey was done in the UK, Germany and Italy to gather information about diagnosis and management of the ataxias in specialist (SAC) and non-specialist settings, utilisation of other primary and secondary health care services, and patients' satisfaction of received treatment.

Results

Patients gave positive feedback about the role of SAC in understanding their condition, ways to manage their ataxia ($p<0.001$; UK) and delivering car adapted to their needs ($p<0.001$; UK), in coordinating referrals to other healthcare specialists, and in offering opportunities to take part in research studies. Similar barriers for patients were identified in accessing the SACs among the selected countries, UK, Germany, and Italy.

Conclusion

This study provides crucial information about the ataxia patients care pathways in three European countries. Patients' satisfaction being significantly better in SAC compared to non-SAC in the UK brings evidence of the unique model of care in SAC, more focused on the management, with a service designed and improved with patients' feedback. The outcomes can be used now for policy recommendations on how to improve treatment and care for people with these very rare and complex neurological diseases across Europe.

Prospective natural history study of SCD patients in Japan using the Japanese version of FARS-ADL

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Shinji Oda [1], ***Dr. Yuji Takahashi*** [2], ***Dr. Hidehiro Mizusawa*** [2]

1. *Department of Neurology, National Center Hospital, National Center of Neurology and Psychiatry,* ***2.*** *National Center of Neurology and Psychiatry*

Background

We developed the Japanese version of FARS-ADL (Friedreich's Ataxia Rating Scale - Activities of Daily Living) as a clinical measure for patients with spinocerebellar degeneration (SCD). FARS-ADL is a 9-item (out of 36 points) measure of activities of daily living for SCD patients and does not require a face-to-face examination. We conducted a prospective natural history study of SCD patients living in Japan by establishing a structured telephone interview system for the Japanese version of the FARS-ADL.

Methods

We extracted cases with a confirmed genetic diagnosis and SARA score below 15 points from J-CAT (Japan Consortium of Ataxias), a Japanese registry of patients with ataxia. We then conducted FARS-ADL telephone interviews every six months with 128 patients with SCD (SCA31: 49, SCA3/MJD: 34, SCA6: 30, SCA2: 5, SCA1: 4, SCA36: 3, SCA8: 2, CANVAS: 1).

Results and Discussion

At the initial telephone interview, the average age was 62.0±11.5 years (mean±SD), disease duration was 8.4±6.2 years, SARA score was 9.3±3.7 points, and FARS-ADL score was 10.4±4.9 points.

Thereafter, FARS-ADL scores were followed up by telephone interviews every six months.

In the study of 100 patients who had been followed up for one year, the FARS-ADL scores were 10.3±5.1 at baseline, 10.5±5.6 at six months, and 12.5±5.4 at one year. Scores for MJD/SCA3, SCA6, and SCA31 also worsened over time, reflecting disease progression.

Conclusion

This study provides valuable longitudinal data on the progression of SCD using FARS-ADL, especially in forms of ataxia other than Friedreich's ataxia (FA). The results suggest that the FARS-ADL telephone interview is an effective and simple tool in prospective natural history studies for SCD.

We are currently developing a PC/smartphone-compatible application, "J-CAT ePRO", which will further simplify and facilitate the accumulation of natural history data in SCD patients.

Retinal nerve fiber layer as a biomarker in Friedreich Ataxia.

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Victoria Profeta [1]***, Ms. Katherine Gunther*** [1]***, Dr. Rachel Kenney*** [2]***, Dr. Laura Balcer*** [2]***, Dr. David Lynch*** [3]

1. *Children's Hospital of Philadelphia,* ***2.*** *NYU,* ***3.*** *Department of Pediatrics and Neurology, The Children's Hospital of Philadelphia*

Introduction: Friedreich Ataxia (FRDA) is a degenerative disease caused by GAA expansions in the *FXN* gene. About 20% of FRDA patients develop significant optic neuropathy. This study used optical coherence tomography (OCT) to measure the peripapillary retinal nerve fiber layer (RNFL) thickness to determine who is at risk for vision loss and to identify the degree to which RNFL values provide markers of disease progression in FRDA.

Methods: Subjects (n=364 distinct adults and children, with 180 follow up), were examined with low contrast Sloan letter chart testing and spectral domain OCT. Data was analyzed by correlations with neurological and visual measures and by linear regression.

Results: In serial examinations, RNFL values decreased over time, consistent with ongoing retinal degeneration. The speed of degeneration was 10 times faster in subjects under 21 compared with those older than 21. Disease duration and RNFL thickness also correlated (R^2 = 0.15) as seen in previous publications, further demonstrating ongoing degeneration. In addition, RNFLs are significantly thin in children with FRDA at presentation compared to age and sex matched controls, suggesting hypoplasia rather than degeneration alone. Modified Friedreich Ataxia Rating Scale (mFARS) and RNFL values correlated significantly in both adult and pediatric patients (R^2 = 0.45) as did FRDA stage and RNFL values (R^2 = 0.343). Finally, GAA repeat length and age predicted RNFL values, but with lower R^2 values than other markers of disease progression.

Discussion: Progressive loss of RNFL thickness is most v severe in children with FRDA vs adults. Overall, RNFL provides a significant marker of both visual and neurologic progression in in FRDA, with R^2 values generally higher than other published imaging approaches. GAA alone is not a reliable predictor of RNFL loss or eventual vision loss suggesting that other factors also contribute to optic neuropathy in FRDA.

Validation of SCACOMS for Use in Patients with Spinocerebellar Ataxia

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Michele Potashman [1], ***Dr. Maggie Heinrich*** [2], ***Dr. Katja Rudell*** [2], ***Ms. Linda Abetz-Webb*** [3], ***Ms. Naomi Suminski*** [2], ***Ms. Rinchen Doma*** [2], ***Dr. Kavita Jarodia*** [2], ***Mr. Chris Buckley*** [2], ***Ms. Mahak Jain*** [2], ***Dr. Melissa Wolfe-Beiner*** [1], ***Dr. Vlad Coric*** [1], ***Prof. Jeremy D. Schmahmann*** [4], ***Dr. Gilbert L'Italien*** [1]

1. *Biohaven Pharmaceuticals, Inc.,* ***2.*** *Parexel International,* ***3.*** *Patient-Centered Outcomes Assessment,* ***4.*** *Department of Neurology, Massachusetts General Hospital and Harvard Medical School*

Background/Objective: Spinocerebellar ataxia (SCA) is a group of rare inherited neurodegenerative disorders characterized by progressive ataxia affecting limb coordination, balance, speech and an array of other symptoms. SCACOMS is a statistically derived composite measure of SCA disease progression consisting of items most sensitive to progression in early stages of disease, with weighting that reflects the item's relative contribution to progression. The goal of this study was to examine the content validity of SCACOMS.

Methods: SCA patients (N=24) and clinicians treating SCA patients (N=2) participated in cognitive interviews to evaluate the patient-relevance, content validity and meaningful changes of SCACOMS. Respondents were asked to comment specifically on the item composition/weights (f-SARA gait item [12%], f-SARA stance item [17%], f-SARA sitting item [8%], f-SARA speech [10%] and Clinician Global Impression of Change [CGI] [53%]). All interviews/analyses were conducted in accordance with standard procedures/methods, noting that methods to obtain feedback on the item weighting is novel – each patient first ranked items based on importance and then used this information to construct a pie diagram of respective weights for each item in SCACOMS.

Results: Based on an examination of the first n=13 interviewed patients, the items comprising SCACOMS were deemed relevant and endorsed. The weights allocated to each item were variable: f-SARA gait item ranged 15-60% (median 30%), f-SARA stance item ranged 4-30% (median 17%), f-SARA sitting item ranged 5-20% (median 10%), f-SARA speech ranged 10-40% (median 25%) and Clinician Global Impression of Change ranged 4-30% (median 13%). Pending analysis include examining the data for the full cohort and by stage of disease (mild, moderate and severe disease) and will be presented at ICAR.

Conclusions: When developing composite measures using statistical measures based on sensitivity to detect disease progression, patient input is critical to validate item selection and respective weights.

Funding: Biohaven Pharmaceuticals, Inc.

Patient Concept Elicitation Interviews: Insights into Spinocerebellar Ataxia Patient Experiences

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Michele Potashman [1], ***Ms. Naomi Suminski*** [2], ***Dr. Katja Rudell*** [2], ***Ms. Rinchen Doma*** [2], ***Ms. Linda Abetz-Webb*** [3], ***Dr. Maggie Heinrich*** [2], ***Dr. Melissa Wolfe-Beiner*** [1], ***Dr. Vlad Coric*** [1], ***Prof. Jeremy D. Schmahmann*** [4], ***Dr. Gilbert L'Italien*** [1]

1. *Biohaven Pharmaceuticals, Inc.,* ***2.*** *Parexel International,* ***3.*** *Patient-Centered Outcomes Assessment,* ***4.*** *Department of Neurology, Massachusetts General Hospital and Harvard Medical School*

Background/Objective: Spinocerebellar ataxia **(**SCA) is a group of rare, dominantly inherited neurodegenerative disorders that cause progressive dysfunction across motor, cognitive and affective domains. The PROM-Ataxia is a validated patient-derived assessment of patient experience with ataxia. Here we performed patient cognitive interviews to further understand patients' lived experience with SCA as part of a larger study examining the content validity of several SCA outcomes measures.

Methods: Semi-structured interviews with 7 SCA patients (SCA3, N=6; SCA1, N=1) were conducted to elicit the signs and symptoms of SCA, including most bothersome and important symptoms, and the impacts of these to activities of daily living (ADLs). Interviews employed an open-concept elicitation phase that was followed by a set of probes designed to query symptoms recommend by clinicians as prominent. Interviews were audio recorded, transcribed, coded and analysed by ATLS.TI, following established qualitative research methods.

Results: A total of 85 concepts reflecting signs, symptoms, or ADLs impacted were reported during the patient interviews (n=66 spontaneously reported and n=18 confirmed with probes). All patients spontaneously reported difficulties with walking and balance. Other signs/symptoms/ADL impacts frequently spontaneously reported (≥50.0% of participants) were falls (N=6/7), tired/fatigued (N=5/7), difficulty working (N=5/7), challenges with social life (N=5/7), difficulty being understood (N=4/7), emotional dysfunction (N=4/7), difficulty driving (N=4/7), and vision impairments (N=4/7). The most bothersome symptoms were difficulties with walking (N=5/7), neuropathy (N=4/7), difficulties being understood (N=4/7) and poor balance (N=3/6). Respondents shared feelings of anxiety, fear of falling, difficulty dealing with condition alone, nervousness during work calls, trauma from falls, embarrassment during coughing spells, laziness, and not having initiative.

Conclusion: Findings from patient cognitive interviews provide additional support for the validity of the PROM-Ataxia, illuminate the SCA lived experience and inform the patient centricity of existing/new outcomes measures.
Study funded by Biohaven Pharmaceuticals, Inc.

Longitudinal Endpoint Optimization to Provide an Assessment of Relevant Drugs in Friedreich's Ataxia (LEOPARD-FA): A Remote, 18-Month Longitudinal Study in Friedreich's Ataxia

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Christina Shupe [1], ***Ms. Jamison Seabury*** [2], ***Ms. Anika Varma*** [2], ***Mr. Spencer Rosero*** [3], ***Ms. Jennifer Weinstein*** [1], ***Ms. Charlotte Engebrecht*** [1], ***Ms. Charlotte Irwin*** [1], ***Ms. Preshetha Kanagaiah*** [1], ***Dr. Jane Larkindale*** [4], ***Ms. Susan Walther*** [5], ***Mrs. Ellen Wagner*** [1], ***Ms. Nuran Dilek*** [6], ***Mr. John Heatwole*** [7], ***Ms. Christine Zizzi*** [1], ***Prof. David Lynch*** [8], ***Ms. Courtney Park*** [9], ***Ms. Mackenzie Wells*** [8], ***Dr. Chad Heatwole*** [6]

1. *University of Rochester Center for Health + Technology,* ***2.*** *University of Rochester School of Medicine and Dentistry,* ***3.*** *University of Utah Spencer Fox Eccles School of Medicine,* ***4.*** *Pepgen,* ***5.*** *Friedreich's Ataxia Research Alliance,* ***6.*** *University of Rochester Department of Neurology,* ***7.*** *Cornell University,* ***8.*** *Children's Hospital of Philadelphia,* ***9.*** *Department of Pediatrics and Neurology, The Children's Hospital of Philadelphia*

Objective: To conduct a remote, longitudinal study with caregivers and patients with Friedreich's Ataxia (FA) in order to: 1) optimize the FACR-HI and FA-HI, two disease-specific outcome measures; and 2) obtain natural history data in FA.

Methods: Previously, we developed disease-specific patient and caregiver-reported outcome measures (the FA-HI and FACR-HI) for FA. Currently, we are conducting an 18-month remote longitudinal study, where participants complete the FA-HI or FACR-HI, the SF-36 or PedsQL, a survey preference questionnaire, and a global impression of change form every 6 months.

Results: The initial development of the FA-HI and FACR-HI involved 202 caregivers and individuals with FA. The instruments underwent beta testing and test-retest reliability testing with 38 caregivers and 30 individuals with FA. Currently, 47 caregivers and individuals with FA are enrolled in our international longitudinal study, with 66.0% identifying as female, a mean patient age of 40 years old (SD = 13), and mean caregiver age of 44 years old (SD = 6). Compared to the SF-36 and PEDS-QL, participants identify the FA-HI and FACR-HI as a better tool to address the most important symptoms in FA. Additional natural history data regarding disease burden progression as measured by the FA-HI and FACR-HI is forthcoming.

Discussion: Survey preference data suggests that the FA-HI and FACR-HI are well suited to measure patient-relevant changes in disease burden over time. Continued assessments will determine longitudinal instrument metrics, and identify which demographic and clinical characteristics are associated with a faster disease progression in FA.

Conclusion: The FA-HI and FACR-HI each measure 18 areas of FA symptomatic health and are designed and validated for use in FA therapeutic trials. Longitudinal evaluations of the FA-HI and FACR-HI will further document their ability to detect changes in FA disease burden over time and determine the instruments' minimum clinically important difference (MCID).

An Overview of the CureDRPLA Global Patient Registry - Collecting Patient Reported Data to Advance Research

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Andrea Compton [1], Mr. Paul Compton [1], Prof. Graham McClelland [2], Mr. Yashodhan Desai [2], Dr. Jeff Carroll [3], Dr. Julie Greenfield [4], Dr. Silvia Prades [5]

1. *CureDRPLA,* ***2.*** *King's College London,* ***3.*** *Department of Neurology, University of Washington, Seattle, WA, 98225,* ***4.*** *Ataxia UK,* ***5.*** *CureDRPLA, Brooklyn, NY, USA*

Methods: DRPLA is an ultra-rare neurodegenerative disorder with juvenile- and adult-onset. It is inherited in an autosomal dominant manner and is caused by expanded CAG repeats in the Atrophin-1 gene. We established the CureDRPLA Global Patient Registry to collect patient-reported data on people with DRPLA. Upon enrolment, participants consent and answer questions about demographics, diagnosis, medical history, activities of daily living, mobility, research, and disease and economic burden. Participants are asked to complete the registry yearly. Ethics committee approval was obtained.

Results: There are 49 participants in the patient registry from 10 different countries. For the juvenile-onset group (n=26), age at symptom onset was 7 ± 4.35 (mean ± SD) years old, number of CAG repeats 68 ± 7.47, and 84.6% experience seizures. At baseline, the activities of daily living (from FARS) score for this group was 22 ± 10.23 and the functional disability rating score (from FA Patient Registry) was 4 ± 1.91. The most common present health concerns are balance problems and epileptic seizures. For the adult-onset cohort (n = 15), age at symptom onset was 44 ± 11.72 years old, number of CAG repeats 60 ± 2.33, and 26.7% experience seizures. At baseline, the activities of daily living score for this group was 13 ± 11.51 and the functional disability rating score was 4 ± 1.66. The most common present health concerns are balance and coordination problems. There are five participants who are asymptomatic and three with insufficient information on symptom onset.

Discussion: This registry is creating a cohort of well-characterised DRPLA patients for participation in future research studies. The annual updates from participants will facilitate a dynamic understanding of disease progression.

Conclusion: This information serves as a crucial resource for advancing the understanding of DRPLA and ultimately guiding the development of therapies to improve patient outcomes.

SPAX-composite: A composite scale to evaluate the progression of patients with spastic and ataxia symptoms

Tuesday, 12th November - 18:10: (Minories) - Poster

Mx. Cécile Di Folco [1], Mx. Charlotte Dubec-Fleury [1], Dr. Bernard C. Brais [2], Prof. Bart van de Warrenburg [3], Dr. Filippo Santorelli [4], Prof. Nazli Basak [5], Prof. Alexandra Durr [6], Dr. Rita Horvath [7], Dr. Stephan Klebe [8], Prof. Matthis Synofzik [9], Prof. Rebecca Schüle [10], Dr. Sophie Tezenas du Montcel [1]

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Background and objectives: Spastic ataxias are a group of over 100 rare neurodegenerative diseases for which available treatment is mostly limited to symptoms relief. Current clinical scales tracking the disease progression are specific to either spasticity or ataxia, and may show limited sensitivity to change especially in multisystemic phenotypes like spastic ataxias, hampering the design of clinical trials. The goal of the present study is to develop a sensitive and valid scale adapted to patients presenting both spasticity and ataxia.

Methods: Longitudinal data from N=127 SPG7 and N=112 ARSACS patients were collected in the multicenter PROSPAX study. Sensitivity to change over 1 year of 30 items from the Scale for the Rating and Assessment of Ataxias (SARA), Spastic Paraplegia Rating Scale (SPRS) and the Activities of Daily Living subscale of the Friedreich's Ataxia Rating Scale (FARS-ADL) was evaluated. Items that demonstrated the highest sensitivity to change were progressively added in a stepwise procedure to build the SPAX-composite scale. The effect size of the SPAX-composite scale was compared to that of the three standard scales. Variability was assessed with bootstrapped confidence intervals. As external validation, correlation with disease stage (FARS-Disease Staging) and disease duration were computed. The structure of the composite scale was analyzed with an Item Response Theory model.

Results: The effect sizes of the three standard scales ranged from 0.0008 (FARS-ADL) to 0.46 (SARA) in SPG7, and from 0.21 (FARS-ADL) to 0.30 (SARA) in ARSACS. With a smaller set of items, the SPAX-composite showed a substantially higher effect size of 0.45 in both genotypes (preliminary results).

Discussion and Conclusion: The SPAX-composite is more sensitive to change and homogeneous across genotypes than the standard scales, allowing a reduction of the required sample size in future clinical trials. A replication cohort is needed to validate this scale.

Funding: EJPRD JTC2023-PROSPAX grant

Exploring Mitochondrial Enzymes in Friedreich's Ataxia: A Pathophysiological Approach

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Lucie Stovickova [1], ***Dr. Hana Hansikova*** [2], ***Dr. Jitka Hanzalova*** [3], ***Dr. Zuzana Mušová*** [4], ***Mr. Valerij Semjonov*** [5], ***Mr. Pavel Stovicek*** [6], ***Dr. Haris Hadzic*** [7], ***Dr. Ludmila Novotna*** [7], ***Mr. Martin Simcik*** [7], ***Dr. Pavel Strnad*** [7], ***Dr. Anastaziia Serbina*** [7], ***Dr. Simona Karamazovova*** [8], ***Dr. Jaroslava Paulasová Schwabová*** [8], ***Prof. Martin Vyhnálek*** [8], ***Prof. Pavel Krsek*** [9], ***Dr. Alena Zumrova*** [9]

1. *Centre of Hereditary Ataxias, Motol University Hospital, Second Faculty of Medicine, Charles University, Prague, Czech Republic,* ***2.*** *Department of Paediatrics and Inherited Metabolic Disorders, First Medical Faculty, Charles University and General University Hospital in Prague, Prague 2, Czech Republic,* ***3.*** *Department of Immunology, Second Faculty of Medicine, Charles University, Motol University Hospital, Prague 5, Czech Republic,* ***4.*** *Department of Biology and Medical Genetics, Second Faculty of Medicine, Charles University, Motol University Hospital, Prague 5, Czech Republic,* ***5.*** *Department of Paediatrics, Second Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic,* ***6.*** *Prague, Czech Republic,* ***7.*** *Second Faculty of Medicine, Charles University, Prague 5, Czech Republic,* ***8.*** *Department of Neurology, Second Faculty of Medicine, Charles University, Motol University Hospital, Prague 5, Czech Republic,* ***9.*** *Department of Paediatric Neurology, Second Faculty of Medicine, Charles University, Motol University Hospital, V Uvalu 84, 15006 Prague 5, Czech Republic*

Methods: This observational study involved 34 genetically confirmed Friedreich's Ataxia (FA) patients and 17 healthy controls. Mitochondrial enzyme activities were measured spectrophotometrically in platelet samples, focusing on Complex I (NADH:Quinone Oxidoreductase, NQR), Complex II (Succinate:Quinone Oxidoreductase, SQR), Complex IV (Cytochrome c Oxidase), Citrate Synthase (CS), and Coenzyme Q10 (Ubiquinone, Q10). Neurofilament Light Chain (NFL) and cardiac markers were also analyzed. Statistical analyses included Welch's t-tests, linear regression models, and chi-square tests.

Results: FA patients exhibited significant reductions in Complex II ($p=0.002$, $N_{FA}=28$, $N_{HC}=16$) and Complex IV ($p<0.001$, $N_{FA}=28$, $N_{Hc}=17$) activities compared to healthy controls. Complex I activity showed a non-significant reduction ($p=0.092$, $N_{FA}=28$, $N_{HC}=17$). No significant differences were found in Citrate Synthase activity ($p=0.771$, $N_{FA}=28$, $N_{HC}=17$). The COX/CS ratio was significantly reduced ($p<0.001$, $N_{FA}=28$, $N_{HC}=17$). Elevated Q10 levels in FA patients were observed but were not statistically significant ($p=0.064$, $N_{FA}=34$, $N_{HC}=16$). NFL levels were significantly higher in FA patients ($p<0.001$, $N_{FA}=33$, $N_{HC}=12$). Cardiac markers, particularly NT-proBNP, indicated a trend towards elevation in FA patients ($p=0.088$, $N_{FA}=34$, $N_{HC}=13$).

Discussion: The study highlights marked mitochondrial dysfunction in FA, particularly in Complexes II and IV. The significant reduction in Complex IV activity underscores its potential role as a biomarker for mitochondrial impairment in FA. An earlier onset of cardiological symptoms, such as chest pain and palpitations, correlates with reduced Complex I activity, highlighting the potential link between cardiac symptoms and mitochondrial dysfunction. Elevated NFL levels suggest ongoing neurodegeneration in FA patients, inversely correlating with disease severity and age.

Conclusion: This analysis reveals mitochondrial impairments in FA, suggesting potential biomarkers for disease progression and therapeutic monitoring. Further studies are needed to confirm these findings and explore targeted treatments for mitochondrial dysfunction in FA.

Digital assessment of upper limb ataxia with Q-Motor: patient meaningfulness, sensitivity to longitudinal change, and treatment responsivity

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Andreas Traschütz [1], ***Mr. Dominik Hermle*** [1], ***Mr. Robin Schubert*** [2], ***Mr. Pascal Barallon*** [2], ***Dr. Winfried Ilg*** [3], ***Prof. Rebecca Schüle*** [4], ***Dr. Ralf Reilmann*** [2], ***Prof. Matthis Synofzik*** [5]

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Background and Methods: Digital-motor outcomes for ataxia may overcome the limited responsiveness of clinical outcomes in upcoming trials, but demonstration of patient meaningfulness and sensitivity to change remain challenging, particularly in the upper limb domain**.** This single-center longitudinal study validated the quantitative motor (Q-Motor) assessment of upper limb ataxia against patient-reported outcomes, and for sensitivity to longitudinal and symptomatic drug-related change as contexts of use. Based on 36 cross-genotype ataxia patients and 20 matched controls, digital measures of finger tapping, diadochokinesia, grip-lift, spiral drawing and target reaching tasks were selected and validated based on (i) correlations with individual upper limb items of the Patient-reported Outcome Measure (PROM)-ataxia and their composite, (ii) 2-week test-retest reliability, and (iii) sensitivity to longitudinal change at 1-year follow-up, stratified by Patient Global Impression of Change (PGI-C). Treatment responsivity was tested in two patients with SCA27B assessed on vs. off treatment with 4-aminopyridine.

Results: Twenty-five digital measures correlated with the upper limb composite of the PROM-ataxia (|rho|=0.4-0.7) and had excellent test-retest reliability (ICC=0.93-0.99). Correlations to individual PROM-ataxia items were specific for the functional impairment a measure was hypothesized to capture. Speed of finger tapping and diadochokinesia, and smoothness of target reaching ($SPARC_{3D}$) detected progression of ataxia at 1-year follow-up (effect size: $|r_{prb}|$=0.38-0.51), and only in patients with worsening PGI-C. Sample size estimations revealed that fewer patients are required to detect longitudinal change with digital than with clinical outcomes ($SPARC_{3D}$: n=33, SARA: n=79, 9-Hole Peg-Test: n=214). Variability measures of target reaching were responsive to treatment with 4-aminopyridine in SCA27B, with changes exceeding the minimal detectable and minimal important change.

Discussion: Q-Motor captures patient-meaningful 1-year longitudinal change and treatment response of upper limb ataxia. Validation in genetically stratified cohorts is ongoing for implementation in clinical trials.

Conclusion: Q-Motor presents a promising digital-motor outcome for upcoming ataxia trials.

Intronic GAA-FGF14 expansions as frequent cause of late-onset ataxia with episodic features: an Austrian cohort.

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Elisabetta Indelicato [1], ***Dr. David Pellerin*** [2], ***Dr. Wolfgang Nachbauer*** [1], ***Dr. Matthias Amprosi*** [1], ***Dr. Bernard C. Brais*** [3], ***Dr. Sylvia Boesch*** [1]

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Background and objectives

The recent description of intronic GAA-repeat expansions in *FGF14* in late-onset ataxia cases set a milestone in the field. Herein, we report the clinicogenetic characteristic of an Austrian cohort with GAA-*FGF14* Ataxia.

Methods

Patients were recruited at the Center for Rare Movement Disorders in Innsbruck, Austria. We screened unsolved cerebellar ataxia cases who fulfilled ≥1 of the followings: i) onset >40 years old, ii) history of episodic symptoms, iii) down-beat nystagmus. Targeted analysis of intronic GAA expansion in *FGF14* was performed at the Montreal Neurological Hospital.

Results

Out of a cohort of 35 patients selected for GAA-*FGF14* expansion testing, 17 (49%) presented at least one expansion in *FGF14* (n=1 $GAA_{200-250}$, n=16 $GAA_{>250}$). Two brothers carried a biallelic expansion (264/264 and 219/277 respectively). Family history was negative in 7/17 patients. The average age at symptom onset was 57 years (range 50-70 years). All patients displayed a, mostly mild, chronic cerebellar syndrome. A down-beat nystagmus was a frequent, but not constant, finding in repeated examinations. Fifteen patients (88%) had a history of episodic symptoms with acute worsening of the balance disorder, occurrence of slurred speech and/or oscillopsia. Reported triggers included caffeine, alcohol, physical exercise and psychological stress. Ten patients received an interval prophylaxis with acetazolamide (n=4) or 4-aminopyridine (n=6), which effectively reduced the frequency and severity of paroxysmal symptoms in 2/4 and 6/6 cases, respectively. Brain MRI showed cerebellar atrophy in 14/16 patients, mostly mild and limited to the vermis. The superior cerebellar peduncle sign was evident in 7/11 MRI.

Discussion and Conclusion

The diagnostic yield of targeted GAA-*FGF14* ataxia testing in patients with onset >40 years old and/or history of episodic symptoms and/or down-beat nystagmus approached 50% in our cohort. Based on the present findings, GAA-*FGF14* expansion analysis should be offered as first-tier genetic testing in this setting.

iPatax: A Reliable and Quantitative iPad® Application for Assessing Cerebellar Ataxia

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Takahiro Nagai [1], ***Dr. Masayoshi Tada*** [1], ***Dr. Tomohiko Ishihara*** [2], ***Dr. Masatoyo Nishizawa*** [1], ***Dr. Osamu Onodera*** [1]

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Background and Objective: The purpose of this study was to develop a novel, quantitative method for assessing the severity of cerebellar ataxia. Existing subjective ataxia scales were found to lack quantitative qualities.
Methods: The iPatax application is an application on an iPad® device that allows the user to track a moving target with a finger for one minute. iPatax records finger movements. The execution time was divided into three segments and the values for each period were used for analysis. Values from the last and first segments were compared to determine training effects representing cerebellar function. Twenty-eight control subjects and 31 patients with cerebellar ataxia participated in the data collection.
Results: The coefficient of velocity variation (CVV) during non-continuous movement was lower in the later segments of controls. The rate of improvement was significantly lower in the ataxia group than in the control group, suggesting that CVV during non-continuous movements reflects the cerebellar motor learning ability. The CVVs during non-continuous circular and linear movements discriminated well between the controls and ataxic patients. The area under the curve (AUC) of this classifier was 0.970. Furthermore, a significant positive correlation was found between CVV during non-continuous linear movement and the SARA score.
Discussion and Conclusion: The results showed that iPatax can effectively discriminate between ataxia and control groups and correlates with severity measures. This app has advantages over existing methods in that it is not influenced by gait function and has a low burden on patients. However, the study has limitations, including the possibility of variability due to participant understanding and motivation. This tool may enhance the precision of diagnosing cerebellar ataxia and could be beneficial in evaluating the efficacy of interventions for spinocerebellar degeneration.

Digital gait measures show promise to track progression of Spinocerebellar Ataxia

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Vrutang Shah [1], ***Mr. Daniel Muzyka*** [1], ***Ms. Hannah Casey*** [2], ***Dr. James McNames*** [1], ***Dr. Mahmoud El-Gohary*** [1], ***Dr. Kristen Sowalsky*** [1], ***Dr. Deleram Safarpour*** [3], ***Dr. Patricia Carlson-Kuhta*** [3], ***Prof. Jeremy D. Schmahmann*** [4], ***Dr. Liana S. Rosenthal*** [5], ***Dr. Susan Perlman*** [6], ***Dr. Fay Horak*** [1], ***Dr. Christopher M. Gomez*** [2]

1. *Clario,* ***2.*** *University of Chicago,* ***3.*** *Oregon Health and Science University, OR,* ***4.*** *Department of Neurology, Massachusetts General Hospital and Harvard Medical School,* ***5.*** *Johns Hopkins University,* ***6.*** *University of California at Los Angeles*

Background and Objective. While digital gait measures have shown greater sensitivity than the Scale for the Assessment and Rating of Ataxia (SARA) in cross-sectional studies, digital gait measures also need to be able to capture longitudinal changes within short time frames (such as 1 year) to be valid disease progression endpoints. Our aim was to investigate gait measures sensitive to longitudinal changes in a multicenter clinical trial of spinocerebellar ataxia (SCA).

Methods. 24 individuals with SCA (age: 53 ± 12 years, disease duration: 7 ± 2 years, SARA Total score: 7 ± 3) participated in the study. Subjects wore 6 inertial sensors (one on each foot, each wrist, sternum, and lower back) in the laboratory. Participants performed a 2-minute walk test (walking back and forth for 2-minutes with a walkway length of 10 m), and SARA at baseline and after 1-year follow-up.

Results. Several gait measures showed a statistically significant change over 12 months compared to baseline and had a higher effect size compared to the clinical measure. For example, foot strike angle decreased over 12 months, and changed more than the SARA total score (effect sizes: -0.57 vs. 0.10). Specifically, foot strike angle was 16.07 ± 4.50 degrees at baseline and reduced to 15.0 ± 5.00 degrees after 1-year follow-up, while SARA total score was 7.33 ± 3.00 and increased to 7.58 ± 3.30 after 1-year follow-up.

Discussion and Conclusions.

Unlike the SARA total score, gait measures assessed by wearable sensors captured the natural progression of SCA within just one year, with foot orientation measures representing a promising outcome for upcoming multicenter interventional trials.

The Friedreich Ataxia Global Clinical Consortium and the UNIFAI Study – advancing understanding of Friedreich ataxia and treatment options.

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Jennifer Farmer [1], ***Mr. Thomas Anthony*** [1], ***Dr. Louise A Corben*** [2], ***Prof. Paola Giunti*** [3], ***Prof. David Lynch*** [4], ***Dr. Caterina Mariotti*** [5], ***Dr. Katherine Mathews*** [6], ***Ms. Caitlin Monette*** [1], ***Prof. Massimo Pandolfo*** [7], ***Dr. Myriam Rai*** [1], ***Prof. Kathrin Reetz*** [8], ***Dr. Christian Rummey*** [9], ***Prof. Jörg B. Schulz*** [10], ***Prof. S. H. Subramony*** [11], ***Dr. . FA Global Clinical Consortium*** [1]

1. *Friedreich's Ataxia Research Alliance, Downingtown, PA,* ***2.*** *Monash University, Clayton, Murdoch Children's Research Institute, Parkville and University of Melbourne, Parkville,* ***3.*** *University College London,* ***4.*** *Children's Hospital of Philadelphia,* ***5.*** *Fondazione I.R.C.C.S. Istituto Neurologico C. Besta,* ***6.*** *University of Iowa,* ***7.*** *Department of Neurology and Neurosurgery, Montreal Neurological Hospital and Institute, McGill University, Montreal, QC, Canada,* ***8.*** *Department of Neurology, University of Aachen,* ***9.*** *Clinical Data Science GmbH,* ***10.*** *Universitätsklinikum RWTH Aachen,* ***11.*** *Department of Neurology and Fixel Center for Neurological Disorders, Gainesville, FL*

Background

The recent approval of omaveloxolone as the first treatment for adults with Friedreich ataxia (FA) highlighted the significant role of data from natural history studies and patient advocacy groups. The FA Global Clinical Consortium (FA GCC) is a network of clinician-researchers and patient advocates dedicated to making patient contributions of data more powerful in understanding FA and advancing treatment options for FA, providing infrastructure to accelerate global research and collaboration, and promoting access to clinical care. The first clinical research study of the FA GCC is the UNIFAI study which is a fusion of two well-established prospective, longitudinal studies: FACOMS and EFACTS, each of which followed >1400 patients for > 10 years, and now has >30 international sites.

Methods

Launching the FA GCC involved establishing a governance structure, sustainable funding model, clinical and data management, harmonization of two previous protocols into UNIFAI, and migration of existing data to a new database. In addition, to a well-defined study protocol investigator training, standard operating procedures for the collection of data and data monitoring was required.

Results

The UNIFAI protocol allows for collection of data through in-person and virtual visits, with core data elements (e.g., demographics, medical and FA history, clinical/functional assessments and patient reported outcomes) and supplemental (e.g., cardiac, vision, digital technology assessments). To date, there have been 420 visits (59 baseline and 361 return) captured through UNIFAI. The study will capture real world data in a harmonized, high-quality manner and further enable our ability to understand the evolving natural history of FA as treatments become available.

Conclusion

The FA GCC and UNIFAI have been established so that clinical studies (small investigator led studies to large multi-center trials) can be implemented in parallel leveraging the established infrastructure and data collection.

Compliance rates of performance and patient-reported outcome measures administered via a mobile health app in Patients with Friedreich Ataxia

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Marcus Grobe-Einsler [1], ***Ms. Vivian Maas*** [1], ***Dr. Maresa Buchholz*** [1], ***Dr. Niklas Weber*** [1], ***Prof. Thomas Klockgether*** [1], ***Dr. Bernhard Michalowsky*** [1]

1. *German Center for Neurodegnerative Diseases (DZNE)*

Background: Friedreich Ataxia (FA) is the most common hereditary ataxia. The PROFA-study investigates patient-reported, health-economic and psychosocial outcomes in FA. The study aims to optimize feasibility of clinical trials utilizing mobile health apps and demonstrate patient acceptance.

Methods: After an on-site baseline visit, patients subsequently receive a series of tasks of the categories (a) self-reported outcome measures (PROs) and (b) performance tests (activities) for six months via the ATOM5 mobile health app. Among the activities, $SARA^{home}$ is utilized as remote assessment of disease severity. All patients recruited until February 2024 were included in this preliminary analysis. We investigated (1) the compliance rates of all tasks and (2) the impact of on-site instruction quality on compliance rates of $SARA^{home}$ at home by evaluating the recorded performance during instructions as good, with errors, or poor.

Results: 43 patients received 2,173 PROs (628 questionnaires, 1,545 activities). Overall compliance was higher for PROs than for activities (89% vs 74%, $p<0.001$). Disease severity at baseline did not correlate with compliance of PROs and activities throughout the study (Pearson $r=0.14$ and $r=0.01$). There was a weak negative correlation between study progress and PRO compliance (Spearman $r=-0.24$), which was reversed for the activities (Spearman $r=0.18$). Instruction quality was moderately correlated with $SARA^{home}$ compliance (Spearman $r=-0.41$), indicating that higher instruction quality was associated with higher compliance rates.

Discussion: Disease severity did not impact compliance rates, especially for the activities, which is remarkable considering the effort required for severely affected patients living with Friedrich Ataxia. The total number of tasks has not severely affected compliance rates throughout the study so far. Still, most patients were in the early study phases during our preliminary analysis.

Conclusion: Using mobile health apps in longitudinal observational studies is feasible and well-accepted by patients. Instruction quality is essential for compliance and response rates.

Remote assessment of ataxia severity in SCA3 patients using SARAhome - Results from a multi-centric observational study.

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Marcus Grobe-Einsler [1], ***Ms. Vivian Maas*** [1], ***Mr. Arian Taheri Amin*** [1], ***Dr. Jennifer Faber*** [1], ***Prof. Thomas Klockgether*** [1]

1. *German Center for Neurodegnerative Diseases (DZNE)*

Background: There are no approved therapies for SCAs, but innovative therapeutics are being investigated in clinical trials. More frequent examinations and knowledge of fluctuations in disease severity are required to conduct these studies. SARA^{home} is a remote assessment for ataxia severity at home.

Methods: Patients with spinocerebellar ataxia type 3 (SCA3) independently recorded SARA^{home} twice daily for two weeks at home, using Aparito's ATOM5 App. Before each assessment, patients responded to a self-rating questionnaire on ataxia severity and influencing factors. All videos were rated by SARA-certified investigators. This study was co-funded by the NAF and Ataxia UK.

Results: 11 of 80 patients did not start home-recordings or recorded only one day and were considered dropouts. The remaining patients had a mean compliance rate of 76%. 35 patients had a compliance rate of ≥90% (at least 26 recorded scores). 15 patients recorded a complete set of 29 videos. Preliminary analysis of available scores indicates considerable fluctuation of ataxia severity of 3 points (sd 3.0) during 14 days. The item-specific fluctuation was 1.1 (sd1.1) for both gait and stance, 1.0 (sd 0.7) for the alternating hands, 0.7 (sd 0.7) for the nose finger test and 1.0 (sd 0.9) for speech disturbance.

Discussion: Ataxia research is transitioning from observational to interventional studies. Remote assessment of ataxia severity using SARA^{home} allows for increased frequency of assessments in large multi-centric trials and detects fluctuations in disease severity. A mean SARA^{home} score from several assessments at home may be used as complement measure of ataxia severity in future clinical trials.

Conclusion: SARA^{home} is a validated clinical scale for remote assessment of ataxia severity in ataxia trials. Feasibility has been demonstrated in a large multi-centric trial. SARA^{home} is integrated into the ATOM5 app by Aparito and available for use in clinical trials.

Diffusion MRI analyses of infratentorial regions in Friedreich's ataxia

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Richard Parker [1], Dr. Kirsi M Kinnunen [1], Dr. Susmita Saha [2], Prof. Christophe Lenglet [3], Prof. Pierre-Gilles Henry [3], Dr. Ian Harding [4], Dr. Louise A Corben [5], Prof. Nellie Georgiou-Karistianis [2], Dr. Marina Papoutsi [1], Dr. Robin Wolz [1], Mx. . TRACK-FA Neuroimaging Consortium [6]

1. *IXICO, plc, London,* ***2.*** *Monash University, Melbourne,* ***3.*** *University of Minnesota, Minneapolis,* ***4.*** *Monash University, Melbourne and QIMR Berghofer Medical Research Institute, Brisbane,* ***5.*** *Monash University, Clayton, Murdoch Children's Research Institute, Parkville and University of Melbourne, Parkville,* ***6.*** *TRACK-FA Study*

Background and Objectives

Diffusion tensor imaging (DTI) metrics, including Fractional Anisotropy (FA) and Mean Diffusivity (MD), can be used to measure changes in white matter (WM) tissue organisation in Friedreich's ataxia (FRDA). Free water (FW) can further characterize tissue changes by quantifying extracellular water, revealing neuroinflammatory processes. Here, we have performed DTI and FW analyses in infratentorial structures known to be affected in FRDA.

Methods

We contrasted 52 FRDA [mean(SD) age=22.9(8.4) years, 52% female] with 28 controls [mean(SD) age=20.7(7.9) years, 64% female] recruited as part of the TRACK-FA study (Georgiou-Karistianis et al., 2022). Regions of interest (ROIs) were bilateral superior cerebellar peduncle (SCP), bilateral cerebellar WM (CWM), midbrain and pons. We also examined (Pearson's) correlation with the mFARS and SARA scores. For both analyses, 2-sided permutation testing was used to ascertain *p*-values (FDR-corrected for multiple comparisons). All tests were adjusted for age, sex and regional volume. Regional volume was computed using FastSurfer for all regions except SCP, which was generated using SUIT.

Results

All metrics (FA, MD, FW) were significantly different between groups for the SCP (all $p<0.006$) with large effect size (all ES>1.2). Only FA was significantly different for the CWM ($p<0.027$, ES=0.6) and the midbrain and pons (both $p<0.001$, both ES=1.0). There were no significant correlations with mFARS, and correlation was low (all<±0.33). The only significant correlation with SARA was for midbrain FA ($p=0.035$, R=0.38).

Discussion and Conclusion

Differences between FRDA and controls in CWM and brainstem are pronounced and affect all structures examined. FA values were lower across all ROIs in FRDA even after adjusting for volume. However, correlation with the mFARS and SARA scores was low, suggesting that they may have limited utility as predictive biomarkers. FW was only significantly different in the SCP, suggesting that extracellular water increases are limited in FRDA.

Longitudinal assessment reveals stage-dependent utility of digital motor markers in SCA1

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Ilse Willemse [1], ***Mr. Teije van Prooije*** [1], ***Ms. Kirsten Kapteijns*** [1], ***Prof. Bart van de Warrenburg*** [1]

1. *Radboud University Medical Center; Donders Institute for Brain, Cognition and Behaviour; Department of Neurology; Nijmegen, The Netherlands*

Objective: To identify sensitive digital gait biomarkers as candidate surrogate outcome measures in future clinical trials in patients with Spinocerebellar Ataxia type 1(SCA1).
Methods: We measured 17 patients with SCA1 and 15 healthy controls at baseline and one-year follow-up. Clinical measures included scales such as SARA and patient-reported outcomes. Subjects walked along a 10-meter path for 30 seconds wearing three opal sensors by APDM. We extracted the top 10 digital gait measures reported by Shah(Mov Disord 2021) complemented with the lateral step variability, and checked for a significant difference between the two groups. For each outcome, we cross-sectionally assessed their relationship to clinical outcome measures and calculated the standardized response means (SRM) to assess one-year responsiveness .
Results: SCA1 patients and controls differed on all gait parameters ($p < .05$). Toe-off angle showed the strongest correlation with ataxia severity (e.g. SARA, $r = -0.66$) and patient-reported outcomes (e.g. PROM-ataxia, $r = -0.76$). Toe-off angle (SRM = -1.15) and variability in toe-off angle (SRM = 0.61) were the only gait measures showing significant responsiveness after one year. For both, responsiveness was not significantly higher compared to SARA (SRM = 1.12). The toe-off angle only outperformed SARA in an early disease subgroup (9 SCA1 patients, SARA < 11 at baseline).
Discussion: In the full cohort of SCA1 patients, who display a relatively rapidly progressive ataxia (2.56 points SARA increase in one year), digital gait biomarkers did not outperform clinical scales in terms of sensitivity to change. The short walking duration may have reduced the reliability of outcomes measuring gait variability.
Conclusion: Sensitivity to change of digital gait biomarkers appears stage dependent in this SCA1 cohort. The toe-off angle seems to be a promising surrogate outcome measure for early-stage SCA1 patients in future clinical trials.

This work is funded by ZonMw(grant number: 40-44600-98-606)

Patient-Informed Research Design: Incorporating RFC1 Ataxia Patient Perspectives into a Natural History Study Proposal

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Celeste Suart [1], ***Dr. Lauren Moore*** [1], ***Dr. Gulin Oz*** [2], ***Dr. Liana S. Rosenthal*** [3], ***Dr. Sheng-Han Kuo*** [4]

1. *National Ataxia Foundation,* ***2.*** *University of Minnesota,* ***3.*** *Johns Hopkins University,* ***4.*** *Columbia University Medical Center*

Background

Numerous studies have demonstrated that meaningful involvement of patients in research increases patient enrollment, decreases attrition, identifies more relevant clinical outcomes, and improves implementation of research findings. We describe a patient engagement initiative to incorporate feedback from patients with RFC1 Ataxia into a natural history study proposal. We collected feedback on three domains – burden and frequency of RFC1 Ataxia symptoms; trial design considerations; and preferences for longitudinal patient engagement. RFC1 Ataxia is an adult-onset autosomal recessive repeat expansion disorder.

Methods

Sixteen patients with RFC1 Ataxia were recruited through social media and targeted emails to the National Ataxia Foundation members. Following a structured interview paradigm, three focus groups were held in March 2024. A qualitative content analysis approach was applied to the data. Triangulation of data from the three focus groups and RFC1 ataxia literature was used to assess data validity.

Results

Most participants identified balance impairments due to cerebellar ataxia, chronic cough, and neuropathy as their most burdensome symptoms. Oscillopsia was also reported as a major burden by participants working full-time. Other challenges impacting participants' quality-of-life included anxiety, fatigue, and fluctuation of symptoms day-to-day. The frequency of symptoms reported by participants mirrors published findings in the literature, apart from arrhythmia which was reported by 38% of participants. Participants found that the proposed study design with annual visits incorporating COA, Video Head Impulse Test (vHIT), EMG, EKG, and MR imaging was generally reasonable. Participants preferred an integrated model of longitudinal patient engagement, including advisory board meetings and a knowledge translation blog.

Discussion

These findings demonstrate that incorporating patient feedback early in the study design process facilitates a comprehensive protocol and discovery of previously overlooked symptoms. Further, they expand our understanding of the lived experience of patients with RFC1 ataxia.

Funding

No specific funding sources were used for this project.

Understanding Dentatorubral-pallidoluysian atrophy (DRPLA) symptoms and impacts on daily life: qualitative interviews with patients and caregivers

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Marielle G. Contesse [1]***, Dr. Rebecca J. Woods*** [1]***, Ms. Mindy Leffler*** [1]***, Dr. Silvia Prades*** [2]***, Dr. Julie Greenfield*** [3]***, Ms. Andrea Compton*** [4]***, Dr. Jeff Carroll*** [5]

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Methods: We aimed to better understand symptoms and the impact on daily life in adult- and juvenile-onset patients with DRPLA and explore patient and caregiver treatment goals and clinical trial participation preferences. This was explored through qualitative interviews with two DRPLA patients and seven caregivers, describing the experiences of 18 patients in total, as some were caregivers of multiple patients. Interview transcripts were coded for themes and reported symptoms were summarized with descriptive statistics.

Results: Adult-onset patients (N = 7) experienced difficulty with ataxia (100%), cognition (100%), fine motor skills (100%), gross motor skills (100%), speech (100%), personality changes (100%), and seizures (57%). Juvenile-onset patients (N = 11) experienced difficulty with ataxia (100%), sleep (100%), speech (100%), jerking/twitching (83%), behaviour (82%), cognition (82%), fine motor skills (82%), gross motor skills (82%), sensory sensitivity (75%), and seizures (64%). When considering aspects of DRPLA to target for future treatment, patients prioritised ataxia/mobility (100%), juvenile-onset caregivers prioritised ataxia/mobility (60%) and independence (60%), and adult-onset caregivers prioritised personality (60%). Almost all patients (93%) would participate in a clinical trial if given the opportunity, though travel to a clinical site could pose a participation barrier for half.

Discussion: While many symptom domains are common across the DRPLA population, this study found differences within each domain based on the age of symptom onset and disease stage.

Conclusion: These findings can be used to inform the selection of outcome measures for future clinical trial designs, ensuring they address the most impactful and prioritised symptoms for patients and caregivers.

The European Spinocerebellar Ataxia Type 3/Machado-Joseph Disease Initiative (ESMI) cohort

Tuesday, 12th November - 18:10: (Minories) - Poster

Prof. Thomas Klockgether [1], ***Dr. Jennifer Faber*** [1], ***Dr. Magda Santana*** [2], ***Dr. Jeannette Hübener-Schmid*** [3], ***Dr. Hector Garcia-Moreno*** [4], ***Prof. Paola Giunti*** [4], ***Prof. Bart van de Warrenburg*** [5], ***Prof. Dagmar Timmann*** [6], ***Prof. Kathrin Reetz*** [7], ***Dr. Heike Jacobi*** [8], ***Prof. Ludger Schöls*** [9], ***Prof. Matthis Synofzik*** [9], ***Prof. Olaf Rieß*** [10], ***Prof. Jon Infante*** [11], ***Prof. Luís Pereira de Almeida*** [2], ***Dr. Mafalda Raposo*** [12], ***Prof. Manuela Lima*** [12]

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Background: Spinocerebellar ataxia type 3/Machado-Joseph disease (SCA3) is worldwide the most common autosomal dominant ataxia. It is caused by CAG repeat expansion mutations in the *ATXN3* gene. Currently, there is no treatment for SCA3, but targeted therapeutic approaches are under development, and the SCA3 field is entering a phase of intense trial activity.
Methods: In 2017, the European Spinocerebellar Ataxia Type 3/Machado-Joseph Disease Initiative (ESMI) started to recruit a longitudinal cohort of pre-ataxic and ataxic SCA3 mutation carriers, and matched controls. All participants undergo a standardized assessment program and biosampling. In a subgroup, MRIs are performed.
Results: In April 2024, the ESMI cohort consisted of 535 participants, 78 of whom were pre-ataxic and 301 ataxic mutation carriers. In 381, one follow-up visit, in 250 two, in 173 three, and in 188 more than three follow-up visits were performed. Using data and materials of the cohort, ESMI initiated research projects that resulted in an in-depth analysis of the time course of neurofilament light chain (NfL) changes in blood and the development of a highly sensitive assay for expanded ATXN3 that is useful as a target engagement marker. Blood transcriptome sequencing identified novel biomarkers capable of tracking disease stages. MRI studies identified a sequence of brain atrophy ascending from the lower brainstem with an early affection of white matter. Comprehensive analysis of the ESMI data allowed to develop the first data-driven staging model of SCA3 that includes an initial *asymptomatic carrier stage* followed by the *biomarker stage* characterized by absence of ataxia, but changes of NfL, as well as pons and cerebellar white matter volumes, finally leading into the *ataxia stage*, defined by manifest ataxia.
Discussion/Conclusions: The ESMI cohort is a highly valuable resource for a deepened understanding of SCA3 and the design of therapeutic, as well as preventive clinical trials.

Modifications of the SARA Score: Implications on Sensitivity to detect Change and other Psychometric Properties

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Christian Rummey [1]

1. *Clinical Data Science GmbH*

Introduction

The SARA score has been used as a reliable measure for ataxias and related diseases for almost 20 years. However, recent clinical trials have employed modified versions of the scale, typically following regulatory guidance. The changes include scoring items on a “no, mild, moderate, severe, unable” paradigm (scores 0-4), effectively collapsing gait, stance and speech items and omitting appendicular function items (SARA items 5-8) entirely. These changes impact the scales’ sensitivity to detect change and other psychometric properties.

Methods

This analysis utilized data from major natural history studies in ataxias, such as EUROSCA and CRCSCA. Instruments included were the full 8-item SARA score, a modified 4-item f-SARA score (derived using a mapping strategy), and all individual axial and appendicular SARA items were included. Yearly changes from baseline and standard deviations were calculated to derive standard response means, focusing on the most responsive subpopulations.

Results

The f-SARA modifications can increase the instrument’s sensitivity depending on the population studied. Collapsing items 1-4 generally reduced sensitivity to change by about 10%. Conversely, omitting appendicular items improved sensitivity by up to 20% in some cases but reduced it in others. The most sensitive items in all scales variants are gait, balance, and speech (independent of the scoring system).

Discussion

The f-SARA score is a sensitive and relevant tool to assess progression in ataxias. It maintains gait, balance, and speech as the key features. However, while removing appendicular function tests can be beneficial for sensitivity, it may also reduce it in other scenarios. These findings underscore the need to carefully consider the modifications’ impact on sensitivity and psychometric properties.

Conclusion

Modifications to the SARA score can improve psychometric attributes but may alter its fundamental properties. These changes should be validated with available natural history and trial data before being used in randomized controlled trials.

Outcome Measures for Pediatric Ataxia - Insights from the FA-CHILD Study

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Christian Rummey [1], ***Dr. Susan Perlman*** [2], ***Prof. S. H. Subramony*** [3], ***Dr. Manuela Corti*** [3], ***Ms. Jennifer Farmer*** [4], ***Prof. David Lynch*** [5]

1. *Clinical Data Science GmbH,* ***2.*** *University of California at Los Angeles,* ***3.*** *Department of Neurology and Fixel Center for Neurological Disorders, Gainesville, FL,* ***4.*** *Friedreich's Ataxia Research Alliance, Downingtown, PA,* ***5.*** *Children's Hospital of Philadelphia*

Background

Assessing disease progression in children with Friedreich's Ataxia (FRDA) is challenging due to the rapid disease progression and often increased non-neurological symptoms. There are no approved treatments for children with FRDA. These two facts underscore the goals of the FACHILD study: To augment and expand the knowledge about the natural history and clinical outcome assessments in this most severely affected population.

Methods

FACHILD enrolled 108 individuals aged 7-18 years with genetically confirmed disease. Outcome measures were the modified Friedreich's Ataxia Rating Scale (mFARS) and its axial component, the Upright Stability Score (USS). In addition to the established assessments, such as the FA-Activities of Daily Living, the timed 25 Foot Walk, and the 9-Hole Peg Board Test, we introduced longer and more complex walking tasks (1-minute, and 6-minute walk, the timed up and go), as well as the Berg Balance Scale (BBS). Longitudinal data was analyzed in relevant subgroups, using linear mixed effect modeling, and contextualized with data from children enrolled in the parallel FACOMS study.

Results

The mFARS proved generally useful in older children, but we also found increased variability and potential training effects in the younger age group, in particular in upper limb scores (FARS B). Gait and Balance scores were more stable over all age and severity subgroups. Among direct functional tests the 1-Minute Walk demonstrated promising properties comparable to mFARS and USS. The BBS showed promising results for early populations.

Discussion and Conclusion

Our findings emphasize that pediatric ataxia requires careful outcome measure selection and evaluation. From our study, the USS, along with the 1-Minute Walk, emerged as the overall most reliable assessments.

These insights are crucial for optimizing trials design and will likely expand into pediatric ataxia populations beyond Friedreich's.

Frataxin Levels Predict Long-term Clinical Progression in Friedreich's Ataxia

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Christian Rummey [1], ***Prof. Ian Blair*** [2], ***Dr. Clementina Mesaros*** [2], ***Prof. Yina Dong*** [2], ***Prof. David Lynch*** [3]

1. *Clinical Data Science GmbH,* ***2.*** *University of Pennsylvania, Perelman School of Medicine,* ***3.*** *Children's Hospital of Philadelphia*

Introduction

Novel therapeutics entering clinical trials are employing various strategies to increase or augment frataxin (FXN) protein levels. More precise knowledge on the direct impact on patient function can increase FXN significance as a surrogate biomarker, significantly accelerating the development of such therapies and help to establish dose levels.

Methods

We utilized four frataxin (FXN) datasets, stemming from two FA natural history studies, two peripheral tissues, two different assays and assessing two FXN isoforms. Blood samples from FACHILD (a pediatric study) were analyzed using a new LCMS methodology (FXN-E from erythrocytes and mature FXN). Additionally, FACOMS samples (blood and buccal swabs) were analyzed using the a lateral flow immunoassay. Protein levels were intercorrelated with age of onset and GAA1, compared between controls, carriers, and patients, as well as between clinical severity groups. Eventually predictive capacity of direct patient function was evaluated.

Results

Data from 87 FACHILD patients, and 428/532 FACOMS participants (blood samples/buccal swabs) were available. Patients showed relative frataxin levels of 15%-25%, while carriers had 50%-80% of control levels. Grouped by clinical severity, patient levels were consistent between datasets, e.g. typical onset patients (AOO 8-14y) had 160% of early onset patients (AOO <7y). FXN levels predicted both age at loss of ambulation and long-term progression slopes in the Upright Stability Score (Section E of the modified Friedreich's Ataxia Rating Scale). Although the FACHILD dataset had only about 20% of FACOMS patients, the LCMS-derived FXN levels predicted function with similar significance.

Discussion

This work provides proof for the direct correlation of FXN levels with long-term patient function. In addition, relative FXN levels between clinical subgroups, as well as carriers and controls provide important guidance for drug development.

Conclusion

This work marks a significant step forward for the use of FXN levels as a clinical biomarker.

Gait adaptability training improves gait speed in Spinocerebellar ataxia patients

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Colette Reniers [1], ***Mrs. Karen Huisman-Venrooij*** [1], ***Prof. Ivan Toni*** [2], ***Prof. Vivian Weerdesteyn*** [3], ***Prof. Bart van de Warrenburg*** [1]

1. *Radboud University Medical Center; Donders Institute for Brain, Cognition and Behaviour; Department of Neurology; Nijmegen, The Netherlands,* ***2.*** *Donders Institute for Brain, Cognition and Behaviour; Radboud University; Nijmegen, The Netherlands,* ***3.*** *Radboud University Medical Center; Donders Institute for Brain, Cognition and Behaviour; Department of Rehabilitation, Nijmegen, The Netherlands; Department of Rehabilitation, Sint Maartenskliniek, Nijmegen, The Netherlands*

Introduction: Spinocerebellar ataxia (SCA) is a rare genetic neurodegenerative movement disorder primarily affecting the cerebellum. So far, there is no available cure for SCA. However, evidence suggests that neurorehabilitation can alleviate symptoms. The most optimal training protocol has not been established and mechanisms that channel the effects of these interventions are incompletely understood. This study aims to investigate the effects of a specific gait training based on the pilot study by Fonteyn et al (2014) in a larger SCA cohort and explore the underlying cerebral mechanisms.

Methods: We included 20 ambulant SCA patients and 18 matched healthy controls. A 5-week, C-mill gait adaptability training protocol was conducted with 10 sessions of 1 hour. We evaluated the effects of training on ataxia severity (SARA), walking speed (comfortable, fast and tandem), and completion time of two functional mobility tests (Timed-Up-and-Go and EFAP obstacle subtask) and a 10 Meter Walking Test. To identify training-related structural brain changes, two T1w structural MRI scans were acquired one week before and within one week after training.

Results: Following training, we found similar improvements in walking speed and task duration for both groups; SARA scores did not change. More specifically, a significant decrease in task duration in both patients (pre 11.6 sec, post 11.0 sec) and controls (pre 9.7 sec, post 9.2 sec), and a significant increase of gait speed in patients (pre 125.1 cm/sec, post 133.9 cm/sec) were detected. These behavioral training effects were not accompanied by detectable structural grey matter changes in the brain.

Conclusion: These results suggest that early-stage SCA patients retain the ability to adapt and learn when exposed to gait adaptability training, and that training effects generalize to relevant gait parameters. The neural substrate mediating this training-induced improvement remains unknown and needs further work.

Internal funding Donders Institute/Radboudumc & funding NWO

Cerebellar mono-system atrophy in the early stage of multiple system atrophy: a study using data from the Japan Consortium for Ataxias

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Atsuhiko Sugiyama [1], ***Dr. Shigeki Hirano*** [1], ***Dr. Yuji Takahashi*** [2], ***Dr. Hidehiro Mizusawa*** [2], ***Prof. Sathoshi Kuwabara*** [1]

1. *Chiba University,* ***2.*** *National Center of Neurology and Psychiatry*

Background and objectives

Multiple system atrophy (MSA) is a progressive neurodegenerative disorder with cerebellar ataxia, autonomic failure, parkinsonism, and pyramidal signs. In cerebellar ataxia-predominant MSA (MSA-C), the median interval from the initial onset to concomitant motor and autonomic manifestations has been reported to be 2 years. Some patients with MSA-C present only with cerebellar ataxia (cerebellar-type mono-system atrophy) for ˃10 years. This study aimed to clarify the frequency and clinical characteristics of cerebellar mono-system atrophy using data from the Japan Consortium for Ataxia (J-CAT).

Methods

Among the cases registered in J-CAT from March 2017 to December 2021, 90 cases that met the diagnostic criteria for MSA as of January 2022 were included. We investigated the frequency and clinical characteristics of patients with cerebellar mono-system atrophy who did not present with parkinsonism, autonomic symptoms, or pyramidal signs other than cerebellar ataxia at the time of J-CAT registration.

Results

Among 90 patients with MSA, 8 (8.9%) had cerebellar mono-system atrophy at the time of J-CAT registration. The age of onset and disease duration in the cerebellar mono-system atrophy group were 55 years and 1.5 years, respectively, and did not differ significantly from those in the other patients with MSA. Furthermore, patients with cerebellar mono-system atrophy had significantly lower Scales for Assessing and Rating of Ataxia and the Unified Multiple System Atrophy Rating Scale scores at J-CAT registration compared with the other patients with MSA.

Discussion and conclusion

Some patients with MSA may initially present with cerebellar mono-system atrophy, which can be challenging to differentiate from other spinocerebellar ataxias. Early in the disease course, patients with cerebellar mono-system atrophy may have a slower disease progression than other patients with MSA.

This work was partly supported by the Japan Agency for Medical Research and Development under Grant Number JP21ek0109532h0001 (Y. Takahashi).

Patient-reported Vision Quality-of-life in Ataxias and Parkinsonian Syndromes and Association with Clinical Oculomotor Findings

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Faye Yang [1], ***Mr. Rohin Manohar*** [1], ***Ms. Anna Luddy*** [1], ***Dr. Nancy Soja*** [1], ***Dr. Anne-Marie Wills*** [2], ***Dr. Albert Hung*** [2], ***Dr. Christopher D. Stephen*** [2], ***Prof. Jeremy D. Schmahmann*** [2], ***Dr. Anoopum Gupta*** [2]

1. *Department of Neurology, Massachusetts General Hospital,* ***2.*** *Department of Neurology, Massachusetts General Hospital and Harvard Medical School*

Background/Objective: Eye movements are well-characterized in ataxias and parkinsonian syndromes. However, there is limited understanding of how oculomotor abnormalities impact vision-related quality-of-life (VQoL). Identifying the functional significance of oculomotor signs could inform symptom management and guide therapeutic development.

Method: For disease versus control analysis, a total of 192 individuals, 97 Parkinson's Disease (PD), 7 Progressive Supranuclear Palsy (PSP), 15 SCA3, 10 SCA6, 7 CANVAS, and 56 controls, completed a 13-item subset of the Visual Activities Questionnaire (VAQ), which assesses VQoL during everyday tasks. VAQ questions measure depth perception, visual acuity/spatial vision, and visual processing speed. A broader set of ataxia participants (n=74) completed VAQ and also had clinical oculomotor assessments of nystagmus, gaze-holding, saccades, and pursuit.

Results: All patient populations had significantly worse VQoL compared to controls. PSP patients reported significantly worse VQoL compared to PD patients in all visual functions: depth perception (Cohen's d=2.42), visual acuity/spatial vision (Cohen's d=1.13), and visual processing speed (Cohen's d=1.98). SCA3 patients reported significantly worse VQoL compared to PD patients in depth perception (Cohen's d=1.11) and visual processing speed (Cohen's d=1.13). No significant differences were observed between SCA3, SCA6, and CANVAS. For ataxia patients, the presence of nystagmus, but not other signs, was associated with worse VQoL compared to individuals without nystagmus (Cohen's d=0.66). For SCA patients (35/74), this association was amplified (Cohen's d=1.25). Multivariate regression utilizing oculomotor signs as predictors for VAQ total confirmed the presence of nystagmus as the sole significant variable in SCAs (p=0.002).

Discussion/Conclusion: Vision quality-of-life is differentially impaired across the studied populations. The finding of nystagmus as a leading contributor to everyday visual impairment motivates targeted development of therapeutics to alleviate this symptom.

Funding: NIH NS117826

Non-polyglutamine CACNA1A disease: natural history and treatment experience in Austria

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Elisabetta Indelicato [1], ***Dr. Wolfgang Nachbauer*** [1], ***Dr. Matthias Amprosi*** [1], ***Dr. Iris Unterberger*** [2], ***Dr. Margarete Delazer*** [3], ***Dr. Katharina Kaltseis*** [4], ***Prof. Gregor Brössner*** [4], ***Dr. Matthias Baumann*** [5], ***Dr. Sylvia Boesch*** [1]

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Methods

Genetically confirmed *CACNA1A* patients were prospectively followed either at the Department of Neurology or at the Department of Pediatrics of the Medical University of Innsbruck.

Results

Our cohort of patients with non-polyglutamine *CACNA1A* mutations comprehends 44 subjects (17 females, 39%, mean age at the first examination 34±22 years), of whom 39 belong to families with multiple affected subjects. Twenty-four patients (55%) were classified as having FHM1, the other 20 patients (45%) as having EA2. Disease onset was in the childhood/adolescence in 33 patients (67%). A developmental delay was the first sign in 10 patients, while in the other 34 the first manifestation were episodic symptoms. Two patients experienced a seizure within a severe migraine attack with cerebral edema, otherwise no convincing history of epilepsy was found in other patients. Thirty-eight patients (86%) displayed non-episodic neurological signs, most frequently a mild chronic cerebellar syndrome. Further non-episodic features encompassed developmental delay, psychiatric symptoms, spasticity, and dystonia. SARA score did not show a significant progression over a 4-year analysis. Gait analysis parameter did show differences only concerning gait velocity in n=12 patients followed up over 4 years. At the time of the last visit, 28 patients required an interval prophylaxis, consisting of acetazolamide (n=14), 4-aminopyridine (n=7), flunarizine (n=3), topiramate (n=2), a combination of flunarizine and acetazolamide (n=1) and galcanezumab (n=1). Medical therapy was effective in reducing the frequency and severity of episodic symptoms.

Discussion

Our cohort displayed the whole spectrum of age-dependent manifestations reported in *CACNA1A* disorders. We encountered a lower frequency of early onset severe phenotypes comparing to other published cohorts recruited in pediatric clinics/through patient organization. We report the first case of FHM1 successfully treated with an anti-CGRP antibody.

Conclusion

We report the real-life distribution of clinical phenotypes and treatment experience in a *CACNA1A* cohort at a European Reference Center.

Medication patterns of spinocerebellar ataxia type 3 patients enrolled in the ESMI cohort

Tuesday, 12th November - 18:10: (Minories) - Poster

Mr. Patrick Silva [1], ***Ms. Marina A. Costa*** [2], ***Mr. João Durães*** [3], ***Ms. Joana A. Ribeiro*** [3], ***Ms. Inês Cunha*** [3], ***Prof. Cristina Januário*** [3], ***Mr. ESMI Study Group*** [4], ***Prof. Luís Pereira de Almeida*** [1], ***Dr. Magda Santana*** [5]

1. *Centre for Neuroscience and Cell Biology, Univ. Coimbra, Portugal; Faculty of Pharmacy, Univ. Coimbra, Portugal; Center for Innovative Biomedicine and Biotechnology, Portugal; Gene Therapy Center of Excellence, Portugal,* ***2.*** *Centre for Neuroscience and Cell Biology, Univ. Coimbra, Portugal; Faculty of Pharmacy, Univ. Coimbra, Portugal,* ***3.*** *Coimbra Hospital and University Centre, Portugal,* ***4.*** *German Center for Neurodegenerative Diseases (DZNE), Bonn,* ***5.*** *Centre for Neuroscience and Cell Biology, Univ. Coimbra, Portugal; Center for Innovative Biomedicine and Biotechnology, Portugal; Gene Therapy Center of Excellence, Portugal; Institute of Interdisciplinary Research, Portugal*

Background: Spinocerebellar ataxia type 3 (SCA3) is the most common dominantly inherited ataxia worldwide. No disease-modifying treatment exists, with disease management focusing on symptom alleviation and maximizing functional capacity while minimizing complications. Guidelines for therapeutic use are scarce, leaving treatment decisions largely to physicians' discretion. To date, no study has reported how symptomatology is managed in SCA3 patients, limiting the harmonization of therapies and the improvement of disease management strategies.

Methods: Medication data were collected from participants enrolled in the multicentric European Spinocerebellar Ataxia Type-3/Machado-Joseph Disease Initiative (ESMI) cohort. Statistical analysis of medications taken by SCA3 mutation carriers and controls was performed. Stratification groups included disease stage, age, and research center.

Results: 373 mutation carriers (50.1% female) and 109 control individuals (60.6% female) were included in the study. A greater percentage (72.1%) of SCA3 mutation carriers were medicated than were controls (46.8%, $p<0.001$), but differences were found only for younger age strata. Compared with controls, SCA3 individuals take more medication for the nervous system, the alimentary tract and metabolism, and the musculoskeletal system, yet no differences between pre-ataxic individuals and controls were observed for any of these classes. Psychoanaleptics and vitamins are the subclasses introduced earlier in the disease course. Analgesic and muscle relaxants are primarily taken by mutation carriers in more advanced disease stages. Not all SCA3 patients who presented with non-ataxic signs were taking recommended medication to alleviate these. Differences in medication patterns across study centers were also observed.

Conclusion: This is the first study exploring real-world medication patterns in patients with SCA3, which we expect to contribute to the development of more standardized and effective treatment approaches to improve the quality of life of SCA3 patients.

Funding: JPND, FCT, and ERDF (COMPETE 2020 and CENTRO 2020).

Oculomotor Biomarkers for Cerebellar Ataxia and Severity in Smooth Pursuit

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Penina Ponger [1], ***Ms. Shimrit Shani*** [2], ***Prof. Yoram Boneh*** [3]

1. *Center for the Study of Movement, Cognition and Mobility, Neurological Institute, Tel Aviv Sourasky Medical Center, Tel Aviv Israel,* ***2.*** *School of Optometry and Vision Science, Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel.,* ***3.*** *The Leslie and Susan Gonda Multidisciplinary Brain Research Center, Bar-Ilan University, Ramat Gan, Israel.*

Background:
There is an unmet need for measures that are clinically significant and capable of monitoring the severity and progression of cerebellar ataxia (CA). Quantitative oculomotor measures offer a straightforward, dependable, and sensitive biomarker for CA. Our research utilizes brief linear smooth pursuit of a moving target to derive various parameters. Analysis of parameters to identify differences from control group and to examine indices relationship with disease severity are presented.
Method:
We recorded the eye movements of 30 CA patients with varying disease severity while participants followed a white disk moving from the center outwards in straight lines in different directions randomly. We developed 6 oculomotor indices to assess the quality of tracking: (1)“Saccadic pursuit” (the saccadic contribution to tracking), (2)Pupil dilation (start of tracking), (3)Initial saccade latency (open-loop phase), (4)Occluder-induced deviation from unoccluded tracking, (5)Vergence stability (the STD of the lag between the eyes during tracking), and (6)Pre-stimulus saccadic inhibition. Differences between groups and correlation of these indices are presented.
Results:
We found a significant deficiency in three oculomotor indices in CA patients compared to controls. Notably, for smooth tracking that requires a tight closed-loop process, the patients were slow to start, applied less anticipatory microsaccade inhibition, used excessive catchup saccades, were inefficient in keeping vergence, and did poorly in coping with occlusion. All 6 indices were significantly correlated with the Scale for the assessment and rating of ataxia score, ($R=0.4\text{-}0.7;p<0.005$). Notably, the oculomotor abnormalities included reduced anticipatory inhibition of movement.
Conclusion:
These findings reveal a notable abnormality in simple visual tracking among CA patients, highlighted by deficiencies in six distinct oculomotor indices. These indices, which reflect both eye movement and inhibition characteristics, show a correlation with CA severity, suggesting their potential as quantitative biomarkers for evaluating treatment effectiveness. Further investigation is required to fully assess this possibility.

Alterations in the Daily Living Gait and Mobility during the Day and Night among Individuals with Cerebellar Ataxia, SCA3: An Exploratory Study

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Penina Ponger [1], ***Ms. Amit Solomon*** [2], ***Ms. Marina Brozgol*** [2], ***Mr. Eran Gazit*** [2], ***Prof. Jeffrey M. Hausdorff*** [2]

1. *Movement Disorders Unit, Department of Neurology, Tel-Aviv Sourasky Medical Center, Tel Aviv, Israel,* ***2.*** *Center for the Study of Movement, Cognition and Mobility, Neurological Institute, Tel Aviv Sourasky Medical Center, Tel Aviv Israel*

Objectives:
Cerebellar ataxia (CA) impairs balance and gait. Previous work has begun to evaluate daily living gait, suggesting large within-bout, step-to-step variability in CA, compared to health controls (HC). In this pilot study, we aimed to examine step counts and daily living physical activity, confirm findings regarding gait variability, explore changes in nighttime mobility, and examine associations with disease severity.
Methods:
11 individuals with SCA3 (age:51.1±11.7yrs; 72% F) and 11 age and sex-matched HC (age:51.9±12.0 yrs;72% F) wore a 3D accelerometer on the lower back for up to 7 days. Previously established methods assessed daytime gait quality and nighttime movement and mobility. The Scale for Assessment and Rating of Ataxia (SARA,range 0-40) rated disease severity.
Results:
SARA scores ranged from 1-12 (mean:6±3). Daily step count, total physical activity, time spent awake at night, and time spent in bed at night did not differ in CA and HC. In unadjusted analyses, CA had fewer long (>120 sec) walking bouts (HC:6.6±4.1;CA:2.3±2.4;p=0.007) and higher measures of step-to-step variability (see figure). CA also had fewer and slower axial rotations of the trunk during the night (presumably during sleep). It took CA about 3x longer to transition from lying position to walking at night and longer to transition from sitting to standing during the day. Measures of gait variability and transition times were correlated with SARA scores (Spearman's rho:0.66-0.81,p<0.05).
Conclusions:
These pilot results suggest that overall physical activity, walking time, and sleep time are similar in CA and HC. In contrast, several aspects of the quality of daily living movement and mobility appear to be markedly altered in CA, during day and night, including during sleep. For example, a high degree of step-to-step variability was observed in CA. These initial findings can be used to guide larger studies and to inform biomarker and intervention research.

Physical Activity and Fitness Levels of Individuals with Ataxia: A Cross-Sectional Study

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Scott Barbuto [1], Mr. Michael Spinner [1], Dr. Sheng-Han Kuo [1], Dr. Lori Quinn [1], Dr. Joel Stein [1], Dr. Seonjoo Lee [1], Dr. Yaakov Stern [1]

1. *Columbia University Medical Center*

Background and Objective: This study investigates the physical activity levels of individuals with ataxia and correlates fitness to ataxia severity.
Design: Observational study
Setting: Outpatient ataxia clinic in a large, tertiary, urban hospital in the US.
Participants: Individuals with cerebellar ataxia (n=42).

Methods: Participants were classified as sedentary or physically active using the International Physical Activity Questionnaire-Short Form (IPAQ-SF). Maximal oxygen consumption (VO_2max) as an indicator of fitness level was measured, and ataxia severity was determined by the Scale for the Assessment and Rating of Ataxia (SARA). Mixed effect models were used to correlate ataxia severity to fitness levels.
Results: Most participants (28 out of 42) lived sedentary lifestyles, and these individuals had poor fitness levels (only 67.3% of their predicted measure). The main barriers to physical activity included lack of energy, lack of time, and fear of falling. There were no differences in age, sex, disease type, disease duration, ataxia severity, fatigue level, and medication use between sedentary and active groups. Measures of VO_2max, maximal work, maximal heart rate, and anerobic threshold demonstrated statistically significant differences between groups whereas maximal respiratory rate and expired ventilation/carbon dioxide production were similar between groups. When adjusting for age, sex, functional mobility status, and disease duration, ataxia severity was inversely correlated with fitness level in the sedentary group. There was no relationship between ataxia severity and fitness level in the fourteen individuals who were physically active.
Conclusions: Lower fitness levels were associated with more ataxia symptoms in the sedentary group. This relationship was not seen in individuals who were more active. Given the poor health outcomes associated with low fitness, physical activity should be encouraged in this population.

Development of Two Regulatory-Grade Patient and Caregiver-Reported Outcome Measures for Friedreich's Ataxia Therapeutic Trials: The FA-HI & FACR-HI

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Charlotte Engebrecht [1], ***Ms. Jamison Seabury*** [2], ***Ms. Anika Varma*** [2], ***Mr. Spencer Rosero*** [3], ***Ms. Jennifer Weinstein*** [1], ***Ms. Christina Shupe*** [1], ***Ms. Charlotte Irwin*** [1], ***Ms. Preshetha Kanagaiah*** [1], ***Dr. Jane Larkindale*** [4], ***Ms. Susan Walther*** [5], ***Mrs. Ellen Wagner*** [1], ***Ms. Nuran Dilek*** [6], ***Mr. John Heatwole*** [7], ***Ms. Christine Zizzi*** [1], ***Prof. David Lynch*** [8], ***Ms. Courtney Park*** [8], ***Ms. Mackenzie Wells*** [8], ***Dr. Chad Heatwole*** [6]

1. *University of Rochester Center for Health + Technology,* ***2.*** *University of Rochester School of Medicine and Dentistry,* ***3.*** *University of Utah Spencer Fox Eccles School of Medicine,* ***4.*** *Pepgen,* ***5.*** *Friedreich's Ataxia Research Alliance,* ***6.*** *University of Rochester Department of Neurology,* ***7.*** *Cornell University,* ***8.*** *Children's Hospital of Philadelphia*

Background and Objective: There is a need for disease-specific, patient and caregiver-reported outcome measures in preparation for future Friedreich's ataxia (FA) therapeutic trials. The objective of this study was to develop and validate two outcome measures, the Friedreich's Ataxia-Health Index (FA-HI) and the Friedreich's Ataxia Caregiver Reported-Health Index (FACR-HI), capable of detecting small but clinically-relevant changes in response to therapeutic intervention over time.

Methods: We conducted qualitative interviews with adults with FA, minors with FA, and caregivers of individuals with FA to determine the most important and impactful symptoms of disease burden. Based upon participant responses, we designed a national cross-sectional survey to determine the prevalence and impact of each symptom item. The questions selected for both the FA-HI and FACR-HI were based on symptoms with the highest impact to the population, the potential to respond to therapeutic intervention, and generalizability. Beta testing was conducted to assess the usability and content validity of both instruments. Subsequent test-retest and known groups analysis were performed to optimize the instruments responsiveness and reliability.

Results: Thirty-nine patients and caregivers participated in the initial qualitative interviews, resulting in the identification of 2,527 direct symptom quotes. Two-hundred and two patients and caregivers of individuals with FA participated in the national cross-sectional study. Beta testing demonstrated that both the FA-HI and FACR-HI were easy to use, comprehensible, and relevant to the patient population. Thirty-eight patients and caregivers participated in test-retest analysis, demonstrating the instruments' high reliability. Known groups analysis determined that both instruments were able to distinguish between differing levels of disease burden.

Discussion and Conclusion: The FA-HI and FACR-HI both contain 18 subscales that represent the multifaceted areas of disease burden in FA, providing researchers with a regulatory-grade tool to detect therapeutic gain during clinical trials.

Funding: This study was funded by Friedreich's Ataxia Research Alliance.

Specialist Ataxia Centres (SACs): Exploring socio-demographic factors leading to continued and discontinued access in the United Kingdom, Italy, and Germany.

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Suzanne Booth [1], ***Dr. Julie Vallortigara*** [1], ***Dr. Julie Greenfield*** [2], ***Prof. Barry Hunt*** [2], ***Prof. Paola Giunti*** [1]

1. *University College London,* ***2.*** *Ataxia UK*

Background: Progressive ataxias are a diverse and rare group of neurodegenerative conditions characterised by unsteadiness, incoordination and slurred speech. Additional neurological features and pathology outside the central nervous system may also be present.[1] Optimal management of these complex conditions requires access to specialist diagnostics and treatment via Specialist Ataxia Centres (SACs). Greater understanding of factors impacting on SAC access is required to maximise utilisation.[2]

Aim: This study explored factors associated with referral to SACs or discontinued access.

Methods: Data was analysed from 550 people with ataxia responding to cross-sectional surveys distributed separately in three European countries at different time points.

Results: Respondents with a genetic diagnosis were observed to have lower representation in SACs in the UK than in Italy and Germany, with this contrast being most prominent with a diagnosis of Friedreich's ataxia. Respondents with Idiopathic Cerebellar Ataxia also had significantly lower proportion of referrals in the UK relative to Germany and Italy. Fewer comorbid conditions, being in employment, and shorter travel time to SACs were associated with SAC attendance or referral. Increasing mobility impairment was associated with discontinued SAC attendance. No significant association was observed between referral source, time since diagnosis or impact of ataxia on daily living and SAC attendance.

Conclusions: Statistically significant differences between SAC and non-SAC respondent cohorts were observed, as well as variations in SAC access between countries. Further opportunities to maximise SAC access and the scope for telehealth were identified.

Speech, swallowing and quality of life in children and adolescents with Ataxia Telangiectasia

Tuesday, 12th November - 18:10: (Minories) - Poster

Prof. Adam P. Vogel [1], ***Ms. Sarah Harriman*** [2], ***Mr. Dylan Edwards*** [1], ***Carine Esther Marc*** [1], ***Ms. Caitlyn Mares*** [1], ***Ms. Tahlia Smith*** [1], ***Dr. Matthew Lynch*** [3], ***Prof. Robert Ware*** [4], ***Prof. David Coman*** [5]

1. *The University of Melbourne,* ***2.*** *Wesley Research Institute,* ***3.*** *Queensland Health,* ***4.*** *Griffith University,* ***5.*** *The University of Queensland*

Objective: To characterise communication and mealtime deficits in A-T.
Methods: Twenty-eight people with genetically confirmed A-T were recruited from the National Ataxia Telangiectasia Clinic in Australia. Ages ranged from 3 to 35 years. A protocol recording speech (set tasks and free speech), assessing oral motor function, and measuring dysphagia via a standardised clinical bedside of swallowing and quality of life were administered. Speech data were analysed acoustically (objective) and perceptually (subjective) to describe performance.
Results: Speech in A-T is characterised by ataxic and hyperkinetic features, including reduced rate, imprecision and nasality issues. Dysarthria severity increased with disease duration. Disease severity was associated with decreased speech naturalness. Phonological processes were prevalent amongst participants, with gliding observed in 75% of participants. Swallowing in A-T was impaired compared to healthy controls.
Conclusion: Age at onset and disease duration appear to influence speech and swallowing severity. Outcomes from this work have informed clinical endpoints in A-T trials.

Development and Evaluation of a Novel Reproductive Educational Tool for Patients with Spinocerebellar Ataxia

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Megan Howard [1], ***Dr. George Wilmot*** [2], ***Dr. Cynthia Jorgensen*** [3], ***Ms. Suzanne Cahn*** [1], ***Dr. Nadia Ali*** [1], ***Ms. Jennifer Pagno*** [4], ***Ms. Ami Rosen*** [1]

1. *Emory University, Department of Human Genetics,* ***2.*** *Emory University, Department of Neurology,* ***3.*** *Emory University, Rollins School of Public Health,* ***4.*** *AdventHealth, Neuroscience Institute*

Methods

Reproductive decision-making can be distressing for patients with hereditary conditions. Research demonstrates that patients with spinocerebellar ataxia (SCA) lack knowledge about available reproductive options due to the scarce number of patient education materials and limited provider knowledge on the topic. To address this gap, we developed an educational handout covering inheritance, pregnancy considerations, and information on various reproductive options for patients with SCA. We evaluated the impact of our tool using a pre- and post-intervention survey measuring objective and self-reported knowledge. We recruited participants through email communication via the National Ataxia Foundation (NAF) and the Coordination of Rare Disease at Sanford (CoRDS), posting our study on the NAF website, and patients at Emory Movement Disorders Clinic.

Results

Forty-six participants completed the pre-intervention study, and twenty-nine participants completed the study in full. After utilizing the educational handout participants' objective knowledge score increased from 54.92% to 72.17% ($p < .001$). The largest increases in objective knowledge were on the topics of in vitro fertilization (IVF) and prenatal testing (amniocentesis and chorionic villus sampling). After utilizing the educational handout, participants' self-reported knowledge score increased from 59.4% to 76.9% ($p < .001$). The largest increases in self-reported knowledge were on the topics of intrauterine insemination (IUI) and surrogacy.

Discussion

Objective and self-reported reproductive knowledge increased after use of our educational tool suggesting the handout is useful for reproductive education and can empower patients in the SCA community. However, one-time exposure to the handout did not provide complete knowledge as measured by the post-intervention scores. This underscores the need for ongoing patient education.

Conclusion

Our novel educational tool effectively increased patient knowledge about reproductive options and may be a useful resource in the counseling and care for patients with spinocerebellar ataxia.

This research study received support from the Georgia Association of Genetic Counselors.

COVID□19 Impacts the Mental Health and Speech Function in Spinocerebellar Ataxia Type 2

Tuesday, 12th November - 18:10: (Minories) - Poster

Mr. Yasmany González-Garcés [1], ***Prof. Luis Velázquez-Pérez*** [2], ***Prof. Roberto Rodriguez Labrada*** [3], ***Prof. Yaimee Vázquez Mojena*** [3], ***Dr. Jacqueline Medrano Montero*** [1], ***Ms. Nalia Canales Ochoa*** [1], ***Dr. Yennis Domínguez Barrios*** [4], ***Prof. Ulf Ziemann*** [5], ***Prof. Georg Auburger*** [6]

1. *Center for Research and Rehabilitation of Hereditary Ataxias.,* ***2.*** *Cuban Academy of Sciences,* ***3.*** *Cuban Center of Neurosciences,* ***4.*** *Cuban Institute of Neurology and Neurosurgery,* ***5.*** *University of Tübingen,* ***6.*** *Faculty of Medicine, Goethe University*

Background: Limited evidence suggests that the SARS-CoV-2 infection can accelerate the progression of neurodegenerative diseases, but this has been not verifed in the spinocerebellar ataxias. The objective of this study is to assess the impact of COVID-19 on the mental health and motor features of spinocerebellar ataxia type 2.

Methods: A follow-up study was carried out in 170 Cuban subjects with spinocerebellar ataxia type 2 subjects and 87 community controls between 2020 and 2021. All subjects underwent a structured questionnaire to assess the risks of exposure to COVID-19, the confirmation of COVID-19 diagnosis, and the Hospital Anxiety and Depression Scale. Moreover, 36 subjects underwent the Scale for the Assessment and Rating of Ataxia.

Results: The risk of exposure to SARS-CoV-2 and the frequency of COVID-19 were similar between the ataxia cohort and the community controls. Within the ataxia group, signifcantly increased of Hospital Anxiety and Depression Scale scores existed at the 2nd visit in both groups, but this increase was more evident for the infected group regarding the depression score. Moreover, a signifcant within-group increase of Scale for the Assessment and Rating of Ataxia score was observed in the infected group but not the non-infected group, which was mainly mediated by the signifcant increase of the speech item score in the infected group. Similar results were observed within the subgroup of preclinical carriers. Our study identifed no selective vulnerability nor protection to COVID-19 in spinocerebellar ataxia type 2, but once infected, the patients experienced a deterioration of mental health and speech function, even at preclinical disease stage.

Conclusions: These fndings set rationales for telehealth approaches that minimize the detrimental efect of COVID-19 on spinocerebellar ataxia type 2 progression and identify individuals as clinical model to elucidate the link between SARS-CoV-2 infection and neurodegeneration.

Poster session I - Emerging and existing therapeutics - clinical research

“Deep breath, hold, huff and cough!” Redesigning the active cycle of breathing technique for use with children with ataxia

Tuesday, 12th November - 18:10: (Minories) - Poster

Prof. Lisa Bunn [1], ***Ms. Munira Khan*** [1], ***Ms. Lisa Bunn*** [2]

1. *University of Plymouth,* ***2.*** *Various*

Objectives: Respiratory muscle function is vital when children develop respiratory tract infections. Inspiratory muscles act to mobilise air behind infected secretions. Expiratory muscles shift secretions out of the lungs with huffs and coughs. Children with ataxia who are less mobile, with restricted breathing mechanics can struggle to effectively clear secretions. Children with ataxia telangiectasia are physiologically more at risk of respiratory infections. This project aimed to create an exercise intervention to optimise respiratory function.

Methods: A qualitative research approach involving focus groups, with two children with ataxia telangiectasia and eight parents, acted to instigate the design of a respiratory training programme.

Results: Focus group findings highlighted concerns that children struggled to engage with respiratory exercises whilst remaining at risk of respiratory infections. Further involvement and engagement with parents of children with ataxia telangiectasia and physiotherapists highlighted the opportunity for ‘Active Cycle of Breathing Technique’ (ACBT) training to target inspiratory and expiratory muscle training. A traditional effective approach used with adults, barriers exist in teaching children this technique; namely how to take and hold deep breaths, perform end inspiratory sniffs, huffs at varying velocities and avoiding a cough until the end of a cycle.

Discussion: A short animated movie was coproduced with Cosmic Kids Yoga, guiding children through the phases of ACBT: Owls visualise relaxed diaphragmatic breathing, frogs perform deep breathing, rabbits demonstrate end inspiratory sniffs. A low cost and widely commercially available respiratory training device licensed for use by children and adults, provided resistance during deep breaths (frogs) and high velocity huffing (dragon fire-breathing) respectively.

Conclusion: The movie is widely available and free to download https://www.youtube.com/watch?v=ujLkszl0xT4 as an adjunct to an existing widely adopted practice. Commercially available, THE BREATHER®, is available for optional use with the movie. A feasibility trial of the intervention with children with ataxia telangiectasia is ongoing.

Prediction of tissue frataxin levels with long term administration of nomlabofusp in adults with Friedreich's ataxia using modeling and simulations

Tuesday, 12th November - 18:10: (Minories) - Poster

Prof. Flavia De Toni [1], Ms. Noreen Scherer [1], Prof. Emily Schapiro [2], Dr. Magdy Shenouda [3], Mr. Mohamed Hamdani [1], Prof. Gopi Shankar [1], Dr. Russell Clayton [1]

1. *Larimar Therapeutics, Inc.,* ***2.*** *A2-Ai,* ***3.*** *Clinilabs, Inc.*

Methods: Plasma nomlabofusp concentrations and skin frataxin concentrations from adults with Friedreich's ataxia (FRDA) before and after short term (< 30 days) subcutaneous administration of 25, 50, 75, and 100 mg nomlabofusp in Phase 1 and 2 clinical studies were used to construct an exposure-response model. Simulations of a population of virtual FRDA patients receiving daily doses of 25, 50, 75 or 100 mg of nomlabofusp were performed (n=100, 100 trials) and skin frataxin profiles over time at each dose were predicted.

Results: The simulations predicted that skin frataxin concentrations should reach steady state at approximately 28 days after daily administrations across all doses. Daily administration of 25, 50, 75, and 100 mg nomlabofusp was predicted to attain a median maximum skin frataxin concentration of 6.22, 9.06, 11.9, and 14.7 pcg/mcg, respectively.

Discussion: In a separate study, the mean skin frataxin concentration in healthy controls with 2 normal frataxin alleles was 16.35 pcg/mcg. Prior published studies indicate that the mean frataxin concentration in asymptomatic heterozygous carriers is 50% of healthy controls. In relation to these findings, 59% of patients with FRDA receiving daily 50 mg nomlabofusp are predicted to achieve skin frataxin concentrations that are equal or above 50% of the concentrations found in healthy controls.

Conclusion: Modeling and simulation using data from short term studies of nomlabofusp administration can be used to predict a potential long term therapeutic dose. Daily administration of 50 mg nomlabofusp is predicted to result in skin frataxin concentrations that are > 50% of concentrations found in healthy controls.

The MOXIe trial of omaveloxolone in Friedreich ataxia: exploring the transient nature of treatment-emergent adverse events

Tuesday, 12th November - 18:10: (Minories) - Poster

Prof. David Lynch [1], Dr. Sylvia Boesch [2], Prof. Martin Delatycki [3], Dr. Paola Giunti [4], Dr. Angie Goldsberry [5], Dr. Chad Hoyle [6], Dr. Katherine Mathews [7], Dr. Seemi Khan [5], Dr. Colin Meyer [8], Dr. Masako Murai [5], Dr. Wolfgang Nachbauer [9], Dr. Susan Perlman [10], Prof. S. H. Subramony [11], Dr. Theresa Zesiewicz [12]

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Background and objectives: MOXIe Part 2 (NCT02255435) was a multicenter, double-blind, placebo-controlled trial to evaluate the efficacy and safety of omaveloxolone 150 mg once daily for 48 weeks in patients with Friedreich ataxia (FA) aged 16-40. A total of 51 patients were randomized to receive omaveloxolone and 52 patients to placebo. We sought to examine the frequency, severity, onset, and duration of treatment-emergent adverse events (TEAEs) in MOXIe Part 2 in patients with FA treated with omaveloxolone compared with those treated with placebo.
Methods: All randomized participants who had received ≥1 dose of the randomized drug (N=103) were included in the safety analyses. The frequency and severity of TEAEs during MOXIe Part 2 were recorded during screening and at Weeks 2, 4, 12, 18, 24, 36, and 48 with a follow-up safety visit 4 weeks after the final dose at Week 52. Median time to onset and duration of TEAEs were examined in participants reporting ≥1 TEAE within 52 weeks of follow-up.
Results: TEAEs occurred in 100% of participants treated in both arms. Most events were mild or moderate in severity. Omaveloxolone-treated participants showed a higher incidence of TEAEs compared to those placebo-treated within the first 12 weeks of treatment;events less frequently reported in omaveloxolone-treated participants after 12 weeks included elevated liver enzymes (alanine aminotransferase and aspartate aminotransferase), headache, nausea, abdominal pain, fatigue, diarrhea, influenza, vomiting, muscle spasms, and decreased appetite. Most TEAEs had a total median duration of approximately one month or less.
Discussion and conclusion: The more commonly reported TEAEs in MOXIe Part 2 experienced by participants who received omaveloxolone compared with placebo were generally reported within the first 12 weeks of treatment and decreased thereafter. These findings provide evidence to guide patient treatment expectations and support the importance of dosing compliance among physicians and patients.
Funding: Biogen

PROTEIN REPLACEMENT THERAPY FOR FRIEDREICH´S ATAXIA: EXPLORING THE POTENTIAL OF BBB-SHUTTLE PEPTIDES

Tuesday, 12th November - 18:10: (Minories) - Poster

Mr. Alejandro Benítez-Troncoso [1], ***Ms. Arabela Sanz-Alcázar*** [2], ***Ms. Marta Portillo-Carrasquer*** [2], ***Dr. Joaquim Ros*** [2], ***Dr. Elisa Cabiscol*** [2], ***Dr. Macarena Sanchez*** [1]

1. *Instituto de Parasitología y Biomedicina López-Neyra, IPBLN-CSIC, Granada, Spain.,* ***2.*** *Departament de Ciències Mèdiques Bàsiques, Universitat de Lleida, Institut de Recerca Biomèdica de Lleida (IRBLleida), Lleida, Spain.*

Methods

Four frataxin (FXN) derivatives modified with Blood-Brain Barrier (BBB)-shuttle peptides were expressed and physicochemically characterized by SDS-PAGE, mass spectrometry and biophysical techniques. Their activity has been evaluated in primary cultures of dorsal root ganglia neurons (nDRG) and patient-derived fibroblasts. FXN deficiency was induced in nDRG by short hairpin RNA interfering sequences. Activity assays assessed internalization capacity and subcellular localization. FXN, nuclear factor erythroid 2-related factor 2 (Nrf2) and mitochondrial complex I and II levels were examined by Western Blot (WB). A human BBB cell-based model, consisting of human endothelial cells co-cultured with bovine pericytes, was used to evaluate the constructs' ability to cross the BBB. The constructs' potential to recover mitochondrial activity was measured using oxidative stress rescue assays and SeaHorse analysis.

Results

The new FXN derivative P1-MLScs-FXN_{81-210} showed differential internalization into nDRG with mitochondrial colocalization regarding MLScs-FXN_{81-210}, TAT-MLScs-FXN_{81-210} and P2-MLScs-FXN_{81-210}. Constructions with BBB-shuttle peptides had higher rate of internalization in patient-derived fibroblast. P1-MLScs-FXN_{81-210} increased Nrf2 and FXN levels in nDRG, as shown by WB. P1-FXN_{81-210} has demonstrated its ability to recover mitochondrial activity in FXN-deficient nDRGs. Evaluation of the transport across BBB cellular models show higher transport of the constructions with BBB-shuttle peptide.

Discussion

We developed and characterized new FXN derivatives modified with BBB-shuttle peptides, demonstrating promising therapeutic potential for the treatment of FA. P1-MLScs-FXN_{81-210} internalize into nDRGs and glial cells with mitochondrial colocalization. Additionally, P1-MLScs-FXN_{81-210} shows a greater effect on affected cells by differentially increasing FXN and Nrf2 levels. The improved transport rates of BBB-shuttle peptide-modified derivatives suggest their potential for effective CNS delivery. P1-MLScs-FXN_{81-210} is able to restore mitochondrial capacity in FXN-deficient nDRGs more effectively than the other constructs.

Conclusion

P1-MLScs-FXN_{81-210} holds significant potential as a novel treatment for FA, given its ability to internalize into nDRG and its superior efficacy in restoring FXN levels and mitochondrial activity.

Pulmonary Dysfunction in Friedreich's Ataxia (FRDA)

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Theresa Zesiewicz [1], ***Dr. Tuan Vu*** [1], ***Dr. Aarti Patel*** [1], ***Dr. Karel Calero*** [1]

1. *University of South Florida*

Objective:

FRDA is a neurodegenerative disease that causes cardiac dysfunction. There is little data on pulmonary involvement in FRDA, and the role it may play in morbidity and mortality. We sought to characterize pulmonary function in FRDA and identify disease variables that may contribute to dysfunction.

Methods:

We evaluated 25 FRDA patients who presented to our multidisciplinary clinic from October 2023 through February 2024. Patients received pulmonary function tests (PFTs) and mFARS (Modified Friedreich's Ataxia Rating Scale) scores. We also reviewed their demographic data, echocardiograms, and electrocardiograms (EKGs).

Results:

The mean age in this cohort of FRDA patients was 31 years (±14), mean age at diagnosis was 19 years (±13), mean total repeats was 1616 (±407), and 54% of patients were non-ambulatory. The mean sitting Forced Vital Capacity (FVC) was 2.83L (±0.93), and the mean percent predicted sitting FVC was 64 % (±19). The mean sitting Forced Expiratory Volume (FEV1) was 1.96L (±0.72), and the mean sitting percent predicted FEV was 55% (±20%). The mean FEV1/FVC was 70% (±13). The average mFARS score in this cohort was 59 (±15), and the mean ejection fraction was 60% (±6%). FVC sitting % predicted and FEV1 sitting % predicted correlated with age at diagnosis ($p = 0.017$ and $p = 0.002$ respectively) and correlated positively with non-ambulation ($p = 0.035$ for both FVC% and FEV1%). FVC sitting % predicted and FEV1 sitting % predicted inversely correlated with mFARS ($p = 0.02$, $p = 0.023$ respectively) and the total number of repeats ($p = 0.029$ and $p = 0.032$).

Conclusions:

In this pilot study, lung function appeared to be more compromised in FRDA patients than would be considered normal for the subjects' age. Pulmonary function correlated significantly with disease severity, age of symptom onset, the total number of repeats, and non-ambulation.

Thirty four months of repositioned drug combination in Friedreich Ataxia: report of two cases.

Tuesday, 12th November - 18:10: (Minories) - Poster

Prof. Pamela Moceri [1], ***Prof. Pierre-Marie Roger*** [2], ***Prof. Emmanuel Flamand-Roze*** [3]

1. *Centre Hospitalier Universitaire de Nice,* ***2.*** *Polyclinique Les Fleurs,* ***3.*** *Assistance Publique des Hôpitaux de Paris*

Background

Friedreich ataxia (FDRA) is related to a low production of frataxin and neurological and cardiological impairments. Drug repositioning led to pilot studies using nicotinamide, thiamine or etravirine, associated with some benefits in patients.

Objectives

To report the safety and the efficacy of a drug combination with a focus on cardiological impairment.

Methods

FDRA were established in the first child at twelve years old in July 2021, and in her sister at ten in December 2021. Genetic analyses showed a large GAA expansion on both alleles in *FXN* gene (> 800). Parents who are physicians prescribed the drug combination. Dosages of nicotinamide was 2.5 g twice a day for the first one (weight 49 kg) and 3.0 g once a day for the second one (weight 33 kg). As the treatment was well tolerated over four months, etravirine was introduced, 150 mg twice-a-day for the first children and 150 mg once a day for the second one. Finally, thiamine 250 mg three times a week was added in July 2022 for both children.

Results

Investigations showed a regression of electrocardiogram abnormalities for the youngest and an improvement of echocardiographic parameters such as global longitudinal strain: from -16 to -25% for the first child, and from -20.6 to -24% for the second one, with stable septal wall thickness and left ventricular mass index. The safety of the combination was assessed in both sisters with successive blood analyses until May 2024 showing no abnormality. In neurological terms, disappearance of falls was observed, associated with stable results of SARA scale in 2022 and 2023.

Discussion

Nicotinamide + etravirine + thiamine over 34 months appeared to curb cardiac disease without adverse effect.

Conclusion

As single drug trials showed only limited improvement in FDRA, we advocate for the evaluation of drug combination in well-designed studies

MR-based electric field modelling in SCA3: towards a better understanding of interindividual variability in cerebellar tDCS effects

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Roderick Maas [1], ***Dr. Jennifer Faber*** [2], ***Dr. Gulin Oz*** [3], ***Prof. Paola Giunti*** [4], ***Dr. Heike Jacobi*** [5], ***Prof. Kathrin Reetz*** [6], ***Prof. Dagmar Timmann*** [7], ***Prof. Thomas Klockgether*** [8], ***Prof. Bart van de Warrenburg*** [9], ***Prof. Dennis Schutter*** [10]

1. *Department of Neurology, Donders Institute for Brain, Cognition, and Behaviour, Radboud University Medical Center, Nijmegen, the Netherlands,* ***2.*** *Center for Neurology, Department of Parkinson, Sleep and Movement Disorders, University Hospital Bonn,* ***3.*** *University of Minnesota,* ***4.*** *University College London,* ***5.*** *Department of Neurology, University of Heidelberg,* ***6.*** *Department of Neurology, University of Aachen,* ***7.*** *Department of Neurology, University of Essen,* ***8.*** *German Center for Neurodegnerative Diseases (DZNE),* ***9.*** *Radboud university medical center,* ***10.*** *Department of Experimental Psychology, Helmholtz Institute, Utrecht University, Utrecht*

Background: Cerebellar transcranial direct current stimulation (tDCS) has recently emerged as a potential therapeutic strategy in degenerative ataxias. Clinical trials have shown inconsistent results, however, and patient characteristics predicting treatment response remain largely unknown.
Objectives: Using advanced computer simulations, we aimed to identify anatomical and clinical factors that underlie the interindividual variability in cerebellar tDCS-induced electric field strength in SCA3. Secondary objectives were to compare field strengths in different cerebellar regions between SCA3 mutation carriers and healthy controls, and to examine the influence of the position of the reference electrode on field characteristics.
Methods: High-resolution brain MRI scans from 37 healthy volunteers and 68 SCA3 mutation carriers spanning the disease spectrum were transformed into individualized tetrahedral volume meshes. Electric field simulations of 2 mA midline cerebellar tDCS were performed using buccinator, frontopolar, and lower neck reference electrodes. Eight regions of interest (ROIs) with a 5 mm radius were defined in the anterior and posterior vermis and cerebellar hemispheres. Multivariable linear regression models were used to identify anatomical and clinical factors predicting field strength in these ROIs.
Results: Field strengths were generally lower in SCA3 mutation carriers than in healthy controls, which was most pronounced in the anterior lobe and with cephalic reference electrodes. In both groups, the frontopolar montage consistently induced the highest field strength and lowest focality, while the lower neck montage invariably caused the lowest field strength and highest focality. Multivariable regression analyses showed that skin-cerebellum distance, SARA score, and “occipital angle” were independently associated with electric field strength.
Conclusion: Skin-cerebellum distance, morphometric posterior fossa features, ataxia severity, and electrode montage predict cerebellar tDCS-induced electric field strength in SCA3. These results will inform the design of future cerebellar tDCS trials in degenerative ataxias.
Funding: National Ataxia Foundation.

Growth and Bone Mineral Density (BMD) in Children with Ataxia-Telangiectasia (A-T) Treated with Intra-Erythrocyte Dexamethasone (EryDex) for 24 months

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Dirk Thye [1], ***Prof. Biljana Horn*** [1], ***Mrs. Maureen Roden*** [1]

1. *Quince Therapeutics*

Objective:
Children with classical A-T have abnormal bone mineral density and growth. Their z-scores fall -2.5 standard deviations (SD) in height (Ht), and -1.5 SD in body mass index (BMI) by 16 years old, compared with unaffected children. Corticosteroids further adversely affect growth and bone health in children. We describe growth and BMD in children with A-T treated for ≥24 months with EryDex.
Methods:
Patients receiving ≥24 months of EryDex, for treatment of neurological symptoms in the ATTeST and Open Label Extension (OLE) studies, are included in this report. Ht, weight (Wt) and BMI measurements were converted to z-scores using LSM calculations from CDC tables for subjects <20 years of age. Baseline and mean change from baseline (mCFB) after 24 months of therapy z-scores for Ht, Wt, BMI, and BMD ± SD are presented.
Results:
Patients' characteristics (N=66) included: mean age (10.3±5.4 years); age <10 years (61%),10-19 (34%), ≥20 (5%); male (56%), female (44%); genetic confirmation of A-T (97%); and mean alpha-fetoprotein level (266±223 ng/mL).
Baseline mean±SD Ht z-score for 63 patients <20 years old, with available normative CDC data, was -0.73±1.38; 24-month mCFB -0.06±0.49. Baseline Wt z-score was -0.97±1.85; 24-month mCFB -0.02±0.71. Baseline BMI z-score was 0.75±1.70; 24-month mCFB 0.03±0.87.
Baseline BMD z-score for 37 patients with available DEXA scans was -0.5±1.2 and mCFB at 24 months was -0.41±0.95.
Discussion
Over 24-month treatment with EryDex, minimal changes in z-scores for Ht, Wt and BMI (<0.1 SD) were observed, compared to baseline. These findings are encouraging, because the natural history study of classical A-T describes annual decline in Ht and BMI z-scores of -0.16 SD and -0.11 SD.[1] In untreated A-T patients, BMD z-scores are also known to decline over time.
Conclusions
24 months of EryDex treatment did not adversely affect growth of children with A-T.

Improvement in Upright Stability Subscale of mFARS with Vatiquinone Treatment in MOVE-FA: a Phase 3, Double-blind, Placebo-controlled Trial

Tuesday, 12th November - 18:10: (Minories) - Poster

Prof. David Lynch [1], Dr. Antoine Duquette [2], Prof. Marcondes França [3], Dr. Susan Perlman [4], Prof. Alexandra Durr [5], Dr. Enrico Bertini [6], Dr. Alejandra Darling [7], Dr. Katherine Mathews [8], Prof. Ludger Schöls [9], Dr. Anne Fournier [10], Prof. Martin Delatycki [11], Prof. S. H. Subramony [12], Prof. Richard Roxburgh [13], Dr. Olivia Zhang [14], Dr. Mark Rance [14], Dr. Christian Rummey [15], Dr. Alana Salvucci [16], Dr. Bert Yao [16], Dr. Lee Golden [14], Dr. Jonathan J. Cherry [14], Dr. Theresa Zesiewicz [17]

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Background and Objective: FA is characterized by progressive neurological damage and loss of ambulation. The Upright Stability Subscale (USS) is the component of the modified FA Rating Scale (mFARS) that assesses functions related to balance, stance, and mobility. Analysis of natural history data shows that disease progression in ambulatory pediatric and adolescent FA patients is primarily driven by declines in the functions assessed in the USS. Here we describe the effect of vatiquinone on the USS in MOVE-FA (NCT04577352), the 72-week placebo-controlled phase 3 study of patients with Friedreich Ataxia (FA).

Methods: The study enrolled 143 subjects with FA aged ≥7 years, mFARS score of 20–70, and the ability to ambulate ≥10 feet in 1 minute. The primary endpoint was placebo corrected change from baseline in mFARS at 72-weeks. The Intent-to-Treat (ITT) population had a mean age of 18.7 and the primary analysis population modified ITT (mITT) included 123 subjects 7-21 years (mean 14.6). USS was collected as part of mFARS, the primary endpoint.

Results: In the placebo population, of the four subscales of mFARS, USS is the only one to demonstrate progression from baseline to week 72, consistent with natural history data. Significant benefit was recorded in USS (-1.26 [p=0.021]) in the mITT population. Vatiquinone treatment also delayed the loss of functional milestones represented by individual items within the USS, specifically items E2B (feet apart eyes closed) and E3A (feet together eyes open). Comparison of rate of disease progression in USS predicted a 42% reduction in disease progression per year in the vatiquinone-treated group.

Discussion and Conclusions: Vatiquinone treatment resulted in clinically meaningful and statistically significant treatment effects on the USS, a sensitive and predictive endpoint for risk of loss of ambulation, prevention of which is a key goal for therapy in ambulatory FA patients.

Long-term safety profile of omaveloxolone in patients with Friedreich ataxia: design of an observational, global, post-marketing registry-based study

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Susan Perlman [1], ***Prof. David Lynch*** [2], ***Dr. Caterina Mariotti*** [3], ***Dr. Katherine Mathews*** [4], ***Ms. Caitlin Monette*** [5], ***Dr. Myriam Rai*** [5], ***Dr. Pamela Dobay*** [6], ***Dr. Susan Eaton*** [7], ***Dr. Jeremy Furtado*** [7], ***Dr. Andres Greco*** [7], ***Dr. Shobhana Natarajan*** [7], ***Dr. Changyu Shen*** [7], ***Dr. Lucy Wu*** [7], ***Ms. Jennifer Farmer*** [5]

1. *University of California, Los Angeles,* ***2.*** *The Children's Hospital of Philadelphia,* ***3.*** *Fondazione IRCCS Istituto Neurologico Carlo Besta,* ***4.*** *University of Iowa,* ***5.*** *Friedreich's Ataxia Research Alliance,* ***6.*** *Biogen International,* ***7.*** *Biogen, Inc.*

Background and objectives: Omaveloxolone is the first therapy approved in the US and EU for the treatment of patients with Friedreich ataxia (FA) aged ≥16 years. The adverse event (AE) profile in the registrational MOXIe study of omaveloxolone included transient increases in aminotransferase levels (ALT, AST, GGT) and slight elevations in BNP. Consequently, monitoring for potential safety concerns of omaveloxolone treatment, including drug-induced liver injury (DILI) and congestive heart failure (CHF), is a regulatory requirement. This post-marketing registry will assess the long-term safety of omaveloxolone in patients with FA under real-world conditions; document and characterize all serious AEs (SAEs) and DILI and CHF AEs; and capture timing and reasons for treatment discontinuation, interruption, and overdose.

Methods: An observational, multinational, registry-based cohort study (SKYCLARYS PASS) of patients with FA who are treated with omaveloxolone will be conducted within the FA-Global Clinical Consortium (FA-GCC) platform for patient enrollment. Study initiation will commence in countries that have marketing authorization for omaveloxolone. Eligible patients are aged ≥16 years with a documented diagnosis of FA, omaveloxolone naive, and enrolled in the FA-GCC UNIFIED Natural History Study (UNIFAI). Study participation is planned for 60 months. Information on all SAEs and AEs related to DILI and CHF and incidence for all events that occurred from study baseline until 5 years or 60 days following treatment discontinuation, whichever comes first, will be collected. The schedule of assessments is based on anticipated visits as per the prescribing label and the UNIFAI study. Exploratory objectives to be assessed in omaveloxolone-treated patients include health-related quality of life over time using the FA-Health Index and Modified Fatigue Impact Scale, as well as healthcare resource utilization.

Discussion: The SKYCLARYS PASS study will help inform real-world, long-term safety and patient experience on omaveloxolone, which can guide healthcare professionals in their clinical decisions.

Funding: Biogen

Cerebellar transcranial direct current stimulation in the Cerebellar Cognitive Affective Syndrome: a randomised controlled trial

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Stacha Reumers [1], ***Dr. Roderick Maas*** [1], ***Prof. Dennis Schutter*** [2], ***Mr. Steven Teerenstra*** [3], ***Prof. Roy Kessels*** [4], ***Prof. Frank-Erik de Leeuw*** [1], ***Prof. Bart van de Warrenburg*** [1]

1. *Radboud University Medical Centre, Donders Institute for Brain, Cognition, and Behaviour, Department of Neurology, Nijmegen,* ***2.*** *Department of Experimental Psychology, Helmholtz Institute, Utrecht University, Utrecht,* ***3.*** *Radboud University Medical Centre, IQ Health science department, Biostatistics Section, Nijmegen,* ***4.*** *Donders Institute for Brain, Cognition and Behaviour, Radboud University, Nijmegen*

Objectives The Cerebellar Cognitive Affective Syndrome (CCAS) encompasses cognitive and affective symptoms in patients with cerebellar disorders, for which no proven treatment is yet available. Our primary objective was to study the effect of anodal cerebellar transcranial direct current stimulation (tDCS) on cognitive performance. Secondary effects on ataxia severity, mood, and quality of life were explored.

Methods A randomized, double-blind, and sham-controlled trial was performed. Thirty-five patients with CCAS were included to receive ten sessions of twenty minutes real (n=18) or sham (n=17) tDCS. Cognitive performance was assessed using the computerized Test of Attentional Performance (TAP) as primary endpoint. Secondary outcomes were ataxia severity, mood, and quality of life. These were evaluated one, three, six, and twelve months post-intervention.

Results Our sample exhibited good tolerance to tDCS and no serious adverse events related to the intervention did arise. No significant tDCS effect was found for cognitive performance, but a small-sized improvement on the TAP was observed in the sham group one month post-treatment (estimate = -0.248, 95% CI = -0.49, -0.01). A positive effect of real tDCS was observed for ataxia severity one month post-treatment, indicated by an improvement on the Scale for the Assessment and Rating of Ataxia (estimate = -0.985, 95% CI = -1.94, -0.03). Prolonged disease duration significantly interacted with more improvement of ataxia severity.

Conclusion tDCS did not prove efficacious for cognitive impairment as part of CCAS, but a moderate effect of tDCS was found for ataxia severity, contributing to the accumulating evidence of tDCS as a therapeutic neuromodulation tool in cerebellar disorders.

Targeted epigenetic approaches rescue Frataxin expression and downstream pathways in Friedreich's ataxia diseased cell types

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Elena Melacini [1], ***Ms. Margherita Rossi*** [1], ***Dr. Agostina Di Pizio*** [1], ***Mr. Giosuè Moscato*** [1], ***Dr. Sharon Muggeo*** [1], ***Dr. Mirko Luoni*** [1], ***Dr. Serena Giannelli*** [1], ***Dr. Vania Broccoli*** [1]

1. *IRCCS San Raffaele Hospital*

Friedreich's ataxia (FA) is a neurodegenerative disorder caused by the Frataxin (FXN) gene silencing, due to the expansion of a GAA triplet in its first intron. Thus, we thought that CRISPR activatory technology (CRISPRa) might represent an innovative and valuable approach to restore FXN expression. To promote the reactivation of *FXN* expression in diseased cell types from FA patients, we employed a lentiviral vector expressing dCas9 fused with the strong VP160 transcriptional activator domain. Multiple sgRNAs targeting *FXN* promoter were tested in FA patient fibroblasts and iPSCs-derived neural progenitor cells (NPCs) and neurons. We demonstrated that dCas9-VP160, when assembled to a specific sgRNA, triggers a rescue of FXN deficiency in all the examined diseased cell types. Indeed, CRISPRa system is able to achieve 2-5 fold increase of FXN mRNA and protein in FA fibroblasts, NPCs and neurons, completely or partially restoring physiological FXN levels, according to the severity of the disease. RNA-seq analysis on patient-derived NPCs treated with the CRISPRa system showed that, upon FXN up-regulation, genes involved in pathways such as Iron Sulphur Clusters assembly and ROS detoxification were increased as well, suggesting the regularization of the biochemical pathways controlled by Frataxin, leading to normalized cellular homeostasis. Our results indicate that the CRISPRa system should be considered a promising tool for *FXN* reactivation with a translational relevance. In this regard, we are testing zinc finger proteins (ZFPs) fused with the VP64 or VP160 activatory domains and targeting *FXN* promoter. Being of human origin and having a small size that allows them to fit into an AAV vector, ZFPs represent an optimal system with straightforward clinical potential. Overall, our data identify innovative targeted epigenetic approaches to overcome the silencing of the endogenous *FXN* gene, paving the way for *in vivo* validation and advancing innovative therapies for patients.
Fundings: GoFar association

Preliminary Safety Data and Outcomes for an open-label single center, single participant study of an experimental antisense oligonucleotide treatment for ATN1 gene mutation

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Jennifer Bain [1], ***Dr. Claire Miller*** [2], ***Ms. Anya Revah-Politi*** [1], ***Dr. Orrin Devinksy*** [2], ***Dr. Julie Douville*** [3], ***Dr. Laurence Mignon*** [3], ***Dr. Sheng-Han Kuo*** [1]

1. *Columbia University Medical Center,* ***2.*** *New York University,* ***3.*** *n-Lorem*

Methods: A seventeen-year-old male with a heterozygous pathogenic trinucleotide expansion (67 CAG repeats) in the atrophin 1 (ATN1) gene was treated with an experimental antisense oligonucleotide (ASO). The mutant ATN1 protein causes dentatorubral-pallidoluysian atrophy (DRPLA), a rare, fatal neurodegenerative disorder. Our patient regressed socially and cognitively at 2 years of age and later manifested with seizures at 9 years. At pre-intervention baseline, he has refractory epilepsy, intellectual disability, anxiety, attention deficit hyperactivity disorder and sleep apnea, with ataxia and dysarthria and requires full support for daily activities.

A library of antisense oligonucleotides (ASOs) were designed to bind and degrade ATN1 mRNA by recruiting RNase H1. The lead ASO, nL-ATN1-002, was administered once in two non-GLP studies, and once monthly for 4 doses in a 13-week GLP study. The FDA approved a research IND to administer the compound to our patient. The local IRB approved the study and Informed Consent was obtained. nL-ATN1-002 was administered intrathecally at 0 (20 mg), 4 (40 mg) and 8 (50 mg) weeks. Primary endpoint is change in mobility (using the Scale for Assessment and Rating of Ataxia, wrist/ankle accelerometers, and gait video assessments). Secondary endpoints are change in seizure frequency, caregiver-reported Quality of Life, safety and tolerability. Exploratory endpoints include cognition, language and communication changes (using the NIH toolbox and the Observer Reported Communication Ability scale).

Results: The ASO administration has been well tolerated with adverse events (not drug dependent) limited to nausea, vomiting and headache after the initial dose. Tremor, coordination and speech show signs of improvement. Seizure frequency and severity are stable.

Discussion: The nL-ATN1-002 ASO was well-tolerated at doses up to 50 mg with improvements in tremor, gait and communication.

Conclusion: Initial treatment for ATN1-related DRPLA with a non-allele specific ASO was safe and well-tolerated with sign of improvement in some endpoints.

Double-blind L-arginine trial for SCA6

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Tomohiko Ishihara [1], ***Dr. Kensuke Ikenaka*** [2], ***Dr. Yuji Takahashi*** [3], ***Prof. Takanori Yokota*** [4], ***Prof. Kinya Ishikawa*** [4], ***Dr. Makito Hirano*** [5], ***Prof. Yoshitaka Nagai*** [5], ***Prof. Osamu Onodera*** [1]

1. *Niigata University,* ***2.*** *Osaka University,* ***3.*** *National Center of Neurology and Psychiatry,* ***4.*** *Tokyo Medical and Dental University,* ***5.*** *Kindai University*

Background and Objective: The molecular pathogenesis of polyglutamine diseases, including SCA6, involves mutant proteins with expanded polyglutamine tracts that undergo conformational changes. Recently, our group found that the chemical chaperone L-arginine inhibited the conformational change of polyglutamine proteins and improved the symptoms of the polyglutamine mouse model (Minakawa EN, Brain, 2020). We conducted a phase II study to evaluate the safety and tolerability of L-arginine and its therapeutic effect in polyglutamine diseases.
Methods: A multicenter, randomized, double-blind, placebo-controlled Phase II study (AJA030-002) enrolled 40 patients with SCA6 diagnosed by genetic testing. Their SARA “walking” score was at least 1 point, and their SARA “total” score was at least 10 points. Oral administration of either 0.38 g/kg/day of L-arginine or a placebo was initiated in the study subjects for a period of 48 weeks. The primary endpoint was the change in the total SARA score from baseline to 48 weeks. Tolerability and safety were evaluated at 48 and 52 weeks.
Results: Forty patients received the study drug and 37 completed the study (18 in the L-arginine group and 19 in the placebo group). SARA total score after 48 weeks of treatment, the difference between the L-arginine and placebo groups was -1.52 (95% CI: -3.10 to 0.06, P=0.0582) (ANCOVA). The two serious adverse events in the L-arginine group included one case of pneumonia (death) and one case of liver dysfunction (recovery).
Discussion and Conclusion The primary endpoint showed a trend difference between the two groups. Although serious adverse events were reported in the L-arginine group, it is crucial to prioritize safety precautions. Based on these results, we conclude that a Phase III trial of L-arginine for SCA6 can be planned with a 48-week observation period and a primary endpoint of “change in total SARA score” as in the present study.

Study Design of a Phase 1b Study Evaluating ASP2016 Gene Replacement Therapy in Adults with Friedreich Ataxia Associated Cardiomyopathy

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Bryan McGill [1], ***Ms. Jill Woloszynek*** [2], ***Ms. Diane Barnes-Glait*** [1], ***Joshua Chang*** [2], ***Dr. Wei Chen*** [1], ***Dr. Amit Desai*** [1], ***Ms. Caitlin Leppert*** [1], ***Ms. Frances Nordgren*** [1], ***Dr. Yusuke Yamaguchi*** [1], ***Dr. Paul Esteso*** [3], ***Dr. Mary Kay Koenig*** [4], ***Dr. Susan Perlman*** [5]

1. *Astellas Pharma Global Development, Inc.,* ***2.*** *Astellas Gene Therapies,* ***3.*** *Boston Children's Hospital,* ***4.*** *The University of Texas McGovern Medical School- Houston,* ***5.*** *University of California at Los Angeles*

Background and Objectives: Friedreich ataxia (FA) is caused by mutations in the frataxin gene and is often characterised by hypertrophic cardiomyopathy (FA-CM). ASP2016 is an investigational AAV8-mediated frataxin gene replacement therapy being developed to treat FA-CM. This is a phase 1b clinical trial to evaluate the safety, tolerability, and preliminary efficacy of ASP2016 in adults with FA-CM.

Methods: Adults aged 18–40 years with genetically confirmed diagnosis of FA, disease onset before 25 years of age, body mass index 17.0–30.0 kg/m^2, and left ventricular ejection fraction ≥40%–<55% will be included. Participants with elevated anti-AAV8 total antibodies and fibrosis >15% myocardial mass will be excluded. Use of omaveloxolone is permitted with some restrictions. Following a 60-day screening and baseline period, participants will receive a single intravenous ascending low or high dose of ASP2016 along with oral prednisolone for 8 weeks, followed by an 8-week taper.

Results: Outpatient observation visits will begin on Day 5 and continue until Month 60. The primary objective is to evaluate the safety and tolerability of ASP2016. Primary endpoints include frequency and severity of treatment-emergent adverse events, including serious adverse events, and change from baseline through Week 52 in clinical laboratory tests, electrocardiograms, and physical examinations. Secondary objectives (endpoints) are to evaluate ASP2016 cardiac transduction (vector copy number), frataxin in cardiac tissue (protein level), preliminary effects of ASP2016 on cardiac function (cardiopulmonary exercise testing and echocardiography) at Weeks 24 and 52, and anti-drug antibodies (anti-AAV8 and anti-frataxin) at Week 52. Exploratory endpoints include change from baseline in cardiac magnetic resonance imaging at Weeks 24 and 52.

Discussion and Conclusion: This study will evaluate the safety and tolerability of systemic AAV8-mediated frataxin gene replacement therapy with ASP2016 in adults with FA-CM and may provide preliminary evidence on ASP2016 efficacy.

Ataxia ACT: A Novel Trial Readiness Service for the Ataxia Community

Tuesday, 12th November - 18:10: (Minories) - Poster

Mrs. Ira Eberhardt [1], ***Dr. Birte Zurek*** [1], ***Dr. Holm Graessner*** [1], ***Prof. Bart van de Warrenburg*** [2], ***Dr. Susan Perlman*** [3]

1. *Ataxia Global Initiative, University Hospital Tübingen,* ***2.*** *Radboud university medical center,* ***3.*** *University of California at Los Angeles*

Developing a successful therapy programme is a complex task that involves numerous challenges. These challenges include significant obstacles surrounding the delivery of pre-clinical studies, selecting trial outcome measures, patient input and perspective in research planning, consideration of further disease specific symptoms, or appropriate statistical and clinical trial design. Indeed, 90% of clinical drug development fail (Sun et al. 2022).

Recognising that this journey is even more challenging for rare diseases with limited numbers of patients who can be enrolled into clinical trials, the Ataxia Global Initiative (AGI) is launching the *Ataxia Advisory Committee for Therapeutics* (Ataxia ACT). This international expert committee helps to de-risk drug development by providing multidisciplinary, independent and objective guidance on the preclinical and development pathway of potential therapies.

The ultimate goal of the Ataxia ACT review is to enable researchers from industry and academia to position their candidate compound along a realistic and well-informed plan to clinical trials, and eventual registration. The reviews and subsequent recommendations are focused on generating meaningful and rigorous data that can enable clear go/no-go decisions and facilitate longer term funding or partnering opportunities. The review process thereby acts to comment on viability, de-risking the process of proceeding on a development programme.

Following the launch of the Ataxia ACT and the first review meeting in the fall 2024, we here present the ACT review process, explain how researchers from academia and industry can benefit from Ataxia ACT and show how this helps to improve the community's chances of successfully bringing new Ataxia drugs to approval and ultimately to market.

ClearSpeechTogether versus standard SLT: a pilot randomised controlled trial of speech intervention for people with MSA-C

Tuesday, 12th November - 18:10: (Minories) - Poster

Prof. Anja Lowit [1], ***Dr. Kaiyue Xing*** [1], ***Prof. Marios Hadjivasssiliou*** [2]

1. *Strathclyde University,* ***2.*** *Sheffield Ataxia Centre*

Background Speech problems are an early and disabling feature of Multiple System Atrophy (MSA). These speech difficulties can have significant impact on people's quality of life, frequently leading to social withdrawal and poor mental health.

Despite the early and relatively severe manifestation of speech deficits, there has been a considerable lack of clinical intervention trials in this population. We recently developed a novel speech intervention (ClearSpeechTogether) that provides intensive, mixed individual / group intervention and is distinct to other approaches in its use of peer supported group session. Pilot data indicates that it can result in speech improvements and improved confidence and communication participation. The aim of this project was to establish feasibility and acceptability of ClearSpeechTogether in patients with MSA-C, and to pilot an RCT comparing it to standard SLT provision.

Methods: 24 patients with clinically probable MSA-C and dysarthria were recruited. Both treatment approaches lasted 6 weeks, those randomised to standard SLT received 1 hour of individual therapy a week, those randomised to ClearSpeechTogether received four individual therapy sessions over two weeks, followed by daily group interaction for the following four weeks which maximises patient benefit and minimises therapist workload. All assessment and intervention sessions took place online via videoconferencing software. Two baseline assessments were taken in the week preceding treatment, followed by one immediate post-therapy assessment and one 8 week follow-up assessment.

Results: We will report findings on feasibility, acceptability and efficacy (speech performance and patient reported outcome measures). In addition, we collected qualitative interview data to capture patient reported benefits or problems.

Discussion: Demonstrating acceptability, feasibility and potential communication benefits in this pilot will set the basis for a larger registered randomised controlled trial, and area of significant need for rare conditions and motor speech disorders in general.

Patients with Friedreich ataxia treated with omaveloxolone in France: an early access program

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Claire ewenczyk [1], ***Ms. Federica Ruscitti*** [2], ***Ms. Elodie Petit*** [1], ***Dr. Valeria Gioiosa*** [3], ***Dr. Marie-Lorraine Monin*** [4], ***Dr. Chloe Angelini*** [3], ***Prof. Cyril Goizet*** [3], ***Dr. Antoine Pegat*** [5], ***Dr. Philippe Petiot*** [5], ***Dr. Sabine Souci*** [5], ***Prof. Caroline Froment Tilikete*** [5], ***Dr. Françoise Bouhour*** [5], ***Dr. Andra Ezaru*** [6], ***Prof. Mathieu Anheim*** [7], ***Prof. Christine Tranchant*** [7], ***Dr. Thomas Wirth*** [7], ***Dr. Cecilia Marelli*** [8], ***Dr. Elisa De La Cruz*** [8], ***Dr. Alix Durand*** [8], ***Dr. Fabienne Ory*** [9], ***Dr. Pascal Cintas*** [9], ***Dr. Frederique Fluchere*** [10], ***Dr. Stephan Grimaldi*** [10], ***Prof. Jean-Philippe Azulay*** [10], ***Dr. Virginie Pichon*** [11], ***Prof. Christophe Verny*** [11], ***Dr. Clarisse Scherer Gagou*** [11], ***Prof. Benoit Funalot*** [12], ***Dr. Elsa Besse-Pinot*** [13], ***Prof. Catherine Sarret*** [13], ***Dr. Lucie Guyant*** [14], ***Prof. Gael Nicolas*** [14], ***Dr. Catherine Vanhulle*** [15], ***Dr. Antoine Bonnevalle*** [15], ***Dr. Anne-Laure Kaminsky*** [16], ***Dr. Mathilde Renaud*** [17], ***Dr. Elisabeth Sarrazin*** [18], ***Dr. Armelle Magot*** [19], ***Dr. Amelie Dos Santos*** [20], ***Dr. Sacha Weber*** [21], ***Mr. Matthieu Benoiton*** [22], ***Dr. Solange Roumengous*** [22], ***Ms. Beatrice Baciotti*** [23], ***Prof. Shahram Attarian*** [10], ***Prof. Alexandra Durr*** [1]

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Background and objectives

Omaveloxolone is the first treatment approved in United States and European Union for the treatment of patients aged ≥16 years with Friedreich ataxia (FA). Since January 2024, omaveloxolone has been available in France through an early access program. For the first time, the French National Registry of Rare Diseases is responsible for collecting data from this program. Our aim is to describe demographic data of patients with FA and baseline data of treated patients over the first 3 months of inclusion in the program.

Methods

Data were collected on April 11, 2024, including age, sex, age at disease onset, and age at diagnosis. For patients treated with omaveloxolone, we also collected age at inclusion in the program and ataxia motor severity using the Scale for the Assessment and Rating of Ataxia (SARA; scored 0-40, with higher scores indicating worse severity).

Results

Currently, 825 patients with FA are included in the database (mean [SD] age: 39.6 [1.2] years; range: 5.5-90 years), with a mean [SD] age at onset of 17.3 [1.2] years and a mean [SD] age at diagnosis of 22.9 [1.4] years. Over the first 3 months of data collection, 117 patients started receiving omaveloxolone in 24 centers throughout France, representing 14.1% of the total cohort. The number of patients in the database initiating omaveloxolone increased linearly over time ($R^2 = 0.96$). Omaveloxolone-treated participants had a mean (SD) age of 35.7 (14.2) years (range: 17-71 years) and a mean (SD) SARA score of 21 (8.4; range: 2-40); 33% received idebenone.

Discussion and Conclusion

The inclusion rate for omaveloxolone-treated patients followed a steady linear pace, owing to the strong involvement of many centers in France. Initially, patients receiving omaveloxolone were selected from those who were still ambulatory. However, the inclusions will be expanded in the coming months.

Calcitriol treatment is safe and increases frataxin levels in Friedreich Ataxia patients.

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Berta Alemany-Perna [1], ***Dr. Jordi Tamarit*** [2], ***Dr. Elisa Cabiscol*** [2], ***Dr. Fabien Delaspre*** [2], ***Mr. Albert Miguela*** [3], ***Ms. Juana Huertas-Pons*** [3], ***Dr. Ana Quiroga-Varela*** [3], ***Mr. Miguel Merchan Ruiz*** [3], ***Mr. Daniel López Domínguez*** [1], ***Dr. Lluís Ramió i Torrentà*** [4], ***Mr. David Genís*** [3], ***Dr. Joaquim Ros*** [2]

1. *Ataxia Unit, Neurology Service, ICS/IAS, Hospital Josep Trueta/Hospital Santa Caterina, Girona/Salt. Department of Medical Sciences, University of Girona,* ***2.*** *Departament de Ciències Mèdiques Bàsiques, Universitat de Lleida, Institut de Recerca Biomèdica de Lleida (IRBLleida), Lleida,* ***3.*** *Neurodegeneration and Neuroinflammacion Group, Institut d'Investigació Biomèdica de Girona (IDIBGI), Girona,* ***4.*** *Neurology Service, ICS/IAS, Hospital Josep Trueta/Hospital Santa Caterina, Girona/Salt. Neurodegeneration and Neuroinflammacion Group (IDIBGI). Department of Medical Sciences, University of Girona*

Background: Calcitriol, the active form of vitamin D (also known as 1,25-dihydroxycholecalciferol), improves the phenotype and increases frataxin levels in cell models of Friedreich Ataxia (FRDA).
Methods: Based on these results, we started a pilot clinical trial in which a dose of 0.25mcg/24h was administered to 15 FRDA patients for a year. Evaluations of neurological function changes (SARA scale, 9-HPT, 8-MWT, PATA test) and quality of life (Barthel Scale and SF-36 quality of life questionnaire) were performed. Frataxin amounts were measured in isolated platelets obtained from these FRDA patients, from heterozygous FRDA carriers (relatives of the FA patients) and from non-heterozygous sex and age matched controls.
Results: Although the patients did not experience any observable neurological improvement, there was a statistically significant increase in frataxin levels from initial values, 5,5 pg/mg, to 7,0 pg/mg after 12 months. Differences in frataxin levels referred to total protein amounts were observed amongst sex and age matched controls (18.1 pg/µg), relative controls (10.1 pg/µg), and FRDA patients (5.7 pg/µg). The treatment was well tolerated by most patients, and only some of them experienced minor adverse effects at the beginning of the trial.
Conclusion: Calcitriol dosage used (0.25mcg/24h) is safe for FRDA patients and it increases frataxin levels. We cannot rule out that higher doses administered longer could yield neurological benefits.
Funding sources: This research was funded by FEDAES (Federación de Ataxias de España) and by Ministerio de Ciencia e Innovación (Spain), grant number PID2020-118296RB-I00.

Current state of alternative and augmentative communication approaches in ataxia

Tuesday, 12th November - 18:10: (Minories) - Poster

Prof. Adam P. Vogel [1], ***Dr. Caroline Spencer*** [2], ***Ms. Katie Burke*** [3], ***Dr. Peter gibilisco*** [1], ***Mr. Scott Blackman*** [1], ***Dr. Jenny Vojtech*** [4], ***Dr. Thayabaran Kathiresan*** [1]

1. *The University of Melbourne,* ***2.*** *Indiana University,* ***3.*** *Tallaght University Hospital,* ***4.*** *Boston University*

The progression of multisystem neurodegenerative diseases such as ataxia significantly impacts speech and communication, necessitating adaptive clinical care strategies. With the deterioration of speech, Alternative and Augmentative Communication (AAC) can play an ever increasing role in daily life for individuals with ataxia. This review describes the spectrum of AAC resources available, ranging from unaided gestures and sign language to high-tech solutions like speech-generating devices (SGDs) and eye-tracking technology. Despite the availability of various AAC tools, their efficacy is often compromised by the physical limitations inherent in ataxia, including upper limb ataxia and visual disturbances. Traditional speech-to-text algorithms and eye gaze technology face challenges in accuracy and efficiency due to the atypical speech and movement patterns associated with the disease.

In addressing these challenges, maintaining existing speech abilities through rehabilitation is prioritized, complemented by advances in digital therapeutics to provide home-based treatments. Simultaneously, projects incorporating AI driven solutions aim to enhance the intelligibility of dysarthric speech through improved speech-to-text accuracy.

This review discusses the complex needs assessment for AAC in ataxia, emphasizing the dynamic nature of the disease and the importance of regular reassessment to tailor communication strategies to the changing abilities of the individual. It also highlights the necessity of multidisciplinary involvement for effective AAC assessment and intervention. The future of AAC looks promising with developments in brain-computer interfaces and the potential of voice banking, although their application in ataxia requires further exploration.

A Pilot Phase 2 Randomized Trial to Evaluate the Safety and Potential Efficacy of Etravirine in Friedreich Ataxia Patients

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Andrea Martinuzzi [1], ***Dr. Gabriella Paparella*** [1], ***Dr. Cristina Straga'*** [1], ***Dr. Nicola Pesenti*** [1], ***Dr. Valentina Dal Molin*** [1], ***Dr. Vasco Merotto*** [1], ***Dr. Alessandra Rufini*** [2], ***Prof. Roberto Testi*** [2]

1. *IRCCS Medea,* ***2.*** *Roma 2 University*

Introduction: A drug repositioning effort supported the possible use of the anti-HIV drug etravirine as a treatment for Friedreich Ataxia (FRDA). Etravirine increases frataxin protein and corrects the biochemical defects in cells derived from FRDA patients. Because of these findings, and since etravirine displays a favorable safety profile, we conducted this pilot open-label phase 2 clinical trial assessing the safety and potential efficacy of etravirine in FRDA patients.

Methods: Thirty-five patients were stratified into 3 severity groups and randomized to etravirine 200 mg/day or 400 mg/day. They were treated for 4 months. Efficacy endpoints were represented by changes in peak oxygen uptake and workload as measured by incremental exercise test, SARA score, cardiac measures, measures of QoL and disability. Data were collected 4 months before the start of the treatment (T-4), at the start (T0), at the end (T4) and 4 months after the termination of the treatment (T+4).

Results: Etravirine was well tolerated. Etravirine completely stopped the progression of the SARA score during the 4-months treatment period, compared to the 4 months pre and post treatment. It increased peak workload, while the improvement of peak oxygen uptake was not statistically significant. No changes in the cardiac measures were observed. Health and QoL measures showed a worsening at the suspension of the drug.

Discussion: In this open trial etravirine significantly improved neurological function and was generally safe and reasonably tolerated. This suggests that etravirine represents a potential therapeutic agent in FRDA deserving testing in a randomized placebo controlled clinical trial.

Poster session I - Cell and animal models

An integrated iPSC-derievd cortical, cerebellar and choroid plexus organoid model of SCA1

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Lisa Julian [1], ***Ms. Negin Imani Farahani*** [1], ***Mr. Alireza Naderi*** [1]

1. *Simon Fraser University*

Introduction: Spinocerebellar ataxias (SCAs) are a group of ~50 genetic disorders, leading to brain tissue degeneration in the cerebellum and often the cerebral cortex. Symptoms include loss of balance, coordination, speech and swallowing difficulties, muscle stiffness and spasms. Current treatments can alleviate symptoms but are not curative. We are using induced pluripotent stem cells (iPSCs) to study cells and tissues of the human brain carrying patient-specific mutations for SCA1.

Objectives: Our goal is to establish a human stem cell-derived model that reflects the multi-region involvement in SCA1 (beginning with cerebral and cerebellar tissues) and that enables identification of biomarkers in CSF fluid, ultimately to develop effective treatments for ataxia patients.

Methods & Results: Here, we generate cerebral, cerebellar and choroid plexus (ChP) organoids from SCA1 patient-derived iPSCs. Using microscopy and biochemical approaches to analyze these tissues, focusing on developmental signatures, morphological and metabolic markers of degeneration to uncover SCA1 disease onset and progression. As cerebral spinal fluid (CSF) biomarker analysis is a clinical priority, we also generated ChP organoids from SCA1 iPSCs to perform CSF biomarker analysis. Preliminary analyses of cerebellar organoids indicates abnormal development of Purkinje cells in SCA1 iPSC-derived tissues, noting altered cell morphology and metabolic function. We also find that ChP tissue and the CSF-like fluid they produce develop normally from SCA1 patients, and are now applying biochemical strategies including mass spectrometry to identify metabolic signatures and protein expression profiles reflective of the SCA1 disease state.

Conclusions: Ultimately this data will provide a link to translational clinical applications and an alternative to the current invasive methods of extracting human CSF. Our long-term goals are to use this approach to elucidate commonalities and differences among SCA subtypes and to understand mechanisms of disease and identify a reliable biomarker that can point to better therapies for SCA patients.

Generating Spinocerebellar Ataxia 27B Model Systems for the Ataxia Community

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Guinevere Spurdens [1], ***Dr. Adriana Rebelo*** [1], ***Christopher Yanick*** [2], ***Dr. David Pellerin*** [1], ***Dr. Matt C. Danzi*** [1], ***Dr. Bernard C. Brais*** [3], ***Dr. Mario Saporta*** [4], ***Dr. Stephan Zuchner*** [1]

1. *Dr. John T. Macdonald Foundation Department of Human Genetics and John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL, USA,* ***2.*** *Department of Neuroscience, University of Miami Miller School of Medicine,* ***3.*** *Department of Neurology and Neurosurgery, Montreal Neurological Hospital and Institute, McGill University, Montreal, QC, Canada,* ***4.*** *Department of Neurology and Neuroscience, University of Miami Miller School of Medicine*

Background: Spinocerebellar Ataxia 27B (SCA27B) is a prevalent adult-onset ataxia caused by an intronic GAA expansion in *FGF14*. Investigating repeat-expansion-disorders has proven to be challenging, largely due to inadequate modeling and cloning complexities. To surmount these obstacles, our study develops SCA27B induced-pluripotent-stem-cell (iPSC) lines and a tailored methodology to assemble a linearized vector replicating the SCA27B locus that will be used to generate a rat model.

Methods: iPSCs were derived from fibroblast lines with *FGF14* alleles of pathogenic length using the Cytotune 2.0 Sendai Virus Reprogramming kit. For cloning vector creation, we employed the pJAZZ vector, capable of cloning up to 2kb of repetitive sequences. PCR amplification of rat homology arms, 508 GAA human repeats from SCA27B patient DNA, and flanking regions enabled SCA27B locus cloning, with precise ligation ensuring seamless integration.

Results: In our study, iPSC lines were generated from SCA27B patient fibroblasts carrying *FGF14* alleles of 9/508, 16/383, and 292/304 repeat units. Morphologically distinct iPSC colonies were isolated and characterized using pluripotency markers and karyotyping. Given the limitations of a narrow gene expression profile, these lines serve as valuable cell models offering potential for diverse neuronal differentiation studies involving GABAergic neurons. We are currently utilizing these models to test expression changes compared to controls and the presence of R-loops as a pathomechanism. These results will be presented at the meeting. Additionally, we successfully assembled a linearized-vector encoding the humanized SCA27B repeat-expansion and flanking regions, confirmed by gel-electrophoresis and linear-amplicon sequencing.

Discussion and Conclusion: With the development of iPSC lines from SCA27B patient fibroblasts, encompassing varying repeat sizes, alongside a linearized vector containing the humanized SCA27B repeat-expansion and its adjacent regions, we have established the groundwork for robust modeling systems. They offer valuable tools for exploring pathophysiology and mechanisms of disease, as well as testing targeted therapeutics.

Ataxia Global Initiative: Model Systems & Preclinical Trials Working Group

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Ronald Buijsen [1]***, Dr. Magda Santana*** [2]***, Mrs. Ira Eberhardt*** [3]***, Dr. Jeannette Hübener-Schmid*** [4]***, Dr. Thorsten Schmidt*** [4]

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Ronald A.M. Buijsen[1,2], Magda Santan[1,3], Ira Eberhardt[1] , Jeannette Hübener-Schmid[1,4], Thorsten Schmidt[1,4]

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The Ataxia Global Initiative (AGI) is a worldwide research platform that has the goal to facilitate the development of therapies for dominantly and recessively inherited ataxias. For the development of therapeutic approaches, pre-clinical analyses in model systems are indispensable.

In this line, the “Model systems & preclinical trials Working group” formed within AGI. The main goal of the “Model systems & preclinical trials” working group is to standardize preclinical research that is essential to establish trial-readiness for ataxias. The topics covered by the group are animal and cellular models as well as pre-clinical biomarkers including the associated methodology (for Animal models e.g. breeding, genotyping, behavioral phenotyping, histology, and biochemical analyses; for Cellular models including patients derived (IPSC-based) neuronal cultures e.g. culturing, differentiation, and functional assays). In line with the aim of AGI to promote the sharing of data and biomaterials, one of the aims of the working group is to create an inventory of existing models and protocols. The group further aims to develop standardized protocols and guidelines based on the collected information. Moreover, appropriate training materials and tools are about to be developed to conduct training sessions for ataxia researchers, patients and the public.

The aim of the working group is to cover pre-clinical research for all forms of inherited ataxias. Everybody working in the field of Model systems & preclinical trials in ataxias is invited to participate.

A novel conditional mouse model with severe depletion of frataxin in the nervous system

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Shreya Kadam [1], ***Dr. Jill Napierala*** [2], ***Dr. Marek Napierala*** [2], ***Dr. Helene Puccio*** [3], ***Dr. Jordi Magrane*** [1]

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Strategies to lower frataxin (FXN) protein levels in mouse models to mimic the consequences of reduced FXN in Friedreich's ataxia (FRDA) individuals range from full *FXN* gene knock-out, to inducible *FXN* gene silencing, to genetically introduce either GAA repeats or FXN point mutations. While all these *in vivo* mouse models replicate certain aspects of the disease, particularly at a molecular and tissue level, their neurobehavioral phenotypes either appear rapidly and are too severe or they have a slow, often mild, progression. This has precluded the effective and rapid testing of pre-clinical therapeutic interventions.

Here we present a novel approach to achieve low levels of mature FXN in neurons and glia of both peripheral and central nervous systems *in vivo*. We generated a nervous system-specific FXN depletion mouse model using the Cre/*loxP*-recombination system and the FXN G127V missense mutation in the mouse *FXN* gene. Severe depletion of FXN levels was achieved throughout the nervous system from neonatal stages to adulthood. G127V/KO mice were viable and displayed progressive neurobehavioral deficits, which affected coordination and balance, as demonstrated by wire-hanging, notched bar, accelerating rotating rod, and footprint tests. Based on these abnormalities, we defined an early disease onset of 10.5 weeks (74 days); we are currently assessing tissue and biochemical disease features at both pre-symptomatic (8.5 weeks, 60 days) and late stages of the disease (26.5 weeks, 186 days).

This novel mouse model of FXN depletion in the nervous system reproduces important pathophysiological and biochemical features of FRDA over a distinctive timescale, while presenting residual FXN levels, and provides the research community with a fast progressive neuropathology *in vivo* FRDA model, but still with a long enough window for therapeutic interventions.

Funding sources: The Friedreich's Ataxia Research Alliance (FARA), Muscular Dystrophy Association (MDA), and National Ataxia Foundation (NAF) to J.M.

Novel SCA3 knock-in mouse model harboring an hyperexpanded CAG repeat shows early-onset motor deficits and CAG repeat instability

Tuesday, 12th November - 18:10: (Minories) - Poster

Mx. Sara Trumza [1], Mx. Anna J. Barget [1], Mx. Michelle Hoang [1], Mx. Aidan Snell [1], Mx. Kristen H. Schuster [1], Mx. Luke L. Nourie [2], Mx. Louisa Liu [1], Dr. Hayley McLoughlin [3], Dr. Henry Paulson [3], Dr. Vanessa C. Wheeler [4], Dr. Maria do Carmo Costa [5]

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Background: Machado-Joseph disease (MJD), or spinocerebellar ataxia type 3, is caused by an expanded polyglutamine-encoding CAG repeat in exon 10 of the human *ATXN3* gene. The mouse *Atxn3* gene is structurally highly similar to its human counterpart, also harboring a short CAA$(CAG)_5$ repeat in its exon 10. SCA3 knock-in (KIN) mouse models, harboring an expanded CAG repeat in the mouse *Atxn3* gene, are amongst the best models to investigate at physiological levels the pathogenic mechanisms and behavioral alterations triggered by the SCA3 mutation.

Methods: Through subsequent mouse crossings of a SCA3 knock-in (KIN) mouse model, previously reported by us, harboring a humanized expanded repeat [$(CAG)_2$(CAAAAG)$(CAG)_{82}$] with 86 triplets in the mouse *Atxn3* gene (SCA3-KIN-Q86), we generated a novel SCA3 KIN mouse with an hyperexpanded CAG tract ranging from 120 to 350 repeats. Here, we assessed the intergenerational and somatic instability of the expanded CAG repeat, neuropathology, motor function and survival of the novel hyperexpanded SCA3 KIN mice.

Results and Discussion: In contrast with the original SCA3-KIN-Q86, a group of heterozygous SCA3 KIN mice harboring in average 175 CAGs show progressive deficits of locomotor and exploratory activities and early death starting around the 60 weeks of age. In addition, these mice show progressive decreased body weight, aggregation of ATXN3 protein and somatic CAG repeat instability in brain areas vulnerable to SCA3 disease in humans. Similarly to humans, SCA3-KIN mice also show an inverse correlation of the CAG repeat size with severity of symptoms with SCA3-KIN-Q230 mice (harboring in average 230 repeats) showing severer deficits of motor and exploratory functions that start at 10 weeks of age than SCA3-KIN-Q175 mice.

Conclusion: The novel hyperexpanded SCA3 KIN mouse model replicates the human SCA3 disease at the genetic, pathological and behavioral levels and, therefore, is a great model to evaluate *in vivo* SCA3-associated toxicity.

Glial cell models of Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS)

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Fernanda Murtinheira [1], ***Ms. Ana Sofia Boasinha*** [2], ***Mr. João Belo*** [3], ***Ms. Luana Macedo*** [2], ***Mr. Tiago Robalo*** [3], ***Dr. Vukosava Torres*** [2], ***Dr. Francisco Pinto*** [2], ***Dr. Adelaide Fernandes*** [4], ***Ms. Patrícia Nascimento*** [2], ***Prof. Mário Rodrigues*** [3], ***Dr. Federico Herrera*** [5]

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Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a neurodegenerative disorder characterized by progressive cerebellar ataxia, spasticity, motor sensory neuropathy and axonal demyelination. It is caused by mutations in the SACS gene, leading to the loss of function of sacsin protein. This protein is involved in chaperone functions, regulation of neuronal intermediate filaments, mitochondrial dynamics, and cytoskeletal regulation. ARSACS has been associated with Purkinje cell dysfunction, but the impact of sacsin deficiency on glial cells has not been investigated. Our research explores how sacsin deficiency impacts glial cells. We found that primary astrocytes, C6 rat glioma cells, N9 mouse microglia, and HMC3 human microglial cells, among other non-neuronal cells, express the sacsin protein. Using CRISPR/Cas9 to create sacsin-deficient C6 and HMC3 glial cell models, we observed disruptions in the distribution of cellular components such as intermediate filaments and membrane organelles and impaired cellular responses to stress and signalling pathways. Proteomic analysis showed changes in protein expression affecting cellular mechanical properties. Atomic Force Microscopy corroborated these results, showing reduced cell body height and increased stiffness.

Our study suggests that sacsin plays a crucial role in glial cells, highlighting the importance of astrocytes and microglia in ARSACS. It also suggests that sacsin-deficient glial cell models could be valuable for understanding ARSACS and related conditions.

We acknowledge the BioISI/FCUL Microscopy Facility, a node of the Portuguese Platform of BioImaging (PPBI-POCI-01-0145-FEDER-022122). FH was supported by a grant from the ARSACS Foundation (Canada). FH and MR were supported by centre grants UIDB/04046/2020 and UID/MULTI/04046/2020 (to BioISI) funded by FEDER funds through COMPETE2020-Programa Operacional Competitividade e Internacionalização (POCI) and national funds through Fundação para a Ciência e Tecnologia (Ref. PTDC/FIS-MAC/2741/2021). FM was supported by PhD fellowships from FCT (Ref. SFRH/BD/133220/2017). This study was supported by the European Union (TWIN2PIPSA, GA101079147).

Dysregulation of the CACNA1A transcription factor protein, a1ACT, may play a critical role in CACNA1A-spectrum disorders

Tuesday, 12th November - 18:10: (Minories) - Poster

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Mutations in the *CACNA1A* gene that lead to a spectrum of neurological disorders may impact the behavior and function of the two co-expressed *CACNA1A* proteins, the a1A, P/Q calcium channel subunit and the transcription factor, a1ACT. a1ACT is essential for survival and cerebellar development. a1ACT translocation to the nucleus is regulated in part by cellular calcium levels. We used transgenic expression of a1ACT to explore its role in *CACNA1A* mutation phenotypes. The R1300Q mutation, seen in patients with ataxia, migraine, and epileptic encephalopathy has abnormally enhanced P/Q channel activity. a1ACT targeted to Purkinje cells of R1300Q mice improves motor coordination, activity levels, and gait, in a dose-dependent manner, as assessed by Rotarod, open field, and DigiGait, respectively. Similarly, the mouse *Cacna1a leaner* mutation, Tg^{la}, produces a truncated channel protein, a reduction in calcium currents, absence of a1ACT, and is lethal in the homozygous state. Mice homozygous for the Tg^{la} *leaner* mutation were unaffected by the presence of one copy of the a1ACT transgene but had improved survival and motor function in the presence of two copies of a1ACT. These findings, demonstrating that expression of a1ACT in Purkinje cells can partially rescue *CACNA1A* mutant mice, suggest that dysregulation of a1ACT signaling may contribute to *CACNA1A* mutation phenotypes. Our studies establish a model to understand the role of a1ACT in the pathogenesis of *CACNA1A* mutations and suggests a route for a potential therapeutic strategy.

Evaluation of FMRpolyG sufficiency to elicit FXTAS phenotypes in vivo

Tuesday, 12th November - 18:10: (Minories) - Poster

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Objective: Fragile X-associated tremor/ataxia syndrome (FXTAS) is a neurodegenerative disorder characterized by ataxia, action tremors, and dementia that stems from a trinucleotide CGG repeat expansion in the 5' UTR of FMR1. CGG repeats are thought to drive neurodegeneration through either CGG RNA mediated sequestration of RNA binding proteins or through repeat-associated non-AUG (RAN) translation of toxic homopolymeric peptides, the most abundant of which is a polyglycine protein (FMRpolyG). This project aims to elucidate the contributions of these two molecular mechanisms to CGG repeat elicited toxicity *in vivo*.

Methods: We generated adeno-associated virus (AAVs) based expression models that separately express FMRpolyG or CGG repeat RNA separately or in combination through intracerebroventricular (ICV) injection in neonate mice, who underwent behavioral, pathological, and molecular/transcriptomic analysis.

Results: Expression of CGG-repeat RNAs that support RAN translation exhibit robust P62+ and ubiquitin positive inclusions that correlate with impaired motor behavioral deficits, widespread astrogliosis, and Purkinje cell loss at 6 months of age - recapitulating many key pathological features observed in FXTAS patients. However, AAVs that make either FMRpolyG in the absence of the CGG-repeat or that express the CGG repeat in a fashion that markedly diminishes FMRpolyG production do not elicit robust disease pathology or behavioral abnormalities**.**

Conclusion/Discussion: We describe a new series of AAV based mouse models of FXTAS pathogenesis that recapitulate key pathological and behavioral findings observed in the human condition. Only RAN translation competent CGG repeats recapitulate these disease relevant features, supporting a synergistic interaction between RNA and protein based molecular mechanisms in disease pathogenicity.

Engineering of patient-derived isogenic neuronal models by CRISPR/Cas9 to unravel the impact of ATXN3 knockout in Spinocerebellar Ataxia Type 3

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Ana Rita Fernandes [1], ***Ms. Diana Adão*** [2], ***Dr. Sara Lopes*** [3], ***Mr. Frederico Pena*** [1], ***Dr. Pasqualino De Luca*** [3], ***Mr. Daniel Henriques*** [4], ***Dr. Liliana Mondonça*** [5], ***Dr. Magda Santana*** [6], ***Dr. Pedro Perdigão*** [6], ***Prof. Luís Pereira de Almeida*** [7]

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Spinocerebellar Ataxia type 3(SCA3) is a fatal neurodegenerative disorder characterized by an aberrant expansion of the CAG trinucleotide in the *ATXN3* gene. The encoded mutant protein is prone to aggregation leading to the formation of toxic species that compromise neuronal viability. Therapeutic strategies targeting the *ATXN3* gene are promising candidates for therapy, however long-term consequences of *ATXN3* abrogation remain elusive due to its involvement in numerous cell mechanisms.

Here, we used CRISPR/Cas9 genome engineering to generate patient-derived pluripotent stem cells(iPSC) isogenic lines that were then differentiated into neuronal cultures to evaluate the effect of *ATXN3* knockout on neuronal development.

A SCA3 iPSC line was edited with a Cas9 endonuclease directed to an early exon of *ATXN3* to disrupt the coding sequence and promote gene silencing. *ATXN3* disruption was confirmed by Sanger sequencing. Following single-cell clone screening, we obtained four distinct lines i)non-edited, ii)hemizygous knockout of wild-type allele, iii)hemizygous knockout of mutant allele or iv)homozygous knockout of both *ATXN3* alleles. Genotypes were validated by high-throughput sequencing and *ATXN3* expression by western blot. Isogenic iPSC lines were differentiated into neuronal cultures using two different methods: a two-step monolayer protocol, consisting in the generation of an intermediate neural progenitor cell (NPC) population further differentiated into neuronal cells; and a rapid neurogenin-mediated directed neuronal differentiation protocol. With the first method, heterogeneous cultures of GFAP-, TUJ1- and MAP2-positive cells were obtained from NPC cultures characterized by a high percentage of SOX1, PAX6 and Nestin positive cells, while with the second, homogeneous neuronal cultures of MAP2- and TUJ1-positive cells were generated within 4 days.

Currently, *ATXN3*-related cell mechanisms, neuronal synaptic activity and global transcriptomics are under investigation. These models will help us understand the contribution of *ATXN3* toxic gain *versus* loss of function in SCA3 progression and the impact of *ATXN3* silencing for potential treatment avenues.

Understanding the regulation of stress responses in a mouse model of SCA3

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Joana Correia [1], Ms. Daniela Monteiro-Fernandes [1], Ms. Sara Guerreiro [1], Ms. Bruna Ferreira-Lomba [1], Ms. Daniela Cunha-Garcia [1], Ms. Patrícia Gomes [1], Dr. Andreia Teixeira-Castro [1], Dr. Sara Duarte-Silva [1], Prof. Patrícia Maciel [1]

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Background and Objective: Spinocerebellar ataxia type 3 (SCA3) is a neurodegenerative disease caused by a CAG triplet expansion in the *Ataxin-3* gene. SCA3 affects mainly motor function, but patients often report mood-related symptomatology. Previous results proved an interaction between glucocorticoid receptor (GR) and ATXN3, pointing GR as potential disease biomarker. A reduction in GR levels was found in CMVMJD135 mice and in post-mortem brain samples from SCA3 patients. At late-stages of disease, SCA3 mice exhibited elevated levels of peripheral corticosterone. Our aim was to gain deeper knowledge on GR mechanisms and the impact of stress on SCA3 progression. Unlike patients, mice are unaware of their disease condition making this model useful to understand disease-intrinsic mood disturbances.

Methods: To evaluate the regulation of the hypothalamic-pituitary-adrenal(HPA)-axis in SCA3, quantification of corticosterone was conducted throughout disease progression (6 to 34 weeks of age) under basal conditions (nadir/zenith) and after acute stress. At 35 weeks of age, mice were submitted to a dexamethasone injection to challenge the HPA-axis. A 6-weeks chronic unpredictable stress (CUS) protocol was applied at an early symptomatic stage, to simulate negative prospects of the disease and assess the interference of stress on motor impairments.

Results: Serum corticosterone elevation in SCA3 mice starts between 26 to 30 weeks-old, but no impairment was observed in the physiological response to an acute stressor. Also, SCA3 mice were able to restore corticosterone levels after challenge with dexamethasone. A transient impact of stress on SCA3 motor phenotype was observed following CUS exposure, but this negative effect did not prevail throughout disease progression.

Conclusions: SCA3 mice exhibited a normal response to acute stressors, suggesting a functional HPA-axis. Additionally, CUS exposure in SCA3 mice did not have a major impact on their motor phenotype indicating that mood deficits are not major determinants of disease severity.

Investigating the Mechanosensory neurons in Friedreich's Ataxia using Stem Cell Models

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Amy Hulme [1], ***Ms. Marnie Maddock*** [1], ***Ms. Sara Miellet*** [1], ***Dr. Anjila Dongol*** [1], ***Dr. Marek Napierala*** [2], ***Dr. Rocio Finol-Urdaneta*** [1], ***Prof. Mirella Dottori*** [1]

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Background: Mechanosensory neurons are essential for everyday functions, such as sitting, walking, and holding objects, as well as detecting internal organ sensations. Loss or dysregulation of mechanosensory neuron functioning is associated with a range of peripheral neuropathies, such as Friedreich's Ataxia (FA), which results in progressive ataxia and eventual loss of mobility. While breakthroughs have been made in the field of FA research, many of the cellular mechanisms underlying FA pathology are still unclear, which limits the development of therapeutics to treat FA.

Methods: To interrogate the cellular mechanisms that result in the degeneration of the mechanosensory neurons in FA, we generated mechanosensory neurons from FA patient-derived induced pluripotent stem cells (iPSCs) and investigated their functional, mitochondrial, and lipidomic profiles.

Results: The FA induced mechanosensory neurons had similar excitability profiles to their corrected isogenic controls, however, displayed impaired responses to mechanical stimulation, which worsened with high frequency mechanical stimulation. These findings are also correlated with observed cellular and morphological changes within the neurons.

Discussion and Conclusion: The functional and cellular characteristics of induced FA mechanosensory neurons is consistent with the mechanosensory impairments that progressively deteriorate in FA individuals. These are the first studies to identify this specific functional impairment in a cellular disease model, which can be applied to further understand the cellular mechanisms leading to degeneration and be used as a platform to test efficacy of novel treatments.

Functional characterization of a mouse model for Spinocerebellar Ataxia Type 44

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Mohamed Fasil Ibrahim [1], ***Dr. Sevda Boyanova*** [2], ***Mr. Yin Chun Cheng*** [1], ***Dr. Peter L. Oliver*** [3], ***Dr. Ed Mann*** [4], ***Prof. Andrea H. Németh*** [1], ***Prof. Esther B. E. Becker*** [5]

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Introduction

The spinocerebellar ataxias (SCA) are a genetically diverse group of autosomal dominant cerebellar ataxias, characterized by progressive loss of motor coordination and abnormal gait. With over 40 SCA subtypes and no current treatments, identifying common pathogenic pathways is critical for developing potential therapies. The metabotropic glutamate receptor subtype 1 (mGluR1) is highly expressed in Purkinje cells (PCs), and enhanced mGluR1 signalling in PCs has increasingly been implicated in multiple SCA subtypes, suggesting that this pathway could be a common therapeutic target. However, the mechanisms underlying ataxia caused by enhanced mGluR1 signalling are unclear.

Objective

The objective of our study is to better understand the role of enhanced mGluR1 signalling in SCA.

Methods

We previously discovered that SCA44 is caused by gain-of-function mutations in the *GRM1* gene encoding mGluR1. Using gene editing, we have recently created the first SCA44 mouse model harbouring one of the identified patient mutations (p. Tyr792Cys). Here, we report on the detailed characterization of these mice including motor and non-motor behaviour, histology and PC function.

Results

SCA44 mice developed progressive loss of motor coordination. PCs were not lost but showed functional deficits including increased mGluR1-mediated synaptic currents. Early on in the disease, SCA44 PCs displayed region-specific, aberrant spontaneous activity patterns including an increased percentage of silent and bursting PCs, and a reduced percentage of tonic firing PCs, which fired with decreased precision.

Discussion

Our findings demonstrate that SCA44 mice recapitulate the progressive loss of motor coordination observed in human SCA patients. Moreover, the observed early deficits in PC activity are consistent with those reported in other preclinical SCA mouse models. This suggests that enhanced mGlur1 signalling might be one of the key pathological drivers in SCA. Further understanding of the underlying mechanisms could pave the way for the development of common therapeutics to treat multiple SCA.

New neuronal model for Friedreich's Ataxia based on direct transdifferentiation of fibroblasts into neurons

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Saúl Herranz-Martin [1], ***Dr. Andrés Vicente-Acosta*** [2], ***Dr. Frida Loria*** [3], ***Prof. Javier Díaz-Nido*** [4]

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Background and objective: Friedreich's ataxia (FRDA) is a rare autosomal recessive neurodegenerative disorder characterized by an early atrophy of the spinal cord and progressive degeneration of the cerebellum. The caused by a GAA triplet repeat expansion within the first intron of the gene encoding the protein frataxin (FXN). This mutation leads to a reduced expression of this protein, whose role is crucial for the biogenesis and repair of FeS clusters. One of the major challenges for the study of FRDA is the lack of robust experimental models that accurately mimic the human disease. In this sense, to better understand the disease as well as to screen some new drugs, new animal and cell models are being developing using different technical approaches. Therefore, in this work, we have developed a novel cell model for FRDA using fibroblasts from FRDA patients and healthy subjects which are directly transdifferentiated into induced neurons (iNeurons).

Results: iNeurons generated from fibroblasts display a neuronal-like phenotype, showing some markers typical of mature neurons such as MAP2 and phosphorylated NF proteins, while a pool of fibroblast-specific genes are fully down-regulated. Moreover, compared to iNeurons obtained from healthy fibroblasts, those generated from FRDA patient fibroblasts show low levels of FXN as well as reduced expression of proteins containing FeS clusters such as Aconitase 2 and some complexes of the mitochondrial electron transport chain, which may compromise the mitochondrial function.

Discussion and conclusion: Our approach to generate a new neuronal model through direct conversion of mature fibroblasts from FRDA patients into neurons is feasible. The resulting FRDA iNeurons recapitulate some of the hallmarks of the disease.

Death by Ferroptosis: A key element and a therapeutic avenue in Drosophila models of Friedreich´s Ataxia

Tuesday, 12th November - 18:10: (Minories) - Poster

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Objectives

Friedreich´s Ataxia (FRDA) is caused by the reduced expression of frataxin. Frataxin deficiency triggers a drastic reduction in the cellular energy production leading to cell death. Mitochondrial dysfunction occurs along with deregulation of iron metabolism, depletion of glutathione and accumulation of lipid peroxides. Remarkably, these molecular signatures recapitulate the process of ferroptosis, a cell death driven by iron.

We aimed to analyse the impact of ferroptosis on the development and progression of the disease in an *in vivo* model of the disease.

Methods

The study is based on the evaluation of the response to both, either ferroptosis induction as well as to ferroptosis inhibition. We have downregulated frataxin in fly tissues using RNA interference constructs. All applied drugs were included in the fly food to ensure treatment during development as well as during aging. We have applied a top-notch approach to directly visualize iron deposit in frataxin-deficient neurons. We used full-field Transmission X-ray Microscopy to generate nanotomographies from neurons isolated from living fly brains at the Synchrotron ALBA.

Results

We corroborated that frataxin-deficient flies display increased levels of ferroptosis markers such as accumulation of lipid peroxides and reduction of glutathione levels. Regarding iron accumulation, our imaging study with soft X rays in the water window and at the absorption edge of the iron clearly showed accumulation of iron in the frataxin-deficient neurons that mainly localized within damaged mitochondria. In agreement, frataxin-deficient flies were more sensitive to ferroptosis induction.

Remarkably, inhibition of ferroptosis by increasing cysteine levels by means of N-acetylcisteine-derived compounds or reducing lipid peroxidation by overexpressing fly or mouse Glutathione Peroxidases greatly improved fly locomotion, brain degeneration and mitochondrial energy production. Activation of the NRF2 pathway and reduction of lipid peroxides underlies all rescues observed.

Conclusion

Our results highlight ferroptosis inhibition as a therapeutic possibility for Friedreich´s Ataxia.

Novel evidence of white matter contribution in Spinocerebellar Ataxia type 3 pathology

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Carina Henriques [1], ***Ms. Marta Silva*** [1], ***Mr. António Silva*** [1], ***Dr. David Rufino-Ramos*** [2], ***Mr. Miguel Monteiro Lopes*** [1], ***Dr. Romina Aron Badin*** [3], ***Dr. Philippe Hantraye*** [3], ***Prof. Luís Pereira de Almeida*** [4], ***Dr. Rui Jorge Nobre*** [5]

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Background: Spinocerebellar ataxia type 3 (SCA3) is a devastating neurodegenerative disorder that belongs to the family of polyglutamine disorders. Despite the CAG repeat expansion beneath SCA3 disease having been discovered 30 years ago, no cure or treatment can still delay its progression. One of the reasons for this lag may be attributed to the clinical heterogeneity among individuals, both phenotypic and neuropathological.

Objectives and Methods: To overcome this gap, we investigated the contribution to the pathology of specific brain regions that have been consistently reported to be the most degenerated in SCA3 patients, the cerebellar cortex (lobule IV-V, VIII and X), deep cerebellar nuclei and the pons. For this purpose, we used lentiviral vectors to deliver human mutant ataxin-3 (mut*ATXN3)*, the SCA3-causing gene, to these specific regions in mice.

Results: We observed that the overexpression of mut*ATXN3* in different hindbrain regions led to the formation of ataxin-3 aggregates and alterations in motor phenotype. Neurons in the pons were more vulnerable to mut*ATXN3* overexpression than in the cerebellum. There was an increase in astrocytes and microglia recruitment that may be behind myelin damage and consequently, white matter loss in the cerebellum. Indeed, white matter loss was the most broadly observed pathological feature upon mut*ATXN3* overexpression in different regions of the hindbrain.

Discussion and Conclusion: This work presents new evidence that white matter changes are a key feature of SCA3 neuropathology. Often overlooked in SCA3 animal models, white matter changes should be considered a biomarker for assessing disease progression and novel therapies.

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MOLECULAR PROFILING OF SENSORY NEURONS DERIVED FROM FRIEDREICH ATAXIA PATIENT- INDUCED PLURIPOTENT STEM CELLS

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Wenyao Yang [1], ***Prof. Bruce Thompson*** [2], ***Ms. Sara Miellet*** [3], ***Ms. Marnie Maddock*** [3], ***Dr. Marek Napierala*** [4], ***Prof. Mirella Dottori*** [3], ***Dr. Faith Kwa*** [5]

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Background and objectives

Friedreich Ataxia (FRDA) is an inherited and incurable disease caused by excessive GAA repeats in the frataxin gene (*FXN*), leading to epigenetic modifications that reduce FXN protein levels. Consequently, cells experience iron accumulation, oxidative stress, inflammation and death. Early symptoms affect motor coordination which is due to the damage to sensory neurons in the spinal cord. Although new treatments show promising outcomes in preclinical studies, these have not been proven effective in clinical trials due to the use of poor disease models. Therefore, models that are physiologically relevant and express the molecular hallmarks of the condition are required for evaluating new drugs. Here, we compare the characteristics of an FRDA model comprising sensory neurons derived from FRDA patient-induced pluripotent stem cells and their respective isogenic control.

Methods

The expression levels of FXN, sensory neuronal markers (*BRN3A*, *PRPH*, *TRKC*) and markers of epigenetic (*HDAC1*, *HDAC3*, *HDAC6*, *DNMT1*), oxidative stress (*NRF2*, *NQO1*, *HO-1*, *GCLM*, GSH/GSSG), and inflammatory (*TNFα*, MCP-1) pathways were examined by quantitative real-time PCR, glutathione and/or enzyme-linked immunosorbent assays.

Results

The isogenic control and patient sensory neurons expressed *BRN3A*, *PRPH*, and *TRKC*. Compared to the isogenic control, the patient sensory neurons showed: 1) a 2.5 to 5.8-fold reduction in FXN expression; 2) a 1.3 to 2-fold decrease in the expression of antioxidant markers; and 3) an increase in the expression of inflammatory and epigenetic markers by at least 1.5-fold.

Discussion and Conclusion

The patient sensory neurons express low levels of FXN and exhibit the molecular hallmarks of FRDA. Therefore, it is a robust in vitro model that can be used in drug screening.

Funding sources

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An induced pluripotent stem cell neuronal model to investigate the disease mechanisms of RFC1-mediated ataxia

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Kayli Davies [1], ***Dr. Haloom Rafehi*** [2], ***Dr. Kiymet Bozaoglu*** [1], ***Prof. Martin Delatycki*** [3], ***Prof. Melanie Bahlo*** [2], ***Prof. Paul Lockhart*** [1]

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Background and Objectives: Cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS) is an autosomal recessive, late-onset, neurodegenerative disease characterized by the triad of cerebellar impairment, sensory neuronopathy, and bilateral vestibular hypofunction. In 2019, biallelic AAGGG expansions in intron 2 of *RFC1* were identified as a major cause of late-onset ataxia and the cause of CANVAS. The molecular mechanisms underlying biallelic *RFC1* expansions are currently unknown and there are no disease modifying treatments available. Therefore, there is a need to develop cellular models to investigate disease pathogenesis and identify potential therapeutic targets. The aim of our study is to generate CANVAS patient-derived induced pluripotent stem cells (iPSCs) and differentiate them into cortical neurons for use in cellular and disease modelling.
Methods: We have generated iPSC lines from three unrelated individuals with CANVAS carrying biallelic *RFC1* expansions. In addition, heterozygous gene corrected isogenic lines were established by deleting one expanded allele with CRISPR/Cas9 technology. Using a lentiviral based NGN2 induction protocol, iPSCs were differentiated into cortical neurons. We performed a differentiation time course experiment, where iPSC-derived cortical neurons were collected at different stages of differentiation and maturation (Days 0, 7, 14, 21, 28) and we performed molecular analyses including RNAseq and immunofluorescence staining.
Results: We successfully generated mature cortical neurons from six iPSC lines (n=3 CANVAS; n=3 isogenic controls). Transcriptomic analysis is ongoing, but preliminary results revealed no reduction in *RFC1* transcript levels in CANVAS lines compared to isogenic controls (adjusted p=0.99) and have identified several differentially expressed genes.
Discussion and Conclusion: We have established reproducible patient-derived cortical neuronal models of CANVAS and are conducting molecular analyses including transcriptomics to identify the underlying disease mechanisms of *RFC1* expansions. These models will lead to mechanistic insights and the identification of new therapeutic avenues for RFC1-mediated ataxia.

Modelling FRDA cardiomyopathy with human iPSC-derived cardiomyocytes and autonomic neurons

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Jarmon Lees [1], ***Mr. Haoxiang Zhang*** [1], ***Mr. Ren Jie Phang*** [1], ***Dr. Jill Napierala*** [2], ***Dr. Marek Napierala*** [2], ***Prof. Mirella Dottori*** [3], ***Prof. Alice Pebay*** [4], ***Dr. Shiang Lim*** [1]

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Background: Friedreich ataxia (FRDA) is a hereditary disease characterised by progressive cardiomyopathy and neurodegeneration. Heart disease is the leading cause of premature death in individuals with FRDA. There is currently no treatment for FRDA heart disease. Although autonomic dysfunction is commonly reported in individuals with FRDA, its contribution to the pathogenesis of FRDA cardiomyopathy remains unclear.
Aims: To assess pathological phenotypes of FRDA patient-specific induced pluripotent stem cell-(iPSC) derived cardiomyocytes and autonomic neurons, and model cell-cell interactions in a 3D multicellular cardiac organoid.
Methods: FRDA-iPSCs and CRISPR-corrected isogenic controls were differentiated into cardiomyocytes and autonomic neurons to assess phenotypes on day 2. Autonomic neurons were also matured for 28 days for electrophysiological assessment by multielectrode array. These cardiomyocytes, autonomic neurons and endothelial cells were also engineered into cardiac organoids for functional assays on day 7.
Results: Compared to isogenic controls, frataxin mRNA and protein levels were significantly lower in FRDA-iPSCs and their derivatives. Cardiomyocytes derived from FRDA-iPSCs exhibited an increased frequency of irregular calcium cycling, increased mitochondrial superoxide production, increased cell death, and hypertrophy. Autonomic neurons derived from FRDA-iPSCs showed increased mitochondrial superoxide production, increased proliferation and apoptosis, and increase electrophysiological activity including longer burst duration and a faster spike rate. FRDA cardiac organoids exhibited prolonged time-to-peak (a surrogate measure of systolic function), a decreased beat rate, and increased cell death. All presented data is $n \geq 3$ and $p<0.05$.
Conclusions and significance: Cardiomyocytes and autonomic neurons derived from FRDA-iPSCs exhibited pathological phenotypes observed in FRDA patients. This is the first pre-clinical evidence that autonomic neurons are negatively impacted by frataxin loss. These findings suggest that autonomic dysfunction contributes to FRDA cardiomyopathy and represents a promising new cellular target for therapy.
Funding: Friedreich Ataxia Research Alliance, National Ataxia Foundation, Australian National Health and Medical Research Council.

Excision of the expanded GAA repeats from Fxnnull::YG8s(GAA)>800 increases frataxin expression

Tuesday, 12th November - 18:10: (Minories) - Poster

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1. *Department of Neurology, University of Texas Southwestern Medical Center, Dallas, TX, USA*

Background: Appropriate mouse models are essential in the processes of drug discovery and therapy development for Friedreich's ataxia (FRDA). The Fxn^{null}::$YG8s(GAA)_{>800}$ (Jax Stock No. 030395) humanized transgenic mouse is one of the best characterized mouse models of FRDA. It is also the only mouse model that expresses human frataxin encoded by the entire human *FXN* gene. It carries a large GAA repeat expansion of ~800 repeat and develops a mid to late onset progressive phenotype. It is the only FRDA mouse model that can be used for testing "human sequence-specific" therapies such as antisense oligonucleotides or CRISPR-Cas9 editing. Control mice (Y47R) were created by random integration of a human transgene containing the entire *FXN* gene with only 9 GAA repeats. The transgene is inserted in a different chromosomal location than that of the $YG8s(GAA)_{>800}$ model, resulting in very high frataxin expression. Progressive weight gain in these animals presents a concern for motor/behavioral analyses. Therefore, to address these issues and generate an appropriate control strain for $YG8s(GAA)_{>800}$ mice, we utilized CRISPR-Cas9 editing to excise the fragment of intron 1 containing 800 GAAs.

Methods: $YG8s(GAA)_{>800}$ embryos were electroporated with Cas9 protein and four gRNAs (two upstream and two downstream of the GAA repeats) to generate the YG8sD(GAA) strain. Embryos were transferred to pseudo-pregnant female mice.

Results: Genotyping and DNA sequencing of seven surviving pups demonstrated that all carried edited alleles. Two males were selected as founders of the YG8sD(GAA) colony and crossed with B6 wild type females to minimize potential off-target effects of genome editing. One of the seven pups was sacrificed, and RNA and protein analyses demonstrated significant upregulation of frataxin expression after GAA excision compared to the $YG8s(GAA)_{>800}$ strain.

Conclusions: Using CRISPR-Cas9, we generated a novel control mouse strain of critical importance to drug discovery and therapy development for FRDA.

Synaptic and dendritic impairments in the Spinocerebellar Ataxia 44 (SCA44) mouse model

Tuesday, 12th November - 18:10: (Minories) - Poster

Mr. Yin Chun Cheng [1], ***Dr. Mohamed Fasil Ibrahim*** [1], ***Ms. Tamara Raad*** [2], ***Mr. Julien Roy*** [1], ***Prof. Andrea Nemeth*** [3], ***Prof. Esther B. E. Becker*** [3]

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Introduction

Spinocerebellar ataxia (SCA) comprises a group of autosomal dominant cerebellar disorders characterised by the gradual deterioration of motor coordination, balance, and speech capabilities. Although SCAs have been traditionally classified as neurodegenerative diseases, emerging evidence highlights that impaired development of cerebellar Purkinje cells (PCs) contributes to disease severity in SCA models. Interestingly, metabotropic glutamate receptor 1 (mGluR1) signalling is key for PC development and has been implicated in various SCAs. In this study, we have used a novel SCA44 mouse model to investigate the synaptic and dendritic developmental abnormalities caused by enhanced mGluR1 function.

Methods

We employed a mouse model for SCA44 (*Grm1*^{Y792C}) that was recently generated in our lab and allows us to study the consequences of constitutively active mGluR1 for PC development and function. We performed immunohistochemistry on cerebellar cryosections from wildtype (WT) and SCA44 mice to examine PC synaptic innervation. Additionally, organotypic slice cultures from WT and SCA44 mice were prepared to investigate PC dendritic development *in vitro*.

Results

We found a significant reduction in climbing fibre (CF) territory and synaptic density in the SCA44 cerebellum, starting during postnatal development. Changes were first apparent in the anterior cerebellum and only much later appeared in the posterior cerebellum. Furthermore, SCA44 PCs exhibited significantly impaired dendritic development in organotypic slice culture experiments, which could be ameliorated by the pharmacological inhibition of mGluR1-TRPC3 signalling.

Discussion and Conclusion

Our findings demonstrate that overactive mGluR1 signalling causes a progressive, region-specific impairment of CF synapse innervation and arrested dendritic development in the SCA44 cerebellum. Since aberrant mGluR1 signalling has been implicated in many other SCAs, our work suggests that enhanced mGluR1 signalling is a key driver of developmental cerebellar abnormalities that might contribute to the pathogenesis in SCA.

Development and characterization of a novel SCA2 patient-derived induced pluripotent stem cell model as a tool to investigate therapeutic strategies

Tuesday, 12th November - 18:10: (Minories) - Poster

Ms. Cristiana Madeira [1], ***Dr. Rebekah Koppenol*** [1], ***Dr. Sofia Calado*** [1], ***Prof. Carlos Matos*** [2], ***Mr. Ricardo Reis*** [2], ***Prof. Luís Pereira de Almeida*** [3], ***Dr. Clévio Nóbrega*** [2]

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Objectives: The main objective was to develop a SCA2-hiPSC model and a CRISPR-based therapeutic approach. **Methods:** SCA2 patient-derived fibroblasts were reprogrammed into human induced pluripotent stem cells (hiPSCs) with the CytoTune iPS 2.0 Sendai Reprogramming Kit. The SCA2-hiPSC line was characterized regarding the absence of the reprogramming vectors' expression, genome integrity, the presence of pluripotency markers, and differentiation capacity into cells of the three germ layers. SCA2-hiPSCs were differentiated into neurons using STEMdiff™ media. Immunocytochemistry assessed neuronal markers expression and 1C2 in SCA2 iPSC-derived neurons. An ImageJ tracing tool was used to evaluate neurites' length/number per neuron. To silence the *ATXN2* gene, a CRISPR-Cas9 complex was designed and validated in the HEK293T cell line. Western blot was performed to compare the levels of ataxin-2 protein between cells with the therapeutic strategy and the control. **Results:** The SCA2-hiPSCs had no reprogramming vectors at passage 10. The hiPSCs line expresses the pluripotency markers: OCT4/Nanog/SOX2/SSEA4 and can differentiate into cells from the 3-germ layers. The SCA2-hiPSCs-derived neurons did not demonstrate well-established neuronal networks and exhibited poor neuronal maturation compared to WT neurons. SCA2 hiPSCs-derived neurons show 1C2 immunoreactivity in the form of aggregates in the cytoplasm and reduced neurite length/fewer branches per neuron compared to WT neurons. Cells with the CRISPR-Cas9 strategy demonstrated lower ataxin-2 protein levels than control cells. **Discussion:** The therapeutic scenario for SCA2 is lacking effective disease-modifying approaches. This work uses hiPSCs-derived neurons as a powerful tool to investigate disease mechanisms and test novel therapeutic strategies in an SCA2 disease model preserving the human genetic background. As a future perspective, we seek to evaluate the impact of the designed CRISPR-mediated approach on rescuing neuronal function. **Conclusion:** Although SCA2 remains an incurable disorder, silencing the pathogenic expression of *ATXN2* could constitute a promising therapeutic approach for the treatment of SCA2 patients.

Characterization of FA iPSC-derived DRG organoid to decipher neurodevelopmental and neurodegenerative mechanisms

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Valentine Mosbach [1], ***Ms. Adèle Hennick*** [1], ***Dr. Marek Napierala*** [2], ***Mr. Lucas Lemarié*** [3], ***Dr. Helene Puccio*** [1]

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Friedreich Ataxia (FA) is an inherited neurodegenerative disease caused by a GAA expansion in the 1st intron of frataxin gene leading to reduced transcription and protein levels. FA is a multisystemic disorder, however, one of the primary affected neuronal sites is the dorsal root ganglia (DRG). DRG contain the cellular bodies of sensory neurons, notably the proprioceptive that are specifically affected in FA. While FA has traditionally been considered to be a neurodegenerative disease, recent studies suggest that a neurodevelopmental component might contribute to the pathology. However, the majority of FA mouse and cellular models do not recapitulate the very low levels of frataxin observed in patients throughout development. New human-derived models of the disease are being developed based on induced pluripotent stem cell (iPSC) technologies. We optimized a recently published protocol to generate from hiPSC 3D-culture of DRG organoids that mimic the major neurogenic steps *ex vivo*. First characterization of this model showed that DRG organoids derived from FA patients-hiPSC present a “giant size” phenotype that appears to correlate with GAA-repeat size. We aim to decipher the neurodevelopmental mechanisms leading to sensory degeneration in frataxin deficient condition using this model.

Glial overexpression of Tspo is protective in a Drosophila model of Friedreich Ataxia

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Estelle JULLIAN [1], Dr. Maria RUSSI [1], Mrs. Ema TURKI [1], Mrs. Margaux BOUVELOT [1], Mrs. Laura TIXIER [1], Dr. Sandrine MIDDENDORP [1], Dr. Elodie MARTIN [1], Prof. Veronique MONNIER [1]

1. *University Paris Cité*

Background and objectives: The translocator protein TSPO is an evolutionary conserved mitochondrial protein overexpressed in various contexts of neurodegeneration. We previously observed that *Tspo* was overexpressed in a Drosophila model of Friedreich Ataxia (FA) generated by CRISPR/Cas9 insertion of approximately 200 GAA in the intron of *fh*, the fly frataxin gene. We aimed to decipher whether*Tspo* overexpression was either protective or deleterious in a context of frataxin deficiency or in a wild-type context.

Methods: We have generated a new Drosophila model of FA with 42 GAA repeats, called fh-GAAs. Effects of *Tspo* overexpression on fly survival was achieved using RU486 inducible drivers, either ubiquitous or targeting specifically neurons or glial cells.

Results: We first characterized phenotypically the fh-GAAs models. Compared to the 200GAA model, the smaller expansion size allowed to obtain viable adults. These flies were short-lived and exhibited locomotor defects, hypersensitivity to oxidative stress and heart dilatation. *Tspo* was also overexpressed in the heads of these flies. We further overexpressed *Tspo* specifically in glial cells and observed improved survival and locomotor function. Finally, we observed that in healthy flies, increased longevity was conferred by *Tspo* glial-specific overexpression, with opposite effects in neurons.

Discussion and conclusion: The new fh-GAAs model of FA exhibited hallmarks of the FA disease and was associated with T*spo* overexpression. We describe protective effects of glial Tspo both in a healthy and in a frataxin-deficient context which suggests that Tspo might be a relevant therapeutic target for FA disease.

The generation and characterization of the fh-GAAs model was funded by the French Friedreich's Ataxia Patient Organisation (Association Française de l'Ataxie de Friedreich, AFAF)

Cerebellar ataxia due to mutations of KIF1C, a new player in oligodendrocyte biology

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Amandine Duchesne [1], ***Mrs. Anne Vaiman*** [1], ***Mr. Clément Beaurieux*** [2], ***Mr. Johan Castille*** [1], ***Dr. Liriope Toupenet*** [2], ***Dr. Florence Jaffrezic*** [1], ***Dr. Carlos Parras*** [2], ***Mrs. Marthe Vilotte*** [1], ***Dr. Corentine Marie*** [2], ***Dr. Jean-Luc Vilotte*** [1], ***Dr. Giovanni Stevanin*** [3], ***Dr. Hamid El Hachimi*** [2]

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Background:
KIF1C is a ubiquitously expressed kinesin-3 motor protein transporting a variety of cargoes, such as vesicles into axons and dendrites, integrins in migrating cells or mRNAs in cell protrusions. *KIF1C* mutations cause spastic ataxia 2 (SPAX2/SAX2) in human and progressive ataxia in bovine, both with cerebellar ataxia as prominent neurological feature. In the bovine model, late demyelination is associated with oligodendrocyte abnormalities.
Methods:
We generated a KO mouse model mimicking the ataxic bovine KIF1C mutation, and used locomotion tests, histopathological and transcriptomics analyses along with oligodendrocyte primary cell culture to unravel the role of KIF1C in myelination and myelin maintenance.
Results:
KIF1C KO mice mimic several features of cattle pathology as shown by locomotion and histopathological analyses. Cerebellum transcriptome study pinpointed an early downregulation of genes involved in gliogenesis and myelination. Not only downregulated, some transcripts encoding myelin proteins are also restricted around the oligodendrocyte nuclei, instead of being expressed along the myelinated tracts. Accordingly, a subset of myelin proteins is differentially expressed.
In vitro studies showed that KIF1C KO oligodendrocytes exhibited a frequent degenerative profile and a weakened reticulated pattern, consistent with an early impairment of gliogenesis and myelination. Furthermore, immunofluorescence confirmed the suspected transport defect of myelin components.
Discussion:
KIF1C seems to be involved in the transport of myelin protein mRNAs in oligodendrocyte processes, either directly, or in interaction with ribonucleoprotein (RNP) granules. To identify exhaustively KIF1C partners (mRNA and protein cargoes), a second mouse expressing V5-epitope tagged KIF1C was generated by CRISPR/Cas9.
Conclusion:
KIF1C is a new player in oligodendrocyte biology, as revealed by in vitro and in vivo analyses. Studying this new oligodendroglial function of KIF1C may contribute to a better understanding of the function of KIF1C in myelin maintenance and may be useful to the development of therapeutic designs for this peculiar cerebellar ataxia.

Early glial cell reactivity followed by neurodegeneration in the cerebellar cortex of a murine model of Friedreich ataxia

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Frida Loria [1], ***Dr. Andrés Vicente-Acosta*** [2], ***Dr. Saúl Herranz-Martin*** [3], ***Dr. María Ruth Pazos*** [1], ***Mr. Jorge Galán-Cruz*** [4], ***Mr. Mario Amores*** [1], ***Prof. Javier Díaz-Nido*** [4]

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Background and objective: Friedreich's ataxia (FRDA) is a genetic neurodegenerative disorder caused by a deficiency of the protein frataxin due to an expanded GAA repeat within the first intron of the *FXN* gene. Frataxin deficiency leads to gradual and progressive degeneration of the cerebellum and the loss of movement coordination and equilibrium, two of the main symptoms observed in people affected by the disease. To study pathological mechanisms and develop possible therapeutic strategies, we need to use animal models that faithfully replicate what occurs in humans. In this work, our objective was to characterize the phenotype of YG8-800 mice (Fxn^{null}::YG8s$(GAA)_{>800}$), which harbor a human *FXN* transgene with more than 800 GAA repeats.

Methods: To characterize the phenotype of this FRDA experimental model, we performed behavioral, biochemical, and immunohistochemistry experiments in YG8-800 mice and the control strain Y47R every 3 months for more than one year.

Results: Compared to age-matched control mice, YG8-800 mice were underweight, and exhibited poor motor coordination, and hair loss. Along with evident atrophy, we detected synaptic alterations and neuronal loss in the cerebellum. Moreover, we observed early glial cell reactivity, predominantly astrocytes, and microglia, preceding neuronal degeneration. This glial activation was accompanied by an increase in the mRNA expression of key proinflammatory cytokines and iron accumulation.

Discussion and conclusion: Our results demonstrate that the YG8-800 mouse model reliably mimics human disease, showing a stronger ataxic phenotype than previous FRDA models, with early microglial and astroglial activation followed by neurodegeneration in the cerebellar cortex.

Mechanisms involved in SCA36 disease

Tuesday, 12th November - 18:10: (Minories) - Poster

Dr. Ana Quelle Regaldie [1], ***Dr. Nicolas Charlet Berguerand*** [2], ***Dr. Edor Kabashi*** [1]

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Background and objectives

Spinocerebellar ataxia type 36 (SCA36) is a type of late-onset dominant ataxia that presents the classic phenotype of SCAs: gait impairment, sensory hypoacusia and involvement of motor neurons(atrophy and lingual fasciculations). It's caused by GGCCTG expansion in the first intron of NOP56 gene. Normal repeat range is between 3 and 14, while in patients the expansion consists of at least 30 repeats. These repeats are spontaneously translated without the need for a start codon or ATG (a process known as Repeat Associated Non-ATG translation), resulting in toxic dipeptide repeats (DPRs) that aggregate in neurons. Bidirectional translation in 6 different ñ reading frames gives rise to 5 distinct DPRs, of which poly-PR and poly-GP are common with Amyotrophic Lateral Sclerosis caused by C9orf72 mutations (C9 ALS). Current research in SCA36 is very limited, so the underlying mechanisms and molecular pathways involved are not known, and although it is suspected to be caused by toxic gain-of-function mechanisms, contribution of different DPRs and loss-of-function mechanisms to the pathology has not been determined yet.

Methods

Zebrafish were used to create models of SCA36 either knocking down nop56 gene (70% of homology with human gene) as injecting constructions of SCA36 DPRs. These models were analysed by confocal imaging, histology, locomotion analysis and immunohistochemistry.

Results

Zebrafish models show neurodegeneration by both haploinsufficiency and DPRs expression. There are also differences in DPR aggregation and solubilization with and without haploinsufficiency. We observed also shared pathways with C9 ALS as we have recently published in a novel zebrafish model.

Discussion and conclusions

In this work we defined some of the mechanisms and pathways involved in SCA36. Study of the mechanisms underlying ataxias is essential for the discovery of potential biomarkers that may be indicative of therapeutic pathways, which may also be common to other expanding diseases.

Multi-omic analysis reveals loss of the ataxia-linked protein DNAJC3 disrupts cell cycle progression

Tuesday, 12th November - 18:10: (Minories) - Poster

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1. *William Harvey Research Institute, QMUL, London, UK,* ***2.*** *University of Wisconsin-Madison*

Background and Objective: Mutations in the molecular chaperone protein DNAJC3 cause a rare syndrome that presents with cerebellar ataxia and diabetes mellitus (Ocansey et al., 2022; Lytrivi et al., 2021; Synofzik et al., 2014). DNAJC3 is a J-domain protein that functions with the endoplasmic reticulum (ER) resident chaperone BiP, implying disruption of ER function may be a key contributor to disease associated with its loss. Moreover, previous, studies using patient-derived fibroblast, revealed that the absence of DNAJC3 protein results in an increase in amyloid precursor protein levels and mitochondrial alterations (Jennings et al., 2021). Here, to explore the consequences of DNAJC3 loss in the context of ataxia we developed an isogenic neuroblastoma-derived knockout (KO) cell model.
Methods: Using CRISPR/Cas9 we generated DNAJC3 KO SH-SY5Y cell lines. To better understand the molecular mechanism of DNAJC3-linked ataxia we then performed a multi-omic comparison between KO cells and isogenic controls. This included transcriptomics, proteomics, and mass spectrometry-based traced analysis.
Results: Our analysis revealed that multiple pathways were disrupted in DNAJC3 KO cells. This included a decrease in the expression of genes associated with cell cycle regulation, which was consistent with impaired growth rate of DNAJC3 KO lines. We also observed metabolic alterations, including increased glucose levels, in DNAJC3 KO cells. This was interesting given the context of diabetes as part of the syndrome associated with DNAJC3 mutations. Surprisingly, we did not identify evidence for significant disruption of ER function.
Discussion and Conclusion: Defining cellular deficits associated with the loss of DNAJC3 is a valuable first step towards understanding molecular mechanism of disease and identification of potential therapeutic strategies for this ataxia.

Age and tissue-dependent iron homeostasis alterations in the FXNI151F mouse model of Friedreich Ataxia

Tuesday, 12th November - 18:10: (Minories) - Poster

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Background and objectives

Friedreich Ataxia (FA) is caused by mutations in the FXN gene which result in low frataxin protein levels in all the tissues. Even though it is well established that frataxin deficiency affects iron metabolism, most studies related to iron metabolism alterations in FA mouse models have been performed in conditional tissue-specific KOs. These models do not fully represent the condition of the disease since FA patients present partial frataxin deficiency in all the tissues. Therefore, our objective is to analyse the progression and tissue specificity of iron-related alterations in a mouse model presenting partial frataxin deficiency in all the tissues (FXN^{I151F}).

Methods

The FXN^{151F} mouse model was recently developed in our laboratory. It is based on the pathological human point mutation I154F. It presents low FXN protein levels in all the tissues and neurological defects resembling FA patients. We analysed several iron-related parameters in the cerebrum, cerebellum, heart and liver from these animals at 10, 21 and 39 weeks.

Results

Iron overload is early observed in the nervous system and later in the liver of FXN^{I151F} mice. Moreover, Iron Regulatory Protein 1 (IRP1) content is decreased in these tissues while the heart presents normal iron and IRP1 levels, increased IRP2 content and decreased aconitase 2 activity. Remarkably, in the liver, IRP1 is phosphorylated and we do not detect general iron-sulfur loss.

Discussion and Conclusion

We conclude that partial frataxin deficiency produces tissue-specific iron deregulation. The heart presents an iron deficiency response at 21 weeks, while IRP1 loss in the nervous system and the liver might be caused by alterations in other signalling pathways.

Funding

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Frataxin bypass by ISCU M141I substitution in mammalian cells

Tuesday, 12th November - 18:10: (Minories) - Poster

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Iron sulfur (Fe-S) clusters are essential co-factors required for the function of a variety of proteins involved in key cellular processes. The conserved multiprotein machinery ISC, localized in the mitochondria in eukaryotes, initiates Fe-S clusters biogenesis by *de novo* assembly of sulfur, provided by a cysteine desulfurase, and iron on the scaffold protein ISCU. One of the major actors of Fe-S biogenesis is the frataxin protein (FXN), which plays a regulatory role on the ISC machinery, increasing Fe-S clusters biogenesis rate by specifically accelerating the persulfide formation and sulfur delivery to the scaffold protein ISCU. In eukaryotes FXN is essential, its absence leading to growth deficit in yeast and embryonic lethality in mice. However, in yeast, a substitution Met to Ile in position 141 of the scaffold protein *Isu1* allowing frataxin deficient yeast (D*Yfh1*) to grow was recently identified. This discovery raises the question if the same substitution in ISCU protein could allow to bypass FXN in mammalian cells. **Methods:** We introduced this mutation at the endogenous ISCU gene locus using a CRISPR-Cas9 system in NC6 L3/L- mice fibroblasts carrying a conditional allele allowing FXN deletion. **Results:** We showed that in dividing cells ISCU M141I substitution can bypass FXN lethality, but survivor clones presented a slower growth, a mitochondrial dysfunction and a deficit in mitochondrial Fe-S clusters proteins. However, survivor clones present a normal cell cycle progression and less DNA damage at the basal level compared to fibroblasts expressing the mutant protein FXN I154F known to decrease Fe-S clusters biogenesis rate by impeding the processing of the FXN intermediary form. **Conclusion:** These results raise the questions of the Fe-S clusters biogenesis rate produce in the ISCU M141I cells and their distribution among the different compartment of cells that requires them.

Investigation of pathological changes in neurodegenerative diseases in an induced pluripotent stem cell-derived cerebellar organoid model

Tuesday, 12th November - 18:10: (Minories) - Poster

Mrs. Alexandra Sándor [1], ***Dr. Kornélia Szebényi*** [2]

1. *Doctoral School of Molecular Medicine, Semmelweis University,* ***2.*** *Institute of Molecular Life Sciences, HUN-REN Research Centre for Natural Sciences*

Spinocerebellar ataxias (SCA) represent a diverse range of neurological disorders, mainly characterised by the slow degeneration of the cerebellum resulting in loss of motor co-ordination. Given the distinct development, cell composition and size of the human cerebellum compared to mice, human model systems are essential for studying neurodegenerative changes underlying SCAs. Human induced pluripotent stem cell (hiPSC)-derived cerebellar organoid systems contain the disease relevant cell types in a tissue-like organization and therefore can provide a relevant model for investigating the molecular mechanisms of ataxias affecting the human cerebellum.

We developed a protocol for the generation of a hiPSC-derived organoid model of the cerebellum. The cell types found in the organoids on days 35 and 50 of the differentiation were characterized by immunocytochemical methods, using markers specific to the developmental stages of the cerebellum. We showed that Kirrel2-positive Purkinje progenitors appear on day 35, from which Calbindin-positive Purkinje neurons develop by day 50. At this point astroglial cells are also present in the cerebellar organoids. In order to prove that the model is also suitable for detecting pathological changes, we treated the organoids with IL-1β, capable of inducing ataxia in mice. IL-1β treatment increased the expression level of the autophagy marker P62.

Overall, it can be concluded that we have created an organoid model of the human cerebellum, which is suitable for examining cell-specific pathological changes and can serve as a platform for the development of therapies targeting them.

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I am grateful to the Doctoral School of Semmelweis University for the financial support to appear at the conference.

The Dutch CureQ consortium: Using iPSC modelling and clinical and biomarker data from patients to predict, delay and cure polyglutamine caused neurodegeneration

Tuesday, 12th November - 18:10: (Minories) - Poster

Prof. Willeke van Roon-Mom [1], ***Dr. Ineke Bolt*** [2], ***Dr. Rob Haselberg*** [3], ***Dr. Jurre Hageman*** [4], ***Dr. Bart Baselmans*** [5], ***Prof. Harrie Kampinga*** [6], ***Dr. Monique Mulder*** [1], ***Dr. Mayke Oosterloo*** [7], ***Dr. Wilianne Vonk*** [8], ***Prof. Bart van de Warrenburg*** [9], ***Dr. Tsjerk Wassenaar*** [4], ***Prof. Eric Reits*** [10]

1. *LUMC,* ***2.*** *ErasmusMC,* ***3.*** *Vereniging van Huntington,* ***4.*** *HanzeHogeschool, Groningen,* ***5.*** *Hogeschool van Amsterdam,* ***6.*** *UMCG, Groningen,* ***7.*** *MUMC+,* ***8.*** *Prinses Maxima Center, Utrecht,* ***9.*** *Radboud university medical center,* ***10.*** *Amsterdam UMC*

Introduction: Spinocerebellar ataxia (SCA) type 1 and 3 and Huntington disease (HD) are caused by CAG repeat expansions within relevant genes. Novel promising therapeutic developments have raised new and urgent questions. 1) the optimal time to start treatment. 2) can we derive patient-tailored predictions. 3) can less invasive strategies be developed to lower mutant protein. 4) can we prediction onset and progression of disease in an ethically sound way.

Methods: We will create isogenic human-derived cells with different CAG repeat lengths for SCA1, SCA3 and HD in work package (WP) 1 and develop robust platforms to characterize amphenotypic landscape whilst differentiating them into 2D 3D models. In parallel, we will generate these landscapes for iPSC-models derived from cells from actual patients with known ages at onset to find landscape-derived parameters predicting disease onset (WP2) combined with clinical, imaging and fluid biomarkers (WP3). These landscapes will be used to evaluate newly-developed therapeutic strategies (WP4). Finally we will study the ethical implications of such improved diagnostics (WP6)

Discussion and Conclusion: With our consortium of academic and biotech researchers, clinicians, ethics experts and patient communities we hope to provide robust and sensitive phenotypic, cell biological readouts for a proper and responsible implementation of patient-tailored prediction of disease onset, disease course and severity. These will aid clinical and personal decision-making on presymptomatic testing and life planning by at-risk individuals.

ASO-induced exon 10 skipping of ATXN3: assessing the functionality of the truncated ΔpolyQ Ataxin-3 protein isoform

Tuesday, 12th November - 18:10: (Minories) - Poster

Mr. Bas Rottgering [1], ***Dr. Chantal Beekman*** [2], ***Mr. Rudie Weij*** [2], ***Dr. Janwillem Testerink*** [2], ***Mr. Ruurd Verheul*** [2], ***Ms. Suzanne Bijl*** [2], ***Dr. Linde Bouwman*** [3], ***Prof. Willeke van Roon-Mom*** [3], ***Dr. Nicole Datson*** [2]

1. *Leiden University Medical Center/VICO Therapeutics BV,* ***2.*** *VICO Therapeutics BV,* ***3.*** *Leiden University Medical Center*

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*) which 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, and 10.4, an ASO developed by the LUMC, bind to exon 10 in *ATXN3* pre-mRNA, inducing skipping of this exon resulting in a shortened mRNA. This mRNA codes for a truncated ATXN3 protein known as ΔpolyQ which lacks the polyQ domain and one of three ubiquitin-interacting motifs (UIMs). Although ΔpolyQ retains partial deubiquitinating (DUB) activity, limited information on the effect of the truncation on ATXN3 protein function is available. Therefore, the aim of this study is to examine the functionality of ΔpolyQ.

Methods

Control iPSCs were transfected with CAS9 and guide RNAs to delete *ATXN3* exon 10 to
establish iPSCs expressing ΔpolyQ. Additionally, the DUB function of ΔpolyQ was assessed by treating ubiquitin chains *in vitro* with recombinant ΔpolyQ followed by quantification using Capillary Western Blot (Wes).

Results

ATXN3 exon 10 knockout iPSC models were generated using CRISPR/CAS9. Presence of the deletion was examined with PCR and verified with Sanger sequencing. Pure clones, homo- and heterozygous for the deletion were obtained through single cell cloning. Additionally, recombinant ΔpolyQ exhibited DUB activity on ubiquitin chains *in vitro*.

Discussion and Conclusion

ΔpolyQ retains partial DUB functionality. However, its overall functionality needs to be further investigated. The generated iPSC models will be differentiated into neurons and cerebellar organoids to examine changes in phenotype resulting from replacement of ATXN3 with ΔpolyQ. This will provide better understanding of the therapeutic potential of exon skipping ASOs for SCA3.

Funding

This research was funded by VICO Therapeutics B.V.

Poster session II - Advances in genetics and diagnostics

Spinocerebellar ataxia type 4

Wednesday, 13th November - 18:00: (Minories) - Poster

Martin Paucar MD, PhD [1], ***Daniel Nilsson PhD*** [2], ***Martin Engvall, MD, PhD*** [3], ***José Laffita-Mesa PhD*** [4], ***Cilla Söderhäll, PhD*** [5], ***Mikael Skorpil MD, PhD*** [6], ***Prof. Patrik Fazio, MD, PhD*** [7], ***Kristina Lagerstedt-Robinson, PhD*** [8], ***Göran Solders, MD, PhD*** [9], ***Andrea Varrone, MD, PhD*** [7], ***Prof. Mårten Risling, MD, PhD*** [10], ***Prof. Hong Jiao, PhD*** [11], ***Inger Nennesmo, MD, PhD, Associate Professor*** [12], ***Anna Wedell, MD, PhD, Professor*** [3], ***Per Svenningsson, MD, PhD, Professor*** [1]

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Background

Spinocerebellar ataxia 4 (SCA4), characterized in 1996, features adult-onset ataxia, polyneuropathy and linkage to chromosome 16q22.1; its underlying mutation has remained elusive.

Objective

To explore the radiological and neuropathological abnormalities in the entire neuroaxis in SCA4 and search for its mutation.

Methods

Three Swedish families with undiagnosed ataxia went through clinical, neurophysiological and neuroimaging tests, including PET studies, and genetic investigations. In four cases, neuropathological assessments of the neuroaxis were performed. Genetic testing included short read whole genome sequencing (WGS), short tandem repeat analysis with ExpansionHunter *de novo,* and long read sequencing.

Results

Novel features for SCA4 include dysautonomia, motor neuron affection, and abnormal eye movements. We found evidence of anticipation; neuroimaging demonstrated atrophy in the cerebellum, brainstem and spinal cord. [^{18}F]FDG-PET demonstrated brain hypometabolism and[^{11}C]Flumazenil-PET reduced binding in several brain lobes, insula, thalamus, hypothalamus and cerebellum. Moderate to severe loss of Purkinje cells in the cerebellum and of motor neurons in the anterior horns of the spinal cord along with pronounced degeneration of posterior tracts was also found. Intranuclear, mainly neuronal, inclusions positive for p62 and ubiquitin were sparse but widespread in the CNS. This finding prompted assessment for nucleotide expansions. A polyglycine stretch encoding GGC expansions in the last exon of the zink finger homeobox 3 (*ZFHX3)* gene was identified segregating with disease, and not found in 1000 controls.

Expanded ATTCT-ATXN10 alleles in healthy Peruvian population

Wednesday, 13th November - 18:00: (Minories) - Poster

Mr. Ismael Araujo-Aliaga [1], ***Ms. Karina Milla-Neyra*** [2], ***Ms. Carla Manrique-Enciso*** [2], ***Dr. Elison Sarapura-Castro*** [1], ***Dr. Maryenela Illanes-Manrique*** [3], ***Mr. Diego Veliz-Otani*** [4], ***Ms. Ana Saldarriaga-Mayo*** [1], ***Mr. Angel Medina-Colque*** [5], ***Ms. Julia Rios-Pinto*** [6], ***Mr. Ivan Cornejo-Herrera*** [7], ***Ms. Andrea Rivera-Valdivia*** [1], ***Mr. Ignacio Mata*** [8], ***Mr. Douglas Loesch*** [4], ***Mr. Leonel Lozano-Vasquez*** [9], ***Mr. Timothy O'Connor*** [4], ***Ms. Birgitt Schüle*** [10], ***Prof. Mario Cornejo-Olivas*** [1]

1. *Neurogenetics Working Group, Universidad Científica del Sur, Lima, Perú. Neurogenetics Research Center, Instituto Nacional de Ciencias Neurológicas, Lima, Peru,* ***2.*** *Neurogenetics Research Center, Instituto Nacional de Ciencias Neurológicas, Lima, Peru,* ***3.*** *Neurogenetics Working Group, Universidad Cientifica del Sur, Lima, Peru,* ***4.*** *Institute for Genome Sciences, School of Medicine, University of Maryland, Baltimore, Maryland, USA,* ***5.*** *Direccion Regional de Salud de Puno, Puno, Peru,* ***6.*** *Universidad Peruana Los Andes, Huancayo, Peru,* ***7.*** *Universidad Privada de Tacna, Tacna, Peru,,* ***8.*** *Genomic Medicine Institute, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio, USA,* ***9.*** *Universidad Peruana de Ciencias Aplicadas, Lima, Peru,* ***10.*** *Department of Pathology, Stanford University School of Medicine, California, USA*

Spinocerebellar ataxia type 10 (SCA10) is a neurodegenerative disorder predominantly affecting Latin Americans with Amerindian ancestry, caused by an ATTCT repeat expansion in the ATXN10 gene. Healthy individuals have 9-32 repeats, whereas SCA10 patients exhibit 280 or more. Recent findings show expansions over 32 repeats in healthy Peruvian Amerindians, with unknown significance. Additionally, the SNP rs41524547 (C>G) is associated with SCA10 in the general population. This study aims to genotype the ATTCT-ATXN10 microsatellite and explore SNP rs41524547 in Peruvian Mestizo and Amerindian subpopulations.

DNA samples from 754 Mestizos and 117 Amerindians were selected based on age, absence of neurological disease, and self-identified ethnicity. ATTCT-ATXN10 repeats were amplified by PCR and RP-PCR followed by capillary electrophoresis. A subset of 21 Amerindians and 44 Mestizos were genotyped for SNP rs41524547 using enzyme digestion. Additionally, global and local ancestry was inferred using RFMix v.2.

A total of 1742 alleles were identified, with the most common being the 14-repeat allele (41.5%), followed by 13-repeat (21.7%) and 15-repeat (14.3%). Expanded alleles (>32 repeats) made up 4.5% (78/1742) of total alleles, with a significantly higher proportion in Amerindians (9.9%) compared to Mestizos (3.7%). SNP genotyping showed 78.4% (51/65) heterozygous (C/G) and 4.6% (3/65) homozygous (G/G). No clear difference was observed between the carriers and non-carriers of the ATXN10 expanded allele.

The presence of expanded alleles in healthy individuals with Amerindian ancestry would complicate the genetic diagnosis of SCA10. The predominance of the G allele in SNP rs41524547 among 65 healthy Peruvians challenges its risk association. Amerindian component would not explain the expanded allele frequency in Peruvians; however sample size could have prevented from detecting any signal. Further analyses are needed to understand ATTCT-ATXN10 repeat as well as the SNP rs41524547 and ancestry analysis.

This work was funded by PROCIENCIA-CONCYTEC (Contract N° 148-2020-FONDECYT).

Spinocerebellar ataxia 27B: frequency in a large, multi-center French cohort

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Céline Bonnet [1], Dr. Virginie ROTH [1], Dr. Marion WANDZEL [1], Dr. Guillemette Clement [2], Dr. Salomé Puisieux [2], Dr. David Pellerin [3], Dr. Bernard C. Brais [4], Dr. Mathilde Renaud [5], French SCA27B Network [6]

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Background and objectives

GAA repeat expansions in the first intron of *FGF14* are a common cause of autosomal dominant hereditary cerebellar ataxia (spinocerebellar ataxia 27B, SCA27B), particularly in late-onset cerebellar ataxia (LOCA). SCA27B is classically characterized by a slowly progressive cerebellar syndrome with episodic onset in more than 2/3 of cases and accounts for 10-30% of cases of unsolved ataxia in European cohorts. Here, we studied the frequency of SCA27B in a large, multi-centre French cohort.

Methods

We employed a validated three-step molecular diagnostic strategy for the screening of SCA27B: fluorescent long-range PCR (fLR-PCR) to determine allele sizes, bidirectional repeat-primed PCR (RP-PCR) to verify the presence and nature of the expansion and LR- PCR products gel electrophoresis and/or Sanger sequencing depending on the profile observed in RP-PCR. We screened a cohort of 761 patients with unsolved LOCA recruited across 40 centers in France.

Results

We identified 215/761 patients (28.3%) carrying *FGF14* (GAA)≥250 expansions including 34/761 (4.5%) with expansions between 250 and 300 GAA (intermediate allele with incomplete penetrance), and 10/761 (1.3%) with biallelic *FGF14* (GAA)≥250 expansions. Further studies are needed to help in the interpretation of these intermediate alleles. We identified non-pathogenic non-GAA expansions and GAA-interruptions.

Discussion and Conclusion

We confirmed that SCA27B is a common cause of LOCA in the French population. Detailed phenotyping of patients appears essential particularly for the interpretation of intermediate alleles.

Beyond Monogenic Disorders: A Case of Mixed SCA8 and STUB1 Phenotypes Through Digenic Inheritance

Wednesday, 13th November - 18:00: (Minories) - Poster

Hailey Segall [1], Morcos Saeed [1], Jenna Lea [1], Dr. Claudia Testa [1], Dr. Jonathan Schisler [1]

1. *University of North Carolina at Chapel Hill*

Background and Objectives: We present a 29-year-old genetic male with hypogonadism, ataxic gait, dysarthria, and cerebellar atrophy, as evidenced by MRI, with no familial precedent. Initial genetic screening identified a variant of uncertain significance (VUS) in the *STUB1* gene (c.646T>C; p.Ser216Pro), linked to SCA48 and SCAR16. The *STUB1* gene product, CHIP (C-terminus of HSC70 interacting protein), possesses multifunctional domains crucial for protein quality control. Our objective was to determine the pathogenicity of the mutation.

Methods: We conducted an exhaustive clinical evaluation, parental genetic testing, and additional SCA repeat expansion assays. Structural and thermodynamic alterations were assessed using AlphaFold2 and DynaMut.

Results: The *STUB1* variant was de novo, suggesting pathogenicity. It's located in the coiled-coil domain, rarely linked to dominant SCA48 mutations. A heterozygous CAG repeat expansion over 100 units in the *ATXN8OS* gene was identified, absent in the maternal genome. This expansion accounts for the cerebellar ataxia and atrophy observed but not the parkinsonism/tremor or hypogonadism, prompting a reevaluation of the VUS. Intriguingly, the p.Ser216Pro mutation is the only one among 19 possible mutations at Ser216 predicted to be pathogenic, with a likelihood score of 0.876. Predictive modeling indicates a ΔΔG of 0.200 kcal/mol and ΔΔSVib of -0.346 kcal/mol/K, suggesting a slight increase in protein stability and reduced flexibility. The proline mutation is predicted to form additional hydrogen bonds and dipole-dipole interactions with neighboring residues.

Discussion and Conclusion: Genetic analysis indicates a digenic inheritance of mixed SCA8 and *STUB1* phenotype, with a novel *STUB1* variant and an *ATXN8OS* CAG repeat expansion. Predictive modeling supports the pathogenic classification of the *STUB1* variant. The mutation disrupts the coiled-coil domain's flexibility, which is crucial for enzyme function and potentially explains the clinical symptoms. This case highlights the complex genetics behind disease expression and the importance of advanced genetic characterization in unusual neurodegenerative diseases.

DNA damage repair genes as modifiers of clinical outcomes in Cuban patients with Spinocerebellar Ataxia type 2

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Luis Enrique Almaguer-Mederos [1], ***Mr. Dany Cuello-Almarales*** [1], ***Dr. Raúl Aguilera-Rodríguez*** [1], ***Mr. Dennis Almaguer-Gotay*** [1], ***Ms. Yanetza González-Zaldivar*** [1], ***Dr. Suzana Gispert-Sánchez*** [2], ***Dr. Georg Auburger*** [2]

1. *Center for the Investigation and Rehabilitation of Hereditary Ataxias,* ***2.*** *Faculty of Medicine, Goethe University*

Background and objectives. DNA damage repair mechanisms have been associated with outcomes of disease severity in Huntington´s disease and spinocerebellar ataxias, and are involved in the pathogenesis of these polyglutamine disorders. Here, we assess the role of 10 single nucleotide variants (SNVs) as candidate modifiers of clinical severity in a large cohort of patients with Spinocerebellar ataxia type 2 (SCA2). **Methods.** A study involving 307 Cuban SCA2 patients was conducted. The *ATXN2* CAG repeat length was determined by PCR followed by polyacrylamide gel electrophoresis. Most SNVs (*MLH1* rs1800734, *MSH3* rs26279, *ERCC2* rs13181, *ERCC3* rs4150407, *ERCC6* rs2228528, *LIG1* Exon 6 A→C, *XRCC1* rs25487, *XRCC3* rs861539, and *OGG1* rs1052133), were assessed by PCR/RFLP; *MLH3* rs175080 was assessed by 4P-ARMS-PCR. The age at disease onset, SARA, and INAS scores were used as clinical outcome variables. Statistics were performed with SPSS software (version 20.0). **Results.** Age at onset was significantly associated with *MLH1* rs1800734, *ERCC3* rs4150407, *LIG1* Exon 6 A→C, and *OGG1* rs1052133. Nominally significant associations were also obtained between the SARA score and *XRCC1* rs25487, *XRCC3* rs861539, and *OGG1* rs1052133. Besides, the INAS score showed nominal significant associations with *MSH3* rs26279 and *MLH3* rs175080. Contributions of SNVs to clinical outcomes varied between 1.1 and 3.3 percent. **Discussion and Conclusion.** It has been shown that mutations in DNA damage repair genes cause late-onset neurodegenerative disorders and that SNVs in DNA damage repair genes modify disease severity in polyglutamine disorders. Evidence for modifier effects of SNVs on clinical outcomes of patients with SCA2 is provided, suggesting that DNA damage repair pathways might be relevant to SCA2 physiopathology. However, further studies in additional SCA2 cohorts are needed to account for the potential effects of population structure. Besides, further functional studies would be valuable to establish the mechanistic role of the relevant SNVs in SCA2 physiopathology.

A novel homozygous KCNJ10 mutation broadens the clinical spectrum of KCNJ10-related disorders

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Jaroslava Paulasová [1], ***Dr. Anna Uhrová Mészárosová*** [2], ***Dr. Ludmila Novotná*** [3], ***Dr. Michaela Kuzmiak*** [4], ***Dr. Dana Šafka Brožková*** [2], ***Dr. Martin Vyhnálek*** [5]

1. *Centre of Hereditary Ataxias, Motol University Hospital, Second Faculty of Medicine, Charles University, Prague, Czech Republic,* ***2.*** *Neurogenetic Laboratory, Department of Paediatric Neurology, Charles University, Second Faculty of Medicine and Motol University Hospital, Prague,* ***3.*** *Department of Pediatric Neurology, 2nd Faculty of Medicine, Charles University and Motol University Hospital,* ***4.*** *1) Center of Hereditary ataxias, Department of Neurology, Charles University, Second Faculty of Medicine and Motol University Hospital, Prague, Czech Republic,* ***5.*** *Center of Hereditary Ataxias, Department of Neurology, Charles University, Second Faculty of Medicine and Motol University Hospital, Prague*

Introduction: Mutations in the *KCNJ10* gene, which encodes the inwardly rectifying potassium channel Kir4.1, cause a complex syndrome characterized by seizures, sensorineural deafness, ataxia, intellectual disability, and electrolyte imbalance, known as SeSAME/EAST syndrome. One patient with non-syndromic early-onset cerebellar ataxia has been described. Until now, hereditary spastic paraparesis has not been considered as part of the clinical spectrum of this channelopathy.

Methods: Genetic and clinical investigations were performed on two sisters (S1 and S2) with early-onset progressive spastic paraparesis (combined with focal dystonia in S2) and their asymptomatic parents, who were referred to the Centre of Hereditary Ataxia at Motol University Hospital. Clinical data, medical histories, neurological examinations, laboratory tests, brain MRIs, and results of other electrophysiological methods and clinical examinations were collected.

Results: Targeted next-generation sequencing identified the previously described homozygous c. 179T>C, p.(Ile60Thr) variant in the *KCNJ10* gene (NM_002241.5) in both sisters (S1 and S2), with the same heterozygous mutation present in the father. In both patients, the clinical picture was dominated by spastic paraparesis, ataxia, and suspected cognitive deficit. Sister S1 had epilepsy starting at 6 months of age, with gait disturbances appearing in the first decade and an episode of upbeat nystagmus accompanied by worsening contractions during pregnancy. Sister S2 observed her first difficulties with running at the age of 11. Audiometry and neuropsychological examinations confirmed perceptual hearing loss and cognitive deficits in both siblings. Electrolyte imbalance/tubulopathy was absent.

Conclusion: Our findings broaden the clinical and mutational spectrum of *KCNJ10*-related disorders and suggest that screening for this gene should be performed in patients suspected of having complex hereditary spastic paraparesis.

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Identification of FXN protomutation alleles explains the unequal population distribution of Friedreich ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

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

1. *University of Oklahoma Health Sciences Center,* ***2.*** *Children's Hospital of Philadelphia*

Background: Friedreich ataxia (FRDA) is typically caused by homozygous inheritance of an expanded GAA triplet-repeat in the *FXN* gene. FRDA is seen in Europe and South Asia, where the frequency of heterozygous carriers is 1%. FRDA is not seen in Sub-Saharan Africans and East Asians. Disease-causing expanded (E) alleles have >100 triplets, and have evolved from non-disease causing Long Normal alleles (LN; 12-30 triplets). LN and E alleles are found in FRDA-susceptible populations. However, Sub-Saharan Africans are the only non-susceptible population with LN alleles that have remarkably not transitioned to E alleles.

Objective: Determine the molecular basis for the unequal global distribution of FRDA.

Methods: The entire *FXN* locus was sequenced in multiple FRDA patients using short-read and long-read technologies. Eurasian and Sub-Saharan African GAA repeat lengths were determined by long-range PCR using genomic DNA from the 1000 Genomes Project. Long-read genomic sequences of a thousand African-Americans and Africans were obtained from the All of Us Research Program. Ancient human DNA sequences were obtained from the Allen Ancient DNA Resource.

Results: Haplotype analysis of Eurasian LN and E alleles showed that E alleles arose at least twice from a subset of LN alleles. These alleles, termed *protomutation* alleles, have 20-30 triplets. All Sub-Saharan African LN alleles are devoid of this key haplotype, and have remained under 20 triplets in length. Protomutation alleles have been present in Europe and Western Asia for thousands of years.

Discussion & Conclusion: E alleles evolved from LN alleles via a key protomutation allele. This occurred exclusively in Eurasia, which explains the current population distribution of FRDA.

Practice Recommendations for Genetic Testing of Hereditary Ataxias

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Sharan Srinivasan [1], ***Ms. Amy Mook*** [1], ***Ms. Wendy Uhlmann*** [1]

1. *University of Michigan*

Background and Objectives: Genetic testing is becoming an invaluable diagnostic tool in the workup of patients with ataxia. However, there is a lack of guidance in the literature for neurologists considering genetic testing for patients and determining when formal genetic counseling is indicated. To fill this gap, we developed practice recommendations for clinical neurologists.

Methods: We completed a literature review and polled clinical neurologists and genetic counselors at several major academic centers with ataxia expertise. We also performed a national patient survey to assess experience with ataxia genetic testing. The genetic testing protocol for Huntington's Disease was adapted to incorporate condition differences to be specific to hereditary ataxias.

Results: We present proposed practice recommendations for genetic testing in patients with suspected hereditary ataxia. Topics covered include addressing different testing methodologies and limitations, informed consent, and insurance implications. Indications for genetic counseling include predictive and prenatal testing. Other special circumstances (e.g. intermediate alleles, anonymous testing requests) are also discussed.

Discussion and Conclusion: These recommendations will be informative for all clinical neurologists, especially those without access to genetics professionals, and help promote equity and efficiency in patient testing. We intend to formalize these recommendations with the input and approval from national physician and genetic counselor leaders in ataxia, in conjunction with the National Ataxia Foundation. Ensuring that both patients and providers are given adequate information to pursue genetic testing confidently and safely is paramount in clinical care.

Genetic diagnostics in ataxia patients - a clinical perspective

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Astrid Nümann [1], ***Prof. Andrea Kühn*** [1], ***Dr. Claudia Dufke*** [2], ***Dr. Tobias Haack*** [2]

1. *Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Neurology, Berlin, Germany,* ***2.*** *U Tuebingen*

Objectives

We aimed to investigate the additional diagnostic benefit of genome and transcriptome sequencing in the investigation of ataxia patients who could not be clearly diagnosed after exome sequencing.

Methods

Genome and transcriptome analyses were carried out on selected patients from the ataxia clinic at the Charité in Berlin. Following the publication of FGF14 repeat expansions as a major cause of late onset ataxia, conventional fragment length testing was supplemented.

Results

In 40 patients with negative exome diagnostics, additional genome diagnostics were carried out and in 5 patients disease-causal variants in known disease genes were identified. We observed alterations in*COQ2* (primary coenzyme Q10 deficiency-1), *DAGLA* (developmental delay, ataxia and complex oculomotor abnormalities) and *SPEN* (radio-Tartaglia syndrome). In two patients, smaller deletions were found to be the cause of the symptoms. Candidate variants in new disease genes were detected in three patients. *FGF14* repeat expansions were identified in 38 out of 85 patients. In three patients with different heterozygous *STUB1* variants, which have been described as recessive in the literature, a transcriptome analysis was performed and evidence of nonsense mediated decay was detected in one patient. This is an indication that this variant may cause autosomal dominant ataxia.

Discussion

The additional analyses significantly improved the diagnostic yield in patients with ataxia. This is particularly important for genetic counseling on disease progression, prognosis and risk of recurrence. Due to the therapeutic influence of FGF14-repeat expansion with 4-aminopyridine and Diamox in some patients, diagnostics should be offered early in the diagnostic work-up.

Conclusion

The diagnostic yield was significantly increased after publication of the FGF14 repeat expansion as the most common cause of late onset ataxia. Genome diagnostics should be favored for complex ataxia patients. In selected cases, a transcriptome analysis may be helpful.

Exploring the genetic architecture of RFC1 CANVAS/spectrum disorder: a genome-wide association study

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Riccardo Curro [1]***, Prof. Henry Houlden*** [2]***, Dr. Andrea Cortese*** [3]***, on the behalf of the RFC1 expansion study group*** [3]

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Objectives

Cerebellar ataxia with neuropathy and vestibular areflexia syndrome (CANVAS) is an autosomal recessive repeat expansion disorder (RED) resulting from biallelic repeat expansions in *RFC1*. The disease exhibits significant heterogeneity in terms of age of onset (AOO) and disease severity. However, the repeat size accounts only for 6% of the variability of AOO, suggesting that additional factors, such as genetic modifiers, may be at play. We aim to conduct a GWA analysis to identify loci harbouring genetic modifiers of AOO and other clinical variables in *RFC1* CANVAS/spectrum disorder.

Materials and methods

DNA samples of *RFC1*-positive patients will be collected from all the collaborating centres. Clinical and demographic variables will be recorded. Genotyping will be conducted using the Illumina Global Clinical Research Array (GCRA). Additional custom content (~ 3,000 single nucleotide polymorphisms, SNPs) encompassing reported SNPs meaningful to other REDs along with SNPs surrounding the repeat locus have been added to the backbone of the standard GCRA (~ 1,204,769 SNPs). Notably, the same arrays are being used to genotype SCA and FGF14 samples in a complementary study.

Long-read sequencing will be used to sequence the *RFC1* repeat locus and flanking regions, as well as the GWAS loci harbouring genetic variations.

Results

To date, 600 DNA samples of *RFC1* positive patients have been already collected from 35 centres across the world and the enrolment is still ongoing.

Conclusions

The proposed study holds promise for enhancing our understanding of the genetic determinants in RFC1 CANVAS/spectrum disorder, thereby facilitating more precise prognostic modelling of the disease. Additionally, it is likely this research will provide seminal information about the disease mechanisms and, therefore, potentially druggable targets. To note, a similar modifier study in GAA-FGF14 ataxia and SCAs is ongoing and analyses will be carried out to identify modifiers shared by these inherited ataxic disorders.

DNA REPAIR GENES - ERCC6, PMS2, FAN1 AND MLH1 - AS POTENTIAL MODULATORS OF AGE AT ONSET AND INSTABILITY OF THE CAG REPEAT IN MACHADO-JOSEPH DISEASE

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Ana Rosa Vieira Melo [1], ***Ms. Daniela Benevides*** [2], ***Dr. Mafalda Raposo*** [3], ***Mrs. Sara Pavão*** [2], ***Dr. Mariana Santos*** [4], ***Prof. Jorge Sequeiros*** [5], ***Dr. Ahmed Sidky*** [6], ***Prof. Darren G. Monckton*** [6], ***Prof. Manuela Lima*** [7]

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Variation in DNA repair genes has been proposed to modulate age at onset (AO) of polyglutamine (polyQ) diseases by acting as somatic instability mediators of the expanded CAG tract. In Machado-Joseph disease (MJD), the size of the expanded allele at the *ATXN3* locus accounts for 50 to 75% of the variation of AO, with only a small fraction of the remaining variation explained by genetic modifiers already identified. In this study, in addition to *FAN1*, which has been reported as a modulator of AO in a Brazilian MJD cohort (n=144) we analyzed variants in *ERCC6*, *PMS2*, and *MLH1*, using 185 blood DNA samples from Portuguese patients, to determine its impact on AO and in somatic expansion of the CAG tract. Genotypes for variants rs2228528 (*ERCC6*), rs1805323 (*PMS2*), rs3512 (*FAN1*), and rs1799977 (*MLH1*) were obtained by Sanger sequencing. The number of CAG repeats and the blood somatic expansion ratio (n=93) were obtained by high-throughput ultra-deep MiSeq amplicon sequencing and were available from a previous study. After adjusting for the number of CAGs in the expanded allele, no effect of the four variants on AO was detected; noteworthy, our sample size calculations indicate a power of 80% to detect at least small differences between groups. Non-replication of the modifying effect of *FAN1* across cohorts might reflect, amongst other factors, differences in the frequencies of the variant analyzed between the Portuguese (GG: 0.447, GC: 0.274, CC: 0.279) and the Brazilian (GG: 0.590, GC: 0.333, CC: 0.077) patients (exact test of population, $p<0.001$). Correlation between somatic expansion ratio and presence/absence of each variant, adjusted for age at blood collection, revealed no differences between groups. Further studies are needed to elucidate the role of DNA repair gene variants in MJD pathogenesis.

DNA enrichment for PCR-free sequencing of long tandem repeat expansions: a new method for neurodegenerative disease diagnostics

Wednesday, 13th November - 18:00: (Minories) - Poster

Mr. Raul Cuellar [1], ***Mr. Yilin Liu*** [2], ***Ms. Helena Merck*** [3], ***Ms. Ana Tomac*** [1], ***Prof. Jesper Wengel*** [4], ***Prof. Edvard Smith*** [1], ***Prof. Peter Savolainen*** [2], ***Dr. Rula Zain*** [1]

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Tandem repeats (TRs), consisting of DNA units of one to six base pairs (Short Tandem Repeats, STRs) or longer sequences (Long Tandem Repeats, LTRs), constitute about 3% of the human genome, with approximately 500,000 TRs mapped. Our group focuses on diagnosing and treating disorders related to trinucleotide repeat expansions, this study focuses on Friedreich's Ataxia (FRDA) .

The project aims to develop a method for enriching GAA • TTC regions of the *FXN* gene, which are implicated in FRDA. Accurate diagnosis and treatment validation of FRDA depend on precisely evaluating the number of repeats. Traditional diagnostic techniques like PCR or Southern Blot are inadequate for analysing these large repeat sequences.

This project employs third-generation PCR-free Nanopore sequencing, which can read large genomic regions but requires significant amounts of target DNA. Therefore, optimizing the enrichment of STR regions is essential. We have developed an enrichment method using a pull-down strategy. By enriching TRs regions and utilizing PCR-free sequencing, the goal is to improve FRDA diagnostic accuracy and treatment validation.

We have developed a method using using genomic DNA (gDNA) from healthy and FRDA cell lines. We have captured and enriched the target gene locus of the *FXN* gene in FRDA and healthy cell lines. The expanded *FXN* locus (FRDA) was enriched to 62 -175 folds from total gDNA, and the healthy *FXN* locus was enriched to 30-50 folds.

The enriched DNA fragments, from either healthy or affected (FRDA), were sequenced on the Nanopore instrument. This demonstrates that the procedure works for PCR-free enrichment and for native DNA sequencing of the expanded and non expanded *FXN* locus. This approach offers a robust framework for molecular diagnostics and gene-directed therapies, promising improved diagnostic accuracy and therapeutic validation for TR-dependent diseases.

Bridging the gap: a prospective trial of short-read genome sequencing and adaptive long-read sequencing for genetic diagnosis of cerebellar ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Haloom Rafehi [1], Dr. Liam Fearnley [1], Dr. Justin Read [2], Ms. Penny Snell [3], Ms. Kayli Davies [2], Mr. Liam Scott [4], Mrs. Greta Gillies [3], Ms. Genevieve Thompson [2], Ms. Tess Field [3], Dr. David J. Szmulewicz [5], Prof. Martin Delatycki [6], Prof. Melanie Bahlo [1], Prof. Paul Lockhart [2]

1. *Walter and Eliza Hall Institute for Medical Research; University of Melbourne,* ***2.*** *Murdoch Children's Research Institute; University of Melbourne,* ***3.*** *Murdoch Children's Research Institute,* ***4.*** *Walter and Eliza Hall Institute for Medical Research,* ***5.*** *Balance Disorders & Ataxia Service, Royal Victorian Eye and Ear Hospital,* ***6.*** *Murdoch Children's Research Institute; University of Melbourne; Victorian Clinical Genetics Service*

Background and Objectives: Repeat expansions (RE) cause seventeen cerebellar ataxias (CA), but diagnostic testing is generally limited and expensive. Standard clinical testing in Australia is only available for six pathogenic RE, with diagnostic rates of ~5%. This research aims to develop the evidence base for Next Generation sequencing technologies as a frontline diagnostic test for CA.

Methods: A cohort of 110 Australian individuals with a clinical diagnosis of CA were recruited and underwent standard clinical testing (Ataxia panel: SCA1, 2, 3, 6 & 7). For research testing ~300 genes associated with CA were analysed, including 17 pathogenic RE. Short-read genome sequencing (GS) was performed with TruSeq PCR-free libraries (Illumina) with variants analysed with ExpansionHunter/exSTRa (RE), SeqR (SNV) and CXGo (CNV). Long-read sequencing was performed using adaptive sampling [Oxford Nanopore Technologies, (ONT)] and variants were assessed using STRaglr/Tandem Genotypes (RE), Clair3 (SNV) and QDNASeq (CNV).

Results: Participants (48F/62M) had CA with mean onset at 56±14 years (range 15-77) and age at testing 68±13 years (range 29-88). All were undiagnosed following standard care. Short-read GS identified pathogenic variants in 36% of the cohort (40/110). This included 33 RE (24x SCA27B, 5x RFC1, 2x SCA8, 1x FRDA and 1x SCA36) and 7 non-RE disorders. Sensitivity and specificity for SCA27B was 100% and 80% respectively. While analysis of adaptive sequencing is ongoing, preliminary results identified RE in 31 individuals. A result was not achieved for two cases positive by short-read GS (1x SCA27B, 1x SCA36). Sensitivity and specificity for SCA27B was 96% and 100% respectively.

Discussion and Conclusion: Implementation of either technology tested in this trial will dramatically improve diagnostic outcomes for CA. Currently, short-read sequencing provides a higher yield and is a mature technology. ONT potentially offers additional clinical utility by determining RE size and sequence,but requires additional optimisation for clinical implementation.

Spatial and cell type specific expression patterns of autosomal dominant ataxias based on distinct clinical phenotypes

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Fred Shen [1], ***Dr. Alessandro Didonna*** [2], ***Dr. Chiranjit Panja*** [1], ***Dr. Puneet Opal*** [1]

1. *Northwestern University,* ***2.*** *eastern carolina university*

Inherited ataxias affect at least 150,000 people in the United States. They share a common ataxia phenotype characterized by poor coordination and gait imbalance. However, inherited ataxias can also have many distinct phenotypes beyond pure ataxia including seizures, cognitive impairment, dystonia, and neuropathy. In this study, we examined whether distinct phenotypes in autosomal dominant ataxia syndromes have any implications for disease pathophysiology.

We used an unbiased systematic approach to create a comprehensive list of genes associated with autosomal dominant ataxias, categorized by distinct clinical features (i.e seizure, dystonia, etc). We then performed gene ontology analysis to dissect unique pathways for different ataxia plus phenotypes as well as transcriptomic analyses using published single cell RNA sequencing and spatial transcriptomic databases. We analyzed the cell type expression of different ataxia genes based on phenotype in mouse cerebellum. We then used a whole brain spatial transcriptomic dataset to examine RNA expression across brain region to ask whether there were distinct spatial expression patterns for ataxia genes based on clinical phenotypes.
Different ataxia plus phenotypes yielded unique GO terms. For example, ataxia + neuropathy was associated with axo-dendritic transport while ataxia + seizures was associated with synapses and nervous system development. We also found that the majority of genetic ataxias do not have preferential expression in purkinje neurons or even the cerebellum. There appeared to be no region specific expression pattern for ataxia genes based on clinical phenotypes.

Autosomal dominant inherited ataxias have a diverse range of phenotypes and heterogenous mechanisms of disease. Although the cerebellum is the most well known brain region implicated in ataxia, our results suggest that dysfunction of other brain regions can also result in disease. This makes sense as many brain regions beyond the cerebellum are involved in coordination and balance circuits.

DIGENIC STUB1 AND TBP REDUCED PENETRANCE EXPANSIONS ARE UNCOMMON IN A UK COHORT

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Jussi-Pekka Tolonen [1], ***Dr. David Sims*** [2], ***Dr. Kevin Rue-Albrecht*** [2], ***Prof. George Tofaris*** [3], ***Dr. Nicholas Beauchamp*** [4], ***Dr. Andrea Cortese*** [5], ***Dr. Arianna Tucci*** [6], ***Prof. Esther B. E. Becker*** [7], ***Prof. Marios Hadjivasssiliou*** [8], ***Prof. Andrea Nemeth*** [7]

1. *University of Oulu,* ***2.*** *Weatherall Institute of Molecular Medicine, University of Oxford,* ***3.*** *Nuffield Department of Clinical Neurosciences, University of Oxford,* ***4.*** *Sheffield Diagnostic Genetics Service, Sheffield Children's NHS Foundation Trust,* ***5.*** *Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London, WC1N 3BG, UK.,* ***6.*** *William Harvey Research Institute, Barts and the London School of Medicine, Queen Mary University of London, London, EC1M 6BQ, United Kingdom.,* ***7.*** *University of Oxford,* ***8.*** *Sheffield Ataxia Centre*

Background and Objectives

Spinocerebellar ataxia type 17 (SCA17; CAG expansion repeats in *TBP*) and type 48 (SCA48; single-nucleotide variants (SNVs) in *STUB1*) are commonly associated with cognitive impairment and late-onset ataxia. Reduced penetrance (RP) is observed for both diseases. Recently, RP alleles in *TBP* (repeat size range 41-49) and heterozygous *STUB1* variants were proposed to co-exist, causing a digenic disorder. Our objective was to determine the prevalence of digenic cases in a UK cohort.

Methods

Ataxia cases with *STUB1* variants were identified in two diagnostic laboratories, and in the 100,000 Genomes (100KGP), a research project containing clinical/whole genome sequencing (WGS) on >85k UK individuals. *STUB1* SNVs were classified as likely/pathogenic using ACMG guidelines. *TBP* repeat sizing was performed by PCR or Expansion Hunter. Additional ataxia cases with*TBP-RP* alleles were also identified.

Results

Of 62 individuals with likely/pathogenic variants in *STUB1* and cerebellar ataxia +/-cognitive impairment, only 1/62 had a *TBP-RP* (41 repeats), detected using PCR. In two cases, TBP was sized using both PCR and WGS. There was limited correlation of the sizes, but neither detected *TBP-RP* alleles. We found that current short-read NGS methods for *TBP* repeat sizing are accurate only at 40 repeats or more.

2 cases with *TBP-RP* expansions detected by PCR had known pathogenic mutations in *CACNA1A*.

7/16 *TBP-RP* expansions identified in the 100KGP ataxia cases had mutations in other known ataxia genes, the remainder had no other mutations identified.

Discussion and Conclusions

We found little evidence for *TBP-RP/STUB1* digenic cases in two UK datasets. 9 cases with *TBP-RP alleles* had pathogenic mutations in other ataxia genes. The role of *TBP-RP* as a modifier remains uncertain and is complicated by difficulties in sizing the repeats. We propose that standardised measures for *TBP* repeat sizing are developed to determine the contribution of *TBP-RP* alleles to neurodegenerative disorders.

The clinical and genetic scenario in GAA-FGF14 ataxia (SCA27B): a multicenter Italian study

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Sara Satolli [1], Dr. Sirio Cocozza [2], Dr. Alessandra Tessa [1], Dr. David Pellerin [3], Dr. SCA27B Network Italian Network [1], Dr. Bernard C. Brais [4], Dr. Filippo Maria Santorelli [1]

1. *IRCCS Fondazione Stella Maris,* ***2.*** *Università Federico II Napoli,* ***3.*** *Dr. John T. Macdonald Foundation Department of Human Genetics and John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL, USA,* ***4.*** *Department of Neurology and Neurosurgery, Montreal Neurological Hospital and Institute, McGill University, Montreal, QC, Canada*

Background and objectives. Autosomal dominant spinocerebellar ataxia due to intronic GAA repeat expansion (RE) in the *FGF14* gene (GAA-*FGF14* ataxia; SCA27B) is a recently identified, potentially treatable form of late onset ataxia. Here, we aimed to characterize the clinical phenotype in Italian GAA-*FGF14* patients collected in a network study of 18 third-level centers.

Methods. We analyzed the clinical, genetic and neuroradiological features of 77 index cases carrying a heterozygous GAA-*FGF14* RE. We also focused on 4-aminopyridine (4-AP) treatment response. Longitudinal clinical records were systematically assessed according to a comprehensive eCRF data form. We assessed disease severity and progression by using the Scale for the Assessment and Rating of Ataxia (SARA), the Friedreich Ataxia Rating Scale functional disability stage (FARS-DS) and functional impairment in terms of mobility aids. Finally, we analyzed longitudinal SARA scores by linear regression over disease duration.

Results. In our cohort, the prevalence of SCA27B was 13.4% (with as high as 38.5% in ADCA. The median age of onset of SCA27B patients was 62 years. Younger presentations in teenagers are possible, however. All symptomatic individuals showed evidence of impaired balance and gait; cerebellar ocular motor signs were also frequent. Episodic manifestations at onset occurred in 31% of patients. Extrapyramidal features (17%) and cognitive impairment (25%) were also reported. Brain magnetic resonance imaging showed cerebellar atrophy in most cases (78%). Pseudo-longitudinal assessments indicated slow progression of ataxia and minimal functional impairment.

Discussion and Conclusion. Consistent with previous reports, Italian GAA-*FGF14* patients present a slowly progressive cerebellar ataxia with predominant impairment of balance and gait and frequent cerebellar oculomotor signs. The high consistency of clinical features in SCA27B cohorts in multiple populations paves the way towards large-scale, multicenter studies.

A Spanish network for genetic studies in unsolved ataxia: description and first studies in SCA27B

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Pablo Iruzubieta [1], ***Dr. Ines Albajar*** [2], ***Dr. Astrid Daniela Adarmes Gómez*** [3], ***Dr. Raquel Baviera*** [4], ***Dr. Idoia Rouco*** [5], ***Dr. Leire Manrique*** [6], ***Dr. Jorge Alonso-Perez*** [7], ***Dr. Germán Moris*** [8], ***Dr. Paula Pérez Torre*** [9], ***Dr. José Gazulla*** [10], ***Mrs. Lorena Garayoa*** [2], ***Dr. Montserrat Ruiz*** [11], ***Dr. Silvia Martí*** [12], ***Dr. Laura Rojas*** [13], ***Dr. Eva Fages*** [14], ***Dr. Inmaculada Pagola*** [15], ***Dr. Virginia García Solaesa*** [15], ***Dr. Jesús Pérez Pérez*** [16], ***Dr. Gonzalo Olmedo*** [16], ***Ms. Berta Alemany-Perna*** [17], ***Dr. María Obon*** [18], ***Dr. Teresa Muñoz*** [19], ***Dr. Francisco Javier Rodríguez de Rivera*** [20], ***Dr. Irene Sanz-Gallego*** [21], ***Dr. Inés García*** [22], ***Dr. Antonio Gutierrez Martínez*** [23], ***Dr. María Eugenia Marzo*** [24], ***Dr. Javier Ruiz-Martínez*** [25], ***Dr. David Pellerin*** [26], ***Prof. Henry Houlden*** [27], ***Dr. Bernard C. Brais*** [28], ***Dr. Elisabet Mondragón*** [25], ***Dr. Ana Vinagre*** [25], ***Dr. Claudio Catalli*** [5], ***Dr. Raul Juntas*** [29], ***Dr. Izaro Kortazar*** [30], ***Dr. Jone Bocos*** [30], ***Dr. Beatriz Castillo*** [31], ***Dr. Solange Kapetanovic*** [31], ***Dr. Miguel Angel Rubio*** [32], ***Dr. María Jesus Sobrido*** [33], ***Dr. Antonio José Mendez*** [34], ***Dr. Victoria Alvarez*** [35], ***Dr. Pablo Mir*** [3], ***Prof. Jon Infante*** [36], ***Dr. Luis Bataller*** [37], ***Dr. Aurora Pujol*** [11], ***Dr. Adolfo Lopez de Munain*** [38], ***Dr. Carlos Casasnovas*** [11], ***Dr. Pilar Camaño*** [2]

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In the last years, new genes and repetitive motif expansions have emerged as the cause of previously unsolved genetic cerebellar ataxia, especially in late-onset ataxia.

In this context, we have created a national network including 30 hospitals from all over Spain to collect clinical data and biosamples from patients with cerebellar ataxia of unknown cause to explore recently described genetic causes as well as search for new genes involved in ataxia. This project was approved by the Euskadi and Bellvitge Ethics Committee and ratified by the local Committees. Our proposed workflow includes screening for GAA expansions in FGF14 (SCA27B) followed by short-read whole genome sequencing and, in selected patients, long-read whole genome sequencing.

At this point, with recruiting still ongoing, we have included samples from 220 patients from 9 hospitals, and 141

patients have been screened for SCA27B. 36 patients were positive for a GAA expansion over 249 repeats, showing a positivity rate of 26%. 64% were men. Mean age at onset was 61 years old (range, 34-73). Mean age at last evaluation was 74 years old (range, 54-88). 30% had an episodic onset (9/30) and 37% showed downbeat nystagmus in examination (11/30). Mean phenotype was pure ataxia (77%, 23/30), followed by ataxia and neuropathy (17%, 5/30).

In conclusion, this initiative aims to create a Spanish network to move forward genetic research in ataxia in Spain and collaborate with other groups internationally. Our first results show SCA27B is a common cause of late-onset ataxia in Spain, explaining around one-quarter of unsolved cases in our country.

This project is supported by the Spanish Ministry of Science and Innovation - Instituto de Salud Carlos III (ISCIII), grant PI23/01090.

SCREENING FOR SCA27B IN A COHORT OF BRAZILIAN PATIENTS WITH UNSOLVED ATAXIAS

Wednesday, 13th November - 18:00: (Minories) - Poster

Mrs. Amanda Dias [1], Mrs. Adriana Mendes Vinagre [1], Ms. Cynthia Silveira [1], Dr. Fabrício Lima [2], Dr. José Luiz Pedroso [3], Ms. Luciana Bonadia [1], Ms. Luiza Corazza [1], Dr. Orlando Barsottini [3], Mr. Thiago Tonholo [3], Prof. Marcondes França Jr [2]

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Spinocerebellar Ataxia 27B (SCA27B) is a recently described neurodegenerative disease, prevalent in Europe and North America, but not yet properly investigated in Latin America. Since it is an expansion disorder, diagnosis requires specific molecular techniques. In this scenario, the aims of this study are: 1. to standardize GAA expansion genotyping techniques in Brazil and 2. to screen a large cohort of adults with unsolved ataxias for SCA27B. To date, 108 patients with ataxia were recruited, including patients negative for SCA1,2,3,6,7 and 8. DNA was extracted from peripheral blood and analyzed using different PCR techniques. Initially, long-range PCR (LR-PCR) was used, resulting in 90 patients with expansions < 250 repeats (negative). Eighteen patients had a single normal allele and were submitted to Repeat-primed PCR (RP-PCR), which confirmed 13 to be negative. Five out of the 108 patients tested positive so far (4.6%). The phenotype was a late-onset slowly progressive ataxia in all cases with paroxysmal symptoms in 3/5 and pyramidal signs in 1/5. None of them had sensory manifestations. The mean age at onset was 55 years. Our results are in line with previous publications, which showed that SCA27B ataxia presents initial symptoms at an advanced age and has slow progression. The prevalence of SCA27B we found (4.6%) was much smaller than in French Canadian (61%) and European (18%) cohorts. In conclusion, LR-PCR followed by RP-PCR is a reliable and fast approach to diagnose SCA27B. The phenotype of Brazilian patients with SCA27B is similar to previous reports, but the frequency is much smaller. Further studies are needed to understand the genetic epidemiology of this new disease beyond Europe and North America.

Defective m6A methylation of EPRS1 mRNA causes progressive ataxia, spasticity, rotatory nystagmus, global developmental delays and hypomyelination

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Grace Yoon [1], ***Dr. Debjit Khan*** [2], ***Dr. Iyappan Ramachandiran*** [2], ***Dr. Kommireddy Vasu*** [2], ***Dr. Arnab China*** [2], ***Dr. Krishnendu Khan*** [2], ***Dr. Fabio Cumbo*** [2], ***Dr. Dalia Halawani*** [2], ***Dr. Fulvia Terenzi*** [2], ***Dr. Isaac Zin*** [2], ***Dr. Briana Long*** [2], ***Dr. Susan Blaser*** [1], ***Dr. Valentin Gogonea*** [2], ***Dr. Ranjan Dutta*** [2], ***Dr. Daniel Blankenberg*** [2], ***Dr. Paul Fox*** [2]

1. *The Hospital for Sick Children, University of Toronto,,* ***2.*** *Lerner Research Institute, Cleveland Clinic*

Objectives: We describe two siblings with severe cognitive and motor impairment due to progressive ataxia, spasticity, and hypomyelination on serial brain imaging, and undertook a multi-omics approach to elucidate the underlying genetic cause of this disorder

Methods: Exome sequencing revealed both siblings were homozygous for a missense single-nucleotide variant (SNV) in *EPRS1* (c.4444C>A; p.Pro1482Thr), encoding glutamyl-prolyl-tRNA synthetase. Extensive molecular and protein studies were conducted to determine the functional impact of the variant.

Results: Patient lymphoblastoid cell lines exhibited normal EPRS1 specific aminoacylation activity, but markedly reduced EPRS1 protein due to dual defects in nuclear export and cytoplasmic translation of variant *EPRS1* mRNA. Variant mRNA exhibited reduced METTL3 methyltransferase-mediated writing of N^6-methyladenosine (m^6A) and reduced reading by YTHDC1 and YTHDF1/3 required for efficient mRNA nuclear export and translation, respectively.

Discussion: We show the variant reduced EPRS1 expression in patient cells by inhibiting m6A modification of requisite mRNA target sites. Importantly, the variant did not alter m6A site sequence, as observed for other disease-associated genetic variants, but instead masked accessibility of variant-distal mRNA m6A sites, thereby reducing their availability for modification. The defect was rescued by antisense morpholinos predicted to expose m^6A sites on target *EPRS1* mRNA, or by m^6A modification of the mRNA by METTL3-dCas13b, a targeted RNA methylation editor. Additional bioinformatic analyses predicted widespread occurrence of SNVs associated with human disease that similarly alter accessibility of distal mRNA m^6A sites.

Conclusion: These results reveal a new RNA-dependent etiologic mechanism by which SNVs can influence gene expression and cause disease, and suggest novel mRNA-targeted therapeutic approaches.

A new cases series suggests that SCA48 (ATX/STUB1) is primarily a monogenic disorder

Wednesday, 13th November - 18:00: (Minories) - Poster

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Introduction A genetic interaction between *STUB1* variants and *TBP* alleles with intermediate or high-normal (TBP_{40}) CAG/CAA repeat expansions was recently suggested. Pathogenic *STUB1* variants were detected in many ataxic carriers of $TBP_{41\text{-}47}$ intermediate expansions, and vice versa, possibly explaining the incomplete penetrance of *TBP* intermediate alleles. Others suggested a more disease-modifying effect of *TBP* expansions, as carriers of intermediate *TBP* expansions had more severe cognitive disturbances.

Methods We systematically determined *TBP* repeat length in 21 ataxic patients carrying a heterozygous *STUB1* variant to test for a genetic interaction between *STUB1* variants and intermediate or high-normal CAG/CAA repeats in *TBP*, associated with ATX-*TBP*/SCA17.

Results 15 out of 21 patients (71%) carried a normal $TBP_{<40}$ allele, four (19%) carried an intermediate $TBP_{41\text{-}42}$ allele, and two carried a high-normal TBP_{40} allele (9.5%). Longer intermediate *TBP* alleles were not found. Five out of six carriers (83%) of both *STUB1* variants and $TBP_{40\text{-}42}$ alleles exhibited marked cognitive impairment.

Discussion We detected intermediate or high-normal *TBP* alleles in a minority of our *STUB1* cohort. However, the co-occurrence of *TBP* high-normal or short intermediate *TBP* alleles was more frequent than expected in the general population. While patients with isolated *STUB1* variants also manifested cognitive disturbances, there was a suggestion that those with $TBP_{40\text{-}42}$ expansions presented with more pronounced cognitive defects.

Conclusion In our cohort, SCA48 was predominantly a monogenic disorder, with most patients carrying an isolated, heterozygous *STUB1* variant and presenting with the typical combined phenotype of ataxia and cognitive dysfunction. Still, co-occurrence of $TBP_{41\text{-}42}$ or high-normal TBP_{40} alleles was relatively frequent and with the suggestion of a modifying effect on clinical expression in some cases. Complementary analysis of *STUB1* or *TBP* length should be considered as combined interpretation is relevant for diagnostic and counselling purposes.

Type and position of repeat interruptions as determinants of disease severity and expansion size in Friedreich ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

Prof. Michel Koenig [1], Dr. Mehdi Benkirane [1], Dr. Cecilia Marelli [2], Dr. Safa Aouinti [1], Prof. Nicolas Molinari [1], Prof. Ariane Choumert [3]

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Background and objectives: In Friedreich ataxia (FRDA), the most important determinant of disease onset and severity is expansion size on the smaller of the two expansions. However, expansion size accounts for only 36–56 % of the variation in age of onset. Interruption motifs in large repeat alleles are well-known contributors to reduced instability and severity modulation in inherited expansion diseases.
Methods: we investigated both the sequence motif of the interruptions and their precise position with respect to the 3' end of the expansion in a cohort of 164 Friedreich ataxia (FRDA) patients with biallelic expansions, as well as 15 non-FRDA patients having a normal/pre-mutation/expansion allele with non-GAA interruption(s) in *FXN*.
Results: The majority of FRDA patients have a few interruptions (5 or less on a single allele) that often affect repeat length (referred to as non-triplet interruptions), while most interruptions in controls are multiple GGA or GAG interruptions (referred to as triplet interruptions), suggesting that multiple interruptions hamper further expansion, and even pathogenicity at least in the small expansion size range. We also identified that interruptions in FRDA patients correlate with size of the smaller of the two expansions, and with age at onset of ataxia, in a relation that depends on the distance of the interruptions to the 3' end of the expansion (referred to as the "depth" of the interruption).
Discussion and conclusion: Both precise position and motif of the interruptions play an important role in expansion size determination and pathogenicity in FRDA. On the basis of these data we recommend the analysis of interruptions not only for research purpose but also for a correct diagnosis of FRDA expansions and pre-mutations.

From Non-progressive Congenital Ataxia to Severe Epileptic Encephalopathy with cerebro-cerebellar atrophy and neuropathy in GEMIN5-related disorders.

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Ginevra Zanni [1], ***Dr. Gabriele Piccolo*** [2], ***Dr. Alessandra Terracciano*** [2], ***Dr. Paola De Liso*** [3], ***Dr. Concetta Luisi*** [3], ***Dr. Marcello Niceta*** [4], ***Dr. Antonio Novelli*** [2], ***Dr. Marco Tartaglia*** [4], ***Dr. Enrico Bertini*** [5], ***Dr. Adele D'Amico*** [6], ***Dr. Daria Diodato*** [6]

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Background and objectives: GEMIN5 is a multifunctional RNA binding protein that controls SMN (Survival of the Motor Neurons) protein complex assembly and pre-mRNA splicing. In the cytoplasm GEMIN5 is involved in RNA transport along the axon and ribosomal translation. Pathogenic variants in *GEMIN5* were identified in patients with developmental delay and ataxia associated with cerebellar atrophy. Three patients presenting a SMA-like phenotype with early death were reported. **Methods**: Here we present two unrelated patients with novel *GEMIN5* missense variants identified by trio-based Whole Exome Sequencing. **Results:** The first patient is a 2,5 year-old boy harboring a novel homozygous GEMIN5 variant (p.V995E) presenting a severe phenotype characterized by severe hypotonia and respiratory distress at birth, epileptic encephalopathy, progressive cerebro-cerebellar atrophy and mixed sensorimotor peripheral neuropathy. The second patient is a 30 year-old man with congenital non- progressive ataxia carrying compound heterozygous GEMIN5 variants inherited from the parents; a known hypomorphic recurrent variant (p. R1016C) and a novel (p. W94R) variant. Neuroimaging studies revealed a moderate global atrophy of the vermis and cerebellar hemispheres. **Discussion and Conclusion**: *GEMIN5* screening should be implemented in patients with phenotype ranging from Nonprogressive Congenital Ataxia (NPCA) to SMN-negative SMA-like features and seizures. Further studies are necessary to better understand the physiopathological mechanisms underlying the extreme phenotypic spectrum of GEMIN5-related disorders, paving ways to variant-tailored therapies

Absence of SCA27B and unique allelic distribution in Peruvian patients with undiagnosed cerebellar ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Ana Saldarriaga-Mayo [1], ***Mr. Ismael Araujo-Aliaga*** [1], ***Ms. Susan Echavarria-Correa*** [1], ***Ms. Marie-Josée Dicaire*** [2], ***Dr. Matt C. Danzi*** [3], ***Mr. Stephan Zuchner*** [3], ***Dr. David Pellerin*** [3], ***Dr. Bernard C. Brais*** [2], ***Prof. Mario Cornejo-Olivas*** [1]

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Spinocerebellar ataxia 27B (SCA27B) is a newly discovered form of hereditary ataxia characterized by the expansion of an intronic GAA repeat in the *FGF14* gene. It is characterized by a late-onset, slowly progressive cerebellar syndrome with frequent episodic symptoms. Although SCA27B has been described in different populations, its prevalence and genetic characteristics in the Peruvian population are poorly understood. The objective is to estimate the frequency of SCA27B in a Peruvian cohort of patients with hereditary ataxias without a definitive diagnosis treated at the Neurogenetics Research Center (CIBN).

DNA samples were obtained from 166 Peruvian individuals with hereditary ataxia without definitive diagnosis, with informed consent. Genotyping of the GAA repeat in the *FGF14* gene was performed using a workflow including long-range PCR, bidirectional RP-PCR, and Sanger sequencing.

No cases with an expanded GAA repeat above 250 were found. Following genotyping, we found no patients carrying a pathogenic expansion of at least 250 GAA-pure repeats. 27 cases (16%) were found to carry a complex [(GAA)n(GCA)m] expansion ranging in size from 310 to 332 triplet equivalents, which is thought to be non-pathogenic for SCA27B. Normal alleles were found in the range of 7 to 127 repeats, the most common being 8 repeats (84/332; 25.3%).

The frequency of SCA27B caused by *FGF14* GAA repeat expansion in our Peruvian cohort is zero, although a large proportion of patients carried a complex non-GAA-pure expansion. The allelic distribution of the GAA repeat among our cohort of undiagnosed Peruvian patients significantly diverges from the European distribution, with no expanded GAA alleles found, unlike other populations where the expansion frequency ranges from 9% to 19%. The absence of SCA27B cases in the Peruvian cohort might be due to the Amerindian ancestry of the patients.

Funding: DP holds a Fellowship award from the Canadian Institutes of Health Research (CIHR).

Further insights on phenotypic spectrum of the recurrent R294H variant in the KCNA2 gene

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Emílie Vyhnálková [1], ***Dr. Anna Uhrová Mészárosová*** [2], ***Dr. Zuzana Mušová*** [1], ***Dr. Zuzana Blichová*** [3], ***Dr. Markéta Vlčková*** [4], ***Dr. Pavel Tesner*** [4], ***Dr. Jaroslava Paulasová Schwabová*** [5], ***Dr. Dana Šafka Brožková*** [2], ***Dr. Martin Vyhnálek*** [3]

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Background: The *KCNA2* gene encodes a potassium channel critical for neuronal excitability. Mutations in the *KCNA2* have been associated with a spectrum of neurological disorders, including epileptic encephalopathy, intellectual disability, and ataxia. Additionally, a recurrent loss-of-function pathogenic variant (c.881G>A, p.Arg294His, R294H) has been identified in patients with hereditary spastic paraplegia (HSP). To date, the variant has been reported in 8 symptomatic individuals: 6 with HSP and 2 with early-onset epilepsy.

Methods: The R294H variant was identified by NGS in two families.

Results: In family 1, all three confirmed carriers (mother and two daughters) presented with a slowly progressive HSP phenotype combined with mild cerebellar ataxia with onset at 11-22 years of age. The same phenotype was present in the deceased mother's mother and is documented in 3 other maternal relatives (not genetically tested). One of them has HSP and epilepsy.

In family 2, the phenotype of complicated HSP is documented in 3 subsequent generations: the proband has had a gait disorder since childhood. Since adolescence, he presents with periodic atypical episodes of impaired mobility and obnubilations. The proband's sister showed HSP phenotype and mild cognitive impairment since childhood. The deceased mother presented with progressive gait disturbance and epilepsy with onset at 30 and 40 years of age, respectively.

4/5 patients showed significant improvement on treatment with eplerenone.

Discussion and Conclusion: We describe five additional carriers of the R294H variant in the *KCNA2* gene from two unrelated families. Variants in the *KCNA2* gene are rare causes of HSP or epilepsy syndromes. The data from our cohort provide further evidence of a broad clinical spectrum and suggest a frequent co-occurrence of epilepsy in patients with a prominent HSP phenotype.

Funding: Grant MH CR No. AZV NU22-04-00097; National Institute for Neurological Research Project No. LX22NPO5107

A short tandem repeat gene panel by the capture-based long-read sequencing

Wednesday, 13th November - 18:00: (Minories) - Poster

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Objective: Tandem repeat disorders (TRDs) result from the pathological expansion of short tandem repeat (STR). Detecting these STR expansions accurately in s single assay remains a challenge. While next-generation sequencing (NGS) has been increasingly used in clinical practice, traditional short-reads are insufficient for genotyping large and complex repeat expansion. Long-read sequencing platforms, such as Oxford Nanopore, offer potential solutions for facilitating the diagnosis of TRDs. In this study, We developed a capture-based enrichment method combined with Oxford Nanopore sequencing to genotype variable STR expansions at once.

Method: We designed an STR capture panel that targets the 1 kb flanking regions of STR sites, covering 48genes with STRs. A total of 30 individuals who have received genetic tests for spinal cerebellar ataxia (SCA) subtypes by GeneScan were enrolled. DNA was collected and enriched using this capture panel and sequenced on the Oxford Nanopore platform.

Results: Each sample yielded approximately 101797.5 reads (range: 25065-225390) with a median read length of 2984.5 base pairs (range: 2410-4142 bp). Aligning the reads to the human reference genome CHM13, the median on-target rate was 37.8% (range: 18.5-61.5%), and the average read depth was 402 (range: 133-1227). We established an analysis pipeline named CLR-STR to genotype the STR expansion number. Compared to GeneScan results, our method achieved an accuracy of 80.7% (67/83), allowing for a margin of one copy. We also successfully detected the pathological allele on ATXN8OS with expansion number of 91 in an individual diagnosed with SCA8.

Discussion: There is still room for improvement in the accuracy rate, including optimizing the capture probe and expansion calling method.

Conclusion: Capture enrichment combined with long-read sequencing is a cost-effective and efficient approach for STR genotyping, offering significant improvements in accuracy and diagnostic capability for TRDS.

Next generation sequencing in hereditary ataxias - A single center experience

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Adriana Meli [1], ***Dr. Vincenzo Montano*** [1], ***Dr. Clara Bernardini*** [1], ***Dr. Antonella Fogli*** [2], ***Dr. Anna Rocchi*** [1], ***Dr. Annalisa Lo Gerfo*** [2], ***Dr. Rossella Maltomini*** [2], ***Dr. Giulia Cecchi*** [1], ***Prof. Gabriele Siciliano*** [1], ***Dr. Maria Adelaide Caligo*** [2], ***Prof. MICHELANGELO MANCUSO*** [1], ***Dr. Piervito Lopriore*** [1]

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This retrospective study conducted at a single Italian adult neurogenetic outpatient clinic aims to evaluate the diagnostic yield of NGS for diagnosis of hereditary ataxias (HA) in 120 patients suspected of HA recruited from November 1999 to February 2024.

A stepwise approach was applied: targeted gene panel (TP) sequencing and/or clinical exome sequencing (CES) were performed in the case of inconclusive first-line genetic testing, such as short tandem repeat expansion (TRE) testing for SCA1-3, 6-8, 12, 17, DRPLA, other forms (FXTAS, FRDA and mitochondrial DNA-related ataxia) or inconclusive phenotype-guided specific single gene sequencing.

A definitive diagnosis was reached in 39.2% of the cases. TRE testing was diagnostic in 30% of patients. The three most common TREs ataxias were FRDA (39,4%), SCA2 (24,2%), and RFC1 (12,1%). In 5 patients the molecular diagnosis was achieved by single gene sequencing: POLG (2), SACS (1), DARS2 (1), MT-ATP6 (1). Of 72 patients with a suspicion of HAs of indeterminate genetic origin, 49 underwent new molecular evaluation using the NGS approach, in 28 of these CES was performed after TP sequencing resulted negative. In 7 of these 9 patients, the diagnosis was made by CES. Causative genes mutations were found in SPG7 (2), CACNA1G (1), EEF2 (1), PRKCG (1), KCNC3 (1), ADCK3 (1), SYNE1 (1), ITPR1 (1).

HA should be suspected when acquired causes of cerebellar ataxia are excluded. NGS analysis, especially CES, allow to achieve a definite diagnosis for about 20% of patients with a clinical diagnosis of HA, but without molecular diagnosis on routine genetic tests.

The molecular diagnosis of HA remains a challenge for the neurologist and NGS analysis is a valid diagnostic tool in the diagnostic algorithm. It would be more appropriate performing CES rather than TP to more likely obtain a molecular diagnosis in case of routine non-diagnostic genetic testing.

Mitochondrial ataxia: the Italian experience

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Clara Bernardini [1], ***Dr. Adriana Meli*** [1], ***Dr. Piervito Lopriore*** [1], ***Dr. Vincenzo Montano*** [1], ***Prof. MICHELANGELO MANCUSO*** [2]

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In this retrospective study, we evaluated the prevalence and features of ataxia in a cohort of 764 patients with either a genetic diagnosis of late-onset (age>16 years) primary mitochondrial disease (PMD).
The source was the data uploaded on the Italian Mitochondrial Diseases registry. We reviewed clinical, neurophysiological, neuroimaging and genetic data.
Ataxia was present in 63 subjects (33 females). Mean age of onset of the PMD in ataxic patients was 36.38 (+/-13.74), while age of onset of ataxic syndrome was 39.86 (+/-16.03). In 7 cases, ataxia was present before the diagnosis of PMD, and in 28 cases the onset of ataxic symptoms coincided with diagnosis of PMD.
We observed cerebellar ataxia in 26 patients, pure sensory ataxia in 10 and spinocerebellar ataxia in 27 cases.
Electroneurography presented an axonal sensory neuropathy pattern in 24 patients and an axonal sensory motor involvement in 12, while it was normal in 10 cases.
Most frequent MRI findings were cerebellar (47.6%), brainstem (14.3%) and global cerebral atrophy (50.8%), white matter hyperintensities (42.9%), lactate peak on spectroscopy (17.5%) and basal ganglia abnormalities (22.2%).
25 patients harbored mtDNA variant (mainly the m.8344A>G in MT-TK and m.3243A>G in MT-TL1) and 3 a single mtDNA deletion. The other patients presented a nDNA gene variant (16 POLG1, 3 OPA1, 2 C10ORF2, 1 AARS2, 1 DARS2, 1 PMPCA).
Given the growing prevalence of PMDs and the relative frequency of ataxic syndrome in these patients, which often occurs at the onset of the disease, it is important to consider mitochondrial etiology in adult-onset ataxia diagnostic flowchart.
Early identification of this etiology can be crucial for addressing any concurrent medical conditions that may arise in PMDs patients, as well as for potential target therapies.

Sequencing of the FGF14 GAA repeat in patients with SCA27B

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Jean-Loup Méreaux [1], ***Mrs. Claire-Sophie Davoine*** [2], ***Mrs. Patricia Legoix*** [3], ***Mr. Sylvain Baulande*** [3], ***Dr. Giulia Coarelli*** [2], ***Mrs. Ludmila Jornea*** [1], ***Mr. Philippe Martin Hardy*** [1], ***Prof. Alexis Brice*** [1], ***Prof. Alexandra Durr*** [2]

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Background and Objective

Heterozygous *FGF14* intronic expansion of at least 250 pure GAA causes frequent SCA27B late-onset cerebellar ataxia with downbeat nystagmus and episodic symptoms. Our objective was to describe more deeply the sequence of this repeat and the flanking regions to explain the clinical variability.

Methods

We sequenced with PacBio long read technology *FGF14* GAA repeat amplicons from 222 individuals with ataxia and 34 controls. They were selected for having an allele of at least 200 GAA, previously sized by capillary migration and assessed by RP-PCR, including 164 individuals with an allele above 250 GAA and considered as being affected with SCA27B. After alignment and quality control, we analyzed the GAA size, purity and interruptions, and the flanking sequence when coverage was at least 5x by allele.

Results

We analyzed 279 alleles with a median coverage of 11x. We found that large repeats above 100 GAA were very unstable with mostly contraction mosaicism. The 62 GAA repeats in alleles of SCA27B individuals were mostly pure but 3 (5%) were interrupted by either GAAA or GGA. All alleles below 27 GAAs carried the polymorphism GTTAGTCATAGTACCCC in the variable region preceding the repeat without mosaicism. We did not find correlations between GAA number or interruptions with age at onset.

Discussion and Conclusion

The yield of high-quality reads was low. We had some discrepancies compared to previous sizing, due to lack of coverage in the sequencing, and due to 3 interruptions not detected by RP-PCR. In addition to the GAAGGA repeats that are not pathogenic, GAAA interruption could play a role in the SCA27B phenotype.

Acknowledgments

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SCA4 GCC expansion in France

Wednesday, 13th November - 18:00: (Minories) - Poster

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Background and Objective

ZFHX3 coding expansion of at least 42 pure CGG causes SCA4 autosomal dominant cerebellar ataxia with sensory and autonomic neuropathy. Only individuals from a common Swedish ancestry were diagnosed with this disease in 2023. Our aim was to screen this recently discovered expansion in France.

Methods

We sized with *ExpansionHunter*, *ZFHX3* CGG repeat in 805 exomes (TWIST capture) from a French research cohort of spinocerebellar degeneration including 654 index cases. We searched for the loss of serine interruption associated with CGG expansion to prioritize the CGG sizing in 34,558 genomes from the French rare diseases *SeqOIA* cohort.

Results

We did not find *ZFHX3* CGG repeat expansions in the exomes although the coverage was very high at the locus (median at 160x). The distribution of normal allele sizes reproduced Sweden control individuals, the most frequent at 21 CGG with a maximum size of 23 CGG. Among the genomes, 14 had the chr16:72787736-CACT-C variant corresponding to the loss of serine interruption (frequency of 0.0002 in the genomes, absent in the exomes), but we did not find a CGG expansion. Interestingly, we identified a pure uninterrupted 19 CGG repeat in an unaffected mother and her child without ataxia.

Discussion and Conclusion

ZFHX3 CGG repeat was well covered to be sized in exomes. SCA4 was not a cause of cerebellar ataxia in our large French cohort. The rare loss of serine interruption could help to prioritize the *ZFHX3* CGG repeat specific calling in sequenced data. A pure CGG repeat exists in a small allele without expansion nor intergenerational instability.

Acknowledgments

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FGF14 GAA repeat expansion is a frequent cause of Episodic Ataxia in Italian patients

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Elisa Sarto [1], Dr. Daniela Di Bella [1], Dr. Stefania Magri [1], Dr. Lorenzo Nanetti [1], Dr. Chiara Pisciotta [2], Dr. Davide Pareyson [2], Dr. Cinzia Gellera [1], Dr. Ettore Salsano [2], Dr. Silvia Fenu [2], Dr. Caterina Mariotti [1], Dr. Franco Taroni [1]

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A heterozygous GAA repeat expansion in intron 1 of the *FGF14* (fibroblast growth factor 14) gene is a relatively common cause of dominant adult-onset spinocerebellar ataxia (SCA27B). Uninterrupted expansions ≥250 GAA are considered pathogenic, with a potential incomplete penetrance (1).

Objectives: 1) to screen for the *FGF14* expansion a cohort of Italian patients with ataxia (n=222) or episodic ataxia (EA) negative for *CACNA1A*/EA2 and *KCNA1*/EA1 pathogenic variants (n=82); 2) to assess the distribution of *FGF14* alleles in a control population (n=183).

Methods. The *FGF14* expansion was analysed by long-range PCR and by bidirectional fluorescent repeat-primed PCR (1,2).

Results. *FGF14* expansions >250 GAA were identified in 24 EA cases from 19 families (19/82=23%, 6 AD, 13 S), and only in 22 patients with chronic-progressive ataxia from 18 families (18/222=8%, 5 AD, 13 S). Interestingly, the expansion was very frequent in the subset of patients presenting with chronic ataxia and down-beating nystagmus (DBN) (8/10, 80%) that was successfully treated with 4-aminopyridine in 5/7 patients (3). Only one uninterrupted allele >200 (GAA=236) but no uninterrupted alleles ≥250 GAA were identified in controls. In both patients and controls, approximately 1% of alleles may have an alternative/interrupted expanded repeat configuration.

Conclusions. *FGF14* expansion accounts for up to 23% of Italian non-EA1/non-EA2 EA families. Patients present with late-onset EA characterized by disequilibrium, gait ataxia, and eye movements abnormalities, followed by a slowly progressive chronic ataxia. Cerebellar atrophy is present in less than 50% of patients. Interestingly, *FGF14* expansion seems to be a frequent cause in patients with DBN. Sequencing of uncertain alleles by long-read sequencing approach is undergoing and is expected to be crucial to determine their pathogenic role.

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Reduction of sacsin levels in peripheral blood mononuclear cells (PBMCs) as a non-invasive diagnostic tool for SACS variants causing ARSACS

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Daniele De Ritis [1]***, Dr. Laura Ferrè*** [2]***, Dr. Jonathan De Winter*** [3]***, Dr. Clémence Tremblay-Desbiens*** [4]***, Dr. Mathieu Blais*** [4]***, Dr. Maria Teresa Bassi*** [5]***, Prof. Nicolas Dupré*** [6]***, Prof. Jonathan Baets*** [3]***, Prof. Massimo Filippi*** [7]***, Dr. Francesca Maltecca*** [8]

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Background and Objectives

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a rare neurodegenerative disease caused by biallelic pathogenic variants in the *SACS* gene encoding for sacsin. ARSACS is present worldwide with more than 200 pathogenic variants identified to date in the *SACS* gene, most of which are missense. Likely, the prevalence of the disease is underestimated due to the lack of a rapid non-invasive diagnostic tool able to validate variants of uncertain significance (VUS). We have previously shown that sacsin is almost absent in patients' fibroblasts regardless of the type of *SACS* variant because sacsin carrying missense pathogenic variants is cotranslationally degraded. Here, we aimed to establish the pathogenicity of *SACS* variants by quantifying sacsin protein in blood samples.

Methods

We developed a protocol to assess sacsin protein levels by Western Blot from peripheral blood mononuclear cells (PBMCs), which can be propagated and cryopreserved. The study includes eight patients (including a novel case) carrying pathogenic variants of different types and positions along the *SACS* gene, and two parents carrying heterozygous missense variants.

Results

We show that ARSACS patients (carrying either missense or truncating variants) almost completely lacked sacsin in PBMCs. Moreover, both carriers of a *SACS* missense variant showed 50%-reduction in sacsin levels compared to controls. We also describe one unusual case of uniparental disomy with a very C-terminal homozygous nonsense variant where the truncated protein was still present, probably because it escaped mRNA nonsense-mediated decay.

Discussion and conclusion

We optimized a non-invasive diagnostic tool for ARSACS in PBMCs based on sacsin protein levels quantification and provide definite evidence that sacsin carrying missense pathogenic variants undergoes cotranslational degradation. The quantitative reduction in sacsin levels in the case of missense VUS allows to define them as pathogenic variants, which can't be accurately predicted bioinformatically.

Fundings

Italian MoH #RF-2019-12370417, ARSACS Foundation.

Co-occurrence of SPG4 and SPG11 Subtypes in a Single Family: A Case Study

Wednesday, 13th November - 18:00: (Minories) - Poster

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Hereditary spastic paraplegia (HSP) is a genetically diverse group of slowly progressive neurodegenerative disorders characterized by spasticity and weakness of the lower limbs. Among the various subtypes of HSP, SPG4 and SPG11 are two distinct forms caused by mutations in the SPAST and SPG11 genes, respectively. SPG4 is inherited in an autosomal dominant manner, while SPG11 in an autosomal recessive manner. This case study reports the rare occurrence of both SPG4 and SPG11 subtypes within a single family, highlighting the clinical, genetic, and diagnostic challenges associated with such co-occurrences.

We examined a family with no history of consanguinity presenting with heterogeneous clinical symptoms indicative of HSP. Genetic analysis revealed the presence of a novel pathogenic SPAST variation in one of the patients, who presented with a pure phenotype, consistent with the SPG4 subtype, and an SPG11 variation in the other one, who presented with a complex phenotype, consistent with the SPG11 subtype.

This unique case underscores the importance of comprehensive genetic testing in families with a history of HSP, as multiple subtypes may coexist and contribute to the clinical variability observed. Our findings also emphasize the need for tailored clinical management and genetic counselling in families affected by multiple HSP subtypes.

In conclusion, the co-occurrence of SPG4 and SPG11 subtypes within a single family presents significant implications for the diagnosis, treatment, and genetic counselling of HSP. This case highlights the genetic complexity of HSP and the necessity for personalized approaches in managing this diverse group of disorders.

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Significance of 20 or fewer CAG repeats in CACNA1A in ataxia.

Wednesday, 13th November - 18:00: (Minories) - Poster

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Background: The pathogenicity of 20 or fewer CAG repeats in the *CACNA1A* gene for ataxia remains unclear. This study aimed to clarify this by analyzing the genetic data of patients with cerebellar ataxia.
Methods: We included patients with suspected spinocerebellar ataxia who underwent genetic testing for SCA6 and CAG repeat analysis in the *CACNA1A* gene and examined the relationship between CAG repeats and age of onset (AOO). A regression line was used to plot the relationship between CAG repeat units and AOO in definite SCA6 cases with 21 or more CAG repeats, and agreement with this line was assessed in cases with 20 or fewer repeats. Multiple regression analysis was used to examine the corrected repeat units of the shorter alleles and AOO in SCA6 diagnosed cases.
Results: This study included 2768 participants. The regression line for cases with 21 or more repeats was within the mean ± SD of AOO for cases with 19 or 20 repeats of the expanded allele but not for cases with 18 or fewer repeats. However, the number of cases with 19 and 20 repeats of the expanded alleles was significantly fewer, and the AOO was younger than those with 21 or more repeats. The AOO of patients with 20 repeats of the expanded alleles, when the shorter allele was 14 or more, matched the regression line. The CAG repeats units in the shorter allele and AOO showed an association in multiple regression by at least 13 alleles.
Discussion and Conclusion: This study shows that 19 and 20 repeats may cause ataxia under certain conditions. In patients with these lengths, the pathogenicity of these alleles may increase if the repeat units of the short allele are greater than 14. In the shorter allele, more than 13 repeats may contribute to AOO.

FGF14 GAA repeat expansion distribution in the Cypriot population

Wednesday, 13th November - 18:00: (Minories) - Poster

Mr. Ioannis Livanos [1], ***Dr. Christina Votsi*** [1], ***Dr. Kyriaki Michailidou*** [1], ***Dr. David Pellerin*** [2], ***Dr. Bernard C. Brais*** [3], ***Dr. Stephan Zuchner*** [2], ***Prof. Marios Pantzaris*** [1], ***Prof. Kleopas Kleopa*** [1], ***Dr. ELENI ZAMBA-PAPANICOLAOU*** [1], ***Prof. Kyproula Christodoulou*** [1]

1. *The Cyprus Institute of Neurology and Genetics,* ***2.*** *Dr. John T. Macdonald Foundation Department of Human Genetics and John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL, USA,* ***3.*** *Department of Neurology and Neurosurgery, Montreal Neurological Hospital and Institute, McGill University, Montreal, QC, Canada*

Background and objectives

Dominantly inherited intronic GAA repeat expansion in the fibroblast growth factor 14 (*FGF14*) gene has recently been shown to cause spinocerebellar ataxia type 27B (SCA27B). Currently, the pathogenic threshold of $(GAA)_{\geq 300}$ repeat units is considered fully penetrant, while $(GAA)_{250\text{-}299}$ is likely pathogenic with reduced penetrance. This study aimed to investigate the frequency of the GAA repeat expansion and the phenotypic profile in a Cypriot cohort with genetically unsolved late-onset cerebellar ataxia (LOCA).

Methods

We analysed this trinucleotide repeat in 155 LOCA patients and 227 non-neurological disease controls. The repeat locus was examined using long-range PCR (LR-PCR) followed by fragment analysis using capillary electrophoresis, agarose gel electrophoresis and automated electrophoresis. A comprehensive comparison of all three electrophoresis techniques was conducted. Appropriate adjustments were made by employing calibrators previously sized by long-read sequencing to determine the size of expanded alleles accurately. Additionally, bidirectional repeat-primed PCRs (RP-PCRs) and Sanger sequencing were carried out to confirm the absence of any interruptions or non-GAA expansions in the expanded alleles.

Results

The $(GAA)_{\geq 250}$ repeat expansion was present in 12 (8%) patients, and the average age at disease onset was 60 years ± 13 years. Notably, one patient carrying a $(GAA)_{287}$ repeat expansion developed ataxia at the age of 25 years. Additionally, 2 (0.9%) controls were identified with the reduced penetrance repeat expansion. All *FGF14*-GAA-positive patients exhibited signs of gait and appendicular ataxia. Nystagmus and dysarthria were present at 58% and 50%, respectively.

Discussion and Conclusion

Our findings indicate that SCA27B represents the predominant aetiology of autosomal dominant cerebellar ataxia in the Cypriot population, as this is the first dominant repeat expansion ataxia type detected. Given our results and existing research, we propose including *FGF14*-GAA repeat expansion testing as a first-tier genetic diagnostic approach for patients presenting with LOCA.

Characterization of RFC1 AAGGG expansion carriers among the Cypriot ataxia patients

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Christina Votsi [1], ***Dr. Paschalis Nicolaou*** [1], ***Prof. Marios Pantzaris*** [1], ***Dr. Georgios Pitsas*** [1], ***Dr. Archontia Adamou*** [1], ***Prof. Kleopas Kleopa*** [1], ***Dr. ELENI ZAMBA-PAPANICOLAOU*** [1], ***Prof. Kyproula Christodoulou*** [1]

1. *The Cyprus Institute of Neurology and Genetics*

Background and Objective: We recently reported the *RFC1* gene repeat distribution in the Cypriot population, diagnosis of ten patients homozygous for the AAGGG pathogenic expansion and a high percentage of heterozygous AAGGG carriers. Through the current study, we aimed to investigate the heterozygous AAGGG patients further, as they were suspected of having a second *RFC1* intragenic variant.

Methods: We studied a cohort of undiagnosed patients (n=26), carriers of the *RFC1* pathogenic AAGGG expansion, presenting with pure ataxia, ataxia with neuropathy, or additional symptoms. We performed whole exome sequencing to investigate the presence of conventional variants, primarily in the *RFC1* coding regions. In the absence of *RFC1* variants, ataxia, spastic paraplegia and neuropathy *in silico* panels were examined.

Results: Our findings exclude a second small-scale *RFC1* variant in the patients studied thus far; ten cases are still under investigation. Interestingly, four of these patients were confirmed with the *FGF14* GAA repeat expansion through a parallel study. For the remaining, *in silico* panel analysis revealed candidate pathogenic/likely pathogenic variants in other genes or excluded all genes examined. Many of these patients remain undiagnosed.

Discussion and conclusion: We report a different genetic diagnosis for several cases and the exclusion of coding variants in the examined panels genes for many cases, including three patients presenting with full-blown CANVAS or 2/3 typical symptoms. This finding indicates the possible existence of other deep intronic *RFC1*/other gene variants, unknown repeat expansions or unknown disease genes. It also remains to be determined if the coexistence of a single allele AAGGG expansion with another gene variant(s) can affect the phenotype severity/diversity.

Our findings indicate that compound heterozygosity for an *RFC1* AAGGG expansion and a small-scale coding variant is unlikely in the Cypriot population. They also expand the Cypriot population's spectrum of ataxia/neuropathy variants.

Spinocerebellar Ataxia (SCA) type 27B can be suspected based on clinical phenotype: The Massachusetts General Hospital (MGH) Ataxia Center Experience

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Leigh Rettenmaier [1], ***Ms. Jinyun Helen Chen*** [2], ***Mr. Jason MacMore*** [2], ***Dr. Anoopum Gupta*** [2], ***Dr. Christopher D. Stephen*** [1], ***Dr. David Pellerin*** [3], ***Dr. Bernard C. Brais*** [4], ***Prof. Jeremy D. Schmahmann*** [2]

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Background:
SCA27B is a late-onset cerebellar ataxia caused by an intronic GAA repeat expansion in fibroblast growth factor 14 gene (*FGF14*). The clinical phenotype is characterized by slowly progressive cerebellar ataxia often with downbeat nystagmus (DBN) and episodic worsening.
Methods:
We screened the MGH Ataxia Center registry (n=3,182) for patients with a) isolated DBN, b) DBN with ataxia, and c) autosomal dominant cerebellar ataxia with normal repeat disorder and exome analysis. Participants were consented and enrolled in the *FGF14* repeat expansion study at the Montreal Neurological Institute.
Results:
We identified 65 patients based on phenotype. Seven are deceased, 7 lost to follow-up. Of 51 patients available, genetic testing is complete in 26, results pending in 6, with 19 planned. Of 26 genotyped cases, 9 have **isolated DBN** causing a vestibular gait disorder: 6 tested positive (287-461 GAA repeats), and normal alleles were found in 2 with lithium exposure and 1 with sudden-onset DBN. Twelve patients have **DBN and cerebellar ataxia**: 6 tested positive (310–415 GAA repeats), 1 has an allele of uncertain pathogenicity (228 GAA repeats) and 5 have normal alleles. Four patients with **autosomal dominant cerebellar ataxia** and prominent episodic worsening precipitated by caffeine, alcohol or fatigue but without DBN tested positive (320-466 GAA repeats) as did 1 presymptomatic sibling. In 21 patients treated with aminopyridine, 14 (67%) experienced subjective and objective improvement.
Conclusion:
18/26 patients (69%) genotyped with clinically suspected SCA27B carry suspected pathogenic *FGF14*-GAA expanded alleles. With 65 living and deceased close phenotypic matches (69% x 65 = 45 patients), SCA27B is likely to be the third commonest genetic ataxia at MGH after SCA3 and Friedreich's ataxia. Pathogenic *FGF14*-GAA expansions may be smaller than currently conceived. This needs further evaluation in larger cohorts. The anecdotal benefit of aminopyridine holds promise for therapeutic trials.

Spinocerebellar ataxia type-7: First report of clinical and molecular findings of two family (Cases Video) in Senegal (West Africa)

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Maouly FALL [1]

1. *Cheikh Anta Diop University of Dakar*

Introduction

Spinocerebellar ataxia type 7 is an inherited neurodegenerative disease caused by a pathogenic expansion of a CAG repeat within the ataxin 7 gene, resulting in an expanded polyglutamine tract in the ATXN7 protein. In Africa, established studies mainly concern populations in South Africa and elsewhere studies are limited to clinical cases. We describe the clinical and molecular findings in two families originating from Senegal.

Methodology

The patients were followed up in the movement disorders outpatient clinic at the neurology department of “Centre Hospitalier National de Pikine” in Dakar. The blood from the patients were collected by venous puncture. The DNA was extracted from blood samples using by LGC Biosearch Technologies DNA extraction services and stored at -80^{o}C and SCA1, 2, 3, and 7 were tested at the institute of Neurology at UCL queen square.

Results

The first family is a large one in which the case control is a 35-year-old man with no particular personal history, who presented with progressive cerebellar syndrome and vision disorders for 5 to 6 years. Some members of his family had ataxia also. The SARA score of 12/40). Fundus shows macular degeneration and brain MRI showed cerebello-vermian atrophy.

The second family is an average family which the control case is a 16-year-old girl who, for the past two years, has presented with cerebellar syndrome (video 2) with a progressive decline in vision as some of her family members. The SARA score was 15/40.

Fundus shows bilateral macular and brain MRI showed moderate cerebellar atrophy. For both families the SCA7 was found.

Discussion and Conclusion

SCA is a common clinical phenotype in sub-Saharan Africa, but is under-reported due to a lack of appropriate facilities. SCA7 is thought to be predominant in Senegal, but this assertion needs to be confirmed by other large-scale cohort studies.

Genetic and acquired pediatric cerebellar disorders and their phenotypic heterogeneity - the PEDIATAX study.

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Katariina Granath [1], ***Dr. Sanna Huhtaniska*** [1], ***Dr. Juulia Ellonen*** [1], ***Ms. Tytti Pokka*** [1], ***Dr. Heli Helander*** [1], ***Dr. Hanna Kallankari*** [1], ***Dr. Jonna Komulainen-Ebrahim*** [1], ***Dr. Päivi Vieira*** [1], ***Dr. Elisa Rahikkala*** [1], ***Dr. Salla Kangas*** [2], ***Prof. Renzo Guerrini*** [3], ***Dr. Minna Honkila*** [1], ***Prof. Terhi Ruuska*** [1], ***Prof. Reetta Hinttala*** [1], ***Dr. Maria Suo-Palosaari*** [1], ***Dr. Jussi-Pekka Tolonen*** [1], ***Prof. Johanna Uusimaa*** [1]

1. *Oulu University Hospital and University of Oulu,* ***2.*** *University of Oulu,* ***3.*** *Meyer Children's Hospital IRCCS and University of Florence*

Background and objectives. Finland has a unique genetic landscape with enrichment of genetic variants that are rare in other European populations. As the epidemiology of pediatric cerebellar disorders (PCDs) in Finland is yet to be described, we designed our cohort study (Pediatric Cerebellar Ataxias – Genetic Landscape of Northern Finland [PEDIATAX]) to evaluate the epidemiology, etiologies, and clinical and neuroradiological characteristics of PCDs in Northern Finland.

Methods. A longitudinal population-based cohort study of children with a movement disorder or a cerebellar malformation (diagnosis ≤16 years; study period 1970-2022) was performed in the tertiary catchment area of the Oulu University Hospital, Finland. The genotype-to-phenotype associations were compared with those of 1007 individuals with matching monogenic etiologies, identified through a literature search. The pathogenicity of variants of uncertain significance (VUS) was assessed *in vitro*.

Results. A total of 107 individuals were included (cumulative incidence 21.9 per 100,000 live births). A defined etiology was identified for 59 individuals, including monogenic (66%), chromosomal (12%), or non-genetic (22%) etiologies. Ataxia was the most common movement disorder. Friedreich's ataxia was uncommon, while ataxias belonging to the Finnish Disease Heritage were overrepresented. The diagnostic yield of next generation sequencing (NGS) in ataxia was 65%. The most frequent clinical features across the PCDs were hypotonia, developmental delay, ataxia, abnormality on brain MRI, and seizures. Results from functional studies on VUS variants were suggestive for their pathogenicity.

Discussion and conclusions. PCDs are a heterogeneous disease group. Age of onset may help to distinguish between different genetic etiologies. The diagnostic yield of NGS has increased over time. Our dataset will support clinicians to recognize PCDs, their co-morbidities, and genetic etiologies. Further data on epidemiology, monogenic and shared disease mechanisms, and natural history of PCDs will be critical for the development of future treatment approaches.

Molecular characterization of Portuguese families with hereditary cerebellar ataxia: follow-up of a population-based survey

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Joana Damásio [1], ***Dr. Clara Barbot*** [2], ***Prof. José Barros*** [1], ***Prof. Jorge Sequeiros*** [2], ***Dr. Mariana Santos*** [3]

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Background and Objectives: A population-based survey was conducted in Portugal to identify families with hereditary cerebellar ataxia (HCA) and hereditary spastic paraplegia (HSP). Following this work, we aim to perform the molecular characterization of HCA families.

Methods: Clinical and family history were collected; and the most frequent causal genes analysed using conventional methodologies. Recently, undiagnosed families were studied by exome sequencing; new repeat expansions detected by PCR/fragment analysis; and new variants validated by functional studies.

Results: Machado-Joseph disease (MJD/SCA3) was responsible for about half of AD-HCA (102/199 families, 51.3%), followed by dentatorubral-pallidoluysian atrophy (8 families, 4.0%), SCA2 and *CACNA1A* variants (6 families each, 4.0%). The most frequent AR-HCA was Friedreich ataxia (72/250 families, 28.8%), followed by ataxia with oculomotor ataxia (AOA; 24 families, 9.6%), with half of these being attributed to *PNKP* variants (AOA4). The recently identified *FGF14*-GAA repeat expansion is suspected in 5 AD-HCA families. Three families initially classified as AR presented heterozygous *de novo* variants in *KIF1A*, *CACNA1A* or *ATP1A3*. The biallelic *RFC1*-AAGGG repeat expansion was identified in 10 families; 6 families had pronounced spasmodic cough. Additionally, in AR-HCA, we highlight the identification of genes commonly associated with HSP in 9 families, namely *KIF1A*, *KIF1C*, *SPG7*, *SPG11*, *FA2H* and *MAG*. The functional impact of new variants, including *KIF1C* and *SPG11* splice variants and a *MAG* missense variant, was validated. From overall, 46 AD-HCA and 22 AR-HCA families still lack a genetic diagnosis.

Discussion and Conclusion: In our Portuguese cohort, MJD/SCA3 and FRDA were the most prevalent causes of HCA. The AR-HCA group exhibited a broad spectrum of genetic causes, including overlapping genes with other disorders. Many families remain undiagnosed, particularly within the AD-HCA group. In addition to recent genomic technologies, functional studies have helped to uncover the molecular basis of HCA.

Neuropathological features of mitochondrial encephalopathy with learning disability, epilepsy, ataxia, deafness associated with NARS2 variants

Wednesday, 13th November - 18:00: (Minories) - Poster

Prof. Stephen Wharton [1]***, Dr. Lucy Darwin*** [2]***, Dr. Bart Wagner*** [2]***, Dr. Emma Blakely*** [3]***, Dr. Nicholas Beauchamp*** [4]***, Dr. Andrew Martin*** [5]***, Dr. Priya Shanmugarajah*** [6]

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Mitochondrial disorders present with diverse neurological presentations reflected in complex neuropathologies. Whilst multiple classical clinico-pathological syndromes are described, general neuropathological patterns can be characterised; these include disorders with predominant grey matter involvement such as Leigh's, MELAS, MERRF and predominant leukoencephalopathy in Kearns-Sayre. With increasing identification of nuclear and mitochondrial gene pathogenic variants, disorders may not fit into classical syndromes making diagnosis challenging, thus the spectrum of neuropathology needs to be further defined. We report a female with history of severe learning disability, epilepsy, sensorineural deafness, ataxia, low BMI. Following a long period of stability presented with subacute weight loss, dystonic posturing, refractory epilepsia partialis continua, she rapidly deteriorated and died aged 26. MRI showed progressive symmetrical T2 signal change, with restricted diffusion in the posterolateral putamen and white matter of both parietal and frontal lobes. At autopsy, brain showed bilateral discolouration and cavitation of frontal white matter. At microscopy the lesions showed white matter destruction with severe myelin loss, less severe axonal loss, gliosis and macrophage infiltration. There was light perivascular T-lymphocytic response. Small lesions were present in lateral putamen, which showed microvascular proliferation. Electron microscopy revealed matriceal electron dense inclusions in mitochondria in cortex and white matter. Appearances were considered in keeping with mitochondrial encephalopathy. Whole genome sequencing of blood revealed heterozygosity for two *NARS2* variants, one pathogenic, the other of uncertain significance. Whilst the pathology is predominantly a leukoencephalopathy, there is grey matter involvement with putaminal lesions. Clinically and neuropathologically the disorder does not fit a classical syndrome. Whilst a causal role for the heterozygous *NARS2* variants, a mitochondrial asparaginyl-tRNA synthetase in this case is not proven. *NARS2* variants have been associated with non-syndromic hearing loss and Leigh's syndrome. This case potentially extends the clinical and neuropathological description of mitochondrial encephalopathies and the spectrum of *NARS2*-associated disorders.

Clinical, radiological and pathological features of a large American cohort of spinocerebellar ataxia (SCA27B)

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Widad Abou Chaar [1], ***Mr. Anirudh N. Eranki*** [2], ***Ms. Hannah A. Stevens*** [2], ***Ms. Sonya L. Watson*** [2], ***Dr. Darice Y. Wong*** [2], ***Ms. Megan Delfeld*** [1], ***Mr. Alexander J. Gary*** [1], ***Ms. Sanjukta Tawde*** [1], ***Ms. Malia Triebold*** [1], ***Dr. Marcello Cherchi*** [1], ***Dr. Tao Xie*** [1], ***Ms. Veronica S. Avila*** [2], ***Prof. Paul Lockhart*** [3], ***Prof. Melanie Bahlo*** [4], ***Dr. David Pellerin*** [5], ***Ms. Marie-Josée Dicaire*** [6], ***Dr. Matt C. Danzi*** [7], ***Dr. Stephan Zuchner*** [7], ***Dr. Bernard C. Brais*** [6], ***Dr. Susan Perlman*** [2], ***Dr. Margit Burmeister*** [8], ***Dr. Henry Paulson*** [8], ***Dr. Sharan Srinivasan*** [8], ***Dr. Lawrence Schut*** [9], ***Ms. Chuanhong Liao*** [1], ***Dr. Vikram G. Shakkottai*** [10], ***Mr. Matthew Bower*** [9], ***Dr. Khalaf Bushara*** [9], ***Dr. John Collins*** [1], ***Dr. H. Brent Clark*** [9], ***Dr. Soma Das*** [1], ***Dr. Brent L. Fogel*** [2], ***Dr. Christopher M. Gomez*** [1]

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Objectives: Spinocerebellar ataxia 27B due to GAA repeat expansions in the fibroblast growth factor 14 (*FGF14*) gene has recently been recognized as a common cause of late-onset hereditary cerebellar ataxia. Here we present the first report of this disease in the US population, characterizing its clinical manifestations, disease progression, pathological abnormalities, and response to 4-aminopyridine in a cohort of 102 patients bearing GAA repeat expansions.

Methods: We compiled a series of patients with SCA27B, recruited from 5 academic centers across the US. Clinical manifestations and patient demographics were collected retrospectively from clinical records in an unblinded approach using a standardized form. Post-mortem analysis was done on 4 brains of patients with genetically confirmed SCA27B.

Results: In our cohort of 102 patients with SCA27B, we found that SCA27B was a late-onset (57 ± 12.5 years) slowly progressive ataxia with an episodic component in 51% of patients. Balance and gait impairment were almost always present at disease onset with 42/102 (41%) of patients progressing to a cane by 11 years [median age 68.5 years (IQR 62 -75)], 47/102 (46%) to a walker by 16 years [median age 73 (68 - 78)], and 18/102 (18%) to a wheelchair by 17 years [median age 74 (68 - 80)]. The principal finding on post-mortem examination of 4 brain specimens was loss of Purkinje neurons that was most severe in the vermis most particularly in the anterior vermis. Similar to European populations, a high percent of patients 21/28 (75%) reported a positive treatment response with 4-aminopyridine.

Discussion and conclusion: Our study further estimates prevalence and further expands the clinical, imaging and pathological features of SCA27B, while looking at treatment response, disease progression and survival in patients with this disease. Testing for SCA27B should be considered in all undiagnosed ataxia patients, especially those with episodic onset.

Multi-omics unravels pseudodominant inheritance of RFC1 by a repeat expansion and truncating variant causing a multisystemic phenotype

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Danique Beijer [1], ***Dr. Andreas Traschütz*** [1], ***Dr. Vicente Yépez*** [2], ***Dr. Wouter Steyaert*** [3], ***Dr. German Demidov*** [4], ***Prof. Stephan Ossowski*** [4], ***Prof. Matthis Synofzik*** [1]

1. *Division of Translational Genomics of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research and Center of Neurology, University of Tübingen, Tübingen, Germany,* ***2.*** *Technical University of Munich,* ***3.*** *Radboud University Medical Center, Nijmegen,* ***4.*** *Institute of Medical Genetics and Applied Genomics, University of Tübingen, 72076 Tübingen, Germany*

Background and objectives: Multi-omics strategies, e.g. transcriptomics and long-read genome sequencing, might allow the identification of genetic causes in rare neurological disease families that remained unsolved despite exome and short-read genome sequencing, thereby disentangling complex inheritance patterns and phenotypes. Here, we demonstrate how transcriptomics and long-read whole genome sequencing allowed solving of particularly complex combinations genetically and phenotypically; namely pseudodominant inheritance of *RFC1* disease caused by a heterozygous repeat expansion and a truncating *RFC1* variant associated with a severe, early-onset multisystemic *RFC1* phenotype.

Methods: Long-read HIFI genome sequencing (LR-GS) was applied on the trio with subsequent calling for structural variants and STRs. Bulk RNA-sequencing was performed on fibroblasts of the proband and assessed for expression outliers using OUTRIDER.

Results: The index patient presented with an early onset, relatively fast-progressive, multisystemic ataxia phenotype (chorea, spasticity, bradykinesia, cognitive deficits). With ataxia reported in the father, an extensive genetic work-up for autosomal-dominant ataxia genes was initiated which remained negative. LR-GS revealed a biallelic AAGGG *RFC1*-expansion in the father, and a heterozygous *RFC1*-expansion in the index. Outlier analysis of complementary transcriptomics showed reduced *RFC1* mRNA levels in the index, not associated with the STR. This led to identification of a frameshift variant in both the transcriptome and LR-GS.

Discussion and Conclusion: Combined transcriptomics and LR-GS allows to solve genetically plus phenotypically particularly complex ataxia families. For example, a combination of pseudodominant inheritance, due to STR combined with a conventional truncating variant, - and with the latter presenting with a particularly complex multisystemic phenotype. The unique *intra*familial observation of *RFC1* disease – with a milder later-onset phenotype caused by biallelic STR vs. a more severe earlier-onset phenotype cause by STR plus truncating variant – suggests that the phenotypic severity of *RFC1* might be (partially) driven by the underlying type of *RFC1* mutations.

CAG Repeat Expansion in THAP11 Is Associated with a Novel Spinocerebellar Ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Hong Jiang [1], ***Dr. Zhao Chen*** [1]

1. *Department of Neurology, Xiangya Hospital, Central South University, Changsha, Hunan, China*

Background and objectives: More than 50 loci are associated with spinocerebellar ataxia (SCA), and the most frequent subtypes share nucleotide repeats expansion, especially CAG expansion. The objective of this study was to confirm a novel SCA subtype caused by CAG expansion.

Methods: We performed long-read whole-genome sequencing combined with linkage analysis in a five-generation Chinese family, and the finding was validated in another pedigree. The three-dimensional structure and function of THAP11 mutant protein were predicted. Polyglutamine (polyQ) toxicity of THAP11 gene with CAG expansion was assessed in skin fibroblasts of patients, human embryonic kidney 293 and Neuro-2a cells.

Results: We identified *THAP11* as the novel causative SCA gene with CAG repeats ranging from 45 to 100 in patients with ataxia and from 20 to 38 in healthy control subjects. Among the patients, the number of CAA interruptions within CAG repeats was decreased to 3 (up to 5–6 in controls), whereas the number of 30 pure CAG repeats was up to 32 to 87 (4–16 in controls), suggesting that the toxicity of polyQ protein was length dependent on the pure CAG repeats. Intracellular aggregates were observed in cultured skin fibroblasts from patients. *THAP11* polyQ protein was more intensely distributed in the cytoplasm of cultured skin fibroblasts from patients, which was replicated with in vitro cultured neuro-2a transfected with 54 or 100 CAG repeats.

Discussion and Conclusion: This study identified a novel SCA subtype caused by intragenic CAG repeat expansion in *THAP11* with intracellular aggregation of *THAP11* polyQ protein. Our findings extended the spectrum of polyQ diseases and offered a new perspective in understanding polyQ- mediated toxic aggregation.

Funding: This study was funded by the National Key R&D Program of China (2021YFA0805200 to H.J.), the National Natural Science Foundation of China (81974176 and 82171254 to H.J.; 82371272 to Z.C.).

The genetic landscape and phenotypic spectrum of GAA-FGF14 ataxia in China: a large cohort study

Wednesday, 13th November - 18:00: (Minories) - Poster

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1. *Department of Neurology, Xiangya Hospital, Central South University, Changsha, Hunan, China*

Background and objectives: An intronic GAA repeat expansion in *FGF14* was recently identified as a cause of GAA-*FGF14* ataxia. We aimed to characterise the frequency and phenotypic profile of GAA-*FGF14* ataxia in a large Chinese ataxia cohort.

Methods: A total of 1216 patients that included 399 typical late-onset cerebellar ataxia (LOCA), 290 early-onset cerebellar ataxia (EOCA), and 527 multiple system atrophy with predominant cerebellar ataxia (MSA-c) were enrolled. Long-range and repeat-primed PCR were performed to screen for GAA expansions in *FGF14*. Targeted long-read and whole-genome sequencing were performed to determine repeat size and sequence configuration. A multi-modal study including clinical assessment, MRI, and neurofilament light chain was conducted for disease assessment.

Results: 17 GAA-*FGF14* positive patients with a $(GAA)_{\geq 250}$ expansion (12 patients with a GAA-pure expansion, five patients with a $(GAA)_{\geq 250}$-$[(GAA)_n(GCA)_m]_z$ expansion) and two possible patients with biallelic $(GAA)_{202/222}$ alleles were identified. The clinical phenotypes of the 19 positive and possible positive cases covered LOCA phenotype, EOCA phenotype and MSA-c phenotype. Five of six patients with EOCA phenotype were found to have another genetic disorder. The NfL levels of patients with EOCA and MSA-c phenotypes were significantly higher than patients with LOCA phenotype and age-matched controls(p<0.001). NfL levels of pre-ataxic GAA-*FGF14* positive individuals were lower than pre-ataxic SCA3 (p<0.001) and similar to controls.

Discussion and conclusions: The frequency of GAA-*FGF14* expansion in a large Chinese LOCA cohort was low (1.3%). Biallelic $(GAA)_{202/222}$ alleles and co-occurrence with other acquired or hereditary diseases may contribute to phenotypic variation and different progression.

Funding: This study was funded by the National Key R&D Program of China (2021YFA0805200 to H.J.), the National Natural Science Foundation of China (81974176 and 82171254 to H.J.; 82371272 to Z.C.; 82301628 to L.W.).

Non-SCA6 cerebellar ataxia with CACNA1A pathological variant - from J-CAT study.

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Yuka Hama [1], ***Dr. Hidetoshi Date*** [2], ***Dr. Yuji Takahashi*** [3], ***Dr. Hidehiro Mizusawa*** [3]

1. *National Center Hospital, National Center of Neurology and Psychiatry,* ***2.*** *Medical Genome Center, National Center of Neurology and Psychiatry,* ***3.*** *National Center of Neurology and Psychiatry*

Objectives
The aim of the study was to characterize the cerebellar ataxia with CACNA1A pathological variants.
Methods
From 2016 through 2022, patients with cerebellar ataxia have been registered in J-CAT nation-wide. Whole genome sequencing (WGS) was conducted for all patients with negative results in the initial screening (SCA1, 2, 3, 6, 7, 8, 12, 17, 27B, 31, 36, CANVAS, DRPLA and HD), and the characteristics of cases with pathogenic variants of CACNA1A was examined.
Results
During the period, 1895 patients were enrolled. WGS was conducted for 1058 patients. We detected 11 pathogenic variants in CACNA1A including 8 known pathogenic variants and 3 novel pathogenic variants in 19 patients, and 55 variants of uncertain significance. Of 11 pathogenic variants, we detected 4 missense, 4 frameshift, and 3 nonsense variants. Of 4 missense variants, 2 were located in the transmembrane domain, 1 in the intracellular loop, and 1 in the extracellular loop. The clinical characteristics of 19 patients were as follows; the mean age of onset was 29 (4-80) in total, 34 in those with missense mutations and 26 with nonsense/frameshift ones. Fourteen (74%) had a family history. The most common initial symptom was gait disturbance in 14 (74%) cases, followed by dizziness in 5 (26%). Four (21%) had cognitive impairment and Three (16%) had deafness. Brain MRI showed cerebellar atrophy in 18 (95%) patients. Compared to SCA6, our cases had a younger age of onset, high frequency of initial symptoms of dizziness, cognitive impairment, and hearing loss.
Conclusion
Our cases with CACNA1A pathological variants had a wide range of age of onset and more frequent non-ataxic symptoms. A considerable number of VUS have been identified, which necessitates us to conduct familial study or functional analysis. We should take CACNA1A pathogenic variants into consideration in the undiagnosed cerebellar ataxia.
Funding None.

Clinical and Genetic Spectrum in a Large Cohort of Hereditary Ataxia in China

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Zhi-Ying Wu [1]

1. *Department of Medical Genetics and Center for Rare Diseases and Department of Neurology, Second Affiliated Hospital, Zhejiang University School of Medicine*

Background and Objectives

The intricate heterogeneity of hereditary ataxia (HA) poses significant challenges for clinicians in diagnosing HA patients for their therapies and descendants. With advancements in genome sequencing and the discovery of novel causative mutations, the detailed clinical and genetic landscape of HA subtypes requires an update. We aim to further and better characterize the clinical phenotypes and genetic features of HA comprehensively, providing new insights into the diagnostic process.

Methods

Totally 1781 Chinese index patients with suspected HA were enrolled between 2008 and 2023. Fluorescence amplicon length and repeat-primed PCR analyses were performed on known causative expansion loci. Subsequently, whole exome sequencing or whole genome sequencing was carried out on those with negative results. Structure variations and copy number variations were also assessed by various NGS-based bioinformatics tools. Genotype-phenotype correlation analyses were conducted under specific genotypes.

Results

In our cohort, the dominant, recessive, and sporadic cases accounted for 74.4%, 5.7%, and 19.9% respectively. 80.3% patients got their genetic diagnosis, of whom 93.3% had pathogenic repeat expansions with SCA3 predominant (1003 index patients). Particularly, few patients were found to carry abnormal expansions in *FGF14*, *BEAN*, *DAB1*, *THAP11* or *ZFHX3*, which were identified more recently. Among the patients due to non-expansion mutations, *SACS*, *SYNE1*, *ADCK3* and *SETX* accounted for the majority. Clinical features of subgroups with ≥10 patients were summarized.

Discussion and Conclusion

This study offers an updated, comprehensive overview of the clinical and genetic features of Chinese HA and reveals significant differences among different populations, which could provide guidance for HA gene therapy research. We also propose a highly efficient diagnostic flowchart suitable for clinical use. Notably, the remaining 19.7% unexplained patients still need to screen for other unknown causative genes.

Funding

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Poster session II - Cerebellar neurodevelopment and cognitive disorders

Cognitive Aging and Cerebellum: a task fMRI study of the older adults

Wednesday, 13th November - 18:00: (Minories) - Poster

Mr. Yicheng Lin [1], ***Dr. Sheng-Han Kuo*** [2], ***Mr. Ching-Po Lin*** [3], ***Mr. Li-Hung Chang*** [4]

1. *Columbia University Irving Medical Center,* ***2.*** *Columbia University Medical Center,* ***3.*** *Department of education and research, Taipei City Hospital, Taipei, Taiwan,* ***4.*** *Institute of Neuroscience, National Yang Ming Chiao Tung University, Taipei, Taiwan*

Background and objectives:
Cognitive aging used to focus on the cerebral cortex. However, there is little research on the cerebellum's role in cognitive aging. Many studies have shown that cerebellar activation when cognition processing and different cognitive functions correspond to different cerebellum regions. Due to the cognitive function decline with aging, we aimed to investigate the differences in cerebellar activation between the older and the younger across multiple domain tasks. We hypothesize that the cerebellum involved in multiple cognitive process in the aging brain to support the declined cognitive function. We expected increased cerebellar activation during multiple tasks in the older compared to the younger.
Methods:
We used task-based fMRI to observe the BOLD activations of the cerebellum in two age groups (older adults and young adults) when performing different cognitive functions. In our preliminary data, we enrolled 11 participants including 4 older (mean age = 69.40 ± 2.07, three men) and 7 younger (mean age = 21.86 ± 2.04, five men) and underwent multiple task fMRI including motor (finger tapping), language (verbal generation), working memory (N back) and visuospatial (Useful Field of View) functions.
Results:
In the motor task, increased BOLD signals were found in the bilateral anterior cerebellum lobe in the younger compared to the older. In working memory and language tasks, increased BOLD signals were found in the bilateral posterior cerebellum lobe in the older compared to the younger. In the UFOV task, there are no significant changes between groups.
Conclusions and Conclusion:
We showed different cerebellar activation patterns across multiple domain tasks in older adults. Our preliminary results suggest that the cerebellum may be involved in specific non-motor tasks with higher BOLD activations in the aging brain to support high cognitive functions. Our study indicates the cerebellar reserve for maintaining cognitive function in aging.
Funding: NSTC112-2926-I-A49A-502-G

Unraveling Genetic Modifiers in Repeat Expansion Disorders: A Collaborative Genome-Wide Association Study (GWAS)

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Renee Lin [1], ***Mr. Theo Dominot*** [2], ***Dr. David Pellerin*** [3], ***Prof. Henry Houlden*** [4]

1. *Department of Neuromuscular disorders, UCL Queen Square Institute of Neurology, London, WC1N 3BG, UK.,* ***2.*** *Pitié-Salpêtrière University Hospital, France,* ***3.*** *Dr. John T. Macdonald Foundation Department of Human Genetics and John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL, USA,* ***4.*** *Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology and The National Hospital for Neurology and Neurosurgery, University College London, London, United Kingdom*

Introduction

Repeat expansion disorders include the spinocerebellar ataxias (SCAs) such as SCA1, SCA2, SCA3, SCA6, SCA7, SCA17, Huntington's disease, Kennedy's disease and other neurological expansion disorders such as SCA27B. These disorders are mainly caused by CAG repeat expansions, leading to stretches of pure glutamine in the respective disease proteins. While the length of the expanded repeat explains 44-75% of the variance in age of onset (AOO) in SCAs, significant variation exists among different genotypes, within the same genotype, and even within families. This variation suggests additional genetic factors may influence the AOO in these disorders. We are undertaking a genome-wide association study (GWAS) and sequencing the repeat tract to identify novel genetic loci and mechanisms that modify AOO and other clinical phenotypes, such as disease progression, in SCAs and other repeat expansion disorders.

Methods

DNA samples from SCAs and other repeat expansion cases will be collected from all collaborating centers worldwide. Clinical and phenotypic data will be recorded. Genotyping will be conducted using the Illumina Infinium Global Research Array-24v1.0 (GCRA) with additional custom content important SNPs such as mismatch repair genes. Illumina MiSeq will be used to sequence the repeat loci tracts and assess somatic instability.

Results

We have collected 1364 SCA1 cases, 1831 SCA2 cases, 2856 SCA3 cases, 955 SCA6 cases, 330 SCA7 cases, 365 Kennedy's cases, and 55 SCA17 cases from 30 centers worldwide, with ongoing enrollment and midway genotyping. We are also part of other study groups aimed at repeat expansion modifiers in Friedreich's ataxia and part of the SCA27B Study Group (SCA27BSG) where we are setting a similar approach.

Conclusion

This proposed study aims to enhance our understanding of the genetic architecture underlying SCA and other expansion disorders, disease development and progression. It will provide crucial insights into disease mechanisms, potentially leading to new therapeutic strategies.

Functional Domain Analysis of the CACNA1A protein, α1ACT, as a transcription factor and mediator of SCA6

Wednesday, 13th November - 18:00: (Minories) - Poster

Mr. Tyler Thaxton [1], ***Ms. Jessica Markman*** [1], ***Mr. David Zhu*** [1], ***Mr. Eric Gama*** [2], ***Dr. Xiaofei Du*** [2], ***Dr. Christopher M. Gomez*** [1]

1. *University of Chicago,* ***2.*** *Department of Neurology, The University of Chicago, Chicago, IL*

The *CACNA1A* protein, α1ACT, is a transcription factor (TF) that mediates cerebellar degeneration when it bears the SCA6 polyglutamine expansion. We have shown that α1ACT activates a set of neuronal genes critical for Purkinje cell development and preferentially binds to A/T-rich gene regions. However, no studies have been performed to understand the motifs important for the function of α1ACT.

Motif analyses of α1ACT predict the presence of several functional domains: 1) a calcium channel I/Q domain known to interact with calmodulin present at the start site of α1ACT; 2) a 11 histidine sequence polyHis tract, a potential localizer to nuclear speckles that follows the polyQ tract, 3) an AT-hook, a minor groove, A/T-rich DNA-binding domain; and 4) four phosphorylation sites identified by MS/MS spectrum analysis. To study the functions of these regions we transfected plasmids expressing native or mutated variants of α1ACT into H293T or human neural progenitor cells and analyzed the transcriptional, proteomic, and cellular phenotypic effects of different domains.

α1ACT co-immunoprecipitated with calmodulin, and deletion of the I/Q domain prevented co-immunoprecipitation. Deletion of the polyHis tract led to a localization loss to nuclear speckles and a more diffuse expression pattern. Mutation of the AT-hook and the I/Q domain led to a decrease in expression of developmental genes when compared to native α1ACT.

These studies confirmed α1ACT exerts its gene regulatory functions via DNA binding with the AT-hook. Additionally, calcium-mediated functions or nuclear translocation of α1ACT may be mediated by its interactions with calmodulin, and the compartmentalization of α1ACT within nuclear speckles via polyHis tract. Taken together, α1ACT acts as a TF regulating the gene expression via the synchronous action of DNA-binding, protein-binding, and phosphorylation domains. Understanding how α1ACT functions will provide a more robust picture of how the protein functions when it bears the SCA6 polyglutamine expansion.

Cerebellar cognitive affective syndrome in gluten ataxia and other neurological gluten sensitivities

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Iain Croall [1], ***Prof. Marios Hadjivassiliou*** [2], ***Dr. Kevin Teh*** [1], ***Prof. David Sanders*** [3], ***Prof. Nigel Hoggard*** [4]

1. *Division of Clinical Medicine, University of Sheffield,* ***2.*** *Department of Neurology, Sheffield Teaching Hospital Foundation Trust,* ***3.*** *Academic Unit of Gastroenterology, Sheffield Teaching Hospitals NHS Foundation Trust,* ***4.*** *University of Sheffield*

Objectives: The role the cerebellum plays in higher cognitive functions is receiving increasing recognition. One significant development is the characterisation of the specific cognitive and social/emotional deficit that patients with cerebellar disorders are prone to, termed the Cerebellar Cognitive Affective Syndrome (CCAS), for which the CCAS Scale (CCAS-S) has been developed to help diagnose. Neurological issues due to gluten autoimmunity most frequently involve the cerebellum (e.g. gluten ataxia). While the physical impact of these conditions is well recognised, there is a lack of research establishing if cognition is also affected and to what extent.

Methods: Patients with diagnosed neurological problems due to gluten sensitivity were invited to complete the CCAS-S, which was administered in all cases by the same examiner (IDC). Incomplete assessments were discarded. Remaining results were categorised by conventional CCAS-S outcomes and also summarised by pass rate per-subtest. Some patients additionally completed the hospital anxiety and depression scale, and in these cases the CCAS-S pass rate was correlated with depression/anxiety score.

Results: 125 patients fully-completed the CCAS-S. The percentages who obtained each overall outcome were as follows: pass (16.0%), possible CCAS (18.4%), probable CCAS (26.4%), definite CCAS (39.2%). The most frequently failed subtests (and their pass rates) were forwards digit span (53.6%), semantic fluency (60%), phonemic fluency (62.4), backwards digit span (62.4%) and affect (71.2%). Pass rate was significantly negatively correlated with depression (N=78, r= -0.235, p=0.038), and trended towards significance (i.e. p<0.01) with respect to anxiety (where N=76).

Discussion/Conclusion: This is the first time this patient group has received detailed cognitive profiling. Pronounced cognitive deficit is prevalent in patients with neurological gluten sensitivity, showing these conditions to have widespread impact beyond the motor/sensory disturbances most often linked with them. Accompanying low mood may in-part be driven by cognitive impairment.

This study was funded by Beyond Celiac

Exploring the genetic background of hereditary ataxias in Hungary with special emphasis on repeat expansion-related ataxias

Wednesday, 13th November - 18:00: (Minories) - Poster

Mrs. Lilla Buzai-Kiss [1], ***Dr. Peter Balicza*** [1]

1. *Institute of Genomic Medicine and Rare Disorders, Semmelweis University, Hungary*

Background:
Repeat expansions are significantly associated with various types of ataxias, including Friedreich ataxia (FRDA), several spinocerebellar ataxias (SCA), and Cerebellar Ataxia with Neuropathy and Vestibular Areflexia Syndrome (CANVAS). However, specific percentages and the phenotypic spectrum of ataxias related to repeat expansions are not readily available in Hungary.
Patients and Methods:
Our NEPSYBANK database includes 1,014 patients with ataxias. Of these, 840 have been tested for SCA, 244 for FRDA, and 78 for CANVAS. Zygosity and repeat sizes were determined using triplet-repeat primed PCR, fragment analysis, and Southern blot techniques. Additionally, 95 patients underwent either a targeted ataxia NGS panel or whole-exome sequencing.
Results:
In our cohort, polyQ ataxias were the most common, affecting 110 patients. The distribution was as follows: SCA1: 71, SCA2: 30, SCA3: 6, SCA6: 2, SCA7: 1, and FRDA: 10. For CANVAS, 8 patients were affected. In the FRDA cohort, 5 patients had heterozygous pathogenic GAA expansions. One case had a rare damaging variant on one allele, and in four cases, the second variant was not identified, though the patients exhibited typical FRDA phenotypes. In the CANVAS cohort, blot analysis detected one pathogenic allele in 4 samples, both alleles in 2 samples, and one uncertain allele expansion in 2 samples. Rare deleterious variants were identified by NGS in some patients, with POLG1, SPG7, SETX, ATM, CYP27A, SACS, and mtDNA being most frequently affected.
Discussion:
The clinical characteristics of hereditary ataxias are diverse, requiring a combination of clinical assessments and molecular genetic testing to determine the specific genetic cause
Conclusion:
PolyQ ataxias were the most prevalent in our cohort, underscoring the need for a broad range of genetic tests, including NGS sequencing. CANVAS is a common cause of adult-onset ataxias, but its molecular diagnosis is challenging and often requires cascade testing.

Spinocerebellar Ataxia 27B (SCA27B) and associated clinical features: the role of cognitive impairment and possible molecular mechanism

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Alessio Sarnataro [1], ***Dr. Chiara Pane*** [1], ***Dr. Martina Gramaglia*** [1], ***Dr. Nunzia Cuomo*** [1], ***Dr. Angela Marsili*** [1], ***Dr. Alessia Bonfini Rendina*** [1], ***Prof. Francesco Saccà*** [2]

1. *Neuroscience, Reproductive and Odontostomatological Sciences (NSRO) Department, Federico II University,* ***2.*** *GENESIS Department, Federico II University*

Objectives

We describe the case of A.M. 79 y.o. male diagnosed with SCA27B after genetic test with 318 GAA repetition. Symptoms started in 2017 with sudden falls and advanced slowly with progressive imbalance perception and the need of double canes after 3 years.

Methods

To assess the patient's clinical features, we performed neurological examination, MRI, EMG, ENG, neurofilaments serum levels and neuropsychological battery assessment.

Results

The neurological examination revealed classical clinical features of the disease such as a wide-based ambulation, downbeat nystagmus, lower limb hyperreflexia, and slight dysmetria (L>R) with a global SARA score 11/40, EMG, ENG, and serum neurofilaments were normal. MRI showed moderate cerebellar atrophy, especially vermis and floccular lobe, and mild cerebral atrophy. Cognitive tests showed a dysexecutive syndrome together with both short and long-term visuospatial memory impairment.

Discussion

The patient showed significant multiple cognitive impairment affecting mainly frontal functions and visuospatial memory, in addition, neurofilaments levels were normal. These two findings together may suggest a primitive correlation between cognitive impairment and SCA27B rather than a secondary neurodegenerative comorbidity. FGF14 is a protein involved in ion channel regulation and synaptic transmission, in mice model FGF14 deficiency led to neurobehavioral disorders and cognitive symptoms due to a likely involvement of the protein in neural networks involving Hippocampus and dentatus gyrus. In people with SCA27A and FGF14 gene deletion, behavioral disturbances and developmental delay with cognitive impairment are observed in humans. One hypothesis is linked to an alternative phosphorylation pattern by GSK resulting in an abnormal complex formation with SCN8A channel and reduced neuronal excitability.

Conclusion

Cognitive impairment may be a core clinical feature of SCA27B with an independent mechanism of action. We suggest assessing cognitive disorders in all the patients with an extended questionnaire battery. Further data need to be collected to clarify the pathogenesis of cognitive decline.

Cerebellar cognitive affective syndrome (CCAS) in patients with spinocerebellar ataxia type 10 (SCA10)

Wednesday, 13th November - 18:00: (Minories) - Poster

Mr. Angel Omar Romero-Molina [1], ***Dr. Gabriel Ramirez-Garcia*** [2], ***Dr. Amanda Chirino-Perez*** [2], ***Dr. Gustavo Padron-Rivera*** [2], ***Dr. Carlos Roberto Hernandez-Castillo*** [1], ***Ms. Diana Laura Torres-Vences*** [2], ***Dr. Juan Fernandez-Ruiz*** [2]

1. *Universidad Veracruzana,* ***2.*** *Universidad Nacional Autonoma de Mexico*

Background: The Cerebellar Cognitive Affective Syndrome Scale (CCAS-S) has been widely used for the detection of CCAS in different pathologies with cerebellar impairment.
Objective: To characterize the performance of SCA10 patients in the CCAS-S.
Methods: This study included 15 patients diagnosed with SCA10 and 14 controls. The Cerebellar Cognitive-Affective Syndrome Scale (CCAS-S), Montreal Cognitive Assessment (MoCA), Scale for the Assessment and Rating of Ataxia (SARA), the Center for Epidemiologic Studies Depression Scale (CES-D), and an articulation speed test were utilized. Total and subcategory scores of the CCAS-S were analyzed. Scores between patients and controls were compared. Diagnostic accuracy of the CCAS-S was assessed using ROC curve analysis. Demographic and clinical data were examined for correlations with CCAS-S scores.
Results: Patients with SCA10 performed worse than controls, with significant differences in phonological verbal fluency, verbal fluency switching, cube drawing, similarities, Go/No-Go, and affective symptoms. The CCAS-S demonstrated effectiveness (AUC (SE) = 0.86 (0.07), $p < 0.0001$), with a Youden's index J at ≤ 82. Positive correlations were found with education, while negative correlations were observed with age, CES-D scores, and SARA scores.
Discussion: Our study highlights the cognitive and affective alterations in SCA10 patients, consistent with findings in other SCAs where verbal fluency is predominantly affected. Adjusting values through articulation speed tests may provide better clarity of symptoms. The sensitivity and specificity of the CCAS-S indicate its utility in assessing CCAS. Additionally, the strong relationship between demographic and clinical variables suggests that patients with greater cognitive impairment also exhibit greater motor impairment.
Conclusion:
The CCAS-S is an effective screening tool for diagnosing CCAS in SCA10 patients. However, this assessment should be complemented with other clinical motor and mood scales, as well as a comprehensive battery of neuropsychological tests to fully reveal the motor and non-motor deficits present in these patients.

Progressive phenotypic deficits in a novel Atp1a3-E818K mouse model of CAPOS syndrome

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Pariya Anongjanya [1], ***Dr. Steven Clapcote*** [1]

1. *University of Leeds*

Background and objectives

Cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss (CAPOS) syndrome is *ATP1A3*-related disorder with an onset usually in childhood. Our current research is focusing on an *Atp1a3*-E818K mouse model in which we are assessing the phenotypic effects of this mutation.

Methods

A battery of behavioural tests was conducted on *Atp1a3*$^{E818K/+}$ (E818K) and wild type (WT) littermates at 2 months and 6 months of age: open field, fear conditioning, rotarod, and gait analysis.

Results

At 2 months of age, E818K mice showed increased ambulation ($p<0.01$) compared with WT mice in the open field but were not significantly different from WT in the rotarod and gait analysis tests.

At 6 months of age, E818K mice still showed increased ambulation in the open field. In fear conditioning, they showed decreased freezing to the context ($p<0.05$) and to the auditory cue ($p<0.05$), suggesting impaired contextual and cued memory and potentially a hearing deficit. In the rotarod test, 6-month-old E818K mice fell from the rod sooner on the first day ($p<0.05$), indicating balance and coordination deficits. They also showed a shorter stride length for both front and back paws in gait analysis, suggesting ataxia.

Discussion

The results indicated no significant difference in 2-month-old E818K mice for most of the tests except for locomotor hyperactivity in the open field. However, 6-month-old E818K mice presented locomotor hyperactivity, contextual and cued memory deficits, and ataxia compared with WT controls. Thus, the E818K mutation has an age-related phenotype that gets progressively worse with age.

Conclusion

The novel E818K mouse model recapitulates some of the symptoms seen in CAPOS, suggesting that it is a valuable tool for future research into the mechanisms and treatment of CAPOS. However, tests of visual and auditory function are required to obtain a fuller assessment.

Social cognition in spinocerebellar ataxias and its association with neurocognition

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Veronika Matuskova [1], ***Ms. Karolina Mikyskova*** [2], ***Dr. Simona Karamazovová*** [3], ***Ms. Natalie Svecova*** [2], ***Dr. Jaroslava Paulasová*** [3], ***Prof. Martin Vyhnálek*** [4]

1. *Center of Hereditary Ataxias, Department of Neurology, Second Faculty of Medicine and Motol University Hospital, Charles University, Prague, Czech Republic,* ***2.*** *Center of Hereditary Ataxias, Department of Neurology, Second Faculty of Medicine and Motol University Hospital, Charles University, Prague, Czech Republic; Charles University, Faculty of Arts, Prague, Czech Republic,* ***3.*** *Centre of Hereditary Ataxias, Motol University Hospital, Second Faculty of Medicine, Charles University, Prague, Czech Republic,* ***4.*** *Department of Neurology, Second Faculty of Medicine, Charles University, Motol University Hospital, Prague 5, Czech Republic*

Background: The cerebellum, traditionally linked to motor coordination, also plays a crucial role in cognitive and affective functions including social cognition. Recent research has suggested a comparable social cognitive impairment in spinocerebellar ataxias (SCAs) to conditions like schizophrenia and autism spectrum disorders, highlighting the need for further exploration. We aimed to assess social cognition impairment in various SCAs using the Story-based empathy task (SET), an innovative test assessing affective and cognitive theory of mind (ToM), and its relationship to neurocognitive impairment.

Methods: We included 32 individuals with genetically confirmed SCAs or Idiopathic late-onset cerebellar ataxia (ILOCA) (including SCA2=10, SCA1=3, SCA17=3; other SCA subtypes=10 and ILOCA=6; age 49.19±14.93; MMSE 27.84±2.10, SARA 10.91±6.93) from the Center of Hereditary Ataxias and 33 healthy controls without any cognitive complaints, neurological or psychiatric comorbidities (HC, age 40.88±10.56; MMSE 28.97±0.88), who underwent a complex neuropsychological examination, including the SET. We analyzed the group differences in SET total score, intention attribution (IA) and emotion attribution subscores (EA) using ANCOVA controlled for age and sex, and the associations with neurocognitive domains and ataxia severity using Pearson correlations.

Results: Patients with SCAs differed from HC in the total SET score and in both IA and EA subscores (all ps<0.01). SET total score and subscores were moderately to strongly correlated with memory, executive functions, attention, language and visuospatial perception (rs=0.38-0.69) but not with global cognition (MMSE). No correlations with SARA were observed.

Discussion and Conclusion: This study highlights a social cognitive impairment in SCAs encompassing both cognitive and affective ToM, which is unrelated to the severity of ataxia symptoms and can be detected using the SET. Given its associations with neurocognition, future studies should elucidate whether this social cognitive deficit is a specific consequence of cerebellar dysfunction or part of a broader cognitive manifestation.

Spatial perspective taking in spinocerebellar ataxias and Friedreich's ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Simona Karamazovová [1], ***Dr. Martina Lazcó*** [1], ***Dr. Jaroslava Paulasová*** [1], ***Dr. Lucie Stovickova*** [1], ***Prof. Jan Laczó*** [1], ***Dr. Michaela Kuzmiak*** [1], ***Prof. Martin Vyhnálek*** [2]

1. Centre of Hereditary Ataxias, Motol University Hospital, Second Faculty of Medicine, Charles University, Prague, Czech Republic,

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Introduction:

Spatial navigation is a complex set of skills required for everyday movement in the environment. Among its key components is spatial perspective taking, the ability to imagine the environment from a different viewpoint. Animal and functional neuroimaging studies have shown that the cerebellum is important for spatial navigation, but studies in patients with cerebellar disorders, including hereditary ataxias, are lacking. This study aimed to investigate spatial perspective taking in patients with autosomal dominant spinocerebellar ataxias (AD-SCA) and Friedreich's ataxia (FRDA).

Methods:

We recruited 23 patients with various AD-SCA, 31 FRDA patients, and 34 age-matched healthy volunteers (HV). We administered the Perspective Taking/Spatial Orientation Test (PTSOT), an established paper-and-pencil test of perspective taking to all participants. In this test, participants were asked to imagine standing at one object and facing another. Their task was to indicate the direction to a third object in a circular diagram.

Results:

The AD-SCA group performed significantly worse than the FRDA and HV groups. Specifically, the mean angular error was 63.02° in AD-SCA compared to 40.97° ($p=.011$) in FRDA and 26.01° ($p <.001$) in HV, and the mean percentage of responses in the correct quadrant of the circular diagram was 49.3% compared to 67.5% ($p =.028$) and 82.8% ($p <.001$), respectively. In the FRDA group, the mean percentage of responses in the correct quadrant was lower than in the HV group ($p=.043$), but the mean angular error did not differ significantly from the HV.

Discussion and Conclusion:

Spatial perspective taking is impaired in SCA and, to a lesser extent, in FRDA. The difference between the groups may be due to more pronounced cerebellar gray matter atrophy in SCA patients compared to FRDA patients. Further research on spatial navigation in patients with cerebellar diseases is needed, as navigation impairment may negatively impact patients' mobility and independence.

Cerebellar cognitive affective syndrome and mood disturbance in Friedreich ataxia: cross-sectional analysis of the TRACK-FA study cohort

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Imis Dogan [1], Dr. Helena Bujalka [2], Prof. Nellie Georgiou-Karistianis [2], Prof. Kathrin Reetz [1], Dr. Louise A Corben [3], Mx. . TRACK-FA Neuroimaging Consortium [4]

1. *Department of Neurology, RWTH Aachen University,* ***2.*** *School of Psychological Sciences, The Turner Institute for Brain and Mental Health, Monash University, Victoria,* ***3.*** *Monash University, Clayton, Murdoch Children's Research Institute, Parkville and University of Melbourne, Parkville,* ***4.*** *TRACK-FA Study*

Background

The Cerebellar Cognitive Affective/Schmahmann Syndrome Scale[1] (CCAS-S) was developed as a cognitive screening instrument for individuals with cerebellar diseases. We utilised the CCAS-S and mood questionnaires to explore the presence of cognitive and affective disturbances in Friedreich ataxia (FRDA) with individuals from the TRACK-FA[2] baseline cohort.

Methods

As part of the TRACK-FA protocol, the CCAS-S and Hospital Anxiety and Depression Scale (HADS) were administered to 96 adults with FRDA (28.7±7.7 years; 48 female; SARA: 15.8±6.9). Sixty-four paediatric participants (14.0±2.5 years; 30 female; SARA: 12.4±6.4) completed the Revised Children's Anxiety and Depression Scale (RCADS). Groups were compared to respective control participants (49 adults; 38 minors) matched for age and gender.

Results

Total CCAS-S performance was lower in adults with FRDA (96.6±9.8) compared to controls (104.7±6.7; $p<0.001$) and correlated with clinical severity ($r=-0.382$, $p<0.001$). Definite CCAS (≥3 failed tests) was observed in 27% of individuals with FRDA, probable CCAS (2 failed) in 21%, possible CCAS (1 failed) in 29%, while 22% did not fail any test. This distribution differed significantly from controls (2/16/49/33%; $p<0.001$). Performances of semantic fluency ($p=0.004$), phonemic fluency ($p<0.001$) and backward digit span ($p=0.002$) showed significant group differences. Semantic fluency was associated with SARA-speech rating ($r=-0.234$, $p=.022$). Adult patients experienced higher levels of depression compared to controls ($p<0.001$). Paediatric individuals showed both elevated anxiety ($p=0.010$) and depression scores ($p=0.009$). Individuals with FRDA scored more frequently than controls above borderline cut-off values for depression (19% vs. 2% of adults, $p=0.004$; 22% vs. 5% of minors, $p=0.027$).

Discussion

Definite CCAS was less frequent than previously reported[3,4], which may be related to the younger and less affected TRACK-FA cohort. Measures of dysarthria in individuals with FRDA should be considered in the interpretation of verbal fluency tasks.[3] Self-report questionnaires could augment screening for the presence of mood symptomatology, particularly in paediatric FRDA.

Poster session II - Cerebellar circuits and function

Neurochemical Abnormalities in the Cerebellum of Friedreich Ataxia Mouse Models

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Elizabeth Mercado Ayon [1], Ms. Ellarie Talgo [2], Mr. Liam Flatley [3], Ms. Jennifer Coulman [3], Prof. David Lynch [3]

1. *University of Pennsylvania, Perelman School of Medicine,* ***2.*** *Childrens Hospital of Philadelphia,* ***3.*** *Children's Hospital of Philadelphia*

Background: Friedreich ataxia (FRDA) is an autosomal recessive neurodegenerative disorder caused by deficiency of frataxin. This deficiency leads to a multisystemic phenotype; however, neurological deficits remain the ubiquitous feature of FRDA patients. FRDA patients' clinical neurological features include progressive ataxia and dysarthria, both of which are controlled to a large degree by the cerebellum. The precise impact of frataxin deficiency on the cerebellum and its Purkinje cells has yet to be fully clarified. In the present work, we utilized the inducible mouse model of FRDA (FRDAkd) and the knock in knock out (KIKO) to examine biochemical and structural properties of the cerebellum and its Purkinje cells.

Methods: To assess overall cerebellum morphology, we stained cerebellum slides with Purkinje cell and astroglia markers. Biochemical analyses were completed using whole FRDAkd and KIKO cerebella homogenates; postsynaptic glutamate receptors and glia glutamate receptors were analyzed via Western blot.

Results: Acute systemic knockdown of frataxin in FRDAkd mice and chronic frataxin deficiency in KIKO leads to a significant decrease in AMPA receptors while glial glutamate transporters are upregulated. Significant astroglia accumulation occurred in KIKO cerebellum, but not in FRDAkd mice. Purkinje cells dendritic arbors in the molecular layer did not change compared to wildtype in both mice. The Purkinje cell postsynaptic receptor NMDAR1 significantly decreased only in the FRDAkd cerebellum while other NMDA receptors subunits, found in non-Purkinje cells, did not change.

Discussion: We observe dysregulated expression of glutamate transporters in the KIKO and FRDAkd mice models of Friedreich ataxia suggesting the importance of frataxin in maintaining Purkinje cells/cerebellar integrity and supporting our previous data that identified cerebellar synaptic degeneration as an important component of FRDA models. These results point to conserved but not identical synaptic features between the models. Such conserved elements represent more likely markers or conceivably targets in human FRDA.

Frataxin Deficiency Disrupts GluR2 Expression in Cerebellar Purkinje Cells

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Elizabeth Mercado Ayon [1], ***Mr. Liam Flatley*** [2], ***Ms. Teresita Mercado Ayon*** [2], ***Ms. Jennifer Coulman*** [2], ***Mr. Jia-Ying (Lloyd) Lee*** [1], ***Prof. Eric Witze*** [1], ***Prof. David Lynch*** [2]

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

Background: Friedreich ataxia (FRDA) is a life-shortening, neurodegenerative disorder caused by frataxin deficiency. FRDA patients' neurological features include progressive ataxia and dysarthria, which are controlled to a large degree by the cerebellum. Cerebellar neuropathology in FRDA patients includes loss of principal neurons and synaptic terminals in the cerebellar dentate nucleus as well as Purkinje neuronal injury; however, the mechanism by which frataxin deficiency impacts the cerebellum remains to be fully elucidated. In the present work, we utilized an inducible mouse model of FRDA (FRDAkd) to examine biochemical results of frataxin deficiency and a possible mechanism that leads to those changes.

Methods: 2-month-old FRDAkd mice were treated with inducing agent for 2 weeks; Behavior and weight were measured. Western blot and immunohistochemistry were conducted to measure the expression and localization of frataxin and AMPA(GluR2) receptors in the cerebellum. Cerebellum was also stained with the Purkinje cell marker calbindin, and cells were counted. To determine if GluR2 was transcriptionally affected, RNA levels were measured using PCR. Analysis of GluR2 post-translational modifications (phosphorylation and palmitoylation) were conducted using antibodies and acyl biotin exchange assays.

Results: Frataxin and GluR2 expression decreased to 50% of control by 2 weeks of induction, while Purkinje cell number did not change. Immunostaining in wildtype cerebellar slides shows that GluR2 was enriched in Purkinje cell somatodendritic region while it was decreased in FRDAkd PCs. GluR2 RNA levels were not changed. GluR2 phosphorylation decreased, yet when normalized to total amount of GluR2, phosphorylation was unchanged. Palmitoylation of GluR2 was disrupted in the FRDAkd cerebellum.

Discussion: These results indicate that frataxin knockdown leads to GluR2 palmitoylation disruption, suggesting the importance of frataxin for AMPA receptor trafficking in the cerebellum. Interestingly, at 2 weeks of induction FRDAkd mice have no behavioral phenotype, thus indicating early biochemical effects caused by frataxin knock down.

Functional Characterization of Cerebellar Purkinje Cells in Friedreich's Ataxia Mouse Models

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Donald Joseph [1], ***Ms. Elizabeth Mercado-Ayon*** [1], ***Mr. Liam Flatley*** [1], ***Dr. Angela Viaene*** [1], ***Dr. Juliette Hordeaux*** [2], ***Dr. Eric Marsh*** [1], ***Prof. David Lynch*** [1]

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

Background and Objectives: Friedreich ataxia (FRDA) is an autosomal recessive disorder caused by expansion mutations in the mitochondrial gene frataxin (FXN), leading to severe neurological phenotypes. Accumulating evidence suggests that neuronal dysfunction, rather than neuronal death, may be the main driver of the neurological phenotypes. However, the mechanisms underlying the dysfunction mediating such neurological phenotypes remain unclear. Here, we aim to fill this gap by investigating the biophysiological basis of neuronal dysfunction in FRDA.

Methods: We used the shRNA-frataxin (FRDAkd) and the FXN knock in-knockout (KIKO) mice in advanced disease stages for biophysiological analyses. Western blotting and immunohistochemical (IHC) methods were used to measure the expression of mitochondrial proteins and density of Purkinje cells (PCs), respectively. Electrophysiological recording techniques were used to measure global synaptic properties in the molecular layer of the cerebellar cortex and intrinsic membrane properties of major cerebellar neurons.

Results: Western blotting analysis revealed disruption in only of three mitochondrial biogenesis proteins measured. IHC analysis demonstrated a modest reduction in PC density in FRDAkd mice, with no change in KIKO mice. Synaptic responses in the molecular layer exhibited concordant impairments in basal hypo-excitability and long-term plasticity. Similarly, both models showed similar impairments in action potential firing properties of cerebellar neurons.

Discussion and conclusion: Overall, the results show minimal or no changes in histological or biochemical processes in the two models. However, biophysiological properties were remarkably concordant. Therefore, these findings implicate cellular dysfunction FRDA pathophysiology and suggest that neurodegeneration might be a late effect or secondary contributor to disease phenotypes. Future work involving modulation of cellular activity would address the causal contribution of these biophysiological impairments to FRDA phenotypes. Nonetheless, it appears that synaptic instability and intrinsic cellular communication deficits are common features in FRDA mouse models, strengthening their attributes as more reliable pathological biomarkers of neurological symptoms.

Circuit-mediated vulnerability in SCA1, it's not only about Purkinje neurons

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Federica Pilotto [1], ***Dr. Christopher Douthwaite*** [2], ***Prof. Sabine Liebscher*** [3], ***Prof. Smita Saxena*** [4]

1. *Institut Neuromyogène (INMG-PGNM), Inserm U1315, CNRS-Université Claude Bernard Lyon 1 UMR5261, 69008 Lyon, France,* ***2.*** *Institute of Clinical Neuroimmunology, Klinikum der Universitaet Muenchen, Ludwig-Maximilians University Munich, Martinsried, Germany,* ***3.*** *University Hospital Cologne, Department of Neurology, Cologne, Germany,* ***4.*** *Department of Physical Medicine & Rehabilitation, NextGen Precision Health, University of Missouri, Columbia, MO, USA*

Spinocerebellar ataxia type 1 (SCA1) is a neurodegenerative disease that mainly affects the cerebellum, impairing motor coordination. It is caused by a polyglutamine (PolyQ) expansion in the Ataxin-1 protein causing cerebellar Purkinje neuron (PNs) degeneration. Early changes in neuronal connectivity within the cerebellar circuit can affect activity-dependent synaptic transmission in the cerebellar cortex of SCA1. We here set out to dissect early alterations in cerebellar circuits at a structural, molecular, and functional level to gain insight into the circuit mechanisms and their molecular underpinnings driving PNs degeneration in SCA1. Using a knock-in mouse model of Sca1 and combining chemogenetics with in vivo two photon imaging in behaving mice and neuron specific OMICS approaches, we investigate early network changes within the cerebellum of pre-symptomatic Sca1 mice. We show that there is a selective hyperexcitability of molecular layer interneurons (MLIs) in the Sca1 rodent model. Moreover, chronic chemogenetic inhibition of mutant MLINs delayed PNs degeneration, reduced pathology, and ameliorated motor deficits in Sca1 mice. Finally, we identified a conserved proteomic signature in Sca1 MLIs that is shared with human iPSCs derived SCA1 GABAergic interneurons. Thus, providing for the first time evidence for cerebellar circuit dysfunction in causally propagating SCA1 pathology.

Resting-state cerebello-cortical connectivity associated with cognitive dysfunction in spinocerebellar ataxia type 2

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Ami Kumar [1], ***Ms. Ruo-Yah Lai*** [1], ***Ms. Zena Fadel*** [1], ***Dr. Ming-Kai Pan*** [2], ***Dr. Sheng-Han Kuo*** [1]

1. *Columbia University Medical Center*, ***2.*** *National Taiwan University College of Medicine*

Background and objectives: Spinocerebellar ataxia type 2 (SCA2), is a hereditary cerebellar degenerative disorder, associated with motor and cognitive symptoms. The constellation of cognitive symptoms due to cerebellar degeneration is named cerebellar cognitive affective syndrome (CCAS), which has increasingly been recognized to profoundly impact the patients' quality of life, including SCA2; however, the brain circuits underlying these cognitive dysfunctions remain elusive.

Methods: To explore the cerebellar circuit dysfunction in SCA2, we utilized a novel technique, cerebello-cortical electroencephalogram (EEG), to investigate the resting-state functional connectivity in different frequency domains in 12 SCA2 patients and 24 age-matched controls. Given that the prefrontal cortex is strongly connected to the cerebellum, we studied the EEG connectivity between the cerebellum and prefrontal cortex. We also conducted correlation analyses to explore the association between this connectivity and the severity of cognitive dysfunction, determined by CCAS scores, in SCA2 patients.

Results: Functional connectivity between posterior cerebellar regions and the prefrontal cortex revealed decreased theta and increased beta connectivity in SCA2 patients, with no differences in delta and alpha connectivity. Interestingly, the theta and beta frequency connectivity correlated with CCAS scores in SCA2 patients.

Discussion and conclusion: Our findings demonstrated distinct abnormal connectivity patterns in SCA2 patients as compared to healthy controls. The correlation of theta and beta frequency connectivity with CCAS scores highlights the dysfunctional circuitry linked to cognitive impairment. Further, our findings show that cerebello-cortical EEG could be a tool to track circuit dysfunction underlying these cognitive symptoms of cerebellar degenerative disorders, paving the way for developing targeted neuromodulation therapeutics.

The Relationship Between Purkinje Cells and Nuclei Cells in Mouse Models for Ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Alyssa Lyon [1]***, Dr. Meike van der Heijden*** [2]

1. *Graduate Program in Translational Biology, Medicine, and Health, Virginia Tech, Blacksburg, VA,* ***2.*** *School of Neuroscience, Virginia Tech, Blacksburg, VA . Fralin Biomedical Research Institute at VTC, Roanoke, VA. Graduate Program in Translational Biology, Medicine, and Health, Virginia Tech, Blacksburg, VA*

Background: Cerebellar dysfunction can cause ataxia but how abnormal signals in the cerebellum lead to abnormal movements is not fully understood. The cerebellum is comprised of inhibitory Purkinje cells (PC) which synapse onto cerebellar nuclei (CN) cells to relay their output to the rest of the brain. The relationship between PC and CN spiking activity during ataxia remains unclear. This project investigates neural signal changes in ataxia mouse models by measuring single-neuron spike train properties in PC and CN. Because PC is inhibitory, we hypothesize an inverse relationship between PC and CN firing rate (FR).

Methods: Using an existing database of single-neuron recordings, we measured spike train properties in PC and CN in ataxia mouse models. The database also has recordings of optogenetically induced, acute changes in PC spike signals that cause ataxia. We used linear regression analyses on different spike parameters to determine which changes in PC spike pattern changes parameters best predict CN changes.

Results: There was no inverse relationship between PC and CN FR in ataxia mouse models. However, we found positive relationships between PC and CN spike irregularity in ataxia models. In the optogenetic analyses, the FR was the only parameter with a significant inverse relationship between PC and CN.

Discussion & Conclusion: Spike activity results suggest spike parameters for spike irregularity best predict CN spike activity based on PC spike activity changes in ataxia mouse models. Optogenetic results allow us to observe the direct effect PC FR has on CN FR. Results indicate FR is the most susceptible to the synchronized activity driven by optogenetic manipulation, as opposed to the parameters measuring spike irregularity in ataxia models. This is crucial information because it provides a better insight into how disease-causing spike properties are propagated through the cerebellar circuit to cause ataxia.

Poster session II - Disease mechanisms

The FGF14-SCA27B GAA•TTC repeat shows a marked somatic expansion bias in the cerebellum

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. David Pellerin [1], ***Dr. Jean-Loup Méreaux*** [2], ***Dr. Susana Boluda*** [3], ***Dr. Matt C. Danzi*** [1], ***Ms. Marie-Josée Dicaire*** [4], ***Mrs. Claire-Sophie Davoine*** [5], ***Mr. David Genís*** [6], ***Ms. Guinevere Spurdens*** [1], ***Dr. Catherine Ashton*** [4], ***Jillian M. Hammond*** [7], ***Mr. Brandon Gerhart*** [8], ***Dr. Viorica Chelban*** [9], ***Dr. Phuong U. Le*** [4], ***Maryam Safisamghabadi*** [4], ***Christopher Yanick*** [10], ***Hamin Lee*** [9], ***Dr. Sathiji K. Nageshwaran*** [11], ***Dr. Gabriel Matos-Rodrigues*** [12], ***Dr. Zane Jaunmuktane*** [13], ***Dr. Kevin Petrecca*** [4], ***Dr. Schahram Akbarian*** [14], ***Dr. Andre Nussenzweig*** [12], ***Dr. Karen Usdin*** [15], ***Dr. Mathilde Renaud*** [16], ***Dr. Céline Bonnet*** [16], ***Dr. Gianina Ravenscroft*** [17], ***Dr. Mario Saporta*** [18], ***Dr. Jill Napierala*** [8], ***Prof. Henry Houlden*** [9], ***Dr. Ira Deveson*** [7], ***Dr. Marek Napierala*** [8], ***Prof. Alexis Brice*** [3], ***Dr. Laura Molina Porcel*** [19], ***Danielle Seilhean*** [3], ***Dr. Stephan Zuchner*** [1], ***Prof. Alexandra Durr*** [20], ***Dr. Bernard C. Brais*** [4]

1. *Dr. John T. Macdonald Foundation Department of Human Genetics and John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL, USA,* ***2.*** *1 Sorbonne Université, Institut du Cerveau - Paris Brain Institute- ICM, Inserm, CNRS, APHP, University Hospital Pitié-Salpêtrière, Paris, France,* ***3.*** *Sorbonne Université, Institut du Cerveau - Paris Brain Institute- ICM, Inserm, CNRS, APHP, University Hospital Pitié-Salpêtrière, Paris, France,* ***4.*** *Department of Neurology and Neurosurgery, Montreal Neurological Hospital and Institute, McGill University, Montreal, QC, Canada,* ***5.*** *Sorbonne Université, Institut du Cerveau - Paris Brain Institute- ICM, Inserm, CNRS, APHP, University Hospital Pitié-Salpêtrière,* ***6.*** *Neurodegeneration and Neuroinflammacion Group, Institut d'Investigació Biomèdica de Girona (IDIBGI), Girona,* ***7.*** *Genomics and Inherited Disease Program, Garvan Institute of Medical Research, Sydney, NSW, Australia,* ***8.*** *Department of Neurology, University of Texas Southwestern Medical Center, Dallas, TX, USA,* ***9.*** *Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology and The National Hospital for Neurology and Neurosurgery, University College London, London, United Kingdom,* ***10.*** *Department of Neuroscience, University of Miami Miller School of Medicine,* ***11.*** *Neurogenetics Program, Department of Neurology, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA, USA.,* ***12.*** *Laboratory of Genome Integrity, National Cancer Institute, NIH, Bethesda, MD, USA,* ***13.*** *University College London,* ***14.*** *Department of Psychiatry, Department of Neuroscience and Department of Genetics and Genomic Sciences, Friedman Brain Institute Icahn School of Medicine at Mount Sinai, New York, NY, USA,* ***15.*** *Laboratory of Cell and Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA,* ***16.*** *Laboratoire de Génétique, CHRU de Nancy, France,* ***17.*** *Centre for Medical Research University of Western Australia and Harry Perkins Institute of Medical Research, Perth, Western Australia, Australia,* ***18.*** *Department of Neurology and Neuroscience, University of Miami Miller School of Medicine,* ***19.*** *Neurological Tissue Brain Bank, Biobanc-Hospital Clínic-FRCB-IDIBAPS, Barcelona, Spain,* ***20.*** *Sorbonne University, Paris Brain Institute, Pitié-Salpêtrière Hospital*

Background: Spinocerebellar ataxia 27B (SCA27B) is a common late-onset autosomal dominant ataxia caused by an intronic GAA•TTC repeat expansion in *FGF14*. Neuropathological studies have shown that the disease process is restricted to the cerebellum. Although the expanded repeat is highly unstable during intergenerational transmission, whether it exhibits somatic instability remains unknown. Here, we conducted an analysis of the *FGF14* GAA•TTC repeat somatic instability across serial blood samples, fibroblasts, induced pluripotent stem cells (iPSCs), and post-mortem brains.

Methods: We determined the GAA•TTC repeat length and expansion index, which measures the degree of somatic expansion, on 156 serial blood samples from 69 individuals, fibroblasts and iPSCs from three SCA27B patients, and post-mortem brain tissues from six controls and six SCA27B patients. We also performed methylation analysis of *FGF14* in the post-mortem cerebellar hemisphere of four controls and four SCA27B patients using targeted long-read

nanopore sequencing.

Results: Blood samples exhibited minimal somatic instability, which did not significantly change over periods of more than 20 years. There was minimal difference in the length of the expanded GAA•TTC tract between blood samples, fibroblasts, and iPSCs. In the brain, the GAA•TTC repeat was remarkably stable across the 15 regions analyzed, except in the cerebellar hemispheres and vermis. The levels of somatic expansion in the cerebellar hemispheres and vermis were, on average, 3.15 and 2.72 times greater relative to other examined brain regions, respectively. The levels of somatic expansion in the brain increased with repeat length and tissue expression of *FGF14*. Furthermore, we found no significant difference in methylation of *FGF14*, its promoters, or the region surrounding the repeat locus between patients and controls.

Discussion and Conclusion: Our study revealed that the *FGF14* repeat exhibits a unique cerebellar-specific expansion bias, potentially accounting for the pure cerebellar involvement in SCA27B.

Abnormal peripheral nervous system myelination during early postnatal development in a Friedreich's ataxia mouse model

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Jordi Magrane [1], ***Ms. Shreya Kadam*** [1], ***Dr. Clementina Mesaros*** [2], ***Dr. Carmen Melendez-Vasquez*** [3]

1. *Feil Family Brain and Mind Research Institute, Weill Cornell Medicine,* ***2.*** *University of Pennsylvania, Perelman School of Medicine,* ***3.*** *Department of Biological Sciences, Hunter College*

Myelin abnormalities in Friedreich's ataxia (FRDA) have been described in both peripheral (PNS) and central nervous systems, although barely studied in animal models for the disease. Clinical evidence suggests that PNS neuropathology is established early during disease process. Therefore, we aimed to study whether loss of frataxin (FXN) in myelinating cells during early postnatal development contributes to PNS pathology in FRDA.

We found that KIKO neonates exhibited specific delays in acquiring sensorimotor skills, including proprioception, during the first two weeks after birth. Because Schwann cell (SC) differentiation and maturation and PNS myelination largely occurs during the first 2-3 weeks of postnatal development in mice, we speculate that abnormal myelination could be responsible for this loss of proprioception.

We now report abnormalities affecting PNS myelin in KIKO mouse neonates. Analyses of sciatic nerves from postnatal day 15 KIKO pups revealed reduced myelin thickness and increased number of unmyelinated sensory axons. We detected a significant decrease in MBP expression, while other myelin-related proteins, such as P0 and MAG, were expressed at control levels. Electron microscopy studies uncovered myelin debris in the SC cytoplasm, uncompacted myelin, axon degeneration, and detachment of SC-axon plasma membranes. Interestingly, nerves from adult KIKO mice presented normal MBP levels, but disrupted myelin sheets (non-compact myelin) were still frequently observed. We are currently analyzing changes in the lipidome of KIKO sciatic nerves at different stages of postnatal development to reveal myelin lipid composition during active myelination and myelin maintenance.

These early abnormalities strongly suggest that SC and myelin are a primary target of FXN deficiency in the KIKO mouse model and contribute to the sensory neuropathy occurring in FRDA. Moreover, our results challenge the notion that impairments observed in the nervous system in FRDA individuals are merely neurodegenerative in nature.

Funding: FARA and MDA to J.M.

Somatic repeat expansions as a therapeutic target for trinucleotide repeat disorders

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Ricardo Mouro Pinto [1], ***Mr. Maheswaran Kesavan*** [2]

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To date, more than 50 disorders have been associated with expanded DNA repeat loci. A hallmark of most, is the fact that these repeats are somatically unstable: i.e. they tend to further expand throughout the life of a patient in an age- and cell-type specific manner. It is now well accepted for a few of these disorders, especially for Huntington's disease, that somatic expansions are actually the primary driver of disease onset and symptom's progression. Naturally, targeting this mechanism to prevent somatic expansions from occurring has gathered broad enthusiasm as a promising therapeutic strategy that may be common to multiple repeat expansions disorders.

However, our understanding of this mechanism, across different repeat loci and disease models is limited. To address that, we developed an *in vivo* platform for systematic genetic screening of somatic instability modifiers. This platform relies on delivering AAVs to KO genes of interest using CRISPR/Cas9. With this strategy we target multiple genes of interest among littermates rather than having to perform independent genetic crosses. It also allows for systematic comparison of genetic modifiers across different disease mouse models since the same AAV reagents are used. Importantly, it also allows for modelling SI in disease-relevant tissues.

We will present progress so far of using this platform to test 60 candidate genes for Huntington's disease, 12 for Friedreich ataxia and 8 for Spinocerebellar ataxia type 1. Importantly, we identified genes that have shared contributions towards somatic instability, but we also identified differences between the different disease models.

This information is critical for rational therapeutic development for these conditions by targeting somatic expansions. We will also report on our progress developing "one-and-done" CRISPR base editing therapeutics targeting this mechanism with potential application for multiple repeat expansion diseases.

Funding sources: NIH; NAF; HDF; HDSA; CHDI; Pfizer Inc.

Uncovering DNA methylation patterns in brain and blood samples of Machado-Joseph disease carriers.

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Luís Teves [1], ***Dr. Ana Rosa Vieira Melo*** [2], ***Dr. Ana F. Ferreira*** [3], ***Dr. Mafalda Raposo*** [4], ***Dr. Maria do Carmo Costa*** [5], ***Dr. Ricardo Ferreira*** [6], ***Prof. Carolina Lemos*** [7], ***Prof. Manuela Lima*** [8]

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Machado-Joseph disease (MJD) is an untreatable autosomal dominant polyglutamine ataxia with marked clinical heterogeneity. Incomplete genotype-phenotype correlations suggest that in addition to the number of CAG repeats, other factors, such as epigenetic modifications, may influence MJD pathogenesis. While specific genomic DNA methylation patterns have been shown to impact the pathophysiology of some neurodegenerative disorders, this is unknown for MJD. In this study, we aimed to identify methylation alterations associated with MJD in blood and in a brain region vulnerable to neurodegeneration. DNA methylation profiles were obtained from 24 blood samples of MJD carriers (7 preclinical carriers; 17 patients) and 24 age-, sex-, and smoking status-matched controls, and from *post-mortem* dentate cerebellar nucleus (DCN) samples of 6 MJD patients and 6 age- and sex-matched controls. We identified 101 differentially methylated positions (DMPs) in the blood (p-value < 0.05 and mean beta difference > 0.1): 34 hypermethylated (mainly in 5' UTR) and 67 hypomethylated. In DCN, using the same cutoff, we identified 1123 DMPs: 545 hypermethylated and 578 hypomethylated, with a large proportion located in gene bodies. In blood, 367 differentially variable positions (DVPs) were detected with MJD subjects showing slightly more variable positions than controls (211 in MJD; 156 in controls). In DCN, no DVPs were detected in MJD and controls. Our results indicate that genomic DNA methylation alterations in MJD are subtle but located in gene regions affecting transcription, which is known to be dysregulated in MJD. Despite the small sample size, methylation changes are more pronounced in the DCN than in the blood. Notably, in DCN, gene ontology enrichment of DMPs highlighted inflammation-related aspects. Ongoing studies are associating DNA methylation profiles with clinical data to elucidate its influence on clinical presentation. Moreover, DNA methylation profiles in preclinical carriers are being analyzed to disclose putative changes occurring before clinical diagnosis.

Exploring The Role of Sacsin and S100B in Cytoskeleton Organization in ARSACS glial model

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Ana Sofia Boasinha Ribeiro [1], ***Ms. Fernanda Murtinheira*** [1], ***Prof. Cláudio Gomes*** [2], ***Dr. Federico Herrera*** [1]

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Autosomal recessive spastic ataxia of Charlevoix□Saguenay (ARSACS) is caused by mutations in the SACS gene, resulting in defective forms of the protein sacsin. While most ARSACS studies have focused on neuronal cells, we observed high levels of sacsin in astroglia. Sacsin knockout (KO) in rat astroglial-like C6 cells leads to accumulation of intermediate filaments (IFs) in the juxtanuclear area and upregulation of the S100B chaperone, which in other models interferes with the formation of toxic protein aggregates. The main goal of this study was to understand the possible relationship between sacsin, S100B and cytoskeleton organization. Sacs-/- C6 cells exhibited S100B accumulation near IF aggregates. siRNA- or pentamidine-induced inhibition of S100B led to increased mitochondrial fragmentation and circularity of IF aggregates. Higher concentrations of exogenous S100B reduced IF circularity and increased IF area distribution throughout the cell. Our results may provide relevant information for the future treatment of ARSACS but also advance our basic understanding of the function of sacsin and S100B proteins.We acknowledge the BioISI/FCUL Microscopy Facility, a node of the Portuguese Platform of BioImaging (PPBI-POCI-01-0145-FEDER-022122). FH was supported by a grant from the ARSACS foundation (Canada). FH and MR were supported by centre grants UIDB/04046/2020 and UID/MULTI/04046/2020 (to BioISI) funded by FEDER funds through COMPETE2020-Programa Operacional Competitividade e Internacionalização (POCI) and national funds through Fundação para a Ciência e Tecnologia (Ref. PTDC/FIS-MAC/2741/2021). PP and FM were supported by PhD fellowships from FCT (Ref. 2022.14141.BD and SFRH/BD/133220/2017, respectively). This study was supported by the European Union (TWIN2PIPSA, GA101079147).

Non-AUG initiated translation of short tandem repeats initiates neurodegenerative cascades that contribute to cerebellar ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Clare Wieland [1], Ms. Amy Krans [2], Ms. Samantha Grudzien [1], Mr. Ross Kaufhold [1], Dr. Sinem Ovunc [1], Dr. Nurun N. Borna [1], Ms. Shannon Miller [1], Ms. Sydney Willey [1], Ms. Kirsten Warcup [1], Dr. Melissa Asher [1], Dr. Sami Barmada [1], Dr. Jay Brito Querido [1], Dr. Connor Maltby [1], Dr. Evrim Yildirim [1], Dr. Peter Todd [1]

1. *University of Michigan,* ***2.*** *University of Michgan*

Objectives: Short Tandem Repeat (STR) expansions cause more than 60 different neurological disorders, including many genetic forms of Cerebellar Ataxia. Many of these expansions reside outside of annotated protein coding exons but are still translated into aggregation-prone proteins that exhibit intrinsic toxicicty and accumulate within intranuclear neuronal inclusions in patient brains. Exactly how STRs trigger repeat associated non-AUG initiated (RAN) translation and how these events contribute to disease pathogenesis remains poorly understood.

Methods: We used a series of reporter based assays and biochemical techniques to selectively measure RAN translation at CGG/CCG, CAG, AAAAG/CCCCT, AAGGG/CCCTT and GGGGCC repeats in both human cell lines and rodent neurons, with validation through endogenous MSD-based assays in patient derived neurons to define RAN translation mechanisms and modifiers. In parallel, we performed studies in *Drosophila,* rodent and human iPSC based disease model systems to define the contribution of specific RAN translation modifiers to disease-relevant phenotypes.

Results: The efficiency of RAN translation differs significantly across STRs and across reading frames within a given STR. RAN translation can occur through both 5' M^7G cap-dependent and and cap-independent mechanisms with a significant influence from surrounding sequence, repeat structure, cell type, and cell state. Modifers of start codon fidelity, RNA helicase activity, RNA trafficking and ribosomal quality control all impact the efficiency of RAN translation, creating feed-forward cascades that integrate with cellular stress pathways, inflammation and mitochondrial dysfunction to influence toxicity in model systems. Selective targeting of RAN translation alleviates some – but not all – repeat associated toxicity in rodent and fly models and in human neurons.

Discussion/Conclusion: Non-AUG initiated translation of STRs contributes to neurodegeneration and is selectively targetable with genetic and pharmacological interventions. These insights provide a provide a path to rational therapy design in genetic forms of cerebellar ataxia.

ATXN3 is A Key Regulator of Neuronal Splicing and Tau Isoform Dynamics

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Liliana Meireles-Costa [1], Dr. Sara Duarte-Silva [1], Dr. Andreia Neves-Carvalho [1], Dr. Joana M. Silva [1], Ms. Daniela Monteiro-Fernandes [1], Ms. Joana Correia [2], Ms. Beatriz Rodrigues [3], Dr. Sasja Heetveld [4], Ms. Ana Rita Ferreira-Fernandes [1], Ms. Daniela Vilasboas-Campos [2], Dr. Bruno Almeida [1], Dr. Joana Pereira-Sousa [5], Dr. Nuno Silva [1], Dr. Natalia Savytska [4], Dr. Jorge Diogo Da Silva [2], Dr. António Salgado [1], Dr. Ioannis Sotiropoulos [1], Dr. Ana Luísa Carvalho [3], Dr. Peter Heutink [4], Prof. Ka Wan Li [6], Dr. Andreia Teixeira-Castro [1], Prof. Patrícia Maciel [1]

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Objectives: This study aimed to investigate the role of ATXN3, a deubiquitylating enzyme mutated in spinocerebellar ataxia type 3 (SCA3), in regulating neuronal mRNA splicing. Specifically, the focus was on how ATXN3 influences splicing factors, particularly SRSF7, and its downstream effects on MAPT exon 10 splicing.

Methods: Neuronal cells with reduced ATXN3 levels were analyzed by proteomics to identify changes in the ubiquitome, focusing on proteins involved in RNA metabolism and splicing factors/regulators. Transcriptomic analysis and reporter minigenes were used to assess global splicing capability in cells with decreased ATXN3. Immunoblotting was used to assess dynamic and steady-state levels of splicing regulators. The study also employed cellular models of expanded polyQ ATXN3 and brain tissues from SCA3 patients to validate the findings.

Results: Reduced ATXN3 levels led to altered polyubiquitylation in RNA metabolism proteins, including splicing factors/regulators, resulting in widespread splicing dysregulation. The deficiency in ATXN3 affected SRSF7 levels, causing the deregulation of a known target of this splicing regulator - MAPT exon 10 splicing - and thus leading to a decreased 4R/3R-tau ratio. Similar splicing alterations were observed in SCA3 cellular models expressing expanded ATXN3 and in SCA3 patient brains, underscoring the relevance of ATXN3 loss of function in the disease process.

Discussion: This study establishes a connection between ATXN3, SRSF7, and tau splicing – known to be perturbed in different neurodegenerative diseases, revealing a novel mechanism that may be involved in SCA3 pathogenesis. These findings highlight the critical role of ATXN3 in maintaining splicing integrity and Tau isoform balance in neurons.

Conclusion: ATXN3 is crucial for regulating neuronal mRNA splicing and Tau isoform ratios through its deubiquitylating activity on splicing factors. Perturbation of this ATXN3 function may be an important contributor for the neurodegenerative process that takes place in SCA3.

Common striatal vulnerabilities in spinocerebellar ataxia type 1 and Huntington's disease

Wednesday, 13th November - 18:00: (Minories) - Poster

Ashley Owens [1], ***Changwoo Lee*** [1], ***Isabelle Kowal*** [2], ***Dr. Neha Gogia*** [1], ***Dr. Lucile Megret*** [3], ***Stephen Gilliat*** [1], ***Luhan Ni*** [1], ***Eunwoo Bae*** [1], ***Dr. Christian Neri*** [3], ***Dr. Janghoo Lim*** [1]

1. *Yale University,* ***2.*** *University of Pennsylvania School of Medicine,* ***3.*** *Sorbonne Université, Paris Brain Institute, INSERM, CNRS, APHP, Paris, France*

Spinocerebellar ataxia type 1 (SCA1) is a dominantly inherited neurodegenerative disease resulting in deficits in motor and cognitive function as well as altered mood. The cerebellum has been extensively studied in SCA1, but the role of extra-cerebellar regions, particularly the striatum, remains relatively underexplored despite evidence of striatal involvement in disease pathogenesis. Here, we investigate the impact of polyglutamine-expanded ataxin-1 in the striatum using a SCA1 mouse model and demonstrate striatal degeneration, loss of spiny projection neurons (SPNs), and astrogliosis. Additionally, we performed single-nucleus RNA sequencing (snRNA-seq) in the striatum of SCA1 mice and found dramatic transcriptomic alterations across cell types, but in particular SPNs. Next, we completed a cross-disease transcriptomic analysis with snRNA-seq data from Huntington's disease mouse models and found striking transcriptomic overlap, suggesting shared molecular mechanisms between different polyglutamine diseases. Finally, we completed an *in* vivo genetic interaction study to identify molecular drivers of the transcriptomic alterations. Our findings highlight the importance of the striatum in polyglutamine disease pathology and provide insights into potential therapeutic strategies for SCA1 and related neurodegenerative disorders.

The effect of ketosis on the FRDA phenotypes

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Lucie Ngaba [1], ***Ms. Miniat Adeshina*** [1], ***Dr. Clementina Mesaros*** [2], ***Mr. Peining Xu*** [2], ***Prof. David Lynch*** [3], ***Prof. Yina Dong*** [1]

1. *Department of Pediatrics and Neurology, The Children's Hospital of Philadelphia,* ***2.*** *University of Pennsylvania, Perelman School of Medicine,* ***3.*** *Children's Hospital of Philadelphia*

Background and objectives

Frataxin deficiency caused OXCT1 reduction compromises ketone body metabolism. This study aims to investigate the impact of ketosis on the Friedreich's ataxia phenotypes in our animal model.

Methods

Frataxin knock-in/knockout (KIKO) mice were crossbred with OXCT1 heterozygote ($OXCT1^{+/-}$) mice to generate KIKO mice with a 50% knockout of OXCT1 ($KIKO/OXCT1^{+/-}$). The cerebellum and skeletal muscle tissue from frataxin knock-in (KIWT), KIKO, and $KIKO/OXCT1^{+/-}$ mice were homogenized and then subjected to Western blot analysis for the evaluation of OXCT1 and frataxin levels. Rotarod analysis was used to measure the neurobehavioral phenotype.

Results

When compared to KIKO mice, OXCT1 protein levels were further decreased in $KIKO/OXCT1^{+/-}$mice throughout all tissues including the cerebellum and skeletal muscle. While OXCT1 deficiency potentiated cell death *in vitro*, a further decrease in OXCT1 *in vivo* in $KIKO/OXCT1^{+/-}$ mice resulted in ketosis as evidenced by elevated levels of serum Beta-hydroxybutyrate (BHB). This ketosis was accompanied by markedly elevated frataxin levels in the homogenates of cerebellum and skeletal muscle from KIKO/$OXCT1^{+/-}$ mice. The increase in frataxin levels was due to enhanced mitochondrial biogenesis rather than increased *FXN* gene expression, as evidenced by significantly increased levels of mitochondrial markers such as TFAM, NRF2, and Sirtuin1. Furthermore, KIKO/$OXCT1^{+/-}$ mice performed better in the rotarod test, indicating that OXCT1 deficiency-caused ketosis also improves behavioral phenotype.

Discussion and Conclusion

Our results demonstrate that OXCT1 deficiency-caused ketosis results in improved frataxin levels and neurobehavioral phenotype in KIKO/$OXCT1^{+/-}$ mice in comparison with KIKO mice. This effect is attributed to increased mitochondrial biogenesis as evidenced by increased mitochondrial biogenesis markers. To conclude, our findings imply that ketone bodies not only serve as an energy source but also control mitochondrial biogenesis via an as-yet-unidentified mechanism, suggesting a potential benefit of a ketogenic diet for treating **Friedreich's ataxia.**

Role of the serotonin receptor 5-HT3/LGC-50 in the suppression of Spinocerebellar Ataxia type 3 (SCA3) pathogenesis

Wednesday, 13th November - 18:00: (Minories) - Poster

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Introduction and Objective: Spinocerebellar ataxia type 3 (SCA3), also known as Machado-Joseph disease, is a neurodegenerative disorder caused by an unstable expansion of CAG trinucleotide repeats in the ataxin-3 gene, leading to an abnormally long polyglutamine tract in the ATXN3 protein, prone to self-associate and form toxic aggregates. Previous studies showed that citalopram (CIT) treatment, an antidepressant that inhibits the serotonin transporter (SERT), ameliorates SCA3 pathogenesis in *Caenorhabditis elegans (C. elegans*) and mouse models. Binding of CIT prevents serotonin (5-HT) reuptake from the synaptic cleft making it more available for signalling through 5-HT receptors (5-HTRs). CIT action on the suppression of SCA3 depends on the 5-HTRs SER-1/5-HT_2R and SER-4/5-HT_{1A}R, the latest being also a possible therapeutic target for the disease. Recently a new 5-HTR was described in *C. elegans*, the LCG-50 receptor, resembling the human 5-HT_3R. Here, we aimed at exploring the potential orthology of both receptors, as well as the role of LGC-50/5-HT_3R in the suppression of SCA3 using *C. elegans* as a model organism.
Methods and Results: Like the human counterpart, LGC-50 is a pentameric ligand-gated cation channel, showing high similarity score and low e-value in 2D by the HHpred (0.336 and $3.8e^{-51}$, respectively). 3D analysis also suggested high homology and similar ligand binding sites (PyMOL). We are currently exploring the role of LGC-50 in the pathogenesis of SCA3, using advanced genetic tools. Surprisingly, preliminary data suggested that the sole expression of LGC-50, while the other *C. elegans* 5-HTRs are mutated- LGC-50 only- in mutant ATXN3-expressing animals, is sufficient to suppress SCA3 pathogenesis in *C. elegans*. We are currently investigating the necessity of this receptor for CIT therapeutic action, as well as understanding the molecular mechanisms underlying these findings.
Conclusion: These preliminary results further support the importance of serotonergic signalling in SCA3/MJD pathogenesis and treatment.

Modifiers of GAA·TTC repeat expansion in FRDA Induced Pluripotent Stem Cells

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Tea Umek [1], ***Dr. Negin Mozafari*** [2], ***Mrs. Salomé Milagres*** [3], ***Mr. Raul Cuellar*** [3], ***Prof. Edvard Smith*** [3], ***Dr. Tanel Punga*** [4], ***Dr. Rula Zain*** [3]

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Friedreich's ataxia (FRDA) is an autosomal recessive disorder characterized by a pathological expansion of GAA•TTC triplet-repeats in the first intron of the *Frataxin* (*FXN*) gene. The repeats expand both intergenerationally and in somatic cells. The mechanisms responsible for repeat expansions are still being studied, however, several genetic modifiers have been identified to facilitate or hinder repeat expansion in a combined mammalian cell and yeast model (Rastokina et al, 2023).

To test these in a more relevant repeat expansion model, we used FRDA induced pluripotent stem cells (iPSCs), since they are characterized by intrinsic repeat expansions. Selected proteins involved in DNA replication and DNA repair mechanisms (i.e., SHPRH, WRN, Rad52, DDX11) were downregulated by a continuous siRNA treatment during several FRDA iPSC passages. Cell pellets were collected after every passage and RNA and DNA were isolated. We confirmed low levels of the siRNA-targeted mRNA using RT-qPCR. To analyse the level of repeat expansion, we amplified the repeat region using PCR. Samples were then run on a Fragment Analyzer to determine the rate of repeat instability. As expected, the downregulation of certain DNA repair enzymes and proteins involved in DNA replication decreases the rate of repeat expansion. This suggests that these are the facilitators. On the contrary, reducing the levels of certain proteins increased the expansion rate. These proteins most likely suppress further genetic expansion.

Identifying modifiers of repeat expansion in this model lays the foundation for further studies. It is known that the repeats expand differently in specific tissues, with the dorsal root ganglia, heart and pancreas having the largest GAA·TTC repeat tracts. To understand tissue or cell type-specific expansion rates, iPSCs should be further differentiated to relevant cells or the use of animal models is needed.

Molecular consequences of spinocerebellar ataxia type 5 mutations in spectrin-repeat domains of β-III-spectrin

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Sarah Denha [1], ***Ms. Naomi DeLaet*** [1], ***Ms. Abeer Abukamil*** [1], ***Ms. Angelica Alexopoulos*** [1], ***Ms. Amanda Keller*** [1], ***Ms. Alexandra Atang*** [1], ***Dr. Adam Avery*** [1]

1. *Oakland Univeristy*

Objectives

Spinocerebellar ataxia type 5 (SCA5) mutations cluster in the actin-binding domain (ABD) and the adjacent spectrin-repeat domains (SRDs) of β-III-spectrin. A common molecular consequence of ABD-localized mutations is increased actin binding. However, little is known about the molecular consequences of the SRDs-localized mutations. It is known that SRDs of β-spectrin proteins interact with α-spectrin to form a β/α-spectrin dimer. In addition, the SRDs of β-spectrin and spectrin-related proteins are known to contribute to actin binding. Here, we tested if the SRD-localized mutations, R480W and E532_M544del, impact β-III-spectrin binding to α-II-spectrin and/or actin.

Methods

To determine whether R480W or E532_M544del disrupts dimerization with α-II-spectrin, we performed cell-based FRET, co-immunoprecipitation and subcellular localization assays. Further, *in vitro* biochemical protein binding assays were performed using Native PAGE. To complement experimental approaches, AlphaFold protein interaction software was used. To determine the impact of the mutations on actin binding, we conducted actin co-sedimentation assays using purified β-III-spectrin proteins.

Results

For the E532_M544del mutation, our cell-based and *in vitro* binding assays showed that the E532_M544del mutant can bind α-II-spectrin. However, FRET and AlphaFold modeling suggested that the side-by-side coupling of β-III/α-II-spectrin is disrupted following the E532_M544del mutation in SRD3. Further, actin co-sedimentation and subcellular localization assays supported that the mutation increases β/α-spectrin actin binding. In contrast, the R480W mutation did not impact dimerization or actin binding. However, in cells expressing the R480W mutant, large, spherical inclusions were present when co-expressed with α-II-spectrin. This suggests that R480W uniquely alters the co-association of β-III-spectrin and α-II-spectrin by a mechanism not yet fully understood.

Conclusion and Discussion

Both R480W and E532_M544del mutations disrupt the co-association of β-III-spectrin and α-II-spectrin in distinct ways, but without causing a strong loss in binding of the proteins. In addition, E532_M544del increases actin binding, an effect previously shown for ABD-localized SCA5 mutations.

Purkinje cell-to-CN neuron synapse and glia interactions in a mouse model of ARSACS

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Brenda Toscano Marquez [1], ***Mr. Tristan Lemonnier*** [1], ***Mrs. Alyssa Abou-Chakra*** [1], ***Dr. R. Anne McKinney*** [2], ***Dr. Alanna Watt*** [1]

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Background and objectives: Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is an early-onset cerebellar ataxia caused by loss-of-function of the *SACS* gene. Using a *Sacs*$^{-/-}$ mice, we and others have shown progressive decrease in the synaptic innervation from Purkinje cells onto large glutamatergic neurons of the cerebellar nuclei (CN). Glutamatergic CN cells are surrounded by perineuronal nets (PNN), a specialized extracellular matrix that interacts with synapses to stabilize and modulate synaptic plasticity. Glia cells are key regulators of both PNN and synaptic functions. In the cerebellum, dissolving PNN enhances GABA release from Purkinje cell terminals affecting synaptic plasticity. We explored whether glia cells of CN and the extracellular matrix of CN neurons are altered in the Sacs$^{-/-}$ mice. Methods: We used immunohistochemical assays to label PNN, Purkinje cells, astrocytes and microglia. We quantify the intensity of PNN, the size and number of Purkinje cell puncta, the activation state of the astrocytes and number of microglia during disease onset (P40) and progression (P90). Additionally, we used transmission electron microscopy to look at the morphology of putative Purkinje cell puncta onto CN neurons. Results: We found a decrease in the intensity of PNN of CN neurons of ARSACS mice compared to litter-matched controls at both time points. This was accompanied by an increase of Purkinje cell puncta size but a decrease in puncta number. At the same time, we observed an increase in activated astrocytes without a change in microglia number. Discussion and conclusion: These findings support a model where the stability of the synapses made by Purkinje cells onto CN neurons in ARSACS mice is impaired at early stages of disease progression and suggests that astrocytes are a key player mediating synaptic changes. Overall, the changes in the CN are likely to contribute to the pathophysiology of the disease.

Understanding SPAX8 molecular nature through NKX6-2 mutations

Wednesday, 13th November - 18:00: (Minories) - Poster

Mr. Pedro Peralta [1], ***Ms. Fernanda Murtinheira*** [1], ***Dr. Vukosava Torres*** [2], ***Prof. Francisco Pinto*** [2], ***Prof. Mário Rodrigues*** [2], ***Dr. Federico Herrera*** [1]

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Background and Objectives: The NKX6-2 transcription factor regulates the fate of neurons, oligodendrocytes, and pancreatic cells, as well as myelin formation and maintenance. Loss-of-function mutations in NKX6-2 cause Spastic Ataxia 8 (SPAX8), characterized by hypomyelinating leukodystrophy. We aim to understand these mutations' impact on NKX6-2 behavior and function, and thus contribute to design therapeutic strategies for this rare and uncurable disorder.

Methods: We recreated six SPAX8-related mutants (K41*, L163V, E189*, Q197*, R200W, W203*) fused to the Venus fluorescent protein, and analyzed their subcellular distribution by Western blotting and fluorescence microscopy. Concurrently, bioinformatic analyses were conducted using AI tools such as AlphaFold 3.0 to investigate NKX6-2's structural properties and interactions.

Results: We observed that SPAX8-related mutations alter the subcellular localization of NKX6-2 and/or form protein aggregates. Fluorescence Recovery After Photobleaching (FRAP) was used to characterize the dynamics of these aggregates, showing they behave as solid, stable structures. Some SPAX8-related mutants were also unstable and displayed low levels of basal expression, independently of previously proposed Nonsense-mediated mRNA decay mechanisms. Bioinformatic analysis identified other possible amino acid residues that could regulate NKX6-2 stability.

Conclusion: Our results suggest that SPAX8 could belong to the superfamily of protein misfolding disorders. Our unique SPAX8 models could enable high-throughput analysis of the mechanisms involved in NKX6-2 dysfunction and the design of personalized therapeutic approaches.

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Sense and antisense RAN protein aggregates accumulate in Friedreich's ataxia brains and iPSCs.

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Lisa E.L. Romano [1], ***Ms. Camille Preston*** [1], ***Dr. Monica Banez Coronel*** [1], ***Dr. Tao Zu*** [1], ***Prof. S. H. Subramony*** [2], ***Dr. Arnulf H. Koeppen*** [3], ***Dr. Laura Ranum*** [4]

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[Background and Objectives] Friedreich's Ataxia (FA) is a recessive and progressive neuro- and cardio-degenerative disorder caused by a GAA•TTC triplet repeat expansion in the frataxin gene (*FXN*). While most therapeutic strategies aim to restore frataxin protein levels, other unexplored molecular mechanisms include repeat-associated non-AUG (RAN) translation, which has been reported in 11 diseases with non-coding or coding expansion mutations.

[Methods] Our lab recently developed antibodies that detect polySer, polyLeu or polyArg repeat motifs. We also developed novel antibodies that specifically target the c-terminal regions downstream of the *FXN* repeat in the polyLeu and polyGlu frames, allowing the detection of locus-specific FA RAN proteins. Using these antibodies, we performed immunohistochemistry (IHC) on postmortem FA and FA carrier autopsy brains. We also developed human-derived pluripotent stem cells (iPSCs) from peripheral-blood-mononuclear cells of FA patients, FA carriers, and healthy controls.

[Results] IHC shows that antisense polySer and polyLeu RAN protein aggregates accumulate in cerebellum from FA (n>5) but not control (n>4) or FA carriers (n>2) autopsy cases. PolySer and polyLeu RAN protein staining is robust in the cerebellar dentate nuclei, Bergman glia, granule cell layer, and cerebellar white matter regions. PolyLeu C-terminal antibodies confirm that RAN proteins are specifically expressed from the *FXN* locus. Additionally, sense polyArg RAN proteins accumulate in the cerebellar dentate nuclei, granular cell layer, and are also found in iPSCs from FA patients. No similar staining was evident in FA carriers or controls.

[Discussion and Conclusion] Sense and antisense RAN protein aggregates accumulate in FA but not FA-carrier autopsy brains and iPSCs. Understanding the role of RAN proteins and their correlation with frataxin levels will provide novel insight into FA disease mechanisms and potential for developing novel therapeutic strategies.

Nuclear ageing in polyglutamine-induced neurodegeneration: a highlight on Machado-Joseph disease.

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Dina Pereira [1], ***Dr. Janete Cunha-Santos*** [2], ***Dr. Ana Vasconcelos-Ferreira*** [3], ***Dr. Joana Duarte-Neves*** [2], ***Dr. Isabel Onofre*** [2], ***Dr. Vítor Carmona*** [2], ***Dr. Célia A Aveleira*** [4], ***Dr. Luisa Cortes*** [4], ***Dr. Sara Lopes*** [5], ***Ms. Diana Lobo*** [6], ***Dr. Maria Inês Martins*** [4], ***Dr. Nélio Gonçalves*** [2], ***Ms. Ana Margarida Salgueiro*** [7], ***Prof. Cláudia Cavadas**** [3], ***Prof. Luís Pereira de Almeida*** [5]

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Introduction: Machado-Joseph disease (MJD) is an autosomal dominantly-inherited neurodegenerative disorder characterized by over-repetition of the CAG trinucleotide of the *ATXN3* gene. Despite the significant advances produced over the last years, the molecular mechanisms involved in MJD are still unclear and no treatment able to modify the disease progression is available. Ageing is the major risk factor for neurodegenerative disorders, and nuclear membrane proteins – lamins, and lamin-processing related proteins, such as ZMPSTE24, have been shown to be altered, not only during normal ageing, but also in neurodegenerative disorders, such as Alzheimer´s disease. Taking this into account, we aimed at investigating the role of ageing in MJD by evaluating the presence of age-related markers in human and animal MJD models.

Methods:MJD patients' fibroblasts, a Neuroblastoma cell line (N2a) overexpressing ataxin-3, and two MJD mouse models (a transgenic mouse model, expressing a truncated form of ataxin-3 in the cerebellum and a striatal lentiviral model, expressing a full-length human mutant ataxin-3) were studied. To mimic accelerated ageing, viral vectors encoding for progerin were co-injected in the striatal lentiviral MJD mouse model. Western Blot, qPCR and immunolabelling were used to characterize and quantify ageing-associated markers and MJD neuropathology.

Results: Decreased levels of lamins B and C, together with decreased ZMPSTE24 levels were identified in the different MJD models. Accordingly, abnormalities in nuclear circularity, a hallmark of ageing, were also observed. Furthermore, overexpressing progerin, the abnormal lamin A generated in Hutchinson-Gilford Progeria Syndrome patients that present premature/accelerated ageing, in the striatal lentiviral MJD mouse model, induced an aggravation of MJD-associated neuropathology.

Conclusion:Our results suggest that ageing is a key player in the context of MJD pathogenesis, unveiling new pathways for the development of future therapies for the disease.

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

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Marta Daniela Costa [1], Ms. Daniela Vilasboas-Campos [2], Mr. Jorge Fernandes [3], Ms. Cármen Vieira [2], Ms. Lídia Nunes [4], Dr. Andreia Teixeira-Castro [3], Prof. Patrícia Maciel [3]

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Background and Objective

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.

Methods

The model was subjected to EMS treatment to introduce random mutations in its genome. Mutants with improved motor phenotype were isolated from the descendant F2 population.

Results and Discussion

Distinct motor behavioural assays were used to quantify the impact of EMS-induced mutations on motor function, resulting in the identification of 45 screening hits. Currently, whole genome sequencing is uncovering candidate gene modifiers in some of the EMS-screening hits linked with chromatin AND RNA binding, G protein-couple receptor and kinase activity, synaptic transmission, transmembrane transport, among others. RNAi-based silencing strategies of these candidate genetic modifiers are being applied to the C. elegans model of MJD/SCA3 to identify the genetic modifiers of MJD/SCA3 in the *C. elegans* model. Additional studies are planned to validate and confirm their relevance as disease modifiers in patients.

Conclusion

The identification of genetic modifiers of SCA3/MJD, using a key aspect of the disease as readout, hold significant promise for uncovering novel genetic factors influencing this disorder. Most important, such genetic modifier pathways can be targets for new drug development, offering new therapeutic possibilities for these patients.

H2BK120ub alterations as a prominent aspect of the Purkinje cells pathology in spinocerebellar ataxia type 7

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Grażyna Adamek [1], ***Ms. Zuzanna Czarny*** [1], ***Mr. Grzegorz Bartyzel*** [2], ***Dr. Anna Samelak-Czajka*** [3], ***Ms. Magdalena Trybus*** [3], ***Dr. Paweł M. Świtoński*** [1]

1. *Department of Neuronal Cell Biology, Institute of Bioorganic Chemistry, Polish Academy of Sciences,* ***2.*** *Department of Automatic Control and Robotics, AGH University of Science and Technology,* ***3.*** *Laboratory of Single Cell Analyses, Institute of Bioorganic Chemistry, Polish Academy of Sciences*

Background: Purkinje cells (PCs) are large neurons providing the sole output from the cerebellar cortex. Selective degeneration and death of PCs are hallmarks of many human neurological disorders, including spinocerebellar ataxias. Spinocerebellar ataxia type 7 (SCA7) is a rare disease caused by CAG repeat expansion in *ATXN7* gene. The protein product of the gene - ataxin-7 is a core component of STAGA transcription co-activator complex that catalyzes the deposition of the H3K9ac mark and deubiquitination of H2BK120ub.

Objectives: The study aimed to characterize epigenetic changes occurring in PCs of SCA7-266Q animals, focusing on histone marks associated with STAGA complex activity. This should lead to a better understanding of the disease pathology and the process of selective neuronal degeneration that remains poorly understood.

Methods: We used anti-H3K9ac and anti-H2BK120ub antibodies to perform CUT&Tag on PCs isolated from symptomatic SCA7 and WT mice. Further, we collected images of PCs for those histone marks using immunofluorescence stainings and ImageStreamX imaging system for pre/early-symptomatic (3- and 5-week-old) and symptomatic (8-week-old) SCA7 animals and corresponding controls. We analyzed collected data using a customized AI tool and CellProfiler software.

Results: Analysis of collected images allowed us to identify H2BK120ub speckles in SCA7 mice nuclei, which were not present in WT controls. The initial differences were already visible in 3-week-old SCA7 animals and changes were progressing in time. We observed no significant changes in H3K9ac nuclei staining. Moreover, genome-wide investigation of H3K9ac and H2BK120ub allowed us to identify epigenetically altered genes in SCA7 mice, with only a few hits found for H3K9ac.

Discussion and Conclusion: We identified a novel nuclear phenotype suggesting the association of H2BK120ub modification and SCA7 progression in PCs. Although understanding its nature and impact requires further investigation, epigenetic changes seem to be an important aspect of the SCA7 pathology.

Deciphering ferroptosis pathways in dorsal root ganglia of Friedreich Ataxia models. Role of GSK3β and LKB1/AMPK in the impairment of NRF2 response

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Arabela Sanz-Alcázar [1], ***Ms. Marta Portillo-Carrasquer*** [1], ***Dr. Fabien Delaspre*** [1], ***Ms. Maria Pazos-Gil*** [1], ***Ms. Luiza Oliveira*** [1], ***Dr. Jordi Tamarit*** [2], ***Dr. Joaquim Ros*** [1], ***Dr. Elisa Cabiscol*** [1]

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Introduction: Friedreich Ataxia (FA) is a rare neurodegenerative disease mainly caused by the presence of homozygous GAA-triplet expansion in the frataxin gene (*FXN*), causing low frataxin (FXN) protein levels. Nevertheless, some FA patients have a point mutation in one *FXN* allele alongside the expansion.

Objectives: Ferroptosis has recently been implicated in FA. However, its role in DRG sensory neurons, the most and earliest affected cells, remains unknown. Therefore, this study aims to explore the impact of FXN deficiency on DRGs and its association with ferroptosis, as well as the regulatory pathways that modulate it.

Methods: We employed two models: i) primary cultures of DRGs neurons from neonatal rats where FXN levels were reduced by lentivirus transduction and ii) DRGs obtained from the mouse model based on the I151F point mutation in the *FXN* gene (equivalent to the human I154F mutation).

Results and Discussion: FXN deficiency induced upregulation of transferrin receptor 1 and decreased ferritin, leading to mitochondrial iron accumulation, a source of oxidative stress. However, activation of NRF2, a key transcription factor in the antioxidant response pathway, was impaired. Decreased total and nuclear NRF2 levels explain the downregulation of both SLC7A11 (which transports cystine for glutathione synthesis) and glutathione peroxidase 4, responsible for increased lipid peroxidation, the main markers of ferroptosis. This dysregulation could be due to the increased levels of KEAP1 and activation of GSK3β, which promote cytosolic localization and degradation of NRF2. Moreover, a deficiency in the SirT1/LKB1/AMPK pathway might also impair NRF2 activity.

Conclusion: This study demonstrated that FXN deficiency in DRG neurons disrupts iron homeostasis, and the intricate regulation of molecular pathways affecting NRF2 activation and the cellular response to oxidative stress, leading to ferroptosis.

Funding: This work was supported by MINECO (PID2020-1182 96RB-100).

Identifying the astrocyte-mediated molecular mechanisms contributing to neuronal vulnerability in Spinocerebellar Ataxia Type 1 (SCA1).

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Alison Chase [1]

1. *Yale University*

Objective: Spinocerebellar Ataxia Type 1 (SCA1), like many other neurodegenerative diseases, is characterized by the initial dysfunction and eventual death of a specific neuronal population, like cerebellar Purkinje cells (PC) or inferior olivary neurons. While previous literature suggests that the mechanisms underlying this selective neuronal vulnerability are intrinsic to the affected neuronal population, recent work demonstrates that non-neuronal glial cells, including astrocytes (AS), oligodendrocytes (OL), and microglia (MG), may promote changes in neuronal health through non-cell autonomous mechanisms. To explore the role of astrocytes in SCA1 pathogenesis, our lab has developed an astrocyte-specific SCA1 model (AS-SCA1), in which we conditionally overexpress mutant ataxin-1 (*ATXN1*^{82Q}) in astrocytes under a *Gfap* promoter using a tTA-TRE system.

Methods: In this study, we utilize conditional mouse genetics, biochemical and behavioral assays, and single-nucleus RNA-sequencing to address the cell-autonomous and non-cell-autonomous mechanisms by which astrocytes causally contribute to SCA1 pathophysiology.

Results: Gene Ontology (GO) enrichment analysis of Bergmann Glia (BG)-specific differentially expressed genes (DEGs) reveals a downregulation of terms related to synaptic transmission and structure, which our work suggests results from loss of function of the glutamate transporter, EAAT1, and BG-specific Ca-permeable AMPARs. GO analysis of PC DEGs indicates an upregulation of terms related to neurotransmission, which, together with downregulated processes related to synaptic structure, hints at PC excitotoxicity and synaptic dysfunction.

Discussion: Our study suggests that mutant *ATXN1* expression in astrocyte populations leads to loss of homeostatic astroglia functions, which, in turn, negatively impacts PC health. In addition to our sequencing data, pathological analysis of this model confirms the loss of BG glutamate transporters, which may underscore the late-stage PC pathology we observe, including thinning of the cerebellar molecular layer and loss of climbing fiber innervation onto PC dendrites. These findings, therefore, suggest a critical role for astrocytes in facilitating neuronal SCA1 pathogenesis.

A Comprehensive Proteomics Analysis of Friedreich's Ataxia Cells

Wednesday, 13th November - 18:00: (Minories) - Poster

Mrs. zeynep ulukutuk [1], ***Dr. Victor Hernandez*** [1], ***Dr. Sara Anjomani Virmouni*** [1], ***Dr. Faraz Mardakheh*** [2]

1. *Brunel University London,* ***2.*** *William Harvey Research Institute, Barts and the London School of Medicine, Queen Mary University of London, London, EC1M 6BQ, United Kingdom.*

Introduction: Friedreich's ataxia (FRDA) is a neurodegenerative disorder caused by a mutation in the frataxin (*FXN)* gene, which depletes the mitochondrial protein frataxin. This causes iron-sulphur cluster protein deficiencies, increased reactive oxygen species, and mitochondrial dysfunction, leading to cell death. FRDA is characterised by neurodegeneration, diabetes mellitus, and cardiomyopathy, which is the main cause of death. There is currently no cure for FRDA. We investigated the impact of frataxin depletion on the expression patterns of proteins in FRDA fibroblast cell lines by global proteomic analysis using a Tandem Mass Tagging method.

Methods: High-resolution quantitative mass spectrometry (MS) was used to identify differentially expressed proteins in FRDA cell lines. The STRING pathway analysis was used for protein-protein interactions. Gene and protein expression levels were assessed by RT-qPCR and western blot/ELISA respectively.

Results and Conclusions: Using LC/MS-MS, we found differentially expressed proteins between FRDA and control samples at $P<0.05$. Majority of these proteins identified to be downregulated were involved in mitochondrial respiratory chain as subunits or assembly factors of Complex I. Protein-protein network analysis of these proteins with frataxin further supports their involvement in the pathophysiology of FRDA. We were also able to identify a novel protein signature, glutamine-fructose-6-phosphate amidotransferase 2 (GFPT2), that was significantly up regulated in FRDA compared to control cell lines. GFAT2 is a rate-limiting enzyme of Hexosamine biosynthesis pathway (HBP) and has been shown to mediate cardiac hypertrophy by HBP-O-GlcNAcylation-Akt pathway. We also assessed the expression levels of HBP/glycosylation related genes in particular GFPT2 in FRDA cardiomyocytes and consistent with our proteomics analysis we found increased expression of GFPT2 in these cells. Therefore, GFPT2 could be served as a therapeutic target of cardiomyopathy, the main cause of death, in FRDA patients. This study provides insight into mechanisms of FRDA molecular disease progression with implications for future FRDA therapy.

Subcellular localization and ER-mediated cytotoxic function of α1A and α1ACT in spinocerebellar ataxia type 6

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Di Wang [1], ***Prof. Shigeomi Shimizu*** [1], ***Prof. Kinya Ishikawa*** [1], ***Dr. Hidehiro Mizusawa*** [2], ***Prof. Kei Watase*** [1], ***Prof. Shinya Honda*** [1], ***Dr. Min Kyoung Shin*** [1]

1. *Tokyo Medical and Dental University,* ***2.*** *National Center of Neurology and Psychiatry*

[Objectives] Spinocerebellar ataxia type 6 (SCA6) is one of the polyglutamine (polyQ) diseases caused by an expansion of CAG repeats encoding polyQ in the α1A voltage-dependent calcium channel gene CACNA1A. CACNA1A encodes two proteins, namely, full-length α1A (an essential pore-forming subunit of the plasma membrane calcium channel), and α1ACT, the carboxyl-terminal region of α1A translated from the second cistron. Importantly, both α1A and α1ACT have polyQ tracts. The α1A-polyQ and α1ACT-polyQ proteins with an elongated polyQ stretch have been reported to form aggregates in cells and induce neuronal cell death, but the subcellular localization of these proteins and their cytotoxic properties remain unclear. We performed this study to clarify these unidentified points.
[Methods] SCA6 knock-in mice with 118 polyQ tracts and SCA6 cellular models were analyzed to clarify α1A and α1ACT localizations using anti-polyQ antibody and various organelle markers. Cytotoxicity on SCA6 cellular models was assessed through ER stress response and apoptosis assays.
[Results] α1A and α1ACT, when the length of polyQ was normal, localized to plasma membrane and nuclei, respectively. In SCA6 model mice and cellular models, α1A-polyQlong localized mainly to Golgi apparatus, a portion of α1ACT-polyQlong localized to the nucleus. Additionally, in cellular models, both of α1A-polyQlong and α1ACT-polyQlong proteins were partially localized to the endoplasmic reticulum (ER) in cellular models inducing the ER stress response and apoptosis.
[Discussion and conclusion] As a new mechanism, α1A-polyQlong and α1ACT-polyQlong have the potential to activate the ER stress response, suggesting that these multiple mechanisms are intertwined and lead to Purkinje cell dysfunction, degeneration, and death in SCA6 pathogenesis. This study showed for the first time that activated ER stress response takes place by both α1A and α1ACT when polyQ tract is expanded. This may offer a unique insight for future therapeutic development against this disease.

Ataxin-2 is modulating clinical symptoms in SCA3

Wednesday, 13th November - 18:00: (Minories) - Poster

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Background and Objective

The CAG repeat length in Spinocerebellar Ataxia Type 3 (SCA3) is inversely correlated to the age at onset of ataxic gait. However, it only explains 50-80% of disease variability. The *Ataxin-2* gene is described as a genetic risk factor for many neurological diseases including SCA3. The focus of the present work is to determine a known 9bp duplication and the intermediated repeat length of ATXN2 in a large European SCA3 cohort as well as analyze SCA3 mouse models and cell models to evaluate the influence of ATXN2 in SCA3 disease pathogenesis.

Methods

By fragment length analyses, we determined the CAG repeat length within ATXN2 and a known 9bp duplication in a large European SCA3 cohort and correlated our data with clinical data like clinical scores (SARA, INAS) as well as disease progression. Additionally, ATXN2 soluble protein expression and protein aggregation were determined in SCA3 mice. Similar biochemical analyses were performed in an ATXN2 and ATXN3 overexpressing cell culture system.

Results

In our large European SCA3 cohort including 356 participants, only 5% of participants demonstrated an intermediated ATXN2 CAG repeat and 4% an ATXN2 9bp duplication. The intermediated ATXN2 repeat was linked to a lower ATXN3 expanded repeat length and elevated non-ataxic symptoms like sensory symptoms and rigidity. Patients with the 9bp duplication demonstrated a reduced disease duration and faster disease progression. SCA3 mice revealed similar ATXN2 soluble protein expression and no co-aggregation with ATXN3. Interestingly, co-expression of an expanded ATXN3 repeat and intermediated ATXN2 repeat revealed both reduced soluble and aggregated ATXN3 levels but decreased cell viability.

Discussion and Conclusion

The known modulator for several neurodegenerative diseases, Ataxin-2, is modulating SCA3 disease pathogenesis by lowering soluble and aggregated ataxin-3 disease protein which is leading to an elevation of specific non-ataxic symptoms in SCA3 patients.

Deciphering the sensory neurodevelopmental component in Friedreich Ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Lucie Hermet [1], ***Ms. Marie Paschaki*** [1], ***Dr. Helene Puccio*** [2]

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Friedreich Ataxia (FA) is a complex condition that impacts various systems in the body, including the cerebellum, spinal cord, sensory neurons, heart and pancreas. One of its main features is difficulties with movement and coordination, known as ataxia, which stems from neuronal damage in the spinal cord and cerebellum. This neuronal damage also affects the ability to sense the body's position in space, a function called proprioception. It is known that sensory neurons are developed and matured during embryogenesis and recent studies suggest that issues in nervous system development could be a component in FA. A knock-in mouse model, the FxnG127V mouse, has been recently generated and shows very low levels of FXN throughout the entire development. By studying these mice, we hope to uncover whether FA has both degenerative and developmental component, focusing on the sensory neurons of the dorsal root ganglia. Understanding this dual aspect could provide crucial insights into the underlying causes of FA and potentially lead to new treatment strategies.

Transcriptomic analysis of iPSC-cardiomyocytes and proprioceptive neurons from Friedreich's ataxia patients identifies pathogenic biomarkers

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Jackson Chan [1], ***Dr. Sotourella Ellina*** [1], ***Dr. Rosella Abeti*** [2], ***Prof. Paola Giunti*** [2], ***Dr. Chiara Dionisi*** [3], ***Dr. Myriam Rai*** [4], ***Prof. Massimo Pandolfo*** [5], ***Prof. Richard Festenstein*** [1]

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Friedreich's ataxia (FRDA) is a multisystem disorder mainly affects proprioceptive neurons (PPNs) in dorsal root ganglion and cardiomyocytes (CMs) in heart. DNA triplet-repeat (GAA) expansion in Frataxin (FXN) gene is the major cause of FRDA which leads to the depletion of FXN mRNA and protein. To identify molecular features that could be used as the potential biomarkers for FRDA in blood to reliably determine the disease phenotype, we differentiated FRDA derived hiPSCs to CMs and PPNs, and performed RNA sequencing. Transcriptomic analysis of FRDA cells versus healthy controls revealed dysfunctional calcium channel pathway in FRDA-CMs and potassium channels in FRDA-PPNs reflecting the defective cardiac and neuronal function in FRDA, respectively. Both of these pathogenic cell types showed the deregulated oxidative phosphorylation and neural crest stem cell developmental process. Consistent with a previous study of FRDA-PPNs, patient derived hiPSC-CMs also show decreased morphological maturity. We compared our pathogenic biomarkers to the previously published peripheral biomarker lists and illustrated that Cystathionine β-synthase (CBS), catalyzes the production of a gasotransmitter with signaling and cytoprotective effects on neurons, and Scavenger Receptor Class B Member 1 (SCARB1), which encodes a high density lipoprotein cholesterol receptor. They could be used as the non-invasive biomarker to reflect the progression of cardiomyopathy and neuropathy in FRDA patients.

Investigating loss of Replication Factor Complex subunit 1 (RFC1) function in CANVAS patients and heterozygous AAGGG expansion carriers

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Ricardo Schnekenberg [1], ***Dr. Riccardo Curro*** [1], ***Dr. Cecilia Perini*** [2], ***Ms. Natalia Dominik*** [1], ***Dr. Bianca Rugginini*** [3], ***Ms. Arianna Ghia*** [1], ***Dr. Stefano Facchini*** [1], ***Prof. Henry Houlden*** [4], ***Prof. Mary Reilly*** [5], ***Prof. Emmanuele Crespan*** [2], ***Prof. James Jepson*** [1], ***Dr. Andrea Cortese*** [5]

1. *UCL Queen Square Institute of Neurology,* ***2.*** *Institute of Molecular Genetics IGM-CNR, Pavia,* ***3.*** *Department of Brain and Behavioral Sciences, University of Pavia,* ***4.*** *Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology and The National Hospital for Neurology and Neurosurgery, University College London, London, United Kingdom,* ***5.*** *Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London, WC1N 3BG, UK.*

Background

CANVAS is a recessively inherited condition caused in most cases by biallelic AAGGG expansions in RFC1. Despite the recessive mode of inheritance, RFC1 transcript or protein expression appear unchanged. Yet, the identification of compound heterozygous null variants causing CANVAS suggests a role of RFC1 function in the disease pathogenesis.

Methods

Here we show that pathogenic AAGGG expansions form stable nucleic acid structures compatible with G-quadruplexes in vitro and lead to transcription inhibition in vitro and in reporter assays in a repeat-length-dependent manner.

Results

We confirmed that RFC1 transcript and protein expression is preserved in bulk post-mortem cerebellar tissue and IPSC neurons. Long-read RNA sequencing did not show changes in RFC1 transcript processing or splicing. Nonetheless, patients derived lymphoblasts showed increased susceptibility to DNA damage, exhibiting reduced survival and earlier activation of apoptosis when treated with the DNA damaging agents cisplatin or oxaliplatin. Furthermore, we found that neuron-specific knock-down of gnf1 - the Drosophila RFC1 orthologue - led to decreased survival, progressive motor impairment and increased neuronal DNA damage in adult flies, and that these phenotypes were exacerbated by cisplatin treatment.

Because of the known toxicity of platin on sensory neurons, and given the key role of RFC1 in DNA damage repair, we speculated that AAGGG expansions might increase the susceptibility to chemotherapy induced neuropathy in humans. Indeed, in a multicentre cohort of subjects who received oxaliplatin for an underlying neoplasm, heterozygous RFC1 expansion carriers showed an increased risk of developing a severe neuropathy compared to non-carriers (25/34, 73% vs 172/336, 52%, p=0.01).

Conclusion

Although the exact mechanisms causing the selective neuronal loss in CANVAS remain unknown, our in vitro, fruit fly, and human data suggest that RFC1 function is relevant to the disease pathogenesis, and that treatment with DNA damaging agents may unmask a hypomorphic effect of AAGGG expansions.

Circadian rhythms are disrupted in Machado-Joseph Disease: an in vitro, in vivo, and clinical study

Wednesday, 13th November - 18:00: (Minories) - Poster

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Introduction:Circadian rhythms are generated by internal timers that regulate behavioural processes and physiological mechanisms. Sleep–wake cycles are their main output and, importantly, sleep problems have been described in Machado-Joseph disease (MJD). However, clock regulation was not yet studied in MJD pathophysiological mechanisms. Therefore, the present study aimed to investigate the clock components and associated circadian disruptions in MJD.

Methods:Motor activity of MJD patients was assessed for 2 weeks using actigraphy. Taking advantage of the YAC-MJD transgenic mouse model, activity was evaluated by conducting wheel-running experiments, circadian-related neuropeptides(VIP/AVP) by immunohistochemistry, core body temperature using telemetric capsules, and clock genes expression by RT-qPCR. The effects of wild-typeATXN3/mutantATXN3 on the clock were evaluated using bioluminescence reporters *Bmal1*/*Per2*-luciferase.

Results:MJD patients exhibited progressive circadian phenotype alterations, demonstrated by a negative correlation between the Circadian Function Index and MJD clinical scales, including SARA score. Homozygous mice presented significantly reduced levels and increased fragmentation of activity, and decreased levels of VIP and AVP in the master clock(SCN/PVN) compared to WT mice. Furthermore, homozygous mice showed a disrupted core body temperature rhythm, including an abnormal increased temperature at the beginning of the active phase. When submitted to a Jet Lag protocol, hemizygous and homozygous mice required 3.00 and 3.42 more days for re-entrainment, respectively, after an abrupt 4-h-phase-advance in light cycle than WT mice. Additionally, hemizygous and homozygous mice exhibited decreased expression levels of clock-controlled genes analysed every 8 h in the hypothalamus and cerebellum. Finally, we found that both wild-typeATXN3/mutantATXN3 drive the promoter of *Bmal1*, but only wild-typeATXN3 can drive the transcription of *Per*2, a capacity that is lost upon PolyQ expansion in mutantATXN3.

Conclusions:Clock regulation is markedly impaired in MJD and this knowledge is crucial to better understand MJD pathophysiology, identify new biomarkers, and develop novel circadian-based interventions to tackle this fatal disease.

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Pathophysiological roles of the liver in Friedreich's ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

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Friedreich's ataxia (FRDA) is characterized by neuronal and cardiac dysfunctions, in which iron overload-associated mitochondrial dysfunction plays a critical role. However, it remains unknown how other tissues are involved in the FRDA pathologies. Here, we aimed to characterize iron status in the multiple organs of the FRDA mouse model. Among whole-body frataxin deficient mice (Fxn^{null}::YG8s(GAA)>800, #030395, The Jackson Laboratory), we observed the animal group developing a short lifespan (1.5 months), with which we characterized iron status and related markers in the liver, brain, heart, and skeletal muscle of FRDA mice (male, 1-month-old) as compared to age-matched C57BL/6J control mice.

In the FRDA mice, we observed that body weight and muscle mass were significantly decreased ($p<0.05$), although the heart mass was not changed. Notably, the liver was characterized by markedly increased levels of iron (non-heme iron; $p<0.05$), while no difference was observed in other tissues except for the brain ($p<0.05$). In the liver, we also detected significantly increased expression of iron uptake protein (transferrin receptor 1; $p<0.05$), although other iron regulatory makers (iron storage (ferritin) and iron export (ferroportin) proteins) were not changed. However, mRNA levels of hepcidin, the master regulator of iron transport, appeared to be elevated in the liver ($p=0.165$). Using a high-resolution respirometry (O2k-Oroboros), we observed that mitochondrial function (basal (Complex I) and maximal (Complex I & Complex I+II) mitochondrial respiration) was largely impaired in the liver of FRDA mice ($p<0.05$).

Our results suggest that liver function can be affected by abnormal iron status and mitochondrial dysfunction, which may cause or worsen FRDA symptoms or early death. However, more studies are warranted to further understand the roles of the liver in FRDA pathology. Furthermore, our study also suggests that the liver could be a novel target for correcting or ameliorating FRDA symptoms.

mitoXplorer and ataxiaXplorer: 2 sister web-tools to understand gene expression dynamics in Ataxias

Wednesday, 13th November - 18:00: (Minories) - Poster

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We want to introduce the web-platforms ataxiaXplorer and mitoXplorer for visual data mining and data integration of -omics data to understand the gene expression dynamics in Ataxias. Centered around manually assembled, and curated interactomes, these two sister platforms allow to perform in-depth data mining of bulk, as well as single-cell omics-data from a mitochondrial (mitoXplorer (https://mitoxplorer3.ibdm.univ-amu.fr), or an ataxia (ataxiaXplorer, https://ataxiaxplorer.ibdm.univ-amu.fr) perspective. Built for non-computational experts, the web-tools are user-friendly, providing highly interactive and visual user interfaces for data mining, including comparative analysis, time-series analysis, enrichment analysis or single-cell sub-clustering and analysis.

We will demonstrate how these web-tools can be used to analyze and integrate gene expression data from bulk- as well as single-nuclei sequencing experiments, using a recently published SCA1 single-nuclei RNA sequencing dataset as a use case (Tajwani, et al., 2024, doi: 10.1016/j.neuron.2023.10.039), and demonstrate their predictive power in identifying key pathways involved in disease onset and progression, and understanding cell-type specific expression dynamics in this disease. Both web-tools are freely available to the research community.

MEG3 is a novel therapeutic target for vascular dysfunction in Friedreich ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

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Introduction: In FRDA, the cardiac microvasculature, primarily consisting of endothelial cells, exhibits signs of disease. We have identified increased expression of the long noncoding RNA maternally expressed gene 3 (MEG3) in endothelial cells, smooth muscle cells, cardiomyocytes and autonomic neurons derived from induced pluripotent stem cells generated from individuals with FRDA. Excitingly, MEG3 has not been previously implicated in FRDA and could represent a promising therapeutic target.

Aim: To investigate whether the knockdown of MEG3 in FRDA endothelial cells and smooth muscle cells could reverse FRDA disease phenotypes.

Methods: MEG3 expression was knocked down in FRDA endothelial cells and smooth muscle cells using a GapmeR antisense oligonucleotide targeting MEG3 or a scrambled sequence as a control. Transduced cells were assessed for their angiogenic potential (endothelial cells), migration (smooth muscle cells), cell viability, mitochondrial superoxide levels and mitochondrial membrane potential.

Results: Treatment of FRDA endothelial cells (10 nM) and smooth muscle cells (50 nM) with a MEG3 GapmeR antisense oligonucleotide effectively reduced MEG3 RNA levels to a degree comparable to those of the isogenic control levels ($P<0.001$) without affecting frataxin RNA expression or cell viability ($P>0.05$). Excitingly, knockdown of MEG3 reversed angiogenic dysfunction in FRDA endothelial cells ($P<0.01$), reversed elevated levels of mitochondrial superoxide ($P<0.01$), and restored mitochondrial membrane potential ($P<0.01$) to the level of the isogenic control. In smooth muscle cells, knockdown of MEG3 reduced the elevated migratory rate observed in FRDA compared to the control ($P<0.001$). All presented data is $n\geq3$.

Discussion and conclusion: Knockdown of MEG3 reverses endothelial cell angiogenic dysfunction, limit smooth muscle cell invasion, and reverse mitochondrial dysfunction in FRDA endothelial cells. These findings suggest that knockdown of MEG3 may be a promising novel therapy for treating vascular disease in FRDA.

Funding: Friedreich Ataxia Research Alliance, National Ataxia Foundation, Australian National Health and Medical Research Council.

Determining the Gene Expression Phenotype of FRDA Cardiomyocytes

Wednesday, 13th November - 18:00: (Minories) - Poster

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Background: Friedreichs Ataxia (FRDA) is a rare neurodegenerative disease most commonly caused by a homozygous trimeric repeat expansion in the first intron of the FXN gene. The major manifestations of FRDA are neuromuscular degeneration and cardiac disease. Hypertrophic cardiomyopathy (HCM), the heart disease in FRDA, accounts for 60% of patient mortality. The pathophysiology of the FRDA heart is elusive as its molecular mechanisms are multifaceted. **Objective:** In order to identify novel therapeutic targets of FRDA associated HCM and better understand its molecular pathophysiology at a single cell level, we are defining a gene expression phenotype (GEP) in FRDA iPSCs differentiated into ventricular cardiomyocytes (iPSC-CMs) for use in functional assessment of genetic perturbations on the cellular phenotype of FRDA. This phenotype will be used for a follow up coupled scRNA-seq CRISPR screen. **Methods**: We propagated nine iPSC lines (three of each genotype: FRDA, isogenic control, and unaffected control) and differentiated them into iPSC-CMs. After enrichment, we carried out both bulk RNA sequencing and single cell RNA sequencing. **Results**: Our iPSCs showed an average differentiation efficiency of 70% and on average composed 95% of our sample after enrichment. We found 405 DEGs between FRDA cardiomyocytes as compared to matched isogenic controls with FDR corrected p-values <0.05 and fold change >2 or <-2. The top 25 DEGs were also determined with FDR p-values $<5E^{-7}$. **Discussion**: Mapping of functional modulators of genetic disease is challenging. The identification of relevant functional phenotypes to use in genetic mapping can be difficult as disease severity is variable. **Conclusion**: Thus, we propose that the GEP of cells can be used as a novel phenotype to enable mapping of modulators of diverse genetic diseases. The identified GEP of FRDA iPSC-CMs is sufficient to be used in our follow up Perturb-seq.

The role of aging in the pathogenesis of SCA1

Wednesday, 13th November - 18:00: (Minories) - Poster

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Background and objective: Neurodegenerative diseases, even when genetically inherited, start in late life. Age thus is an important neurodegeneration risk factor, but why this is the case is still unclear. To address this shortcoming, we focused on Spinocerebellar ataxia type 1 (SCA1), a disease caused by a CAG expansion in the ATXN1 gene.

Methods and results: We turned to SCA1 knock-in mice ($ATXN1^{154Q/2Q}$) for our experiments. Using immunohistochemistry and western blotting we identified upregulation of the histone variant phosphorylated gamma H2AX, a marker of double stranded DNA breaks. DNA damage triggers the upregulation of two cyclin dependent kinase inhibitors p16 and p21, which mediate senescence. We found both these to be also elevated in SCA1 mice. There was a corresponding increase in senescence associated beta galactosidase staining along with the elevation of specific cytokines and chemokines that define the senescence associated secretory phenotype.

We next used the P16-3MR mouse line to deplete senescent cells. These mice are transgenic for a P16 promoter driving the expression of a tri modal fusion cassette encoding luciferase, red fluorescent protein (RFP) and herpes simplex virus-1 thymidine kinase. The luciferase and RFP serve as reporters of P16 upregulation (and hence senescence), while the herpes thymidine kinase serves as a death trigger upon ganciclovir (GCV) delivery. After crossing with $ATXN1^{154Q/2Q}$ mice, we observed an upregulation of RFP in both neurons and astrocytes reminiscent of our staining with p16. More importantly we observed an improvement in motor phenotype upon GCV delivery. To broaden our findings to the human context, we found evidence for DNA damage in autopsy samples of SCA1 patients.

Conclusion: These studies provide compelling evidence that SCA1 mice undergo premature senescence in SCA1 and that senolysis mitigates the disease phenotype.

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Mutant ATXN1 expression in microglia impacts Spinocerebellar Ataxia Type 1 phenotypes in mice

Wednesday, 13th November - 18:00: (Minories) - Poster

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Background and Objective

Spinocerebellar Ataxia Type 1 (SCA1) is autosomal dominant inherited neurodegenerative disease caused by a CAG trinucleotide repeat expansion in the *ATXN1(ATAXIN1)* gene and characterized by progressive loss in motor and cognitive function, and premature death. Microglia are the resident immune cells in the brain involved in synaptic pruning, clearing and promoting cell death. While activated microglia are present in SCA1 mouse models, and microglia gene expression is altered in both patients and mouse models, causes and effects of microglial activation in SCA1 remain poorly understood. Our objective is to understand how mutant *ATXN1 (mATXN1)* expression in microglia contributes to microglial activation and disease pathogenesis in SCA1.

Methods

We crossed conditional SCA1 model, *f-ATXN1*$^{146Q;2Q}$ mice with microglia and macrophage specific Cre line, *Lyve1*^{CRE} mice to delete *mATXN1* expression in microglia. We enriched microglia using magnetic bead isolation (Miltenyi) and used RT-qPCR to validate selective depletion of *mATXN1* in microglia. Rotarod and Barnes maze were used to assess motor and cognitive phenotypes in WT, *f-ATXN1*$^{146Q;2Q}$ and *f-ATXN1*$^{146Q;2Q}$*;Lyve1*^{CRE} mice. Barnes maze unbiased strategy analysis (BUNS) provided insight into strategy development. Synaptic quantification was performed using immunohistochemistry and the Puncta Analyzer ImageJ plugin.

Results

We confirmed reduction of *mATXN1* in isolated microglia in *f-ATXN1*$^{146Q/2Q}$*;Lyve1*^{CRE} mice but not in other cell-types. We found ameliorated performance on rotarod and in Barnes maze and improved strategy development in *f-ATXN1*$^{146Q/2Q}$*;Lyve1*^{CRE} mice. We are currently quantifying inhibitory and excitatory synapses.

Discussion

Our work highlights the impacts of *mATXN1* expression in microglia on SCA1 behavioral phenotypes. Further investigation into the role of SCA1 microglia is necessary to understand the mechanism underlying these behavioral outcomes.

Conclusion

These results implicate microglia as contributors to motor and cognitive phenotypes seen in SCA1 mice.

Implications of single lysine residues within the polyglutamine protein ataxin-3 in the pathogenesis of Machado-Joseph disease

Wednesday, 13th November - 18:00: (Minories) - Poster

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Background and objectives: Lysine residues are one of the main sites for posttranslational modifications (PTMs) in a protein, and this can influence pathophysiological processes. Lysine ubiquitination within the Machado-Joseph disease (MJD) protein ataxin-3 is implicated in its cellular function and toxicity. Despite previous efforts, individual roles of specific lysine residues within ataxin□3 in the molecular pathogenesis of MJD are not entirely understood. We thus aimed at understanding the role of specific lysine residues in the MJD pathogenesis.

Methods: By analyzing wild-type or polyQ-expanded ataxin-3 retaining single lysine (K) residues (K8, K85, K117, K166, or K200) of otherwise lysine-free (K0) variant via western blotting, microscopy and filter retardation, we assessed the impact of specific sites on MJD molecular hallmarks.

Results: We demonstrate that residues K8 and K85 are essential for maintaining soluble and aggregated ataxin-3 levels. K85 restores the intracellular distribution of ataxin-3, whereas presence of either K8 or K85 leads to significantly higher protein stability. Moreover, ataxin-3 is degraded via the proteasome regardless of the presence of lysine residues. Finally, ataxin-3 K0 presents increased toxicity and binding to polyubiquitin chains, whereas reintroduction of K85 normalizes its catalytic function.

Discussion and conclusion: We show that ataxin-3 K0 and single-lysine ataxin-3 K8 or K85 reproduce physiological characteristics of unmutated polyQ-expanded ataxin-3. The catalytic activity of ataxin-3 towards polyubiquitin chains depends on its two ubiquitin-binding sites (UbS), and we demonstrate that K85, located between them, restored ataxin-3's binding to polyubiquitin chains. K85 is also located inside of a nuclear export signal, and its sole presence recovers ataxin-3's intracellular distribution. This suggests a decisive physiological role of K85 on ataxin□3's localization and activity. Our data highlights the relevance of residues K8 and K85 of ataxin-3 and encourages further investigation on whether modulating potential PTMs of these sites serve as a therapeutic target for MJD.

The spinocerebellar ataxia type 1 protein ataxin-1 is a substrate of calpain-mediated proteolytic fragmentation

Wednesday, 13th November - 18:00: (Minories) - Poster

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Objectives: The role of disease protein fragments in neurodegenerative disorders, specifically in polyglutamine (polyQ) diseases, is an intensively investigated topic summarized within the Toxic Fragment Hypothesis. Its significance is well-described in Huntington's disease and spinocerebellar ataxias (SCAs) such as SCA3 and SCA17, where proteolytic cleavage and mis-splicing generate toxic fragments. However, a disease-modulating contribution of protein fragmentation to the molecular pathogenesis of SCA1 has previously been rejected. In our study, we focused on the potential involvement of calcium-activated calpain proteases in the cleavage of the SCA1 disease protein ataxin-1.

Methods: Combining in silico, in vitro, and cell-based approaches, we tested whether ataxin-1 is cleaved by calpains. Through western blotting, filter retardation analysis, and microscopy, we analysed ataxin-1 fragmentation, subcellular localization, and aggregation. Mass spectrometry (MS) was employed to precisely determine calpain cleavage sites. Observations made in overexpression cell models were validated in SCA1 patient-derived induced cortical neurons (iCNs).

Results: We established ataxin-1 as a novel substrate of calpain-mediated proteolysis and detected polyQ-containing N- and C-terminal fragments under both induced and baseline conditions, in overexpression cell models and patient-derived iCNs. This proteolysis appeared to be mainly calpain-1-mediated. Using a combinatorial strategy, we identified two cleavage hotspots flanking the polyQ stretch, giving rise to a number of ataxin-1 fragments with distinct intracellular localizations.

Discussion: In contrast to previous reports, our findings clearly present ataxin-1 as a substrate of proteolytic fragmentation. This process is executed by calpains and can be modulated by their activation or inhibition. However, other unknown proteases or truncating mechanism might additionally contribute to ataxin-1 cleavage, and the exact pathological involvement of these fragments to SCA1 demands further scrutiny.

Conclusions: Our study underlines the relevance of disease protein cleavage as a central and shared mechanism in polyQ disorders, paving the way for developing therapeutic strategies across these still incurable diseases.

Activity-Induced Inhibitors of Death: A Potential Therapeutic Target for SCA2 and SCA3

Wednesday, 13th November - 18:00: (Minories) - Poster

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Methods: Activity-induced inhibitor of death (AID) genes are a group of 9 pro-survival genes that have been found to be neuroprotective in several neurological disorders. Our goal is to explore the potential of modulating AID expression to mitigate disease progression in mouse models of SCA2 and SCA3, the most prevalent ataxias worldwide. Wildtype (WT), and transgenic mice for SCA2 and SCA3 disease were divided into 2 groups. A group not stimulated and a second group in which the animals are stimulated for 1 hour in the rotarod apparatus. The cerebellum was collected to analyse transcription and translation levels of the AID genes. Additionally, the altered genes were modulated in a lentiviral mouse model of SCA2 and SCA3 to investigate their role in both diseases.

Results: Our findings suggest that the activation of key transcription factors necessary for AID gene expression is reduced in SCA2 and SCA3 models. We found an impaired induction of *AID1* and *AID2* genes upon motor stimulation in diseased mouse models. Modulation of these genes in lentiviral mouse models of SCA2 and SCA3 is still in progress.

Discussion: These findings support the hypothesis that transcriptional dysregulation of these genes underlies SCA pathogenesis. To further understand the circuitry alterations associated with these disorders, we investigated whether alterations in AID gene expression was cell-type-specific. When analysing the reestablishment of these genes in SCA2 and SCA3 mouse models, we expect to observe a reduction in the number of aggregates found, as well as a reduction in the volume of neuronal maker loss.

Conclusion: This work indicates that transcriptional deregulation of AID gene expression could be a promising target for SCA therapy.

Exploring the role of 5' UTR-mediated regulation of ataxin-1 protein expression using splice-modulating antisense oligonucleotides and CRISPR

Wednesday, 13th November - 18:00: (Minories) - Poster

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Objectives: SCA1 is a neurodegenerative disease caused by a CAG repeat expansion in exon 8 of the *ATXN1* gene. The 5' untranslated region (5'UTR) of the *ATXN1* gene contains 7 exons and is long compared to the average human coding gene, suggesting that it could play a role in translational regulation. Previous research using *ATXN1* 5'UTR overexpression cDNA reporter constructs showed that the 5'UTR is able to modulate ataxin-1 protein levels[1,2]. Here, we aim to elucidate the role of the *ATXN1* 5'UTR in the modulation of the ataxin-1 protein.

Methods: To this end, different exons in the 5'UTR will be removed in a human neuroblastoma SH-SY5Y cell line using splice-switching antisense oligonucleotides (ASOs) and CRISPR gene editing. 17 ASOs targeting different exons in the 5'UTR of the *ATXN1* pre-mRNA were tested for their ability to remove the 5'UTR exons. Additionally, a dual sgRNA CRISPR approach was used to delete different exons present in the *ATXN1* 5'UTR on DNA level in the SH-SY5Y cells. To assess effects on both RNA, DNA (exon removal) and protein (abundance) levels, (q)PCR and Western blot analysis were performed.

Results: Efficient exon removal for each of the respective exons present in the 5'UTR was observed after ASO transfection and CRISPR gene editing. Preliminary results on Western blot analysis on the most efficient ASOs and sgRNAs showed little or no significant differences in ataxin-1 protein levels after efficient removal of the different 5'UTR exons in the SH-SY5Y cell line.

Discussion and conclusion: Results suggest that the 5'UTR does not influence ataxin-1 protein levels in an endogenous setting in the SH-SY5Y cell line. Results emphasize that caution should be taken in interpreting results from overexpression studies when studying protein modulation.

[1]Manek et al., 2020 PMID: 31381977

[2] Nitschke et al., 2019 PMID: 32763910

A peptide derived from TID1S rescues frataxin deficiency and mitochondrial defects in FRDA cellular models

Wednesday, 13th November - 18:00: (Minories) - Poster

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Background and objectives

There is currently only one medication, omaveloxolone, available for the Friedreich's ataxia (FRDA) patients, and it is limited to patients 16 years of age and older. This necessitates the development of new medications. The study of frataxin protein regulation might yield new approaches for FRDA treatment.

Methods

HEK293 cells and primary cultured human skin fibroblasts were used for TID1 knockdown and overexpression. Western blot analysis was performed to evaluate the amounts of frataxin and TID1. Co-immunoprecipitation and *in vitro* binding assays were utilized to study the interaction of frataxin and TID1. Mitochondrial morphology was imaged using immunofluorescence.

Results

Tumorous imaginal disc 1 (TID1), a mitochondrial J-protein cochaperone, was identified as a binding partner of frataxin that negatively controls frataxin protein levels. TID1 interacted with frataxin both *in vivo* in mouse cortex and *in vitro* in cortical neurons. Acute and subacute depletion of frataxin using RNA interference markedly increased TID1 protein levels in multiple cell types. In addition, TID1 overexpression significantly increased frataxin precursor but decreased intermediate and mature frataxin levels in HEK293 cells. In primary cultured human skin fibroblasts, overexpression of TID1S resulted in decreased levels of mature frataxin and increased fragmentation of mitochondria. This effect is mediated by the last 6 amino acids of TID1S as a peptide made from this sequence rescued frataxin deficiency and mitochondrial defects in FRDA patient-derived cells.

Discussion and Conclusion

Our results demonstrate that TID1S physically interacts with frataxin and negatively modulates frataxin levels. This effect is mediated by the final six amino acids of TID1S as a competing peptide derived from this sequence rescues frataxin deficiency and mitochondrial defects in FRDA patient-derived cells. To conclude, our findings offer the peptide TID1S448–453 as a small molecule therapy target for FRDA due to its small molecular weight and ease of modification.

Uncovering the function of a group of conserved amino acids in eukaryotic frataxins

Wednesday, 13th November - 18:00: (Minories) - Poster

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Friedreich's ataxia is a rare cardio-neurodegenerative disease caused by mutations in the frataxin gene. Frataxin is an indispensable mitochondrial protein highly conserved among species. Several functions have been described for frataxin, including iron-sulfur biogenesis promotion, ferroxidase and antioxidant activity. We have recently identified a conserved group of amino acids denominated "cluster 3" (Y143, S158, S161, and E189 in human frataxin) exclusively found in eukaryotic frataxins and bacterial CyaY proteins from the *Rickettsia* genus.

Objectives: uncover the function of cluster 3 by analyzing properties and activities of frataxin variants with mutations in this cluster, comparing them to the native protein.

Methods: native (WT) mature human frataxin (mFXN) and mutant versions (E189A, Y143I, and Y143I E189A) were expressed into *Escherichia coli* BL21 cells and purified. To investigate the contribution of cluster 3 to mFXN structure, thermal stability profiles were assessed using thermal denaturation and intrinsic fluorescence emission analysis.

Results: the melting temperature (Tm) for mFXN WT, E189A, Y143I, and Y143I E189A were determined to be 65°C, 47°C, 61°C, and 49°C, respectively. Mutants E189A and Y143I E189A exhibited faster denaturation rates. Intrinsic fluorescence emission analysis revealed no major differences between the variants at 20°C, while at 50°C the mFXNs carrying the E189A mutations presented lower fluorescence intensity.

Discussion: results from the Tm assay and the intrinsic fluorescence emission analysis indicate that mFXN structure remains minimally altered at 20°C across variants, while the E189A mutation significantly decreases thermal stability. The Y143I mutation has a minimal effect on mFXN thermal stability.

Conclusions: it can be concluded that glutamate E189 from cluster 3 has a critical role in maintaining the structural integrity and thermal stability of frataxin. Ongoing research is addressing the potential contribution of Y143 in the antioxidant properties of this protein.

This work was supported by FARA - Award for Innovative Mindset (AIM) 2023.

SCA48 Mutations Reveal a Role for FKBP51 Mediated Proline Isomerization in Regulating the CHIP/HSP70 Interaction

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Ran Ming [1], ***Mr. Ryan Rodriguez*** [1], ***Dr. Erik Soderblom*** [1], ***Dr. Matt Scaglione*** [1]

1. *Duke University*

The ubiquitin ligase C-terminus of HSC70 Interacting Protein (CHIP) is a protein quality E3 ubiquitin ligase that plays a critical role in suppressing protein aggregation and neurodegeneration. Mutations in CHIP cause the neurodegenerative disease spinocerebellar ataxia type 48 (SCA48). Here we find that mutations in the tetratricopeptide repeat (TPR) domain of CHIP that cause SCA48 promote the TPR domain to form an alternate conformation. We find that a similar structural change is also induced by proline isomerization of the TPR domain of CHIP by the prolyl isomerase FKBP51. In this alternate structure, the affinity of CHIP for the C-terminus of chaperones is markedly decreased. We further find that this structural change is reversible and that the structural defects associated with SCA48 mutations are reversible. Together these data identify prolyl isomerization as a posttranslational modification that regulates the association of CHIP with chaperones. Our findings also suggest that the structural defects caused by mutations to the TPR domain of CHIP that cause SCA48 are reversible.

Evolutionary and expression analyses of ATXN3 paralogs raise new possibilities to study genetic modifiers in Machado-Joseph disease

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Daniela Felício [1], ***Dr. Maria Inês Martins*** [2], ***Ms. Andreia Pinto*** [2], ***Ms. Inês P. D. Costa*** [3], ***Prof. António Amorim*** [4], ***Prof. Jorge Sequeiros*** [5], ***Dr. Mariana Santos*** [6], ***Dr. Alexandra M. Lopes*** [7], ***Dr. Susana Seixas*** [3], ***Dr. Sandra Martins*** [3]

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Previous studies on spinocerebellar ataxia type 1 (SCA1) demonstrated that ataxin-1 like (*ATXN1L*), a copy of *ATXN1* was able to partly compensate the partial loss-of-function of ATXN1 and suppress neuropathology in mice. Duplicates of other genes involved in polyglutamine SCAs and their functional role in disease remain vastly unexplored. In this work, we sought to investigate the evolution of *ATXN3*, responsible for Machado-Joseph disease (MJD/SCA3) when expanded. A conserved retrocopy, *ATXN3L*, appears to encode a protein shown *in vitro* to cleave ubiquitin substrates more efficiently than ATXN3.

To study *ATXN3* copies, we retrieved highly homologous sequences from 33 representative assemblies of primates and analysed their diversity, local synteny and selective constraints in the reconstructed phylogenies. We also analysed *ATXN3L* mRNA expression by qPCR.

Our results support the presence of five retrocopies of *ATXN3* in primates. Along with the known *ATXN3L* retrogene (herein *ATXN3L1*) originated in Haplorrhini (~70 MYA), we identified *ATXN3L0*, *ATXN3L2* and *ATXN3L3* that resulted from retrotransposition events in Euarchontoglires (~87 MYA), Simiformes (~45 MYA), and Cercopithecidae (~20 MYA) ancestors, respectively. Lorisoidae species present a separate retrocopy of *ATXN3* (~40 MYA). Moreover, species-specific *ATXN3* retrocopies and tandem duplicates of *ATXN3L1* were found in some primates.

ATXN3L1 seems to be under purifying selection (ω=0.41) throughout primate evolution as the parental gene (ω=0.22), preserving the protein sequence and function. *ATXN3L0* and *ATXN3L2* accumulated nonsense/frameshift mutations, which probably turned them into processed pseudogenes. *ATXN3L3* appears as a younger retrocopy likely to be undergoing pseudogenization. We detected *ATXN3L1* expression in several human tissues, including the brain, although in much lower levels than those observed for *ATXN3*.

The human retrocopies with the highest homology, *ATXN3L1* and *ATXN3L2*, open new opportunities to investigate potential disease modifiers in MJD/SCA3, but their functional characterization and additional genotype-phenotype association studies will be crucial to assess their relevance.

Climbing fiber synaptic loss contributes to reduced cerebellar rhythm and cerebellar ataxia symptoms

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Ilaria Balbo [1], ***Dr. Chih-Chun Lin*** [1], ***Dr. Wen-Chuan Liu*** [2], ***Dr. Yi-Mei Wang*** [3], ***Mr. Christopher Driscoll*** [1], ***Dr. Ke-Chu Fang*** [2], ***Ms. Ting-Yu Liang*** [2], ***Mr. David Ruff*** [1], ***Dr. Phyllis Faust*** [1], ***Dr. Sheng-Han Kuo*** [1], ***Dr. Ming-Kai Pan*** [2]

1. *Columbia University Medical Center,* ***2.*** *National Taiwan University College of Medicine,* ***3.*** *Cerebellar Research Center, National Taiwan University Hospital*

Background and objectives: Despite there are a variety of genetic and non-genetic causes of cerebellar ataxia, these causes shared a strikingly similar clinical symptoms: imbalance, frequent falls, and loss of motor control. These shared symptoms suggest common alterations in brain circuits, which could be targeted for treatment across different causes of cerebellar ataxia. Prior findings in mice show that cerebellar degeneration often starts with a pruning of climbing fiber (CF) innervation on Purkinje cells (PCs). However, whether this synaptic loss is observed in human cerebellar ataxia and the role of CF-PC synaptic activity in these symptoms is unclear. We aimed to study the relationship between CF-PC synaptic activity, ataxia symptoms and disrupted cerebellar physiology.

Methods: We studied postmortem human cerebellar tissues from patients with cerebellar ataxia to determine CF-PC synaptic distribution. Using optogenetic and chemogenetic methods, we determined the role of CF-PC synapses in WT and spinocerebellar ataxia 1 (SCA1) mouse models. We also used in vivo recording in mice to determine the cerebellar physiology.

Results: We found patients with cerebellar ataxia have a loss of CF-PC synapses. Similarly, SCA1 mice exhibited CF-PC synaptic loss and reduced cerebellar rhythm. In addition, we found optogenetic inhibition of CF-PC synaptic transmission in WT mice sufficiently to cause reduced cerebellar rhythm and ataxia-like behaviors. Conversely, chemogenetic activation of CFs in SCA1 mice improved cerebellar rhythm and reduced ataxia-like behaviors.

Discussion and conclusion: CF-PC synaptic loss contributes to disturbed cerebellar physiology and cerebellar ataxia symptoms. Manipulation of CF function could be a therapeutic strategy for cerebellar ataxia.

Primary cilia deficiency in the spinocerebellar ataxia type 1 (SCA1) mouse model.

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Parvathi Satheesh [1], ***Dr. Jan Tůma*** [1], ***Dr. Jiří Moravec*** [2]

1. *Department of Pathophysiology, Faculty of Medicine in Pilsen, Charles University,* ***2.*** *Biomedical Center, Faculty of Medicine in Pilsen, Charles University*

Background and objective: The primary cilium, a non-motile organelle that protrudes from almost every vertebrate cell in interphase, plays an essential role in numerous developmental and physiological processes. Dysfunction of primary cilia is associated with several diseases, including neurodegeneration. Spinocerebellar ataxia 1 (SCA1) is a neurodegenerative disorder characterized by the progressive degeneration of cerebellum resulting in motor deficits. It is caused by an expanded CAG repeat mutation in the *ATXN1* gene. Despite its potential significance, the role of primary cilia in SCA1 pathogenesis remains unexplored.

Methods: To address this gap, we established in vitro primary fibroblast culture from SCA1 and wildtype mice, then assessed them for cell viability and mitochondrial respiration using MTS assay and Seahorse analysis, respectively. Subsequently, the fibroblasts were cultured in serum free condition to enhance primary cilia assembly, then immunostained against primary cilia marker to quantify primary cilia. Furthermore, we conducted a proteomic analysis on cerebellar tissue of SCA1 and wildtype mice.

Results: We demonstrated decreased cell viability and reduced mitochondrial respiration in the SCA1 mutant cells. Quantification of primary cilia indicated significant reduction in the number of primary cilia in SCA1 fibroblasts as compared with wildtype fibroblasts. Proteomic analysis revealed diminished cerebellar levels of ciliogenesis-related proteins in SCA1 mice cerebellum.

Discussion and conclusion: Our results herein demonstrate a novel finding of a possible deficit in primary cilia biogenesis and/or maintenance in SCA1 mouse mutants, evidenced by the decreased number of primary cilia in SCA1 fibroblasts and reduced levels of primary cilia associated proteins in mice cerebellar tissue. The decreased cell viability and mitochondrial respiration are significant cellular phenotypes in SCA1 cells offering insights into primary cilia mediated signalling pathways. Understanding the significance of primary cilia deficiency in the pathogenesis of SCA1 is an integral aspect of our ongoing research.

Funding: Cooperatio and GAUK #70124

Assessment of the clinical interactions of GAA repeat expansions in FGF14 and FXN

Wednesday, 13th November - 18:00: (Minories) - Poster

Mr. Brandon Gerhart [1], ***Dr. David Pellerin*** [2], ***Dr. Matt Danzi*** [2], ***Dr. Stephan Zuchner*** [2], ***Dr. Bernard C. Brais*** [3], ***Dr. Gabriel Matos-Rodrigues*** [4], ***Dr. Andre Nussenzweig*** [4], ***Dr. Karen Usdin*** [5], ***Ms. Courtney Park*** [6], ***Dr. Jill Napierala*** [1], ***Dr. David Lynch*** [6], ***Dr. Marek Napierala*** [1]

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Background and Objectives:
The number of GAA repeats in the *FXN* gene is a major but not sole determinant of the clinical presentation of Friedreich ataxia (FRDA). The objective of this study was to establish whether the length of the GAA repeat tract in the *FGF14* gene, which is associated with another neurodegenerative disorder (SCA27B), affects the clinical presentation (age of onset, mFARS score) of FRDA patients.
Methods:
The number of GAA repeats in the *FXN* and *FGF14* genes was determined using PCR in a cohort of 221 FRDA patients. Next, we compared absolute lengths of the *FGF14* GAAs to *FXN* GAAs, followed by correlative analyses to determine potential effects of *FGF14* GAA length on age of onset and clinical presentation (mFARS) of FRDA.
Results:
We found no significant correlation between the size of the GAA repeats in *FXN* and *FGF14* loci in our FRDA cohort. Moreover, the number of GAAs in *FGF14* did not affect the clinical presentation of FRDA even in the small number of cases where a long *FGF14* allele was present.
Discussion:
Despite both molecular and clinical similarities between FRDA and SCA27B, the length of the GAA repeats in the *FGF14* gene, including potentially pathogenic alleles, did not influence the clinical presentation of FRDA.

Funding:

This work is supported by National Institutes of Health (NIH), National Institute of Neurological Disorders and Stroke (NINDS), and Friedreich's Ataxia Research Alliance (FARA).

Quantitative Co-expression and Pathway Analysis Reveal the Shared Biology of Autosomal Recessive Ataxia Genes

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Jalil-Ahmad Sharif [1], ***Dr. James Timmons*** [1], ***Prof. Paul Chapple*** [1]

1. *William Harvey Research Institute, QMUL, London, UK*

Background and objectives

Mutations in >60 genes are associated with autosomal recessive cerebellar ataxia (ARCA). The cellular pathways in which these genes function remain ill-defined. Identification of molecular pathways or upstream regulators common across multiple ataxias would increase our understanding of the pathophysiological mechanisms and potentially identify therapeutic targets. Quantitative network analysis, using large datasets, is a potentially robust way to identify functional pathways, yet it has not been applied to ataxias at scale.

Methods

We have used co-expression analysis to identify functional modules of genes co-expressed with each causative ARCA gene in human brain tissues. Specifically, we optimised existing large-scale transcriptomic data from three separate human brain data sets (>1,000 samples), two of which are cerebellum (unpublished, Kang 2011, Trabzuni 2013). Optimisation included reannotation, signal filtering, data scaling and then application of Multiscale Embedded Gene Co-expression Network Analysis (MEGENA) framework (Song and Zhang, 2015), to define robust network structures.

Results

Following validation steps, we have confirmed our strategy produced valid co-expression modules for genes with known biology. For example, OXPHOS genes were detected in the same module. Moreover, for modules containing ARCA genes, that have been extensively studied, ontology analysis of the module identified terms related to their known function. This included that the FXN gene (causing Friedreichs Ataxia) module linked to 'heme metabolic process'. Novel pathways related to multiple ARCA genes were also identified.

Discussion and Conclusion

We have identified functional modules of genes that co-expressed with causative ARCA genes in human brain. Defining ARCA gene co-expression modules provided new insights into their underlying biology, revealing novel molecular links between different ARCAs. This should improve molecular classification of these diseases as well as highlight therapeutic targets, facilitating drugs discovery and repositioning for rare ataxias.

Funding Source

Wellcome Trust funded HARP DTP

Human frataxin, the Friedreich ataxia deficient protein, interacts with mitochondrial respiratory chain

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. DAVIDE DONI [1], ***Dr. FEDERICA CAVION*** [1], ***Prof. MARCO BORTOLUS*** [2], ***Dr. ELISA BASCHIERA*** [3], ***Dr. SILVIA MUCCIOLI*** [1], ***Dr. GIULIA TOMBESI*** [1], ***Dr. FEDERICA D'ETTORRE*** [1], ***Dr. DANIELE OTTAVIANI*** [1], ***Dr. ELENA MARCHESAN*** [1], ***Prof. LUIGI LEANZA*** [1], ***Prof. ELISA GREGGIO*** [1], ***Prof. ELENA ZIVIANI*** [1], ***Prof. ANTONELLA RUSSO*** [4], ***Prof. MILENA BELLIN*** [1], ***Prof. GEPPO SARTORI*** [5], ***Prof. DONATELLA CARBONERA*** [2], ***Prof. LEONARDO SALVIATI*** [3], ***Prof. PAOLA COSTANTINI*** [1]

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Background and Objective. Friedreich ataxia (FRDA) is a rare, inherited neurodegenerative disease caused by an expanded GAA repeat in the first intron of the *FXN* gene, leading to transcriptional silencing and reduced expression of frataxin. Frataxin participates in the mitochondrial assembly of Fe-S clusters, redox cofactors of the respiratory complexes I, II and III. To date it is still unclear how frataxin deficiency culminates in the decrease of bioenergetics efficiency in FRDA patients' cells.

Methods. Oxygen consumption studies by Seahorse flux analyzer; morphometric analyses by TEM, to assess mitochondrial ultrastructure; immunofluorescence/PLA, to explore the interaction between FXN and mitochondrial respiratory chain; EPR spectroscopy, to evaluate Fe-S clusters content.

Results. We explored the potential interaction of frataxin with the Fe-S cluster-containing respiratory complexes I, II and III. Using healthy cells and different FRDA cellular models we found that frataxin interacts with these three respiratory complexes. Furthermore, by EPR spectroscopy, we observed that in mitochondria from FRDA patients' cells the decreased level of frataxin specifically affects the Fe-S cluster content of complex I. Remarkably, we also found that the frataxin-like protein Nqo15 from *T. thermophilus* complex I ameliorates the mitochondrial respiratory phenotype when expressed in FRDA patient's cells.

Discussion and Conclusion. To gain novel insights into the function of frataxin in the mitochondrial pathophysiology, and in the upstream metabolic defects leading to FRDA disease onset and progression, here we explored the potential interaction of frataxin with the Fe-S cluster-containing respiratory complexes I, II and III. Our data point to a structural and functional interaction of frataxin with complex I and open a perspective to explore therapeutic rationales for FRDA targeted to this respiratory complex (Doni D. *et al.*, Cell Death Dis 14, 805 (2023) - doi: 10.1038/s41419-023-06320-y).

This work was supported by grant from FARA to Paola Costantini (2021)

Calpain-derived TBP fragments contribute to the molecular pathogenesis in Spinocerebellar ataxia type 17

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Rana Dilara Incebacak Eltemur [1], ***Mr. Florian Fath*** [1], ***Ms. Chrisovalantou Huridou*** [1], ***Ms. Priscila Pereira Sena*** [2], ***Dr. Ana Velic*** [3], ***Prof. Boris Macek*** [3], ***Dr. Nicolas Casadei*** [2], ***Prof. Olaf Rieß*** [2], ***Prof. Huu Phuc Nguyen*** [1], ***Dr. Jonasz Jeremiasz Weber*** [1]

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Background and Objective: Spinocerebellar ataxia type 17 (SCA17) is a neurodegenerative disorder caused by the expansion of a polyQ tract in the transcription factor TATA box-binding protein (TBP). Although precise molecular pathomechanisms caused by polyQ-expanded TBP are still unknown, our previous study unveiled calcium-dependent calpains as important modulator in SCA17. Calpains were reported to be overactivated in SCA17 cell and animal models resulting in elevated cleavage of the disease protein TBP. Here, we determined the exact calpain-dependent cleavage sites and shed light on the role of emerging TBP fragments in the disease mechanism of SCA17.
Methods: We used *in silico*, *in vitro* and cell-based approaches to map and characterize calpain-dependent TBP fragments. Cleavage sites identification was performed by mass spectrometry (MS). By overexpressing corresponding TBP fragment constructs in cell models, we investigated their aggregation, degradation and localization as well as their impact on calpain system activation and cell viability. Global cellular perturbations caused by the presence of TBP fragments were analyzed by omics-based approaches.
Results: We confirmed the calpain-mediated cleavage of TBP, resulting in polyQ containing N-terminal fragments and C-terminal counterparts. MS analysis confirmed a major cleavage site after threonine 106. Overexpression of TBP fragment constructs in cells showed excessive turnover of C-terminal fragments, whereas N-terminal fragments were highly aggregation prone. C-terminal fragments were found to localize mainly in the nucleus. Interestingly, the DNA-binding domain containing C-terminal fragment was shown to activate calpains. Importantly, quantitative MS analysis demonstrated alterations in the proteome of cells overexpressing TBP fragments, potentially causative of the observed calpain overactivation.
Discussion and Conclusions: With this study, we reinforced the relevance of calpains in the molecular pathogenesis of SCA17. Calpain-dependent TBP fragments were found to play an active role in cellular toxicity. Thus, understanding their role in pathological processes represents an attractive opportunity for therapeutic approaches.

RNA-sequencing analysis of repeat-expansion ataxias reveals differential transcriptomic signatures across brain regions and disease pathologies

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Zhongbo Chen [1], ***Dr. Jonathan Brenton*** [2], ***Dr. Emil Gustavsson*** [2], ***Dr. Regina Reynolds*** [2], ***Ms. Clarissa Rocca*** [2], ***Prof. Catriona McLean*** [3], ***Dr. Arianna Tucci*** [4], ***Dr. Zane Jaunmuktane*** [2], ***Prof. Henry Houlden*** [5], ***Prof. Mina Ryten*** [6]

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Pathogenic repeat expansions form the underlying genetic aetiology of a heterogeneous group of prevalent hereditary ataxias. Despite promise of RNA-based therapeutics in development, there is currently limited understanding of the biology of disease at the RNA level. We generated and analysed RNA-sequencing data from brain tissue of individuals with ataxia for transcript usage, differential and cell-type-specific gene expression, as well as splicing, to transcriptomically profile these disorders in order to gain further mechanistic insights.

We compared post-mortem paired cerebellar and frontal cortex tissue bulk RNA-sequencing data from 23 donors with ataxia and 22 sex-, age-matched controls from two brain banks. Repeat expansion sizes were quantified from DNA: spinocerebellar ataxia (SCA) 1, n=3; SCA2, n=3; SCA6, n=3; SCA7, n=2; SCA17, n=1; Friedreich's ataxia, n=4. Seven samples had no known molecular diagnosis despite whole exome sequencing and screening for novel repeat expansions including in *FGF14* and *RFC1*. We studied transcriptional changes at both the causative Mendelian gene and at the transcriptome-wide level.

Using this approach, we found: (i) differing transcriptional signatures between cerebellar and frontal cortices with unexpected extra-cerebellar involvement even in 'pure' ataxia syndromes (SCA6); (ii) distinct expression profiles between diseases including molecularly undiagnosed cases; (iii) activation of immune and inflammatory pathways was a feature of all ataxias; (iv) downregulation of genes with neuronal cell-type-specific expression and upregulation of genes expressed within glial-specific cell types in disease; (v) transcriptome-wide splicing dysregulation in ataxia; (vi) lack of differential expression of the causative Mendelian gene except for a downregulation of *FXN* in Friedreich's ataxia suggesting that overall gene expression is not a critical component of pathogenesis.

This study provides a map of transcriptional changes in ataxia to understand the mechanisms of disease. The results highlight immune pathways as well as the role of non-neuronal cells as early and potentially important therapeutic targets.

Poster session II - Imaging

Quantifying the T2 Hyperintense Dentate Sign in neurodegenerative ataxias: Findings in SCA14 and AOA2

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Peter Pieperhoff [1], Dr. Tanja Schmitz-Hübsch [2], Dr. Michael Scheel [3], Dr. Maria Rönnefarth [4], Dr. Sarah Doss [5], Prof. Matthis Synofzik [6], Prof. Ludger Schöls [7], Prof. Thomas Klockgether [8], Prof. N. Jon Shah [9], Prof. Friedemann Paul [2], Dr. Andreas Deistung [10], Prof. Katrin Amunts [11], Dr. Susanne Greschus [12], Prof. Dagmar Timmann [13], Dr. Martina Minnerop [14]

1. *Institute of Neuroscience and Medicine (INM-1), Research Center Jülich, Jülich, Germany,* ***2.*** *NCRC-Neuroscience Clinical Research Center, Charité–Universitätsmedizin Berlin, corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin,* ***3.*** *NCRC-Neuroscience Clinical Research Center and Department of Neuroradiology, Charité–Universitätsmedizin Berlin, corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, 10117 Berlin,* ***4.*** *Department of Neurology with Experimental Neurology, Charité–Universitätsmedizin Berlin, corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin,* ***5.*** *Department of Neurological Sciences, University of Nebraska Medical Center, Omaha, NE 68198,* ***6.*** *Division of Translational Genomics of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research and Center of Neurology, University of Tübingen, Tübingen, Germany,* ***7.*** *Department of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research, and Center for Neurology, University of Tübingen, Tübingen, Germany,* ***8.*** *Department of Neurology, University Hospital Bonn, Bonn. German Center for Neurodegenerative Diseases (DZNE), Bonn.,* ***9.*** *Institute of Neuroscience and Medicine (INM-4), Research Center Jülich, 52425 Juelich; Department of Neurology, Faculty of Medicine, RWTH Aachen University,* ***10.*** *University Clinic and Outpatient Clinic for Radiology, Department for Radiation Medicine, University Hospital Halle (Saale), University Medicine Halle, Halle (Saale), Germany,* ***11.*** *Institute of Neuroscience and Medicine (INM-1), Research Centre Jülich, Jülich; Cécile and Oskar Vogt Institute of Brain Research, Medical Faculty & Heinrich Heine University, Düsseldorf,* ***12.*** *Department of Radiology, Waldkrankenhaus, Bonn,* ***13.*** *Department of Neurology and Center for Translational Neuro- and Behavioral Sciences (C-TNBS), University Hospital Essen, University of Duisburg-Essen, Duisburg, Germany,* ***14.*** *Institute of Neuroscience and Medicine (INM-1), Research Centre Jülich, Jülich; Dept of Neurology, Center for Movement Disorders and Neuromodulation, University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf, Düsseldorf*

Introduction

Previous work of our group revealed bilaterally T2-hyperintense dentate nuclei (DN) as supporting feature for the classification of PRKCG variants/SCA14, while T2 signal generally decreases during normal aging. Altered dentate signals in T2/FLAIR were also described in AOA, SPG7, SCA48 and in anecdotal reports of other degenerative disorders. Here we aimed to quantify this sign in SCA14 and AOA2 patients in comparison to healthy controls (HC).

Methods

To obtain averaged mean intensity values of the DN in 3D-T2-weighted MR images obtained at 3T(1mm^3 voxel size), individual T2-weighted images were registered to T1-weighted images which were non-linearly registered with the Julich Brain Atlas to enable the transformation of the DN maps of this atlas onto each subject's T2-weighted images. To account for signal variance, mean intensities within the DN were adjusted by using the mean intensities of the cerebellar white matter (DN/WMc-ratio).

Results

DN/WMc-ratios obtained in 20 SCA14 (9 male, disease duration (dd)=17±13y) and nine AOA2 (7 male, dd 18.7±9.7y) were elevated ($p<0.001$), indicating increased T2-signal intensity of DN over WMc, while DN/WMc-ratios in age- and sex-matched HC were close to one. The WMc intensity did not significantly differ between HC and patient groups.

Conclusion

T2-hyperintense DN were seen by inspection in all SCA14 and AOA2 patients of our sample, but it may evade routine brain MRI ratings, as its detection is enhanced by isotropic 3D-T2-weighted imaging. We propose the estimation of DN/WMc-T2-signal-ratios as an observer-independent quantification of the T2-hyperintense dentate sign that may help to further investigate specificity and potential for ataxia classification. Comparison to SCA 1,2, 3 and 6 and FRDA MR data are currently underway. Histopathological correlates of this sign are yet unknown but may reflect specific pathology, e.g., “pure” Purkinje cell dysfunction, shared by SCA14 and AOA2 - with possible reactive gliosis of the dentate nucleus.

The Importance of Accurate Intracranial Volume (ICV) Estimation for Normalization of Longitudinal Neuroimaging in Rare Neurodegenerative Diseases

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Antoine Cossa [1], ***Mr. Abdessamad Hammouche*** [2], ***Ms. Audrey D'Abrigeon*** [1], ***Ms. Mónica Ferreira*** [3], ***Ms. Sahar Elouej*** [1], ***Dr. Annette Merdes*** [4], ***Dr. Thomas Klockgether*** [5], ***Dr. Jennifer Faber*** [6]

1. *Servier, Paris-Saclay campus, Gif-sur-Yvette,* ***2.*** *Servier, Suresnes,* ***3.*** *German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany,* ***4.*** *Servier Forschung GmbH, München,* ***5.*** *German Center for Neurodegenerative Diseases (DZNE), Bonn,* ***6.*** *German Center for Neurodegnerative Diseases (DZNE), Bonn*

Introduction

Tracking volumetric changes of brain subregions over time is crucial for assessing disease progression, aging effects as well as the impact of therapeutic interventions. However, individual factors have an influence on the variability of such volumes. Along with sex and age, intracranial volume (ICV) is used to adjust for differences in head size. Normalization with these variables reduces confounding effects and enhances precision. Thus, reliable ICV estimation methods are key, especially in rare diseases for which data is scarce. In this work, we evaluated several commonly used methods for ICV estimation.

Methods

In total 146 longitudinal T1-weighted MRIs from 36 SCA3 mutation carriers, 11 healthy controls and 2 non-carrier first-degree relatives enrolled in the ESMI cohort (spanning up to 6 visits) were analyzed. ICV was estimated with several versions of FreeSurfer (6.0, 7.3.2 and 7.4.1), as well as with FastSurfer (2.2) and SAMSEG (included in FreeSurfer 7.4.1). FreeSurfer and FastSurfer use a registration-based estimated Total Intracranial Volume (eTIV) while SAMSEG uses a segmentation-based TIV (sbTIV).

Results

SAMSEG sbTIV and FastSurfer eTIV had the highest longitudinal stability and the lowest mean intra-patient standard deviation (SD) (respectively 4.64 and 7.32mL) while FreeSurfer 6.0 had the highest SD (21.38mL). Pearson's correlation analyses showed the highest correlation coefficients (CC) between SAMSEG and FastSurfer (r=0.99). The lowest CC was observed between FreeSurfer 6.0 and both SAMSEG and FastSurfer (r=0.83).

Discussion & Conclusion

Both SAMSEG sbTIV and FastSurfer eTIV demonstrated similar and consistent ICV estimations compared to all tested versions of FreeSurfer. This study highlights that the choice of the ICV method should be carefully considered as its use as a normalization variable can influence downstream analyses. Continued development, validation and quality control of ICV estimation methods are essential for more accurate assessments of volumetric brain changes across patients and over time.

Funding

Servier.

Eye and brain imaging longitudinal biomarkers correlate with progression of motor symptoms in the ARSACS mouse model

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Valentina Gigliucci [1], ***Dr. Su-Chun Huang*** [2], ***Dr. Giorgio Boschetti*** [3], ***Dr. Alessandra Scaravilli*** [4], ***Dr. Valerio Castoldi*** [2], ***Dr. Paola Podini*** [5], ***Dr. Angelo Quattrini*** [5], ***Dr. Sirio Cocozza*** [4], ***Prof. Letizia Leocani*** [6], ***Dr. Francesca Maltecca*** [3]

1. *Mitochondrial Dysfunctions in Neurodegeneration Unit, Division of Neuroscience, IRCCS Ospedale San Raffaele, Milan, Italy,* ***2.*** *Experimental Neurophysiology and MAGICS Center, Institute of Experimental Neurology, Division of Neuroscience, IRCCS Ospedale San Raffaele, Milan, Italy,* ***3.*** *Mitochondrial Dysfunctions in Neurodegeneration Unit, Division of Neuroscience, IRCCS Ospedale San Raffaele, Milan, Italy and Vita-Salute San Raffaele University, Milan, Italy,* ***4.*** *Department of Advanced Biomedical Sciences, University of Naples Federico II, Naples, Italy,* ***5.*** *Experimental Neuropathology Unit, Division of Neuroscience and Institute of Experimental Neurology, IRCCS Ospedale San Raffaele, Milan, Italy,* ***6.*** *Experimental Neurophysiology and MAGICS Center, Institute of Experimental Neurology, Division of Neuroscience, IRCCS Ospedale San Raffaele, Milan, Italy and Vita-Salute San Raffaele University, Milan, Italy*

Background and Objectives: Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS) is caused by mutations in the *SACS* gene encoding for sacsin. It is characterized by childhood-onset cerebellar ataxia followed by pyramidal tract signs and peripheral neuropathy. Disease severity and age of onset vary greatly, hindering to objectively measure and predict clinical progression. Thickening of the Retinal Nerve Fiber Layer (RNFL) is distinctive of ARSACS patients, as assessed by Optical Coherence Tomography (OCT), while conventional brain MRI findings include both supra- and infratentorial changes. Since longitudinal imaging studies in ARSACS patients are not available to define these changes as biomarkers of disease progression, we aimed to address this issue in the ARSACS mouse model.

Methods: We performed longitudinal retinal OCT and brain MRI in the *Sacs*$^{-/-}$ ARSACS mouse model, alongside motor and coordination assessment in the beam walking test. We also investigated visual function and the molecular mechanisms underlying RNFL increased thickness by histology and immunofluorescence.

Results: We demonstrated that RNFL thickening by OCT gradually increases in the early stages of pathology in the *Sacs*$^{-/-}$ mouse model, reflecting the progression of motor impairment, and later reaches a plateau when thinning of the posterior *corpus callosum* becomes detectable by MRI. Mechanistically, we unveiled that RNFL thickening is associated with aberrant accumulation of non-phosphorylated neurofilament H (npNFH) and GFAP. We also uncovered mild signs of myelin pathology coherent with increased latency of visual evoked potentials (VEPs), and altered retinal activation by photopic electroretinography (pERG).

Discussion and Conclusions: We show that both RNFL thickening and MRI changes are biomarkers of disease progression in the *Sacs*$^{-/-}$ mouse model. Our data gathers knowledge instrumental to clinical studies, holding potential as readout for treatment efficacy.

Funding: This work was supported by the Italian Ministry of Health and the Ataxia Charlévoix-Saguenay Foundation.

MR Spectroscopy Supports a Beneficial Effect of CoQ10 on the Cerebellum in Friedreich's Ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

Mrs. Manar Ibdali [1]***, Prof. Marios Hadjivassiliou*** [2]***, Dr. Santosh Mordekar*** [3]***, Dr. Paul Armitage*** [4]***, Dr. Daniel Connolly*** [5]***, Prof. Nigel Hoggard*** [4]

1. *University of Sheffield, School of Medicine and Population Health,* ***2.*** *Department of Neurology, Sheffield Teaching Hospital Foundation Trust,* ***3.*** *Department of Paediatric Neurology, Sheffield Children's Hospital Foundation Trust,* ***4.*** *University of Sheffield,* ***5.*** *Department of Paediatric Radiology, Sheffield Children's Hospital Foundation*

Background and Objective:
Studies investigating the value of the antioxidant CoQ10 in Friedreich's ataxia (FRDA) have yielded inconsistent results, but despite this many patients take supplements: the objective of this study was to identify if there were any changes on MR spectroscopy (MRS) in response to commencing CoQ10 supplements in treatment naïve patients.

Methodology:
Retrospective clinical and imaging data were extracted from Sheffield Teaching and Sheffield Children's Hospitals NHS Foundation Trusts. Four adult and four child subjects had MRS data before and after commencement of CoQ10.

Results:
Preliminary results show an increase in NAA/Cr (N-acetyl aspartate/Creatine) ratio levels in the vermis of six subjects (four adults; two children) and the hemisphere of four subjects (three adults; one child). NAA/Cr ratio levels remained stable in the vermis of one child and hemisphere of another. Intervals between spectroscopy pre and post CoQ10 ranged from 13 to 72 months.

Discussion:
FRDA is a progressive condition caused by a mutation in the FXN gene, encoding for the protein, frataxin. The deficiency of frataxin causes mitochondrial dysfunction and an increase in free radicals causing oxidative stress. MRS has been used in our Ataxia Centre to investigate and monitor the biochemical health of the cerebellum in patients with ataxia for 15 years. N-acetyl aspartate is a biochemical marker of healthy neurons expressed as a ratio to creatine which is a widely used biochemical reference in MRS. The genetic ataxias are typically expected to show slowly declining NAA/Cr ratios. To our knowledge this is the first objective evidence of the positive effects of CoQ10 on NAA/Cr ratios in the cerebellum using MRS.
Conclusion:
Our results showed consistent improvements in NAA/Cr levels on commencing CoQ10 supplements, tending to support its use. Further investigation is warranted to establish how these improvements reflect clinical symptoms.

Neocortical functional hyperconnectivity in a mouse model of spinocerebellar ataxia type 8 (SCA8)

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Angela Nietz [1], ***Dr. Laurentiu Popa*** [1], ***Dr. Russell Carter*** [1], ***Dr. Laura Ranum*** [2], ***Dr. Timothy Ebner*** [1]

1. *University of Minnesota,* ***2.*** *University of Florida*

Background: Spinocerebellar ataxia type 8 (SCA8) is caused by a bidirectionally transcribed trinucleotide repeat expansion in the *ATXN*-8 and *ATXN*-8OS genes. SCA8 patients and our SCA8 mouse model develop progressive motor symptoms with cerebellar, brainstem, and neocortical pathology. Patients also have cognitive and psychiatric symptoms leading to the hypothesis that SCA8 alters neocortical neural dynamics and network functional connectivity (FC).

Methods: We used transgenic mice expressing the human mutated ATXN-8/8OS gene and non-transgenic controls to assay neocortical neural activity throughout SCA8 disease. Using transparent polymer skulls in conjunction with cortex-wide expression of the calcium sensory GCaMP6f we chronically monitored neural activity simultaneously across the mouse neocortex. Spatial Independent Component Analysis was used to segment the dorsal neocortex, followed by canonical correlation and network analyses to assess FC changes.

Results/Conclusions: Neocortical FC shifts to a hyperconnected state in mice expressing the SCA8 mutation compared to their non-transgenic counterparts throughout disease progression. SCA8 mice show a greater number and strength of network connections globally, and increased efficiency and community number. The changes are driven by specific areas, including primary sensory and higher visual cortices. Therefore, SCA8 neocortical networks assume a more random configuration with loss of integrative and processing specificity. Network connectivity in SCA8 can be used to reliably predict animal genotype using a generalized linear model (GLM) demonstrating these changes are robust and may drive SCA8 cognitive dysfunction.

AN INVESTIGATION OF WHITE MATTER INVOLVEMENT IN PARADIGMATIC FORMS OF SPASTIC ATAXIA: RESULTS FROM THE INTERNATIONAL PROSPAX STUDY

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Alessandra Scaravilli [1], ***Dr. Ilaria Gabusi*** [2], ***Dr. Gaia Mari*** [2], ***Dr. Matteo Battocchio*** [2], ***Dr. Sara Bosticardo*** [2], ***Dr. Simona Schiavi*** [2], ***Dr. Benjamin Bender*** [3], ***Dr. Christoph Kessler*** [4], ***Dr. Bernard C. Brais*** [5], ***Dr. Roberta La Piana*** [6], ***Prof. Bart van de Warrenburg*** [7], ***Dr. Mirco Cosottini*** [8], ***Prof. Dagmar Timmann*** [9], ***Dr. Alessandro Daducci*** [2], ***Prof. Rebecca Schüle*** [10], ***Prof. Matthis Synofzik*** [11], ***Dr. Filippo Maria Santorelli*** [12], ***Dr. Sirio Cocozza*** [1]

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ABSTRACT

Background

Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS) and Spastic Paraplegia Type 7 (SPG7) are paradigmatic spastic ataxias (SPAX) with suggested white matter (WM) involvement. Aim of this work was to thoroughly disentangle the degree of WM involvement in these two conditions, evaluating both macro- and microstructure via the analysis of diffusion MRI (dMRI) data.

Material and methods

In this multi-center prospective study, ARSACS and SPG7 patients and Healthy Controls (HC) were enrolled. All participants underwent a standardized dMRI protocol and an extensive clinimetrics evaluation, including the Scale for the Assessment and Rating of Ataxia (SARA). Differences in terms of WM volume or global microstructural WM metrics were probed, as well as the possible occurrence of a spatially defined microstructural WM involvement via voxel-wise analyses, and its correlation with patients' clinical status.

Results

Data of 37 ARSACS (M/F=21/16;33.4±12.4 years), 37 SPG7 (M/F=24/13;55.7±10.7 years) and 29 HC (M/F=13/16;42.1±17.2 years) were analyzed. While in SPG7 only a mild mean microstructural damage was found compared to HC, ARSACS patients present a severe WM involvement, with a reduced global volume ($p<0.001$), an alteration of all microstructural metrics (all with $p<0.001$), without a spatially defined pattern of damage but with a prominent involvement of commissural fibers. Finally, in ARSACS, a correlation between microstructural damage and SARA scores was found ($p=0.004$).

Conclusions

In ARSACS, but not SPG7 patients, we observed a complex and multifaced involvement of brain WM, with a clinically meaningful widespread loss of axonal and dendritic integrity, secondary demyelination and, overall, a reduction in

cellularity and volume.

A NOVEL IMAGING INDEX FOR THE NEURORADIOLOGICAL DIAGNOSIS OF ARSACS: RESULTS FROM THE INTERNATIONAL MULTI-CENTER PROSPAX STUDY

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Alessandra Scaravilli [1]***, Dr. Davide Negroni*** [1]***, Dr. Claudio Senatore*** [1]***, Dr. Lorenzo Ugga*** [1]***, Dr. Mirco Cosottini*** [2]***, Dr. Ivana Ricca*** [3]***, Dr. Benjamin Bender*** [4]***, Dr. Andreas Traschütz*** [5]***, Prof. Nazli Basak*** [6]***, Dr. Atay Vural*** [7]***, Prof. Bart van de Warrenburg*** [8]***, Prof. Alexandra Durr*** [9]***, Dr. Giulia Coarelli*** [9]***, Dr. Roberta La Piana*** [10]***, Prof. Dagmar Timmann*** [11]***, Prof. Rebecca Schüle*** [12]***, Prof. Matthis Synofzik*** [5]***, Dr. Filippo Maria Santorelli*** [3]***, Dr. Sirio Cocozza*** [1]

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Background

Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS) and Hereditary Spastic Paraplegia type 7 (SPG7) represent the most common genotypes of spastic ataxia (SPAX). So far, their MRI features have only qualitatively been described, and a pure neuroradiological differential diagnosis between these two conditions might be hard to achieve.

Objectives

Test the performance of MRI measures to discriminate between ARSACS and SPG7 (as an index of common SPAX disease).

Methods

In this prospective multi-center study, 3D-T1-weighted images of 59 ARSACS (35.4±10.3 years, M/F=33/26) and 78 SPG7 (54.8±10.3 years, M/F=51/27) patients of the PROSPAX consortium were analyzed, along with 30 controls (45.9±16.9 years, M/F=15/15). Different linear and surface measures were evaluated. A ROC analysis was performed, calculating the AUC and corresponding diagnostic accuracy parameters.

Results

The pons area proved to be the only metric increased and exclusively in ARSACS patients ($p=0.02$). Other different measures were reduced in ARSACS and SPG7 compared to controls (all with $p\leq0.005$). A cut-off value equal to 1.67 of the pons-to-superior vermis area ratio proved to have the highest AUC (0.98, diagnostic accuracy 93%, sensitivity 97%) in discriminating between ARSACS and SPG7.

Conclusions

The evaluation of the pons-to-superior vermis area ratio can discriminate ARSACS from other SPAX patients, as here exemplified by SPG7. Hence, we here propose this ratio as the Magnetic Resonance Index for the Assessment and Recognition of patients harboring SACS mutations (MRI-ARSACS), a novel diagnostic tool able to identify ARSACS patients and useful to discriminate ARSACS from other different SPAX undergoing an MRI.

The pattern and staging of brain atrophy in SCA2: MRI outcomes from ENIGMA-Ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Jason Robertson [1], ***Prof. Carlos Hernandez-Castillo*** [1], ***Prof. Ian Harding*** [2]

1. *Dalhousie University,* ***2.*** *QIMR Berghofer Medical Research Institute, Brisbane, Queensland*

Spinocerebellar Ataxia Type 2 (SCA2) is a degenerative neurogenic disorder defined by progressive cerebellar ataxia. Atrophy of the cerebellar cortex, pons, and spinal cord are well-defined features of SCA2. However, it remains unclear how the pattern and magnitude of atrophy evolves over time, and how brain changes map onto symptom severity. Addressing these knowledge gaps is critical for identifying biomarkers, defining biological stages/subtypes, and fully defining whole-brain disease manifestation.

We perform a retrospective, multisite analysis of regional brain volume using automated quantification of T1-weighted MRI data in a large group of SCA2 patients (n=110) and healthy controls (n=128) from 10 sites worldwide. Correlations with SARA scores and cross-sectional profiling of atrophy patterns at different disease stages were also undertaken.

Atrophy in SCA2 was greatest (Cohen's d>1.5) in the cerebellar white matter (WM), brainstem, and corticospinal tracts. Large effects (d>0.8) were also observed throughout the cerebellar grey matter (GM); posterior lobe regions mapping onto non-motor functions showed the greatest changes, followed by motor control areas. In cerebral subcortical regions, the nucleus accumbens, caudate, and thalamus also showed moderate (d>0.5) atrophy.

SARA scores correlated with atrophy in cerebellar WM, brainstem, and (pre)motor tracts of the cerebrum. Significant correlations in cerebellar GM were evident in both motor (lobules I-IV, VIIIa/b) and non-motor regions (lobule IX, Crus II). Atrophy is relatively restricted to subtentorial regions early in the disease, but increasingly impacts the cerebral cortex in later disease stages.

This work provides a comprehensive characterisation of the pattern and spread of neurodegeneration in SCA2, based on the largest sample size of MRI images ever aggregated in this disease. We demonstrate not only that symptom-relevant atrophy in the cerebellum and brainstem continues to progress over time, but also that the cerebral cortex – particularly the motor system – becomes increasingly impacted in later disease stages.

Quantitative muscle ultrasound in SCA3: potential imaging biomarkers to evaluate peripheral nervous system degeneration

Wednesday, 13th November - 18:00: (Minories) - Poster

Mr. Almir Banda [1], Prof. Bart van de Warrenburg [2], Prof. Nens Van Alfen [1], Dr. Roderick Maas [1]

1. *Department of Neurology, Donders Institute for Brain, Cognition, and Behaviour, Radboud University Medical Center, Nijmegen, the Netherlands,* ***2.*** *Radboud university medical center*

Background: SCA3 is characterized by widespread degeneration of peripheral nervous system (PNS) components, but PNS imaging biomarkers are lacking. Muscle ultrasound is a sensitive technique to detect abnormalities in other disorders affecting lower motor neurons.

Objective: To investigate if muscle ultrasound can provide measures of PNS degeneration in SCA3.

Methods: Quantitative muscle ultrasound was used to evaluate the echo intensity (EI) and thickness of 23 bulbar, upper limb, abdominal, and lower limb muscles in ataxic and pre-ataxic SCA3 mutation carriers. Individual muscles were considered abnormal if z-scores for EI and thickness were ≥ 2 and ≤ −2, respectively. At the patient level, an examination was classified as abnormal when EI z-score was >3.5 in 1 muscle, >2.5 in 2 muscles, or >1.5 in 3 muscles. Finally, a fasciculation screening was performed for each muscle.

Results: Twenty-two ataxic and eight pre-ataxic SCA3 mutation carriers participated so far in this study. Muscle ultrasound was classified as abnormal/uncertain/normal in 72.7%/9.1%/18.2% of ataxic individuals and 25.0%/25.0%/50.0% of pre-ataxic individuals. The most commonly affected muscles in terms of abnormal EI and thickness in *patients* were the gastrocnemius (36.4%) and first dorsal interosseus (FDI, 27.3%), respectively. Both z-scores correlated with SARA and FARS ADL score. Fasciculations were frequently found in the FDI (70.0%), rectus femoris (55%), tibialis anterior (45%), and gastrocnemius muscles (45%). In *pre-ataxic individuals*, the biceps brachii was the most commonly affected muscle in terms of abnormal EI (25.0%) and thickness (37.5%). Fasciculations were present in the tibialis anterior, gastrocnemius, and rectus femoris muscles in 25%.

Conclusion: A quantitative muscle ultrasound screening protocol reveals abnormalities in the majority of ataxic and part of pre-ataxic SCA3 mutation carriers. Muscle EI, thickness, and fasciculations may serve as biomarkers of PNS degeneration in SCA3.

This study is ongoing. In November, we will have data from 40 individuals.

Automated Dentate Nucleus Segmentation from QSM Images Using Deep Learning

Wednesday, 13th November - 18:00: (Minories) - Poster

Mr. Diogo Hideki Shiraishi [1], ***Dr. Susmita Saha*** [2], ***Dr. Isaac Adanyeguh*** [3], ***Dr. Helena Bujalka*** [4], ***Dr. Sirio Cocozza*** [5], ***Dr. Louise A Corben*** [6], ***Dr. Manuela Corti*** [7], ***Dr. Andreas Deistung*** [8], ***Prof. Martin Delatycki*** [9], ***Dr. Imis Dogan*** [10], ***Ms. Jennifer Farmer*** [11], ***Prof. William Gaetz*** [12], ***Prof. Nellie Georgiou-Karistianis*** [13], ***Dr. Simon Graf*** [8], ***Dr. Marina Grisoli*** [14], ***Prof. Pierre-Gilles Henry*** [15], ***Mr. Gustavo Jarola*** [16], ***Dr. James Joers*** [3], ***Dr. Christian Langkammer*** [17], ***Prof. Christophe Lenglet*** [18], ***Mr. Jiakun Li*** [19], ***Dr. Camila Lobo*** [20], ***Prof. Eric F. Lock*** [19], ***Prof. David Lynch*** [21], ***Prof. Thomas Mareci*** [22], ***Prof. Alberto Martinez*** [23], ***Dr. Serena Monti*** [24], ***Dr. Anna Nigri*** [14], ***Prof. Massimo Pandolfo*** [25], ***Dr. Myriam Rai*** [11], ***Prof. Kathrin Reetz*** [10], ***Prof. Timothy P. Roberts*** [12], ***Dr. Sandro Romanzetti*** [26], ***Dr. David A. Rudko*** [27], ***Dr. Alessandra Scaravilli*** [5], ***Prof. Jörg B. Schulz*** [28], ***Prof. S. H. Subramony*** [7], ***Prof. Dagmar Timmann*** [29], ***Prof. Marcondes França*** [23], ***Dr. Ian Harding*** [30], ***Dr. Thiago Rezende*** [20], ***Mx. . TRACK-FA Neuroimaging Consortium*** [31]

1. *Department of Neurology, University of Campinas,* ***2.*** *Monash University, Melbourne,* ***3.*** *Center for Magnetic Resonance Research and Department of Radiology, University of Minnesota, Minneapolis, MN,* ***4.*** *School of Psychological Sciences, The Turner Institute for Brain and Mental Health, Monash University, Victoria,* ***5.*** *Department of Advanced Biomedical Sciences, University of Naples Federico II, Naples, Italy,* ***6.*** *Monash University, Clayton, Murdoch Children's Research Institute, Parkville and University of Melbourne, Parkville,* ***7.*** *Department of Neurology and Fixel Center for Neurological Disorders, Gainesville, FL,* ***8.*** *University Clinic and Outpatient Clinic for Radiology, Department for Radiation Medicine, University Hospital Halle (Saale), University Medicine Halle, Halle (Saale), Germany,* ***9.*** *Monash University; Murdoch Children's Research Institute; University of Melbourne; Victorian Clinical Genetics Service,* ***10.*** *Department of Neurology, University of Aachen,* ***11.*** *Friedreich's Ataxia Research Alliance, Downingtown, PA,* ***12.*** *Department of Radiology & Program in Advanced Imaging Research, Children's Hospital of Philadelphia, Philadelphia, PA,* ***13.*** *Monash University,* ***14.*** *Department of Neuroradiology, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy,* ***15.*** *University of Minnesota,* ***16.*** *Department of Neurology, School of Medical Sciences, University of Campinas (Unicamp), Campinas, Brazil,* ***17.*** *Department of Neurology, Medical University of Graz, Graz, Austria,* ***18.*** *University of Minnesota, Minneapolis,* ***19.*** *Division of Biostatistics & Health Data Science, School of Public Health, University of Minnesota, Minneapolis, MN,* ***20.*** *University of Campinas,* ***21.*** *Children's Hospital of Philadelphia,* ***22.*** *Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, Fl,* ***23.*** *Department of Neurology, University of Campinas, Campinas, Sao Paulo,* ***24.*** *Institute of Biostructures and Bioimaging, Italian National Research Council, Naples, Italy,* ***25.*** *Department of Neurology and Neurosurgery, Montreal Neurological Hospital and Institute, McGill University, Montreal, QC, Canada,* ***26.*** *Department of Neurology, RWTH Aachen University, Aachen; JARA-BRAIN Institute Molecular Neuroscience and Neuroimaging, Forschungszentrum Jülich GmbH and RWTH Aachen University, Aachen,* ***27.*** *Department of Neurology and Neurosurgery, McGill University; McConnell Brain Imaging Centre, Montreal Neurological Institute and Hospital; Department of Biomedical Engineering, McGill University, Montreal, QC,* ***28.*** *Universitätsklinikum RWTH Aachen,* ***29.*** *Department of Neurology, University of Essen,* ***30.*** *Monash University, Melbourne and QIMR Berghofer Medical Research Institute, Brisbane,* ***31.*** *TRACK-FA Study*

Background/Objective: The dentate nuclei (DN) are the largest deep nuclei and the primary efferent site of the cerebellum. Abnormalities in the DN are reported in several neurological disorders, especially in the inherited cerebellar ataxias where the DN plays a central role in the pathogenies of such diseases. Direct *in vivo* assessment of DN is possible via quantitative susceptibility mapping (QSM) images. However, automated first-generation solutions suffer from accuracy, reproducibility and generalizability. Therefore, we propose to develop a DN segmentation tool using deep learning (DL) applied to QSM images.

Materials and Methods: We gathered cerebral QSM images from 132 healthy controls and 170 individuals with Friedreich's Ataxia or multiple sclerosis from nine datasets worldwide. Experienced annotators performed the anatomical tracings of the DN, which underwent a robust quality control process. Several architectures, including U-Net, Swin UNETR, and nnU-Net, were tested and compared a priori to create the two-step approach composed by a cerebellum localization and DN segmentation models.
Results: The anatomical annotation protocol showed high intra-rater reproducibility (average ICC 0.906) and inter-rater reliability (average ICC 0.776). The nnU-Net framework performed best during the DL architecture exploration. The final pipeline (cerebellum localization plus DN segmentation) achieved good performance when compared to the manual ground-truth annotations (left/right DN Dice 0.898±0.031/0.894±0.036). Similarly, in external validation dataset, our algorithm outperformed the leading existing automated tool (left/right DN Dice 0.863±0.038/0.843±0.066 vs. 0.568±0.222/0.582±0.239). In addition, the measures showed a superior correlation index with manual annotations and resolved effectively in both isotropic and anisotropic QSM sequences.
Discussion/Conclusion: We provide a state-of-the-art tool that accurately and efficiently segments the DN from brain QSM images. Such model can be applied in observational, natural history, and treatment trials to aid biomarker discovery. This tool is available as source-code in the GitHub repository.

High-resolution nerve ultrasound in SCA3: small nerves as early indicators of peripheral nervous system degeneration

Wednesday, 13th November - 18:00: (Minories) - Poster

Mr. Antony Pop [1], Prof. Bart van de Warrenburg [2], Prof. Nens Van Alfen [1], Dr. Roderick Maas [1]

1. *Department of Neurology, Donders Institute for Brain, Cognition, and Behaviour, Radboud University Medical Center, Nijmegen, the Netherlands,* ***2.*** *Radboud university medical center*

Background: Peripheral nervous system (PNS) involvement is common in SCA3, but PNS imaging biomarkers are lacking. We hypothesized that individuals with SCA3 have smaller upper and lower limb nerves due to degeneration of dorsal root ganglia and the resulting axonal loss of their peripheral sensory projections.
Objective: To investigate if nerve ultrasound can capture PNS degeneration in SCA3 and serve as biomarker of disease status and progression.
Methods: High-resolution nerve ultrasound was used to measure cross-sectional areas (CSAs) of the median, ulnar, superficial radial, tibial, and sural nerves at 22 sites in SCA3 mutation carriers. CSAs were compared with reference values from healthy controls (n=4186 for the upper extremities, n=1001 for the lower extremities). Nerves were considered too small if the CSA at the particular site was below the lower bound of the 95% CI in healthy adults. The same nerves were examined for sensory axonal loss using conduction studies (NCS).
Results: Twenty-two ataxic and nine pre-ataxic SCA3 mutation carriers participated so far in this study. Abnormally small nerve CSAs were found at multiple sites, in particular the median nerve in the mid upper arm (pre-ataxic 8/9, ataxic 19/22), ulnar nerve in the distal forearm (pre-ataxic 8/9, ataxic 16/22), superficial radial nerve in the forearm (pre-ataxic 9/9, ataxic 21/22), tibial nerve in the popliteal fossa (pre-ataxic 6/9, ataxic 15/22), and sural nerve in the calf (pre-ataxic 3/9, ataxic 10/22). Compared with sensory NCS, ultrasound was more sensitive to detect abnormalities in the arms, while the opposite was true for the legs.
Conclusion: High-resolution ultrasound reveals pathologically small upper and lower limb nerves in ataxic and pre-ataxic SCA3 mutation carriers. Nerve CSAs, especially in the upper extremities, may serve as early markers of PNS degeneration in SCA3.
This study is ongoing. In November, we will have data from 40 mutation carriers.

A Longitudinal, Multimodal MRI Characterization in Cuban SCA 2

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Sandro Romanzetti [1], ***Prof. Kathrin Reetz*** [2], ***Dr. Imis Dogan*** [2], ***Prof. Evelio Gonzalez*** [3], ***Prof. Roberto Rodriguez Labrada*** [3], ***Prof. Luis Velázquez-Pérez*** [4]

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Background and Objectives:
This study, a German-Cuban collaboration, aims to develop and validate novel clinical and imaging biomarkers for autosomal-dominant spinocerebellar ataxia type 2 (SCA2) in pre-symptomatic and symptomatic conditions.
Methods:
We characterized SCA2 through baseline and follow-up multimodal MRI. Anatomical MRI (tensor-based morphometry) assessed morphological changes, diffusion-weighted imaging (DWI) examined white matter degeneration, and resting-state functional MRI combined with clinical measurements. Correlations were made with clinical and genetic disease characteristics, neuropsychological parameters, and movement data.
Results:
Significant reductions in volumes of cerebellar white matter, medulla, and pons were found, modulated by clinical status (preclinical vs. clinical), along with significant decreases in fractional anisotropy (FA). Degeneration of cerebellar structures and brainstem, particularly pontine volumes, was evident in both preclinical and clinical SCA2 patients compared to controls.
Discussion and Conclusion:
Volumetric data indicate notable degeneration in cerebellar and brainstem structures in SCA2 patients, corroborating previous findings (Reetz et al., ACTN 2018; 5(2): 128). FA measurements showed statistically significant degenerations in the same structures identified by TBM analysis. These findings highlight the potential of imaging biomarkers in tracking SCA2 progression and evaluating therapeutic interventions. This research, supported by a grant from the German Federal Ministry of Education and Research (BMBF 01DN18022), emphasizes the importance of international collaborations in addressing complex neurological disorders.
Funding:
Funded by a Research Grant to Support Collaboration of German-Cuban Projects from the German Federal Ministry of Education and Research (BMBF 01DN18022), as part of the “German-Cuban collaboration to develop clinical, electrophysiological and imaging markers for spinocerebellar ataxia type 2 (CARE).”

MICROSTRUCTURAL DAMAGE OF THE CORTICOSPINAL TRACT IN ARSACS: RESULTS OF A PROFILOMETRY MRI ANALYSIS OF DATA COLLECTED WITHIN THE PROSPAX STUDY

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Alessandra Scaravilli [1], ***Dr. Gaia Mari*** [2], ***Dr. Ilaria Gabusi*** [2], ***Dr. Matteo Battocchio*** [2], ***Dr. Sara Bosticardo*** [2], ***Dr. Simona Schiavi*** [2], ***Dr. Benjamin Bender*** [3], ***Dr. Christoph Kessler*** [4], ***Dr. Roberta La Piana*** [5], ***Prof. Bart van de Warrenburg*** [6], ***Dr. Mirco Cosottini*** [7], ***Prof. Dagmar Timmann*** [8], ***Dr. Alessandro Daducci*** [2], ***Prof. Rebecca Schüle*** [9], ***Prof. Matthis Synofzik*** [10], ***Dr. Filippo Maria Santorelli*** [11], ***Dr. Sirio Cocozza*** [12]

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ABSTRACT

Background

Spasticity represents a core clinical feature of Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS) patients. Nonetheless, its pathophysiological substrate is poorly investigated. We assessed the microstructural integrity of corticospinal tract (CST) using diffusion MRI (dMRI) via profilometry analysis, to understand its possible role in the development of spasticity in ARSACS.

Materials and Methods

In this multi-center prospective study, data of 37 ARSACS (M/F=21/16; 33.4±12.4 years) and 29 controls (M/F=13/16; 42.1±17.2 years) acquired within the PROSPAX consortium were collected from January 2021 to October 2022 and analyzed. Differences in terms of global CST microstructural integrity were probed, as well as a possible spatial distribution of the damage along the tract via profilometry analysis. Possible correlation between clinical severity, including the Spastic Paraplegia Rating Scale (SPRS), were also tested.

Results

A significant global involvement of the CST was found in ARSACS compared to controls (all tests with $p<0.001$), with a spatially defined pattern of more pronounced microstructural integrity loss occurring right below and above the pons, a structure that was also confirmed to be thickened in these patients ($p<0.001$). A bilateral negative correlation emerged between the microstructural integrity of the CST and clinical indices of spasticity expressed via SPRS ($p=0.02$ for both CSTs).

Conclusion

A clinically meaningful microstructural involvement of CST is present in ARSACS patients, with a spatially defined

pattern of damage occurring right below and above a thickened pons. An evaluation of the microstructure of this bundle might serve as a possible biomarker in this condition.

Substantia nigra degeneration in spinocerebellar ataxia 2 and 7 using neuromelanin-sensitive imaging

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Giulia Coarelli [1], ***Dr. Lydia Chougar*** [2], ***Dr. François-Xavier Lejeune*** [3], ***Dr. Pia Ziegner*** [2], ***Dr. Rahul Gaurav*** [2], ***Dr. Emma Biondetti*** [2], ***Mrs. Rania Hilab*** [2], ***Dr. Alain Dagher*** [4], ***Prof. Alexandra Durr*** [2], ***Prof. Stéphane Lehéricy*** [2]

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Background and objectives: Spinocerebellar ataxias (SCA) are autosomal dominant neurodegenerative diseases with widespread lesions across the central nervous system. Although ataxia is the main clinical sign, patients may also present with parkinsonism. We aimed to characterize substantia nigra degeneration in SCA type 2 and 7 using neuromelanin-sensitive imaging.

Methods: SCA2 (n=15) and SCA7 (n=15) ataxic and preataxic expansion carriers and ten healthy controls (HC) were recruited (NCT04288128). Volume and signal-to-noise (SNR) values of the substantia nigra were extracted from neuromelanin-sensitive images. ROC curves were used to determine the metrics that best separated SCA participants from HC. Correlations between imaging measurements, clinical variables, and plasma neurofilaments light chain (NfL) levels were investigated.

Results: SCA2 participants (110.19±1.31) had lower SNR values in the substantia nigra than HC (113.21±1.35; p=0.0003) and SCA7 subjects (112.51±2.07; p=0.003). SCA7 participants did not differ from HC in terms of signal. Substantia nigra volume was not different between groups. In ataxic subjects, substantia nigra volume was lower in patients with SCA2 (0.13±0.04; p=0.06) and SCA7 (0.10±0.03, p=0.02, p=0.02) compared to HC (0.17±0.04). Signal decrease was detected at the preataxic stage in SCA2, but not in SCA7. SCA2 carriers showed involvement of the associative and limbic nigral territories. SNR (AUC=0.94-0.96) and substantia nigra volume (AUC=1) achieved good discrimination of SCA2 and SCA7 participants, respectively, against HC. Significant correlations were observed in SCA7 between SN volume and estimated time to onset, CAG repeats, severity scores, and plasma NfL levels.

Discussion and Conclusions: Neuromelanin-sensitive imaging provides evidence of nigral degeneration in SCA type 2 and 7 that can be detected in the early stages of SCA2. Future studies on larger samples and longitudinal datasets will investigate the dynamics of progression of substantia nigra neurodegeneration from the presymptomatic stage and determine whether such biomarkers could be relevant outcome measures in therapeutic trials.

Diffusion-weighted imaging shows altered but static white matter integrity in Spinocerebellar Ataxia type 1

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Kirsten Kapteijns [1], ***Mr. Teije van Prooije*** [1], ***Mr. Hao Li*** [1], ***Dr. Anil Tuladhar*** [1], ***Prof. Tom Scheenen*** [2], ***Prof. Bart van de Warrenburg*** [1]

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Background:
Previously, MRI-based volumetric analysis in SCA1 has shown macrostructural white matter changes in specific brain areas. Here, we investigated the white matter microstructure using diffusion-tensor MRI (DTI) to evaluate the integrity and connectivity alterations of both infra- and supratentorial white matter in SCA1.
Methods:
Symptomatic SCA1 patients and matched controls underwent repeated 3T MRI examinations at baseline, after 1 year, and after 2 years.. The scanning protocol included an anatomical 3D T1 MPRAGE acquisition and DTI with 2 diffusion weightings in 64 directions. SARA score was evaluated by the same trained assessor at each visit as a measure of disease severity. Processing was done through FSL DTIFIT (v. 6.0.5). We performed tract-based spatial statistics (TBSS) and extracted patient-specific fractional anisotropy (FA) and mean diffusivity (MD) for specific regions of interest, including the cerebellar peduncles.
Results:
Twenty-seven ataxic SCA1 patients and twenty-one controls were included; twenty-two patients completed the full longitudinal assessment. TBSS showed significant areas of lowered FA and raised MD in SCA1 patients, most notably in all three cerebellar peduncles and posterior corpus callosum (CC; threshold $p<.05$). Cross-sectionally the cerebellar inferior and superior peduncles' FA and MD values correlated strongly with individual SARA scores ($p<.001$). Longitudinally, only part of the anterior CC showed significant loss of FA after 2-years.
Discussion and conclusion:
DTI was able to capture WM integrity loss in both infra- and supratentorial brain areas in SCA1. Changes were most pronounced in the cerebellar peduncles, and while correlated with disease severity, showed no sensitivity to change over a 2-years period. This suggests that DTI has limited utility as an outcome measure for disease progression in interventional trials in symptomatic SCA1 stages. Changes in the anterior corpus callosum will be further investigated with tractography.
This work is funded by ZonMw (40-44600-98-606)

Brainstem Substructure Atrophy in Late-Onset GM2-Gangliosidosis

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Olivia Rowe [1], Dr. Rangaprakash Deshpande [1], Dr. Neha Godbole [2], Dr. Elizabeth Haxton [2], Prof. Florian Eichler [3], Prof. Jeremy D. Schmahmann [3], Dr. Robert Barry [1], Dr. Christopher D. Stephen [3]

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Background and Objectives:
Late-Onset GM2-Gangliosidosis (LOGG) are a heterogenous group of rare, neurodegenerative lysosomal disorders including late-onset Tay-Sachs (LOTS) and Sandhoff disease (LOSD). Cerebellar atrophy is common, even in the absence of clinical ataxia, particularly in LOTS, including pontocerebellar atrophy. We sought to assess for brainstem substructure atrophy in LOGG, including LOSD.
Methods:
Ten LOGG patients (7 LOTS, 3 LOSD) and 7 age-matched healthy controls had structural MRI brain imaging. A FreeSurfer (v7.1) brainstem substructure module was used for segmentations, including the pons, medulla, superior cerebellar peduncle (SCP), midbrain, and total brainstem. Volumes were normalized by estimated total intracranial volume. Clinical ataxia severity was assessed with the Brief Ataxia Rating Scale, Friedreich's Ataxia Rating Scale and Scale for the Assessment and rating of Ataxia.
Results:
There were differences in brainstem volume in LOGG vs. controls, in the pons (12,478.41±1,531.53 vs. 15,412.04±2,767.97 mm^3, p=0.0135) and SCP (173.15±27.70 vs. 266.33±67.41 mm^3, p=0.0009) but not the midbrain. Within the LOTS group, there was also SCP atrophy vs. controls (171.40±30.74 vs. 266.33±67.41 mm^3, p=0.0042). Cerebellar volume was relatively preserved in LOSD compared to the SCP. The LOSD group was too small for independent comparisons. There were no significant correlations between the SCP/pons and clinical scales or disease duration.
Discussion:
We found prominent atrophy of the pons and SCP in LOGG, which did not correlate with disease severity or disease duration. Cerebellar volume was relatively preserved in LOSD but in the LOTS patients, the pontocerebellar atrophy profile was dominated by cerebellar atrophy, as previously reported, compared to the SCP. Future studies, with larger sample sizes, should stratify these two subtypes when investigating their imaging features.
Conclusion:
We demonstrated brainstem volume loss in LOG, particularly LOTS, whereas cerebellar volume was relatively preserved in LOSD, highlighting the clinical and radiological differences between these subtypes.

Progressive Ataxia and Palatal Tremor due to Bilateral Hypertrophic Olivary Degeneration: A Case Report and Literature Review

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Bowen Song [1], ***Dr. Scott Eggers*** [1]

1. *Mayo Clinic*

Objective: To report a patient with progressive ataxia and palatal tremor (PAPT) due to bilateral hypertrophic olivary degeneration.

Methods: Case Report and Literature Review

Background: Hypertrophic olivary degeneration (HOD) is a rare form of multi-synaptic degeneration which leads to hypertrophy of the inferior olivary nucleus (ION) seen on brain magnetic resonance imaging (MRI) as a result of disruption within the dento-rubo-olivary pathway or Guillain-Mollaret triangle (GMT). GMT is composed of contralateral dentate nucleus, the ipsilateral red nucleus and the ipsilateral ION. Unilateral HOD often has an identifiable cause. In contrast, bilateral HOD often has no specific identifiable causes.

Results: A 55-year-old previously healthy man presented with a 1-year history of progressive unsteadiness without dysarthria, dysphagia, dysphonia, or ear clicking. There was no oscillopsia or visual symptoms. Neurologic examination showed a 2-3 Hz tremor of the soft palate synchronous with a low amplitude binocular pendular torsional nystagmus. No other cerebellar signs were identified. T2 weighted image on brain MRI showed asymmetric bilateral HOD, left more than right. No structural etiologies such as infarction, hemorrhage, cavernous malformation, or space-occupying lesions were identified on MRI. CSF analysis showed no pleocytosis, normal protein and glucose level.

Discussion: Unilateral HOD often has a visible causative lesion. Here we report a case of PAPT with MRI findings of asymmetric bilateral HOD. Most common structural etiologies have been ruled out. Upon literature review, genetic etiologies such as spinocerebellar ataxia type 20, Alexander disease, or POLG gene mutation could present with an insidious course of progressive ataxia[1]. Genetic evaluation is in progress. HOD has also been reported as a paraneoplastic syndrome[2]. Scrotal ultrasound, CT chest, abdomen and pelvis did not demonstrate any underlying malignancy. In conclusion, HOD should be in the differential for patients with isolated progressive ataxia and MRI should be obtained.

NucAI: A Deep Learning Framework for Analyzing Nuclear Phenotypes in Degenerating Neurons

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Grazyna Adamek [1], Mr. Grzegorz Bartyzel [2], Ms. Zuzanna Czarny [1], Dr. Anna Samelak-Czajka [3], Mr. Michal Kurkowski [1], Dr. Paweł M. Świtoński [1]

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Introduction:
Spinocerebellar ataxia type 7 (SCA7) is a neurodegenerative disorder marked by cerebellar and retinal degeneration. As SCA7 progresses, Purkinje cells (PCs) become notably susceptible to dysfunction and death, yet the underlying mechanism remains elusive. Increasing evidence highlights the nucleus as a key organelle linked to ataxia, making it a crucial target for mechanistic studies. However, identifying new nuclear phenotypes through immunofluorescence imaging is challenging due to difficulties in recognizing differentiable features in image datasets.
Objectives:
The project aimed to create an AI-powered tool, NucAI, capable of analyzing numerous images of mouse PC nuclei obtained with imaging flow cytometry to identify key pathological features distinguishing between healthy and diseased states.
Methods:
We developed NucAI, an auto-encoder architecture with a fully connected classification module using the open-source PyTorch machine learning framework. The gradient class activation mapping (GradCAM) method was used for visual feedback on the specific features the network focused on during classification. High-throughput imaging flow cytometry was performed on cerebellar nuclear extracts from SCA7-266Q and wild-type (WT) mice, with PC nuclei selected based on the expression of the RanBP2 protein.
Results:
To test NucAI phenotype recognition capabilities, we trained it on images of SCA7-266Q and WT PC nuclei stained for aggregated ataxin-7, achieving over 90% validation accuracy. GradCAM correctly identified aggregates as the primary distinguishing factor. For the discovery phase of potential new phenotypes associated with mutant ataxin-7 toxicity, we trained NucAI on images stained for the H2B120Kub histone modification, achieving 92% validation accuracy. GradCAM revealed H2B120Kub speckled patterns in the nucleoplasm of SCA7 nuclei.
Discussion and Conclusions:
NucAI represents a new generation of data analysis approaches where the machine learning process is utilized not for predictive capabilities but for explainability of the learned data. The novel nuclear phenotypes uncovered by NucAI may provide new insights into SCA7 pathology.

Neuroimaging Biomarkers for Disease Progression in Friedreich Ataxia: A Cross-Sectional Analysis of TRACK-FA

Wednesday, 13th November - 18:00: (Minories) - Poster

Prof. Nellie Georgiou-Karistianis [1], ***Dr. Louise A Corben*** [2], ***Prof. Eric F. Lock*** [3], ***Dr. Helena Bujalka*** [1], ***Dr. Isaac Adanyeguh*** [4], ***Dr. Jonathan J. Cherry*** [5], ***Dr. Manuela Corti*** [6], ***Prof. Martin Delatycki*** [7], ***Dr. Imis Dogan*** [8], ***Ms. Jennifer Farmer*** [9], ***Prof. Marcondes França*** [10], ***Dr. Anthony S. Gabay*** [11], ***Prof. William Gaetz*** [12], ***Prof. Ian Harding*** [13], ***Dr. James Joers*** [4], ***Ms. Michelle A. Lax*** [14], ***Mr. Jiakun Li*** [3], ***Prof. David Lynch*** [15], ***Prof. Thomas Mareci*** [16], ***Prof. Alberto Martinez*** [10], ***Prof. Massimo Pandolfo*** [17], ***Dr. Marina Papoutsi*** [11], ***Dr. Richard Parker*** [11], ***Dr. Myriam Rai*** [9], ***Prof. Kathrin Reetz*** [18], ***Dr. Thiago Rezende*** [10], ***Prof. Timothy P. Roberts*** [12], ***Dr. Sandro Romanzetti*** [18], ***Dr. David A. Rudko*** [19], ***Dr. Susmita Saha*** [1], ***Prof. Jörg B. Schulz*** [18], ***Prof. S. H. Subramony*** [20], ***Dr. Veena G. Supramaniam*** [11], ***Prof. Christophe Lenglet*** [4], ***Prof. Pierre-Gilles Henry*** [4]

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Background

TRACK-FA [1] is a natural history study, representing the largest-ever longitudinal, multi-national, multimodal investigation combining neuroimaging and clinical data from adults and children with Friedreich ataxia (FRDA) and matched controls. Its primary objective is to create a comprehensive dataset, allowing for the validation of potential neuroimaging biomarkers and their integration into clinical trials. Here, we present cross-sectional findings from the baseline cohort.

Methods

Between February 2021-August 2023, a total of 169 eligible individuals with FRDA and 95 matched controls (age range 5.1-42.9 years; Friedreich's Ataxia Rating Scale functional staging score ≤5) were recruited and evaluated during the initial of three annual study visits. The assessment battery included harmonized multimodal techniques, including Magnetic Resonance Imaging (MRI) and Spectroscopy (MRS), alongside clinical assessments (including SARA, mFARS). Primary outcome measures (POMs) were brain and spinal cord morphometry; brain and spinal

cord microstructure (measured with diffusion MRI); brain iron levels (measured with quantitative susceptibility mapping); and spinal cord biochemistry (using MRS).

Results

Significant group differences were observed in most POMs. Within the FRDA group, most POMs exhibited significant associations with clinical measures. Sub-analyses revealed that in the adult FRDA group (but not the pediatric group), several POMs were significantly linked to GAA1. Notably, for several POMs, age-dependent trends observed in controls during childhood were either absent or attenuated in FRDA.

Discussion & Conclusion

Cross-sectional results demonstrated significant group differences in various regions of the cerebellum and spinal cord, across measures relating to morphometry, microstructure, and neurochemistry. Many differences were associated with clinical measures, while others exhibited age-related variations. These findings hold promise for defining novel biomarkers of disease progression for adults and children with FRDA, which could be seamlessly integrated into clinical trials to evaluate the effectiveness of innovative therapeutics.

Reference

[1] Georgiou-Karistianis et al. PLoS One 17(11):e0269649 (2022).

Funding: FARA; TRACK-FA consortium.

Poster session II - Emerging and existing therapeutics – preclinical research

Understanding how cells can adapt to lipoate deficiency

Wednesday, 13th November - 18:00: (Minories) - Poster

Pallavi Joshi [1], ***Dr. Owen Skinner*** [2], ***Fangcong Dong*** [1], ***Alex Guo*** [3], ***Dr. Vamsi Mootha*** [1]

1. *Harvard Medical School and Massachusetts General Hospital,* ***2.*** *Northeastern University,* ***3.*** *Broad Institute of MIT and Harvard*

Objectives: Lipoate is a conserved mitochondrial cofactor essential for the activity of several central metabolic enzymes including pyruvate dehydrogenase, a-ketoglutarate dehydrogenase, the glycine cleavage system, and multiple amino acid dehydrogenase complexes. Mutations in most lipoate containing enzyme complexes have been associated with various pediatric forms of mitochondrial disease that present with hypotonia, seizures, and ataxia. Moreover, lipoate can be deficient in human disorders of Fe-S cluster biogenesis – including Friedreich's ataxia – as this co-factor is required for sulfur donation and electron transfer in the biosynthesis of lipoate. We recently discovered and reported that hypoxia is an environment in which lipoate is dispensable in cultured human cells. Here we sought to investigate the mechanism.

Methods: We utilized a combination of cell culture, immunoblotting, oxygen consumption rate analysis, quantitative proteomics, metabolomics, and labeled metabolite tracing to study adaptations in various oxygen tensions that could allow lipoate deficient cells to proliferate.

Results: Our experiments reveal that hypoxia (1% O_2) rewires cellular metabolism to allow cell proliferation in the absence of lipoate. In normoxia, loss of the lipoate synthesizing enzyme LIAS results in slow cell growth, activates the integrated stress response and alters both the cellular proteome and metabolome. However, all these changes are reversed in hypoxia without restoring lipoate enzyme functionality. We identify activation of the HIF transcriptional pathway as sufficient for growth rescue. Furthermore, we have found metabolites important for LIAS deficient cell growth in hypoxia that point to the altered use of key pathways under low oxygen tensions. These results are consistent with a HIF-dependent re-wiring of metabolism that allows cells to survive without lipoate containing TCA cycle enzymes.

Discussion/Conclusion: Our work shows that under certain environments, lipoate is dispensable. These results could be important in defining new therapies for patients with either primary or secondary lipoate deficiency.

Analysis in Drosophila melanogaster models of modulation of Glutamate Metabolism as a Therapeutic Approach in PolyGlutamine-based disorders

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Juan Antonio Navarro Langa [1], ***Ms. Anna-Lena Horsch*** [2], ***Ms. Elisabeth Martinez*** [3], ***Prof. María Dolores Moltó*** [4], ***Pilar González-Cabo*** [5], ***Prof. Federico V. Pallardó*** [5]

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Objectives

Polyglutamine (polyQ) diseases represent a group of autosomal dominantly inherited neurodegenerative diseases. The common feature of these diseases is an abnormal expansion of CAG repeats resulting in a long polyQ tract upon translation into protein. This polyQ tract leads to protein misfolding and various downstream effects, eventually culminating in neuronal cell death. To date, all these disorders lack effective treatment. Importantly, glutamate excitotoxicity is commonly described as a hallmark of polyQ diseases however little is known about the impact of its manipulation on disease´s phenotypes.

Methods

Drosophila melanogaster has been widely used as a model organism for studying the pathology of polyQ diseases. In this work, we used *Drosophila* to model Spinocerebellar Ataxia Type 3 (SCA3) and Huntington's Disease (HD), by expressing toxic polyQ variants in fly neurons. Furthermore, we have used new fluorescent sensors (iGluSnFR) to evaluate extracellular glutamate.

Results

Expression of Ataxin3 and Huntingtin proteins resulted in a pronounced impairment of locomotion and drastic shortening in lifespan. Furthermore, the expression of these polyQ proteins elicited substantial alterations in the expression of genes associated, among others, with chaperone response, autophagy or apoptosis. Remarkably, and given the critical role of glutamate excitotoxicity in polyQ diseases, our molecular and histological analysis indicate that glutamate metabolism is also strongly altered in fly models. Furthermore, we have manipulated genetically and pharmacologically, glutamate metabolism in SCA3 and HD fly models and observed that it has the potential to restore locomotion impairment to control levels, normalize the expression levels of heat shock proteins, as well as of certain genes related to glutamate metabolism and reduced cell death.

Conclusion

Our findings revealed novel potential targets in the glutamate metabolism for treatment of these diseases.

Funding

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Targeting Necroptosis for Neuroprotection in STUB1 Ataxias

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Kaitlan Smith [1], ***Dr. Jonathan Schisler*** [1]

1. *University of North Carolina at Chapel Hill*

Objectives: Coding mutations in the *STUB1* gene, which encodes CHIP (C-terminus of Heat shock protein 70 – Interacting Protein), underlie two forms of ataxias: Spinocerebellar Ataxia Autosomal Recessive 16 (SCAR16) and Spinocerebellar Ataxia 48 (SCA48). Our research and other studies suggest that necroptosis—a pro-inflammatory form of cell death—may contribute to the cerebellar degeneration observed in SCAR16 and SCA48 patients. The receptor-interacting protein kinases 1 and 3 (RIPK1/3), critical players in necroptotic activation, are known substrates of CHIP. Loss of CHIP function leads to RIPK1/3 upregulation, implicating necroptosis as a crucial neurogenerative pathway in *STUB1* ataxias. **We hypothesized that inhibiting RIPK1 could be neuroprotective and prevent cerebellar degeneration in preclinical models of *STUB1* ataxias.**

Methods: We established a panel of preclinical mouse models of *STUB1* ataxia to explore how CHIP dysfunction impacts neurodegeneration and ataxic severity. In these models, we administered long-term (28 weeks) intraperitoneal injections of Necrostatin-1s (Nec-1s), a RIPK1 inhibitor. Additionally, we assessed motor coordination and cognitive function through a year-long battery of behavioral assays.

Results: STUB1 disease mutations increased motor and cognitive dysfunction in mice, accelerating aging. Remarkably, mice treated with Nec-1s exhibited improved motor coordination, cognitive function, and overall viability compared to untreated mice.

Discussion and Conclusion: Pharmacologically targeting the necroptotic signaling pathway in preclinical mouse models of *STUB1* ataxias offers neuroprotection by mitigating ataxic severity. These findings highlight the potential of druggable targets within the CHIP-regulated necroptotic pathway as promising therapeutic candidates for ataxia. Our study underscores the relevance of CHIP and CHIP-dependent pathways in the quest for effective ataxia treatments.

Dentatorubral-pallidoluysian atrophy (DRPLA): design, de-risking and validation of ASO-induced ATN1 knockdown therapy

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Joanna Korecka [1], ***Dr. Tojo Nakayama*** [2], ***Dr. Lisseth Burbano*** [2], ***Ms. Aleksandra Krzywanska*** [1], ***Dr. Boxun Zhao*** [2], ***Ms. Charlotte Oettgen*** [1], ***Mr. Ali Rahman*** [2], ***Mr. David Ball*** [1], ***Ms. Renata DiDonato*** [2], ***Ms. Aliza Ben-Varon*** [3], ***Dr. Aubrie Soucy*** [2], ***Dr. Claudia Lentucci*** [2], ***Dr. Hien Zhao*** [4], ***Dr. Jeff Carroll*** [5], ***Dr. Vikram Khurana*** [1], ***Dr. Timothy Yu*** [2]

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

Methods: We designed 2'MOE ASOs to induce RNase H1-mediated knockdown of *ATN1* as potential gene-targeted therapeutics for DRPLA. We performed high throughput *in vitro* screening in BE(2)M-17 neuroblastoma cells, followed by microwalking and secondary screening in human iPSC neurons. Lead candidates were further tested for *in vivo* acute tolerability via ICV injection in mice. Utilizing DRPLA patient iPSC-derived neurons, we screened ASOs for acute calcium oscillation shift and long-term neuronal toxicity. ASO functional rescue capacity was tested in DRPLA-specific neuronal activity phenotypes.

Results: A total of 707 *ATN1*-targeting ASOs were synthesized and efficacy tested in BE(2)M-17 neuroblastoma cells. Out of these, 80 ASOs induced a ~50% *ATN1* knockdown in human iPSC-derived neurons when delivered via gymnosis at 1μM. Furthermore, 41 ASOs were assessed for *in vivo* tolerability, with 10 molecules showing no acute behavioral changes in mice. 12 lead ASOs were tested in DRPLA-patient iPSC neurons for calcium oscillation shifts and were found to correlate with the observed acute toxicity in mice. Long-term neuronal survival assays further eliminated potential toxic ASO sequences while confirming that induced *ATN1* knockdown does not affect human neuronal survival *in vitro*. This was supported by the intact viability of CRISPR/Cas9-induced *ATN1*-knockout cultures. DRPLA patient iPSC-derived cortical neurons exhibit increased calcium uptake and hyper-synchronization. Three ASO compounds were found to reverse DRPLA-specific alterations in calcium homeostasis.

Conclusions & Discussion: This study establishes a paradigm for de-risking ASO therapeutics for neurodegenerative diseases by combining *in vivo* and patient-matched iPSC modeling.

Funding: CureDRPLA

Introduction of Premature Stop Codons Within The ATXN2 Repeat Induces Therapeutically Relevant Changes in Expression

Wednesday, 13th November - 18:00: (Minories) - Poster

Mr. Michael Kuckyr [1], ***Dr. Defne Audrey Amado*** [2], ***Dr. Beverly L. Davidson*** [3]

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Spinocerebellar Ataxia Type 2 (SCA2) is an autosomal dominant disorder impacting up to 1 in every 50,000 individuals. SCA2 occurs when the poly-glutamine region within exon 1 of the *ATXN2* gene expands beyond 33 CAG repeats (*mATXN2*). *mATXN2* creates toxic RNA and protein products that cause cerebellar degeneration leading to ataxia, dementia, and premature death. Previous work in our lab has observed that excising the repeats or disrupting the gene using AAV delivered CRISPR-Cas9 can result in genomic integration of AAV at the nuclease cut site. AAV integrations can disrupt gene expression and create toxic genomic events. To avoid these integration events, we are applying cytosine-base editing (CBE) which utilizes a Cas9 Nickase and cytosine deaminase to insert premature termination codons (PTC) by C : T conversion. Preliminary data in HEK293 cells after transfection with CBE achieved 47% editing of the *ATXN2* repeat, resulting in a 33% reduction of RNA levels and an 80% reduction of protein. In a model N2A reporter line containing *ATXN2* exon 1 at various repeat lengths we observed a 35% reduction and 17% reduction of the reporter protein at 58 and 108 CAG repeats, respectively. Currently there are no approved therapies for the treatment of SCA2, and thus far clinical trials targeting *ATXN2* have focused on the use of ASOs and miRNAs, which have a lower target specificity then guide directed Cas9. This work demonstrates the potential of CBEs for the treatment of SCA2 by inserting PTC within *mATXN2* length pathogenic repeats. Future work is focused on translating this method into the 127Q and 72Q SCA2 mouse models by AAV and LNP delivery of CBE and guides.

A feasibility study of a novel home-based complex intervention for children with ataxia telangiectasia

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Munira Khan [1], ***Ms. Lisa Bunn*** [2], ***Mx. The A-Team Collaborative*** [2]

1. *University of Plymouth,* ***2.*** *Various*

Background: Allied health therapies and exercise present a mainstream management option for people with ataxia telangiectasia (A-T) and have the potential to offer interventions to optimise fitness and general health. However, despite the need for allied health inputs, this area remains under-researched for A-T population. This study was therefore designed with a view to provide a viable resource to a) families to self-manage A-T related symptoms at home, and b) therapists to base their interventions on.

Objective: To determine the feasibility and acceptability of a complex home-based exercise intervention for children with A-T.

Methods: A delayed start randomised controlled trial design was used and participants were enrolled into either early start or delayed start group. The intervention was an eight-week programme of four yoga and two breathing exercise weekly home-based sessions. Feasibility was measured using the following parameters- recruitment and retention of participants, acceptability of the programme, and efficacy in terms of health and well-being. The latter were assessed using pre- and post-intervention questionnaires, SARA, spirometry, EQ-5D-Y, PEDI-CAT, and semi-structured interviews. Descriptive analyses were employed to summarize the quantitative data and framework method was used to analyse the qualitative data.

Results: Seven children (female n=4, male n=3; mean age=6.6 years; range=4-10 years) participated in the study. Rates of consent and retention were 64% and 57%, respectively. The engagement rate ranged between 58-100%. The participants experienced physical, functional, and emotional benefits.

Discussion: The findings suggest that the exercise intervention is feasible and acceptable. The participants and their parents found the exercises helpful in improving balance, strength, and coordination.

Conclusion: This study has explored the feasibility and acceptability of a complex home-based exercise intervention for children with A-T. Larger scale trial is warranted to establish the effectiveness of the intervention.

Aripiprazole-related compounds reduce cellular abundance of toxic ATXN3 in models of MJD/SCA3

Wednesday, 13th November - 18:00: (Minories) - Poster

Mx. Louisa Liu [1], Mx. Michelle Hoang [1], Dr. Mathivanan Packiarajan [2], Mx. Madison R. Salvato [1], Mx. Emily D. Shaw [1], Mx. Anna J. Barget [1], Ms. Daniela Vilasboas-Campos [3], Mx. Minahil Raheel [1], Dr. Jason Rech [2], Prof. Patrícia Maciel [4], Dr. Andreia Teixeira-Castro [4], Dr. Andrew White [2], Dr. Maria do Carmo Costa [1]

1. *Department of Neurology, Michigan Medicine, University of Michigan,* ***2.*** *College of Pharmacy, Michigan Medicine, University of Michigan,* ***3.*** *Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus de Gualtar, Braga, Portugal; ICVS/3B's - PT Government Associate Laboratory, Braga, Guimarães, Portugal.,* ***4.*** *Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal*

Background: Disease-modifying therapies are lacking for fatal Machado-Joseph disease (MJD)/Spinocerebellar ataxia type 3 (SCA3). Our long-term goal is to identify small molecules that are effective to reduce the abundance of toxic ATXN3 protein in brains of MJD patients/carriers and hopefully alleviate disease progression. We identified aripiprazole as a drug that reduces the amount of human mutant AXN3 in brains of MJD/SCA3 mice. Here, we aimed to develop novel compounds structurally related with aripiprazole that are more potent to decrease the abundance of mutant ATXN3 in neuronal cells.

Methods: To develop novel therapeutic compounds for MJD/SCA3, we carried out structure activity relationship studies building on the chemical structure of aripiprazole. We tested 36 commercially available aripiprazole-related molecules in a human MJD/SCA3 cell line and found two molecules more potent than aripiprazole. Next, we synthesized six compounds and discovered that two compounds are more potent than aripiprazole to selectively decrease levels of mutant ATXN3 in human MJD/SCA3 midbrain neurons. We further found that one of these molecules specifically decreases oligomeric/aggregated ATXN3 species in brains of MJD/SCA3 mice and reduces the number and size of ATXN3-aggregates and improves locomotion defects in MJD/SCA3 *C. elegans*.

Results and Discussion: Two novel molecules showed increased potency in reducing levels of ATXN3 in MJD/SCA3 neurons comparing with aripiprazole. One of these molecules shows *in vivo* efficacy in reducing brain ATXN3 aggregation and mitigating locomotion defects.

Conclusion: A novel aripiprazole-related compound may show therapeutic potential for MJD/SCA3, and potentially other SCAs or neurodegenerative proteinopathies.

CAG-trageted bivalent-RNAi-based strategy to lower mutant polyQ ATXN3 in SCA3

Wednesday, 13th November - 18:00: (Minories) - Poster

Prof. Maciej Figiel [1], ***Mrs. Żaneta Kalinowska*** [1], ***Dr. Ewelina Jesion*** [1]

1. *Institute of Bioorganic Chemistry Polish Academy of Sciences*

Background: In spinocerebellar ataxias (SCAs) 1-3, 6,7 & 17, the expanded CAG repeat in the mutant causative genes encode toxic proteins containing long polyglutamine (polyQ) tracts, leading to multiple pathogenic consequences. Therefore, targeted suppression of the mutant proteins is the most direct and promising strategy for developing effective therapies. The most current strategies include targeting gene transcript with oligonucleotide (ON)-based molecules, which form a duplex with their target RNA to prevent translation through diverse cellular mechanisms. Previously, efficient and long-term downregulation was obtained for huntingtin using unique chemically modified siRNAs delivered to cells by a divalent scaffold. **Methods:** We explored the divalent approach for therapeutic CAG targeted strategy *in vivo* to test the lowering of ATXN3 proteins and the possible allele-selective effect by designing CAG-directed siRNAs containing chemical modifications and fluorescent labeling with Cy3. We tested the bivalent formulation for its relative potency in entering the cells on Amnis imaging FACS and by imaging the slices prepared from the injected brains. We used a single injection into the cerebrospinal fluid or intraparenchymal injection into the striatum of CAG-targeted divalent reagents in the brain of humanized SCA3 (Ki150Q/21Q) mouse models with mutant 150 CAGs and normal 21 CAGs allele. **Results:** We demonstrated that human fibroblasts effectively take up the bivalent CAG-targeted reagents after 1h of incubation. We also demonstrated that the cells throughout the brain, after injection of reagents into CSF, and the cells in the striatal parenchyma, effectively take up the reagent. We obtained a lowering of mutant and normal ATXN3 protein levels in the striatum of Ki150Q/21Q brain after injections of regents into the cerebrospinal fluid or intraparenchymal. Conclusions and discussion: Our CAG-targeted bivalent reagents can lower the mutant polyQ protein ATXN3 level with large 150 polyQ; however, the lowering also occurred for ATXN3 protein containing 21 polyQ domain.

Assessing the therapeutic effect of the multimodal antidepressant vortioxetine in a mouse model of spinocerebellar ataxia type 3

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Stéphanie Oliveira [1]***, Ms. Patrícia Araújo*** [2]***, Ms. Cármen Vieira*** [2]***, Ms. Daniela Monteiro-Fernandes*** [2]***, Ms. Sara Guerreiro*** [2]***, Mr. Jorge Humberto Fernandes*** [1]***, Dr. Sara Duarte-Silva*** [2]***, Prof. Patrícia Maciel*** [2]***, Dr. Andreia Teixeira-Castro*** [2]

1. *Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus de Gualtar, Braga, Portugal; ICVS/3B's - PT Government Associate Laboratory, Braga, Guimarães, Portugal.,* ***2.*** *Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal*

Background and objectives: Spinocerebellar ataxia 3 (SCA3) is the most common dominantly inherited form of ataxia with no available disease-modifying treatment. Previously, we demonstrated that the modulation of serotonergic signaling, through the selective serotonin (5-HT) reuptake inhibitor citalopram, strikingly improved motor function and suppressed ATXN3 aggregation in SCA3 *C. elegans* and mouse models. Here, we aimed at testing the safe and clinically approved multimodal antidepressant vortioxetine (VORT) as a novel therapy to mitigate SCA3. This multitarget drug can potentially further enhance serotonergic signaling in the brain. The inhibition of the 5-HT transporter SERT, together with the antagonism of 5-HT_3 receptor potentiates the increase in extracellular 5-HT produced by SERT blockade. This in combination with the activation of 5-HT_{1A} hetero-receptors and desensitization of autoreceptors responsible for the negative feedback of 5-HT production would potentially show additional benefit to SCA3.

Methods: SCA3 male and female mice were treated chronically with VORT, through food supplementation (10 mg/kg/day). Pre-symptomatic treatment started at 5 weeks of age and lasted 27 weeks. Health status, neurological signs, and motor behavior performance were tested throughout the experiment.

Results: VORT was well tolerated. No differences in food consumption were observed between SCA3 vehicle- and VORT-treated male and female mice. Treatment led to an increase in body weight, but only in wild-type animals. Regarding motor behavior, VORT did not impact on motor balance and coordination of SCA3 male mice, only leading to transient improvements in clasping evaluation. Instead, SCA3 female mice transiently improved motor balance performance upon treatment. Impact of VORT treatment in SCA3 mice neuropathological hallmarks is currently being assessed.

Discussion and conclusions: Overall, these findings showed a restricted impact of VORT treatment in the motor behavior of SCA3 mice, suggesting no increased beneficial effects of SERT and 5-HT receptors simultaneous modulation at least in the conditions tested.

Targeting the GAA expansion using cell-penetrating peptides to deliver CTT oligonucleotides

Wednesday, 13th November - 18:00: (Minories) - Poster

Mr. Juan Alberto López García [1], ***Dr. Macarena Sanchez*** [2]

1. *1. Instituto de Parasitología y Biomedicina López-Neyra, IPBLN – CSIC, Granada, Spain,* ***2.*** *Instituto de Parasitología y Biomedicina López-Neyra, IPBLN-CSIC, Granada, Spain.*

Background and objectives. Friedreich Ataxia (AF) is an autosomal recessive neurodegenerative pathology caused by an expansion of the GAA triplet in *FXN* gene, leading to reduced levels of the protein. The use of several types of oligonucleotides $(CTT)_n$ targeting the GAA expansion has been proposed to reverse *FXN* gene silencing. To enable these oligonucleotides (ONs) to cross the blood-brain barrier (BBB) for effective delivery, we propose their conjugation to cell-penetrating peptides (CPPs).

Methods. The design and synthesis of the different ONs includes the incorporation of locked nucleic acids (LNA) and phosphorodiamidate morpholino oligo (PMO) monomers and other chemical modifications to increase their stability as phosphorothioate linkage (PS). All the ONs incorporate an azide group at the 3'-position. The synthesis of the proposed peptides has been carried out manually by solid-phase peptide synthesis. All peptides include an alkyne group that will allow the incorporation of the chosen ON. Therefore, conjugation is carried out by Cu(I)-catalyzed 1,3-dipolar cycloaddition (CuAAC) between the azide incorporated in the oligos and the alkyne incorporated in the peptides. All conjugates are characterized by HPLC, MALDI-TOF and UREA-PAGE.

Results. To date, work has focused on optimization of the conditions for reactions between oligonucleotides and peptides, achieving positive results in reactions with LNA oligos and PMOs. However, the conjugation with LNA oligos containing PS linkages has required further optimization to prevent possible desulfurization that we are dealing with.

Discussion and Conclusion. We have demonstrated that CuAAC is a suitable method to obtain oligonucleotide-peptide conjugates, although in some cases we need an optimization of the chemical process to scale up the reactions.

Funding sources. This work is part of the project ProyExcel_00365, funded by the Junta de Andalucia; 'Proyectos de I+D+i', oriented to the Challenges of the Andalusian Society (2021), and the patient associations FEDAES and ASOGAF.

Establishment of a Novel Assay to identify drugs that enhance protein homeostasis: towards treatment of spinocerebellar ataxias

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Daniela Vilasboas-Campos [1], Mr. Jorge Humberto Fernandes [1], Dr. Marta Daniela Costa [1], Dr. Joana Pereira-Sousa [2], Ms. Joana Lopes [1], Ms. Liliana Meireles-Costa [1], Ms. Bruna Ferreira-Lomba [1], Dr. Jorge Diogo Da Silva [1], Mr. Fábio Conceição [3], Dr. Marta Costa [1], Prof. Fernanda Proença [3], Prof. Patrícia Maciel [1], Dr. Andreia Teixeira-Castro [1]

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Background/Objectives: The accumulation of misfolded proteins into aggregates causes cellular toxicity, known as proteotoxicity, leading to long-term detrimental effects and neurodegeneration. This study addresses the lack of effective therapeutic strategies for neurodegenerative diseases (NDs) associated with proteotoxicity, such as Spinocerebellar Ataxias (SCAs). We aim to identify molecules with proteostasis-enhancing activity that could serve as broad-spectrum neuroprotective drugs. To achieve this, we conducted a large-scale drug screening using the nematode Caenorhabditis elegans. This allows a cost and time-efficient screening of small-molecules while providing valuable biological and pharmacological insights.

Methods: We established an automated assay to screen for molecules that enhance cellular protein folding and homeostasis capacity, thereby reducing proteotoxic stress at the whole organism level. We used motor activity, a nervous system-dependent function, following a proteome-disrupting heat-shock (HS) stimulus, as a readout for this assay. Our protocol involved exposing wild-type animals to different durations of HS at 37ºC, followed by automated measurement of their motor activity.

Results and Discussion: We found that an HS duration of 60 minutes was the most effective in allowing potential treatment effects to be detected, as it caused a significant yet recoverable loss of movement, with potential for recovery using previously known proteostasis-enhancing drugs. Using this newly established protocol, we validated the pharmacologic and genetic modulation of serotonergic signaling and mTOR inhibition as strategies that counteract HS-mediated proteotoxic damage, with activity in models of SCAs. Currently, we are screening a library of novel compounds, mainly with imidazole and chromene-based structures. So far, we tested approximately 400 compounds and found that 19 significantly reduced the recovery time to half of the animal's final activity. These hit compounds are being tested in models of SCA3.

Conclusion: This novel assay will identify molecules preventing aging and disease-associated deterioration of proteostasis, providing new drug candidates for treating several SCAs.

Engineering ARMMs with Engagers to Direct Biodistribution to Specific Neurons as a Therapeutic Strategy for Friedreich Ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Wendy Zhao [1], Ms. Zahra Vahramian [1], Mr. Brandon Gerhart [2], Dr. Mike Thomas [1], Dr. Shu-lin Liu [1], Ms. Jennifer Badji [1], Mr. Kevin Le [1], Dr. Silvia Piccinotti [1], Dr. Qin Yu [1], Dr. Jill Napierala [2], Dr. Joseph Nabhan [1]

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The pathological insufficiency of Frataxin (FXN) in Friedreich's ataxia (FA) is caused by bi-allelic expansion of an intronic trinucleotide sequence, GAA, in the *FXN* gene. The expanded repeat region drives formation of heterochromatin and limits potentiation of transcription and access to the transcription start sequence. Consequently, FXN expression in patient cells is reduced by >70% compared to healthy non-carrier levels. FA is a multi-system disease that primarily manifests in sensory proprioceptive neurons and cardiomyocytes. Various strategies such as gene replacement with AAV or FXN protein or mRNA supplementation to treat the loss of proprioceptive functions in FA have encountered significant challenges for therapeutic development.

Methods

Vesigen has been developing a novel approach to potentially treat the major component of FA pathology. We explored the use of human ARMMs (ARRDC1-Mediated Microvesicles) as a non-viral and proprioceptor-targeted delivery vehicle for Cas9/gRNA protein complexes to excise the pathogenic repeat expansion in *FXN* and to consequently activate FXN expression.

Results and Discussion

We screened and identified a pair of gRNAs that can efficiently excise the GAA repeat-containing region and demonstrated that ARMMs-mediated delivery of Cas9 and 2 gRNAs leads to successful excision of the GAA repeat containing region in a) a cell line containing the non-pathogenic expansion, b) FA iPSC-derived neurons containing bi-allelic pathogenic expansions, and c) primary neurons from the YG8sR mouse model. Additionally, we developed a strategy, currently under evaluation *in vivo*, to target ARMMs to proprioceptive neurons in the dorsal root ganglia.

Conclusion

Our non-viral approach provides the foundational evidence for a disease-modifying strategy for FA that could potentially significantly improve the quality of life across the FA patient population.

Spliceosome‐mediated RNA trans-splicing prevents the pathogenic phosphorylation of ataxin-1

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Lotte Schoen [1], Dr. Leanne Jones [2], Prof. Maria Elena Avala [3], Dr. Spyros Petrakis [4], Dr. Jean-Marc Gallo [5], Dr. Ronald Buijsen [1], Prof. Karen Anthony [2]

1. *Leiden University Medical Center,* ***2.*** *University of Northampton,* ***3.*** *Instituto de Investigaciones en Ingeniería Genética y Biología Molecular,* ***4.*** *Institute of Applied Biosciences (INAB), Centre for Research and Technology Hellas (CERTH), Thessaloniki, Greece,* ***5.*** *King's College London*

Background and objectives

Spinocerebellar ataxia type 1 (SCA1) is caused by an expanded CAG repeat in the *ATXN1* gene, resulting in an elongated polyglutamine (PolyQ) tract in the ataxin-1 protein. The exact pathogenic mechanism is not understood but phosphorylation of ataxin-1 at S776 is critical for the stabilisation and neurotoxicity of polyQ-expanded ataxin-1. Our objective is to evaluate the therapeutic potential of preventing pathogenic phosphorylation of ataxin-1 using spliceosome-mediated RNA *trans*-splicing (SMaRT).

Methods

SMaRT creates a hybrid mRNA through a *trans*-splicing reaction between an endogenous target pre-mRNA and an exogenously delivered RNA trans-splicing molecule (RTM). We designed and constructed a FLAG-tagged RTM to substitute S776 for alanine. SMaRT was performed via lentiviral transduction in SH-SY5Y cells stably expressing mutant YFP-ataxin-1(82Q) and patient iPSC-derived neuronal cultures. *Trans*-splicing and S776 phosphorylation were analysed by a combination of RT-PCR, qPCR, sanger sequencing, immunoprecipitation and western blotting.

Results

SMaRT successfully edited the substitution from S776 to A776 in SH-SY5Y cells and patient-derived neuronal cultures at both the RNA and protein level. A dose dependent effect was observed with increasing lentiviral concentrations. Importantly, a significant reduction (37%) in S776 phosphorylation was observed as well as a significant reduction in the intensity of nuclear ataxin-1 aggregates in YFP-ataxin-1(82Q) SH-SY5Y cells.

Discussion and conclusion

These results demonstrate the potential of SMaRT to prevent a pathogenic phosphorylation event and provides proof-of-concept for *in-vivo* pre-clinical development. Here, we demonstrate utility of SMaRT for a 3' exon replacement. However, SMaRT strategies can be designed to replace 5', internal or 3' regions of an mRNA making this is an attractive versatile approach for genetic diseases.

Funding sources: Ataxia UK

Metformin decreases RAN proteins and improves behavioral phenotypes in SCA8 BAC mice.

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Lisa E.L. Romano [1], ***Dr. Setsuki Tsukagoshi*** [2], ***Ms. Tala Ortiz*** [2], ***Ms. Camille Preston*** [2], ***Ms. Duo Chen*** [2], ***Ms. Elaine Ames*** [2], ***Dr. Tao Zu*** [2], ***Dr. Monica Banez Coronel*** [2], ***Dr. Laura Ranum*** [1]

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[Background and Objectives] CTG•CAG expansion mutations cause a number of neurodegenerative and neuromuscular diseases, including myotonic dystrophy type1 (DM1) and multiple forms spinocerebellar ataxia (SCA). In SCA8, the expansion mutation is located in the overlapping *ATXN8/ATXN8OS* genes. Repeat-associated non-AUG (RAN) translation, which has been reported in 11 diseases including SCA8, is highly regulated by the double-stranded RNA-dependent protein kinase (PKR) pathway. RAN proteins are substantially reduced by metformin, an FDA-approved drug and novel PKR inhibitor.

[Methods] Immunohistochemistry was used to test whether all six predicted RAN proteins accumulate in SCA8 human autopsy brains. SCA8-BAC transgenic mice, which express ATXN8/ATXN8OS using the endogenous human promoters, were used to test the effects of metformin on RAN protein reduction, behavioral phenotypes (DigiGait, rotarod, open field), and histopathological phenotypes.

[Results] IHC shows that sense (polySerine, polyGlutamine, polyAlanine) and antisense (polyLeucine, polyCysteine, polyAlanine) RAN proteins accumulate in cerebellum from SCA8 (n>5) but not control (n>5)) autopsy cases. Metformin-treated SCA8 mice showed improved rotarod (n>15/group, p=0.0017) and DigiGait (brake, n>15/group, p=0.002) performance compared to untreated SCA8 mice and non-transgenic littermates. IHC studies showed a dramatic reduction of RAN proteins and neuroinflammatory markers (GFAP and Iba-1).

[Conclusion and Discussion] Most therapeutic strategies for repeat expansion disorders focus on targeting only the sense transcripts, neglecting the potential contribution of the antisense transcript and antisense RAN proteins, which are now known to accumulate across the polyGln SCAs (see Banez et al., abstract). These data show metformin reduces RAN protein load and improves behavior in SCA8 mice. We are currently analyzing additional histopathological and molecular phenotypes. Metformin is an FDA-approved drug, able to target sense and antisense RAN proteins. If effective, it could be rapidly moved into clinical trials to test its efficacy as a safe and affordable treatment for patients with this devastating disorder.

Leriglitazone as a new therapeutic strategy for the treatment of Friedreich Ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

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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 (FXN) gene that results in reduced levels of FXN, a mitochondrial protein. FA is considered a multisystem disorder affecting several organs, particularly the nervous system and heart.

Objectives: Although the first drug to treat FA has been approved very recently, its effects on the evolution of symptoms are limited; therefore, the study of other treatments is essential. Our goal is to analyze the effect of leriglitazone, a peroxisome proliferator-activated receptor gamma agonist, which has reached the end of phase II (human safety and efficacy trial).

Methods: We employed two models: (i) cultures of fibroblasts derived from patients and (ii) cultures of dorsal root ganglia (DRG) neurons from neonatal rats with reduced FXN levels by lentivirus transduction.

Results and Discussion: FA patient-derived fibroblasts and rat DRG neurons showed an accumulation of mitochondrial iron and superoxide levels, which were reverted by leriglitazone treatment. This increase in mitochondrial iron and superoxide levels leads to lipid peroxidation and a decrease in the GSH/GSSG ratio, both markers of ferroptosis, which are also ameliorated by the treatment. Moreover, in these models, we found a decrease in respiration, but with the treatment, the maximal respiration of FXN-deficient cells achieved the levels of the control cells. Surprisingly, we found a discrepancy between our two models regarding Nrf2. While Nrf2 levels increased in patient' fibroblast, they decreased in frataxin-deficient DRG neurons, compared to their respective controls. In both cases, leriglitazone treatment increased Nrf2 levels.

Conclusion: Leriglitazone might have the potential to be used as a new drug to treat FA.

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DNA targeting ONs increase frataxin expression and prevent GAA•TTC repeat expansions.

Wednesday, 13th November - 18:00: (Minories) - Poster

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Friedreich's ataxia (FRDA) is a progressive, autosomal recessive disorder caused by homozygous expansion of GAA•TTC repeats in the first intron of *Frataxin* (*FXN)* gene. This expansion leads to non-B-DNA triplex formation and reduced frataxin expression, resulting in disease. This study aimed to evaluate the effect of oligomers in modulating frataxin expression and preventing GAA•TTC expansions. We designed and evaluated the effect of single-strand anti-gene oligonucleotides (AGOs) targeting non-B-DNA structures at expanded GAA•TTC repeats as a therapeutic strategy.

We used FRDA-patient derived primary fibroblasts with varying GAA•TTC repeat lengths, treated with lipofectamine-mediated transfection or gymnotic delivery . *Frataxin* mRNA and protein levels were measured via RT-qPCR and western blotting. A shuttle plasmid system replicable in human cells was used to study large-scale repeat expansions in mammalian cells.

GAA AGOs significantly enhanced FXN expression in the tested cell lines. Oligonucleotide length and LNA content determined AGO efficiency in upregulating FXN mRNA and protein expression, with longer AGOs more effective at lower doses. Moreover in our experimental model system, we observed GAA•TTC repeat expansions, marking our system the first genetically tractable system for studying such expansions in human cells. LNA-DNA mixmer oligonucleotides and peptide nucleic acids (PNAs) interfered with triplex formation, preventing repeat expansions in human cells. These suggest triplex formation by GAA•TTC repeats stalls replication fork progression, leading to expansions during fork restart.

Our study demonstrates that AGOs targeting non-B-DNA structures at *FXN*-expanded repeats can upregulate FXN expression and prevent expansions. This highlights AGOs' potential as therapeutic approach for FRDA contrasting with RNA-targeting antisense oligonucleotides. Our system also elucidates the mechanism of GAA•TTC repeat expansions, providing a model for future research.

This work was supported by Hjärnfonden, Swedish Research Council, Swelife-Vinnova, CIMED, Region Stockholm, EU Horizon 2020, National Institute of General Medical Sciences, and NovoNordisk.

Novel SMART AAV Vectors for Friedreich ataxia Gene Therapy

Wednesday, 13th November - 18:00: (Minories) - Poster

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Objectives: Gene therapy is currently at the forefront as a potential approach to successfully treat Friedreich ataxia (FA). However, a major issue is to identify the most suitable viral vector that is safe and can specifically transduce diseased cells to deliver the therapeutic gene, Frataxin. Here we describe the use of organoids/cells derivatives from FA patient-derived induced pluripotent stem cells (iPSC) for high-throughput functional screening of a large cohort of existing AAV variants to identify safe 'SMART' viral vectors that will efficiently deliver Frataxin to disease-relevant tissues while avoid delivery to liver.

Methods: The AAV Testing kit, consisting of >64 barcoded AAV variants (including both natural and bioengineered variants), was screened in sensory neurons, cardiomyocytes and cerebellar organoids derived from three different FA iPSC lines. Ten AAV variants were selected from the initial screen and further validated in the stem cell platforms as well as in human tissue-derived liver organoids. Evaluations for each variant were assessed at the DNA (cell entry) and RNA (transgene expression) levels.

Results: Of the top ten selected AAV candidates, at least two AAV variants showed efficient and selective targeting of disease-relevant cell types, including sensory neurons and cardiomyocytes, with lower transduction efficiency in liver cells. These top AAV are currently being assessed for Frataxin delivery to FA cells.

Discussion: To our knowledge this is the first time such comprehensive analyses are performed using stem cell-based platforms for FA gene therapy. Our data shows distinct and variable transduction efficiencies of AAV variants across different cell types, which highlights the value of using in vitro models to fast track screening processes.

Conclusion: These studies are highly promising for advancing FA gene therapy, particularly for targeting FA-affected tissues.

Funding: Medical Research Future Fund Stem Cells Therapies Mission, FARA USA and FARA Australia.

Omaveloxolone: A Potential Novel Therapeutic Approach in DRPLA.

Wednesday, 13th November - 18:00: (Minories) - Poster

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Dentatorubropallidoluysian atrophy (DRPLA) is a rare, autosomal dominant progressive neurodegenerative disease characterised by cerebellar ataxia, epilepsy, myoclonus, chorea, dementia, and psychiatric manifestations. DRPLA is caused by a variant in the *ATN1* gene (which normally encodes for a protein named atrophin 1). The mutation involves a DNA segment known as CAG trinucleotide repeat of at least 48 repeats that changes the structure of the atrophin 1, generating the so-called polyQ tracts, which interfere with normal cell functions. The altered protein accumulates in neurons, forming inclusions that increase oxidative stress and mitochondrial dysfunction and ultimately cause neuronal death. At present, there is no cure for this disorder.

Our study aimed to clarify the physiopathology in DRPLA cellular models and validate the efficacy of a small molecule named Omaveloxolone (Omav) as a potential pharmacological approach. Omav activates the cell's antioxidant pathway: the nuclear factor erythroid 2-related factor 2 (Nrf-2) pathway.

We have previously shown that Omav protects cells from oxidative stress and mitochondrial dysfunction in another form of ataxia (Friedreich's Ataxia), which Omav is now the first approved treatment.

We have used live imaging techniques to assess the levels of oxidative stress and mitochondrial dysfunction in the SH-SY5Y neuroblastoma cell line (transiently transfected with pcDNA-Atrophin-1 65Q [as disease model] and related control) and DRPLA patients' fibroblasts under Omav treatments. Immunohistochemistry and Immunoblot techniques were used to verify the formation of inclusions in our models and the activation of the Nrf2 pathway.

Our results show that DRPLA cellular models have increased oxidative stress and mitochondrial dysfunction, but this can be reverted by Omav administration. We have also seen the reduction of polyQ inclusions and the activation of the Nrf2 pathway.

Our approach aims not to resolve the disease's mutation but to tackle the disease's pathophysiology by restoring cellular function and counteracting neuronal death.

Butyrate Mitigates Inflammation and Metabolic Dysregulation in Friedreich's Ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

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Background and Objectives. Friedreich's ataxia (FRDA) is an autosomal recessive spinocerebellar ataxia caused by mutations in the frataxin (FXN) gene, leading to progressive neurodegeneration. Neuroinflammation and diabetes are common aspects of the disease, contributing to its pathology. This study investigates the anti-inflammatory and neuroprotective effects of butyrate (BUT) in FRDA, focusing on its potential therapeutic role.

Methods. Using the KIKO mouse model of FRDA and wild type (WT) mice, we evaluated the effects of BUT on inflammation and oxidative stress in the cerebellum and visceral white adipose tissue (vWAT). Mice were treated with BUT for four months. A series of omic analyses followed by qPCR, Western blot, and cytofluorimetric analyses validations were carried out. The effects of BUT were also investigated at the systemic level by evaluating possible anti-diabetic effects via monitoring glycaemia and lipidemia. In parallel, we utilized in vitro models where FXN was downregulated in 3T3-L1 adipocytes and BV2 microglial cells via RNA interference.

Results. In the KIKO mouse model, BUT treatment significantly reduced pro-inflammatory cytokines and oxidative stress markers in both the cerebellum and vWAT and was able to lower insulin resistance and cholesterol levels. These findings were corroborated in vitro, where FXN-deficient adipocytes and microglial cells displayed heightened susceptibility to inflammation and lowered glucose uptake, which BUT treatment effectively mitigated.

Discussion and Conclusion. Our findings demonstrate that BUT exerts substantial anti-inflammatory, neuroprotective, and anti-diabetic effects in both in vivo and in vitro models of FRDA. These results suggest that BUT holds promise as an adjuvant therapy to alleviate symptoms and manage the complex pathology of Friedreich's ataxia. Further investigation into BUT's role in FRDA treatment is warranted.

Target identification with NGS for a personalized, allele-specific treatment strategy in SCA3

Wednesday, 13th November - 18:00: (Minories) - Poster

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Background: Spinocerebellar ataxia type 3 (SCA3) / Machado-Joseph disease (MJD) is an inherited neurodegenerative disease caused by CAG repeat expansion within a coding region of *ATXN3*. While the pathogen- associated protein has an increased tendency to aggregate, the wild-type protein fulfills essential functions as a deubiquitinase. Antisense oligonucleotides (ASOs) would be able to specifically target disease-associated variants of the mutant (pre-)mRNA and thus reduce the toxic protein concentration while preserving the wild-type protein.

Methods: 79 SCA3 patients from three cohorts (Germany, UK, Portugal) were sequenced using allele-specific targeted multiplexed Oxford Nanopore technology (ONT).

Results: Pairwise linkage disequilibrium (LD) statistics identified more than 200 potential targets for an allele-specific treatment approach. More than 75% of all patients across all three cohorts could be considered for an allele-specific treatment approach if variants from different haplotype blocks were targeted in an allele-specific manner. As a proof-of-principle study, we have shown that SNPs from different blocks can effectively be targeted with gapmer ASOs and that the mutant protein can be specifically reduced by up to 70% in SCA3 iPSC-derived cortical neurons with a single ASO application.

Discussion & Conclusion: These results show that LD plots are a useful tool to identify and increase the number of potential targets for an allele-specific treatment approach and that allele-specific lowering targeting different variants of the same allele may be a reasonable approach to enable allele-specific treatment for as many patients as possible feasible.

Funding: Multiplexed, targeted allele-specific sequencing with ONT was kindly supported by “Ataxia UK”. We also thank the “Deutsche Heredo-Ataxie-Gesellschaft (DHAG)” for supporting our pilot study with allele-specific ASOs.

Preclinical Efficacy, Biodistribution, and Safety of ASP2016 AAV Gene Therapy in a Mouse Model of Cardiomyopathy in Friedreich Ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

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Background and Objectives: Friedreich ataxia (FA) is a monogenic disease caused by frataxin (FXN) protein deficiency leading to progressive neurodegeneration, and in most patients, severe cardiomyopathy.
Methods Objectives: ASP2016 is an intravenously delivered, rAAV8-based vector expressing the *FXN* gene under the PGK promoter (rAAV8-hPGK-hFXN). Efficacy, biodistribution, and safety of ASP2016 for treating FA-associated cardiomyopathy were evaluated in a conditional knockout (KO) mouse model of FA at dose levels of 1x, 3x, 10x, 30x, and 100x.
Results: Administration of ASP2016 to symptomatic KO mice resulted in dose-dependent improvements in early mortality and ejection fraction (EF) compared with vehicle-treated KO mice. At 4 weeks post-dose the mean EF decreased from 51% to 10% in vehicle-treated KO mice, while vehicle-treated WT mice maintained at 66%. Following ASP2016 treatment the mean EF increased to 26% at the 1x dose, 44% at the 3x dose, and plateaued at 57% for doses ≥ 10x. Cardiac injury biomarkers myosin light chain and cardiac troponin were also reduced, as well as histopathologic myocardial lesions. Transgene DNA and human frataxin expression were dose-dependently detected in the hearts of ASP2016-treated KO mice. At 12 weeks post-dose, KO mice that received the two highest doses had slight to minimal axonal degeneration in the sciatic nerve (30x, 3/10; 100x, 10/10), with one showing signs of neurodegeneration in the dorsal root ganglion. These histological changes did not have clinical manifestations. Additionally, minimal Kupffer cell hypertrophy and hepatocyte necrosis were observed in livers of ASP2016-treated KO mice but were considered non-adverse given the low incidence and severity. There were no significant differences in serum ALT/AST levels between ASP2016-treated KO mice versus vehicle-treated WT mice at 4 weeks post-dose.
Discussion and Conclusion: Overall, there was dose-dependent efficacy in ASP2016-treated KO mice. These data support the investigation of AAV-based gene replacement therapy for cardiomyopathy in FA.

Investigating the effects of butyrate for the treatment of Machado Joseph Disease

Wednesday, 13th November - 18:00: (Minories) - Poster

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Machado Joseph disease (MJD), interchangeably known as spinocerebellar ataxia type 3 (SCA3), is the most common dominantly inherited SCA in the world. Countries with high prevalence of MJD include Brazil, Portugal, and Aboriginal communities located in the northeast Arnhem land of Australia. MJD is characterised by the progressive loss of movement, dysarthria, dystonia, rigidity, oculomotor abnormalities, leading to a wheelchair dependent life and ultimately, death. MJD is caused by inheritance of an ATXN3 gene with increased length of the trinucleotide (CAG) repeat, translating into a long chain of glutamine residues (polyQ tract) within the ataxin-3 protein. Usually ataxin-3 acts as a deubiquitinating enzyme essential for degrading faulty proteins, however mutant ataxin-3 has an intrinsic property to misfold, oligomerise and form aggregates. In MJD, over accumulation of these aggregates puts chronic pressure on neurones, leading to neuronal loss and disease development. Currently, MJD has no cure. Emerging evidence, including from our team, have found that treatment with sodium butyrate can have protective effects in experimental models such as the transgenic MJD zebrafish. Butyrate is a natural metabolite produced by the microbial fermentation of undigested carbohydrate in the colon. Functions of butyrate include maintenance of intestinal barrier integrity, inhibit pro-inflammatory cytokines, energy source to colonocytes, autophagy inducer, and HDAC inhibitor. This study has compared the effect of eight different butyrate producing treatment candidates on the amount of butyrate present within plasma, feces, and brain tissue of treated mice, through the use of gas-chromatography mass spectrometry (GCMS). Findings from this study will allow selection of the best candidate to investigate as a potential treatment for MJD.

Targeting Frataxin Dysfunction in Friedreich Ataxia: Nanobody-Based Approaches for Fe-S Cluster Biogenesis Enhancement”

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Maria Florencia Pignataro [1], ***Dr. Natalia Fernández*** [2], ***Ms. Maria Florencia Paván*** [3], ***Mr. Julián Grossi*** [4], ***Dr. Martín Noguera*** [4], ***Ms. Alba Garay*** [5], ***Dr. Rafael Molina*** [6], ***Ms. Antonella Vila*** [4], ***Ms. Naira Rodríguez*** [4], ***Dr. Hernán Gentili*** [4], ***Dr. Juan Antonio Hermoso*** [5], ***Dr. Lorena Itatí Ibañez*** [7], ***Dr. Javier Santos*** [8]

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In Friedreich Ataxia (FA), reduced frataxin (FXN) protein levels lead to cellular dysfunction, primarily by impairing Iron-sulfur (Fe-S) cluster production via the ISC machinery. FXN is the kinetic activator of the NFS1-ISCU-ISD11-ACP complex, crucial for efficient Fe-S cluster biogenesis. In this work, we propose the quaternary addition of llama nanobodies (NB) targeting FXN in order to improve FXN conformational stability and function in cellular models.

After llama immunization, we obtained NB libraries, from which anti-FXN NBs were selected by phage display. Interaction was assessed using SEC-FLPC, BLI and NMR. NB candidates were transfected in HEK-293T cells. ISC-NBs interaction was evaluated by co-immunoprecipitation assays and by measuring the effect over Fe-S related activities. Trojan versions of the NB_4a7 were purified from E. Coli and characterized. We investigated the impact of Trojan NB transduction on mitochondrial metabolism in FRDA fibroblasts through immunofluorescence, mitochondria isolation and OCR evaluation.

NBs showed high levels of expression and mitochondrial localization in HEK-293T and HeLaKyoto cell lines. NB overexpression in HEK-293T cells did not significantly alter cell viability, Fe-S cluster dependent enzymatic activities (aconitase and Succinate dehydrogenase) nor mitochondria oxygen consumption rates. Also, mature FXN levels were not significantly altered. We found that NBs interact with FXN and other ISC proteins in a cellular context. TAT_CAMP_4A7 and TAT_COX8_4a7 could be expressed in E.coli. Interestingly, we found that trojan versions of NBs are able to enter FRDA fibroblasts and modulate Fe-S cluster dependent enzymatic activities.
Our results suggest that NBs can be expressed and interact with endogenous FXN in a human cell line, without altering Fe-S related activities. Importantly, Trojan NBs can be transduced to FRDA cells, localized in the mitochondria and discretely affect Fe-S related activities. These novel tools may enhance our comprehension of these

complex catalytic processes.

FARA, CONICET and University of BuenosAires

Circuit Modulation as a Pharmacomimetic of Cardiovascular Exercise in Spinocerebellar Ataxia.

Wednesday, 13th November - 18:00: (Minories) - Poster

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Background and Objectives: Spinocerebellar Ataxias (SCAs) are dominantly inherited degenerative disorders resulting in dysarthria, impaired coordination, and gait disability. Motor symptoms largely derive from aberrant firing of cerebellar Purkinje neurons (PCs) due to dysfunction in specific ion channels, which represent viable drug targets. To advance target discovery, we looked to the single clinically effective intervention in human SCA patients: robust cardiovascular exercise. Exercise is a mainstay of clinical care and improves motor function, but the mechanisms driving benefits in SCAs remain unclear, and precise delineation may allow pharmacological intervention to 'mimic' exercise in disabled patients.

Methods: We studied *Atxn*$^{154Q/2Q}$ knock-in mice (an SCA1 model) engaged in voluntary cage wheel running alongside WT littermates and sedentary controls. Mice were exercised at different time points for varying periods of time. Effects of intervention were assessed using behavioral phenotyping (open field, balance beam, and rotarod), cerebellar slice electrophysiology, histological evaluation, and transcriptional profiling.

Results: Voluntary cage wheel running was most effective when started at a pre-symptomatic stage, resulting in a complete rescue of motor ataxia. Cerebellar slice recordings showed improved PC firing and transcriptional analysis demonstrated rescue of ion channel expression and splicing defects known to occur in this mouse model. To mimic these effects, we developed a potassium channel activator that rescues motor ataxia in SCA1 mice.

Discussion and Conclusion: In order to uncover novel therapeutic targets, it is crucial that we identify the driving mechanisms of effective clinical measures, such as cardiovascular exercise. Here, we show that exercise may elicit benefits in motor ataxia through correction of dysfunctional cerebellar circuitry. Thus, drugs that modulate ion channel activity may serve as a pharmacomimetic of exercise for these devastating disorders.

Therapeutic efficacy of chronic 5-HT1A receptor agonism by NLX-112 (befiradol) in Spinocerebellar Ataxia Type 3 mice

Wednesday, 13th November - 18:00: (Minories) - Poster

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Spinocerebellar Ataxia Type 3 (SCA3) is caused by an expansion of a polyglutamine tract in the ATXN3 protein, resulting in progressive motor dysfunction. No effective disease-modifying therapy is available, however, recent studies of drugs targeting the serotonergic signaling pathway revealed promising results. NLX-112 (a.k.a. befiradol/F13640), a highly-selective 5-HT_{1A} receptor (5-HT_{1A}R) full agonist, ameliorated motor dysfunction and reduced mutant ATXN3 aggregation in a *Caenorhabditis elegans* model of SCA3. Here, we assessed the therapeutic potential of NLX-112 in SCA3 transgenic mice, using the 5-HT_{1A}R partial agonist tandospirone (TD) as a reference drug.

NLX-112 (0.625 and 5 mg/kg/day) and TD (20 and 80 mg/kg/day) were administered chronically in the drinking water (DW) for 34 weeks prior to disease onset or by twice-daily intraperitoneal injections (NLX-112 only) for 14 weeks starting after disease onset. Motor function tests and standard immunostaining techniques were employed, in addition to the examination of drug exposure and animals' welfare.

The drugs were safe and well tolerated by the mice. Both doses of NLX-112 were detected in plasma and brain samples, whereas TD was only detected at the highest dose in plasma. NLX-112 treatment, initiated prior to, as well as after symptoms onset, led to significant improvement of motor function, not achieved with TD. NLX-112 also elicited neuroprotective effects, reducing dopaminergic cell loss and astrogliosis in the substantia nigra. Interestingly, while NLX-112 exposure diminished the number of pyknotic cells in the spinal cord (SC), it increased nuclear ATXN3 aggregation in the deep cerebellar nuclei, suggesting that the most relevant mutant ATXN3 toxic species might be more soluble and/or smaller than the microscopically visible aggregates (resolution limit of ~0.2mm).

In conclusion, NLX-112 enhanced motor function and reduced neuropathological biomarkers in SCA3 mice, suggesting that full agonist activation of serotonin 5-HT_{1A}R is a promising therapeutic strategy for treatment of SCA3 patients.

Validation of AAV-PHP.eB-mediated CYP46A1 systemic delivery: a promising non-invasive gene therapy for Spinocerebellar Ataxia Type 3

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Beatriz Serra [1], Dr. Sónia Duarte [2], Mr. João Almeida [2], Dr. Sara Lopes [2], Dr. Françoise Piguet [3], Prof. Nathalie Cartier [4], Dr. Sandro Alves [4], Prof. Luis Pereira de Almeida [2], Dr. Rita Perfeito [2]

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Spinocerebellar Ataxia Type 3 (SCA3) or Machado-Joseph disease (MJD) is the most prevalent autosomal dominant SCA in the world leading to severe clinical manifestations and premature death. SCA3 is caused by a CAG-repeat expansion in the MJD1 gene, resulting in an expanded polyQ tract in the coding region of the ataxin-3 protein that gains a toxic function, accumulates in neurons and promotes the formation of insoluble intranuclear inclusions. Deregulation of brain cholesterol homeostasis has been associated with neurodegenerative disorders, including SCA3. We previously showed that intracerebellar injection of AAVs encoding CYP46A1 (the key enzyme involved in brain cholesterol turnover), into MJD mouse models was neuroprotective, reducing mutant ataxin-3 accumulation and alleviating motor impairments associated with the disease.

Here, we aimed to investigate whether systemic delivery of CYP46A1 would relieve the disease phenotype and neuropathology in a transgenic SCA3 mouse model, with established pathology.

Post-symptomatic MJD mice were injected with 5×10^{11}vg of PHPeB-AAV-CYP46A1 through retro-orbital injection. Motor assessment was performed every 3 weeks until 9 weeks post-administration. Animals were sacrificed 10 weeks after treatment and expression of CYP46A1 in the cerebellum, mutant ataxin-3 aggregate levels and neuropathology of treated mice were investigated.

We observed significantly increased levels of cerebellar CYP46A1 in treated animals, indicating that the PHPeB-AAV effectively reached and transduced the brain. Importantly, results revealed an improvement in motor performance 9 weeks post-AAV-PHPeB-CYP46A1 injection along with mutant ataxin-3 aggregates' reduction in treated SCA3 mice. Data obtained by immunofluorescence suggested an increase in calbindin levels with down-regulation of neuroinflammation mediated by astrocytes in the cerebellum of CYP46A1-treated mice. Preliminary data evidenced autophagy activation after CYP46A1 delivery, with altered LC3B-II and P62 markers.

These results corroborate the beneficial role of CYP46A1 and brain cholesterol metabolism in MJD and provide valuable insights to consider non-invasive delivery of genes as a relevant therapeutic approach.

Nanobody-Frataxin Interaction in the Precise Macromolecular Context

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Maria Florencia Pignataro [1], Ms. Alba Garay [2], Dr. Rafael Molina [2], Dr. Juan Antonio Hermoso Domínguez [2], Dr. Martin Aran [3], Dr. Lorena Itatí Ibañez [4], Dr. Javier Santos [1]

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Background and objectives:

Iron-sulfur (Fe-S) clusters are essential cofactors and hundreds of proteins require such cofactors to work. In eukaryotic cells, the biogenesis of most Fe-S clusters occurs in the mitochondria. The process involves the L-Cys desulfurase supercomplex which is kinetically activated by frataxin. We propose modulating the frataxin stability and the supercomplex function through nanobody-frataxin interaction.

Methods:

Nanobodies were selected by phage display, expressed in *E. coli*, and purified. The interaction with frataxin was characterized by chromatography, interferometry, and NMR. The structures of the nanobody-frataxin complexes were resolved by X-ray diffraction.

Results:

The nanobody-frataxin interaction was strong with a slow dissociation equilibrium and high affinity (K_D= 1-30nM). Furthermore, nanobody interaction stabilized the pathogenic frataxin variant G130V, characterized by an increase in Tm value of 15 °C compared to the G130V variant alone. The binding surface of ^{15}N-frataxin in solution was inferred by the identification of residues exhibiting high chemical shift perturbation. Nanobodies 4A7, 6B1, 16C10, and 29F7 bind in a similar frataxin region, comprising loop 1, the b-turn b2-b3, and the C-terminal of helix a2. Nanobody 28F6 binds to an extended region, including residues of the helix a1 of frataxin. X-ray diffraction of the complexes showed that nanobodies 4A7, 6B1, and 16C10 bind to frataxin in a similar fashion, whereas the nanobody 29F7 interacts in a different way. We mapped the nanobody-frataxin complexes on the structure of the L-Cys-desulfurase supercomplex, showing only a slight clash for the case of nanobodies 4A7, 6B1, and 16C10.

Discussion and conclusion:

The conformational analysis suggests that some nanobodies may bind to frataxin without significantly altering the supercomplex topology. Thus, they may bind to frataxin and stabilize its functional conformation in the structural context.

Funding sources:

Friedreich's Ataxia Research Alliance. CONICET. UBA.

Serotonin transporter as a modifier of proteostasis in SCA3: insights from C. elegans

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Joana Pereira-Sousa [1], Dr. Stéphanie Oliveira [2], Mr. Jorge Humberto Fernandes [2], Dr. Jian Li [3], Prof. Patrícia Maciel [4], Dr. Andreia Teixeira-Castro [4]

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Introduction and objective: Spinocerebellar Ataxia type 3 (SCA3) or Machado-Joseph disease is a polyglutamine disease for which no treatment is available. We previously showed that treatment with citalopram, a serotonin transporter (SERT) inhibitor that blocks serotonin reuptake, attenuates core deficits of SCA3 animal models, including motor behavior, and neuropathology. Ablation of the SERT ortholog in a *Caenorhabditis elegans* (*C. elegans*) model of mutant ATXN3 proteotoxicity, *mod-5,* strikingly rescued motor impairments and suppressed mutant ATXN3 aggregation, mimicking drug action. Here, we aimed at exploring the molecular determinants underlying these findings.
Methods: to further investigate the mode-of-action of serotonin signaling modulation on the suppression of SCA3 pathogenesis, we performed a transcriptomic analysis, by RNA-seq, in mutant ATXN3-expressing animals in the background of *mod-5* ablation in *C. elegans*.
Results: we identified 998 differentially expressed genes (DEG) in four conditions tested [wild-type (WT), *mod-5*, mutant ATXN3 (AT3q130) and double mutant (*mod-5*; AT3q130)], 409 of which were exclusively present in the disease context (WT vs mutant ATXN3) and disease modification (mutant ATXN3 vs *mod-5*; AT3q130) comparisons. Strikingly, all 409 DEGs varied in opposite directions in this latter comparison. Unsupervised cluster analyses revealed that the *mod-5* ablation completely shifts the transcriptional profile of mutant ATXN3 animals towards the wild-type cluster probably by enhancing defense responses – genes involved in response to biotic stimulus, immune system, and stress responses were enriched. Specifically, several pathways were altered upon *mod-5* ablation in mutant ATXN3-expressing animals, including the ubiquitin-proteosome system, heat-shock response, extracellular proteostasis, and many lysosomal genes, with the biological relevance of the players currently being evaluated.
Conclusion: Given the impact on protein homeostasis pathways, this data further supports the modulation of serotonergic signaling as a promising therapeutic strategy for SCA3 and perhaps other ataxias.

Omaveloxolone improves contractile function and calcium signaling in the heart but does not extend lifespan in a mouse model of Friedreich's ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Lili Salinas [1], ***Mr. Francisco Figueroa*** [1], ***Ms. Claire Montgomery*** [1], ***Dr. Gino Cortopassi*** [1], ***Dr. Elena Dedkova*** [1]

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Objectives: Friedreich's ataxia (FA) is a cerebellar ataxia characterized by a GAA trinucleotide repeat expansion in the first intron of the FXN gene resulting in a depletion of the protein frataxin. This disorder results in ataxia; however, the lethal component is cardiomyopathy. Omaveloxolone (OMAV) was recently approved by the FDA for FA treatment, however, less is known about its effects on the heart. The goal of this study was to determine OMAV effects on cardiac structure, function and survival in the cardiac specific FXN knockout (FXN-cKO) mouse model of FA.

Methods: FXN-cKO mice were treated with either vehicle or 24mg/kg OMAV daily starting at 3 weeks of age until survival endpoint (survival study) or for 5 weeks (cross-sectional) study. In cross-sectional study, cardiac structure and function were evaluated by *in vivo* echocardiography, then samples were collected for histology, gene and protein expression, and biochemical assays.

Results: OMAV improved cardiac parameters by echo, increasing fractional shortening (+79%), ejection fraction (+50%), stroke volume (+19%), and cardiac output (+16%) while decreasing LV diameter (-63%) and volume (-80%) at systole compared to vehicle-treated KO mice. Improvement in cardiac function correlated with increased aconitase activity and ATP levels in OMAV-treated hearts which led to the increased expression of mitochondrial Ca^{2+} uniporter and SERCA pump. Elevated heart failure markers such as NPPB, ALDH1A3, and GDF15 were significantly decreased by OMAV. Despite these significant improvement in cardiac function, OMAV did not protect animals against premature death. Furthermore, OMAV treated females died even sooner as compared to vehicle-treated littermates. OMAV had no protective effects against increased fibrotic area or hypertrophy in FXN-cKO hearts.

Conclusion: Despite significant improvements in cardiac function, OMAV failed to increase survival in FXN-cKO mice and even accelerated death in FXN-cKO females. More research must be done on the long-term impact of OMAV in females.

Leveraging Machado-Joseph Disease-associated microRNA dysregulation to develop microRNA-based therapeutics

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Diana Santos [1], Ms. Micaela Pintor [2], Ms. Maria inês Santos [1], Mr. Rafael Baganha [3], Dr. Rui Jorge Nobre [4], Prof. Luís Pereira de Almeida [5], Dr. Sonia Duarte [6]

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Machado-Joseph disease or Spinocerebellar ataxia type-3 (MJD/SCA3) is a genetic neurodegenerative disorder caused by a CAG over repetition in the ATXN3 gene. MJD patients have a premature death and no cure is yet available, with therapies limited to ease patient symptoms. MicroRNA (miRNA) dysregulation is recognized in MJD, however, whether this is a cause or a consequence of the neurodegenerative process is still unclear. This work aimed to identify dysregulated miRNAs contributing decisively to MJD pathology and develop efficient miRNA-based therapeutic strategies.

MiRNA expression profiling was evaluated by Small RNAseq in the cerebella of a transgenic (Tg-Q69) MJD mouse model. Tg-Q69 mice were submitted to bilateral stereotaxic injection in the cerebellum with adeno-associated viral vectors encoding for miRNAs. Balance and motor coordination were assessed over time and brains were processed for PCR, WB, and IHC analyses. *In silico* prediction of miRNA expression regulation by small molecules and a luciferase assay were conducted to identify miRNA-specific small molecule modifiers. MiRNA levels were assessed and validated by qRT-PCR.

Importantly, many differentially expressed miRNAs were identified in Tg-Q69 mice. Of the top 60 dysregulated miRNAs, 30 were highly conserved in mice/humans, and over 10 appeared significantly altered over time in Tg-Q69. Reversion of two abnormally downregulated miRNAs in Tg-Q69 mice resulted in significant upregulation and improvement in motor performance for one of those miRNAs at 9 weeks post-injection. In addition, eight small molecules were identified as promising miRNA-modifiers, whose therapeutic potential is currently under evaluation both *in vitro* and *in vivo*.

Overall, novel dysregulated miRNAs implicated in MJD pathogenesis were identified. Restoration of altered miRNA levels through gene therapy showed promise as a therapeutic strategy for MJD. Furthermore, we successfully identified FDA-approved drugs as promising miRNA modifiers in an attempt to develop a miRNA-targeted pharmacological approach.

Generating Cerebellar Neuronal Progenitors for cerebellar transplantation in Spinocerebellar ataxia type 3 (SCA3)

Wednesday, 13th November - 18:00: (Minories) - Poster

Mr. Daniel Henriques [1], ***Mr. Ricardo Moreira*** [2], ***Mr. Frederico Pena*** [1], ***Dr. Pedro Perdigão*** [3], ***Prof. Agnete Kirkeby*** [4], ***Prof. Luís Pereira de Almeida*** [2], ***Dr. Liliana Mondonça*** [3]

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Spinocerebellar ataxia type 3 (SCA3) is an autosomal dominant ataxia. Cell therapy holds the promise of treating SCA3 patients who usually present extensive neuronal death at diagnosis. Transplanting the correct type of cells is a critical step to the success of cell replacement strategies and here we describe the generation of cerebellar neuronal progenitors (CNPs) to improve engraftment success.

CNPs were generated through a protocol adapted from Nolbrant and colleagues (Nolbrant et al. Nat. Protoc. 2017). Characterization of CNPs consisted in evaluating specific cerebellar markers at different time points (t=0, 9, 11, and 16 days) through RT-qPCR, immunocytochemistry, and flow cytometry. CNPs were differentiated for 30 days and characterized for the presence of cerebellar neurons by immunocytochemistry and functional neurons by single-cell calcium imaging. CNPs were transplanted into the cerebellum of NOD SCID mice to evaluate cell survival and neuronal differentiation.

Cerebellar markers were found increased in CNPs, namely mRNA levels of granule cell progenitors (ZIC1, 10.000-fold), Purkinje cells (FOXP2, 100-fold), Gabaergic neurons (PTF1A, 4.2-fold) at day 16, and glutamatergic neurons (ATOH1, 17.8-fold) at day 11. ZIC1 and ATOH1 expression was also found enhanced through immunocytochemistry and flow cytometry assay, which revealed 9.8- and 71-fold levels increase, respectively. CNPs were able to differentiate into cultures containing functional neurons which responded to KCl stimulus and exhibited a significant decrease in responding cells percentage in the presence of $CdCl_2$ (voltage-gated calcium channels inhibitor). Importantly, the presence of cerebellar markers such as PCP2 (Purkinje cells), ATOH1, ZIC1, and GABRA6 (granule neurons), was detected. Upon cerebellar transplantation, CNPs survived up to 2 months, and differentiated into MAP2 and NeuN positive neurons, with some S100B positive astrocytes.

These findings suggest a successful generation of cerebellar neuronal progenitors. Further *in vivo* testing is required to evaluate the potential of these cells to promote cerebellar regeneration.

Investigating small molecules that target the Integrated Stress Response and mTORC1 pathways in a mouse model of Friedreich's Ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Erin Seifert [1], ***Ms. Brittney Blackburne*** [1], ***Dr. Yuexing Yuan*** [1], ***Ms. Victoria Jackson*** [1], ***Dr. Louise A Corben*** [2], ***Prof. Martin Delatycki*** [3], ***Dr. Kimberly Lin*** [4]

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Background. Two mouse models of Friedreich ataxia (FRDA) reveal an activation of the Integrated Stress Response (ISR), mTORC1 signaling or both in Frataxin (FXN)-depleted heart and skeletal muscle (SM). High specificity small molecules targeting these pathways are available: Rapamycin suppresses mTORC1 signaling and ISRIB inhibits the ISR.

Methods. We studied mice with Doxycycline (Doxy)-inducible shRNA-driven whole-body knockdown (KD) of FXN and littermate controls (Ctrl); KD and Ctrl mice were treated with Doxy. Mice were 9 weeks old when FXN KD was initiated, and studied after 10 and 18 weeks of Doxy (FXN KD of ~80% and 95% respectively). At 13 weeks of Doxy, KD and Ctrl mice were randomized into Rapamycin, ISRIB, or relevant vehicle-treated groups. Outcomes: heart function (echocardiography), exercise capacity (treadmill), Rotarod (coordination, strength), SM mass, Western blot analysis of ISR and mTORC1 signaling, and markers of autophagy, in heart and SM.

Results. We confirmed that Rapamycin and ISRIB had the expected suppressing action on their respective pathways. Rapamycin fully prevented the decline in exercise capacity in KD mice, possibly due to increased autophagic flux in SM, but had little impact on other parameters. ISRIB had, overall, less effect than Rapamycin. In KD mice, ISRIB was associated with a significant, but small, improvement in heart diastolic function and hypertrophy, and SM mass, but did not impact treadmill or Rotarod performance.

Discussion and Conclusion. Rapamycin to suppress mTORC1 activation may have a positive effect on exercise, by mitigating a decline caused by FXN depletion. Though ISRIB treatment has some beneficial effects, suggesting that ISR activation is maladaptive, the effect size was small. An important next step is to understand stress response activation in individuals with FRDA; to this end, analysis of circulating markers of ISR/mTORC1 activation is being conducted in plasma samples adults with FRDA. Funding: FARA, NIH.

Peptide bioconjugation to anti-gene oligonucleotides as a therapeutic approach for Friedreich's ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

Mrs. Salomé Milagres [1], ***Mrs. Eliza Filipiak*** [1], ***Dr. Malgorzata Honcharenko*** [1], ***Mrs. Mónica Lopes*** [2], ***Dr. Kristina Karalè*** [2], ***Dr. Ulf Tedebark*** [3], ***Prof. Roger Strömberg*** [4], ***Prof. Edvard Smith*** [5], ***Dr. Rula Zain*** [5]

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Friedreich's ataxia (FRDA) is an autosomal progressive neurodegenerative disorder caused by GAA•TTC repeat expansions in the first intron of the *Frataxin (FXN)* gene. These expansions tend to form non-B-DNA structures, such as H-DNA, stalling *FXN* transcription. Epigenetic modifications, including DNA methylation and histone deacetylation, contribute to *FXN* silencing and heterochromatin formation. Reduced *FXN* transcription results in a deficiency of the encoded protein, Frataxin (FXN). Since FXN is an essential mitochondrial protein, cells highly dependent on mitochondrial function, like neurons, pancreatic β-cells, and cardiomyocytes, are most affected, resulting in comorbidities like ataxia, diabetes, and heart failure.

Most therapies for FRDA focus on increasing *FXN* transcription, FXN replacement or reverse mitochondrial dysfunction using antioxidants. However, aiming at the downstream effects of the expanded repeats is a suboptimal approach. Our novel strategy targets the expanded chromosomal DNA using therapeutic oligonucleotides to prevent repressive H-DNA formation. We demonstrated that phosphorothioate-modified LNA/DNA mixmer anti-gene oligonucleotides (AGOs) can invade double-strand DNA, disrupt H-DNA formed at GAA•TTC repeat sites (Bergquist, H. et al., 2016) and confirmed that this results in frataxin mRNA and protein upregulation in FRDA patient-derived cells (Mozafari, N. et al., 2024).

To enhance their therapeutic effect, we bioconjugated AGOs to different cell-penetrating peptides (CPP) and endosomal escape peptides (EEP). By improving the uptake and endosomal escape, therapeutic oligonucleotides can be administrated in lower doses, leading to potentially less off-targeting effects. Patient-derived cells were treated with peptide-conjugated (PC-AGOs) and *FXN* mRNA levels were assessed using RT-qPCR. We show that, compared to non-conjugated oligonucleotides, PC-AGOs greatly enhance *FXN* upregulation at lower doses in a non-cytotoxic fashion, demonstrating for the first time the bioconjugation to AGOs as a potential therapeutic approach for FRDA.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska Curie grant agreement No 956070.

Mechanistic Insights into MSC Therapy for Machado-Joseph Disease: Nerve Growth Factor as a central effector?

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Inês Barros [1], Mr. António Silva [2], Ms. Daniela Gonzaga [3], Ms. Diana Lobo [1], Dr. Dina Pereira [1], Dr. Sonia Duarte [1], Dr. Sara Lopes [1], Dr. Rita Perfeito [1], Prof. Clévio Nóbrega [4], Dr. Maria do Rosário Faro [1], Dr. Luísa Cortes [5], Dr. Margarida Caldeira [5], Dr. Tatiana Catarino [5], Dr. Rui Jorge Nobre [6], Prof. Luís Pereira de Almeida [7], Dr. Catarina Miranda [1]

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INTRODUCTION: Spinocerebellar ataxia type 3 (SCA3) is caused by CAG trinucleotide overexpansion in the *MJD1/ATXN3* gene, translating into an abnormal ataxin-3 protein, and leading to neuronal dysfunctions. Repeated Mesenchymal Stem Cells (MSC) therapy decreases ataxin-3 levels in the cerebellum of SCA3 mice and improves their phenotype and neuropathology (Miranda et al., 2018). Here, we aimed at clarifying the mechanisms underlying the neuroprotective effects of MSC in SCA3.

METHODS: We assessed MSC modulation on mechanisms impaired in SCA3 - autophagy, mitophagy, mitochondrial biogenesis and synaptogenesis, through protein analysis by WB or ICC in MSC and SCA3 neuronal cells co-cultures, or by WB or ^{1}H-MRS in transgenic SCA3 mice cerebella after intravenous or intracerebroventricular MSC administration.

RESULTS: In SCA3 *in vitro* models, mutant ataxin-3 (72Q) hampered autophagosome maturation and axonal mitochondrial transport, which MSC corrected. MSC intravenous therapy alleviated p62 accumulation, restored the levels of Mfn2 and p-DRP1, and re-established the levels of p-Parkin and glutathione in the cerebellum of SCA3 transgenic mice, thereby restoring autophagy, mitophagy, mitochondria biogenesis and energetic levels. To further characterize the MSC effectors on SCA3 we performed two panels of exploratory analyses. First, by using the Proteome Profiler Mouse Phospho-RTK Array Kit (R&D Systems), we determined that the levels of the tyrosine-kinase receptors (Trks) Mer, ErbB4, PDGF-Ra, PDGF-Rb, SCF-R, TRK-a and TRK-b were corrected by MSC *in vitro*. Secondly, by using the Olink® Target 96 Neuro Exploratory Panel, we found that EZR, Lrpap1, NGF, TNFRSF12A, RGMA, Gfra1, Flrt2, HAGH, Scarf2, Mstn, ADAM23, and TRKB levels in the CSF were restored by MSC on SCA3 mice treated intracerebroventricularly. Finally, an *in silico* analysis (String Interaction Network) evidenced NGF as the pivotal MSC's effector, nominating it as a strong candidate for mediating MSC-mediated neuroprotection.

CONCLUSION: In conclusion, MSC address multiple molecular mechanisms, including but not limited to NGF secretion.

TARGETING SPHINGOLIPID-METABOLISING ENZYMES IN FRIEDREICH'S ATAXIA (FRDA)

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Zenouska Ramchunder [1], ***Dr. Ester Kalef Ezra*** [2], ***Dr. Saqlain Suleman*** [1], ***Dr. Sandor Szunyogh*** [3], ***Dr. Adamo Valle Gómez*** [4], ***Dr. Sara Anjomani Virmouni*** [1]

1. *Brunel University London,* ***2.*** *University College London,* ***3.*** *University of Oxford,* ***4.*** *University of Balearic Islands*

Background and Objectives: Friedreich's ataxia (FRDA) is an autosomal recessive neurodegenerative disorder, caused by a GAA repeat expansion mutation within the *FXN* gene. This leads to reduced frataxin expression, resulting in increased reactive oxygen species (ROS), mitochondrial dysfunction, and consequential cell death. Altered sphingolipid metabolism and related enzymes have been reported in multiple neurodegenerative diseases. We aim to investigate the role of sphingolipid metabolism in FRDA pathogenesis and the effects of targeting relevant sphingolipid-metabolising enzymes as a novel therapeutic approach for FRDA.

Methods: Experiments were conducted *in vitro* using human cell lines (FRDA and control fibroblasts, iPSC-derived cardiomyocytes and sensory neurons) and mouse cerebellum tissues (FRDA YG8sR>800 and Y47R control mice). Gene and protein expression levels were assessed using qRT-PCR, Western blotting, and ELISA. Mitochondrial dysfunction was assessed using flow cytometry. Short hairpin RNA (shRNA) was employed to target relevant sphingolipid-metabolising enzymes in FRDA human fibroblasts.

Results: We have identified altered sphingolipid metabolism in FRDA models, particularly ceramide (Cer) and sphingosine-1-phosphate (S1P). We have also found altered expression of sphingolipid-metabolising enzymes, in particular lipid phosphate phosphatase (LPP), which affects both Cer and S1P levels, in FRDA models. Targeting this enzyme has shown to increase *FXN* gene expression, reduce ROS, and improve mitochondrial dysfunction, using both a small molecule inhibitor and shRNA.

Discussion and Conclusion: The results have shown that altered LPP expression is apparent across FRDA human fibroblast cell lines, mouse tissues, and iPSC-derived cardiomyocytes and sensory neurons, suggesting that sphingolipid metabolism is involved in FRDA pathogenesis. Furthermore, targeting LPP using different mechanisms has shown to increase *FXN* gene expression and improve mitochondrial dysfunction, which further supports a potential role for LPP signalling in the pathogenesis of FRDA. These findings suggest that targeting the ceramide/S1P axis could be served as a potential therapeutic target for FRDA.

Role of sulfide metabolism in a model of autosomal recessive ataxia type 2 (ARCA2)

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Débora Goncalves [1], ***Dr. Helene Puccio*** [1]

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Autosomal recessive ataxia type 2 (ARCA2) is a rare neurodegenerative disorder characterized by cerebellar ataxia and exercise intolerance. ARCA2 is due to biallelic mutations in COQ8A, a mitochondrial protein involved in CoQ_{10} (CoQ) biosynthesis. Constitutive *Coq8a*$^{-/-}$ knockout mice reproduced most hallmarks of human disease, including cerebellar ataxia with degeneration of Purkinje neurons (PN), exercise intolerance, and modest reduction of CoQ in some tissues. Here we present results of water-soluble reduced Ubiquinol-10 ($CoQ_{10}H_2$) treatment in *Coq8a*$^{-/-}$ mice and preliminary results suggesting that sulfide metabolism could be altered at early disease stages. **Methods:** Treatment with Ubiquinol-10 was given to *Coq8a*$^{-/-}$ mice in drinking water at concentration of 600 mg/Kg in pre-symptomatic stage starting at 7 weeks old and in post-symptomatic stage starting at 15 weeks old until 50 weeks. Motor coordination was assessed in different time points using rotarod. To investigate onset alteration of sulfide metabolism, we measured protein levels and activity of sulfide quinone reductase (SQR) in liver and PN of *Coq8a*$^{-/-}$ mice at 10 weeks. **Results and Discussion:** Our preliminary data indicate that SQR protein levels were increased in liver while decreased in PN and that SQR activity was reduced in the liver at 10 weeks in *Coq8a*$^{-/-}$mice, suggesting alterations in sulfide metabolism at pre-symptomatic stage, probably as a consequence of CoQ deficiency. Supporting this hypothesis, chronic $CoQ_{10}H_2$ supplementation partially improved motor coordination in *Coq8a*$^{-/-}$ mice. **Conclusion:** These results suggest early onset in sulfide metabolism alterations in *Coq8a*$^{-/-}$ mice and partial therapeutic efficacy of $CoQ_{10}H_2$ supplementation in a chronic treatment protocol. In perspective, we will determine if the accumulation of sulfide is one of the first alterations in ARCA2, which could be a good pharmacological target.

Inhibition of NLRP3 Inflammasome Activation by YA-101 Reduces IL-1β Production: Implications for Multiple System Atrophy Therapy

Wednesday, 13th November - 18:00: (Minories) - Poster

Prof. Yufeng Jane Tseng [1], ***Dr. Da-Zhong Luo*** [1], ***Dr. Bo-Han Su*** [1], ***Dr. Olivia A Lin*** [1], ***Dr. Ting-Fang Lo*** [1], ***Dr. Yi-Ching Huang*** [1], ***Dr. Chih-Chi Li*** [1], ***Dr. Wan-Hsun Wu*** [1], ***Mrs. Lisa Kang*** [1]

1. *Yoda Therapeutics Inc.*

Background and Objectives:
Multiple System Atrophy (MSA) is a rare, rapidly progressing neurodegenerative disorder with an urgent need for effective treatments. The pathogenesis of MSA involves the accumulation of misfolded α-synuclein in oligodendrocytes, which activates the NLRP3 inflammasome, leading to IL-1β release. Inhibitors targeting the NLRP3 inflammasome have potential therapeutic value for MSA. This study investigates the efficacy of YA-101, a novel compound, in inhibiting NLRP3 inflammasome activation and reducing IL-1β production.
Methods:
To evaluate the inhibitory effect of YA-101 on inflammation and NLRP3 inflammasome, an *in vivo* model was established using lipopolysaccharide (LPS) to prime the mice, followed by YA-101 treatment. Specifically, wild-type mice were exposed to LPS for 4 hours to induce NLRP3 inflammasome activation. Subsequently, YA-101 was administered, and IL-1β levels were measured 25 minutes post-treatment using ELISA. The experimental groups included a control group, an LPS-only group, and an LPS plus YA-101 treated group.
Results:
The results demonstrated a significant elevation of IL-1β levels in the LPS-treated group, confirming successful inflammasome activation. Treatment with YA-101 resulted in a marked reduction of IL-1β levels compared to the LPS-only group. This suggests that YA-101 effectively inhibits the NLRP3 inflammasome, thereby reducing IL-1β production.
Discussion and Conclusion:
YA-101 demonstrates significant inhibitory effects on IL-1β production in an LPS-induced inflammation model. The observed results highlight the potential of YA-101 as a potent inhibitor of NLRP3 inflammasome. The ability of YA-101 to attenuate the inflammatory response positions it as a promising candidate for therapeutic intervention in neurodegenerative diseases, such as MSA, where NLRP3 inflammasome plays a critical role. These findings provide a foundation for further studies to explore the therapeutic potential of YA-101 in neuroinflammatory and neurodegenerative diseases. Future investigations will focus on elucidating the underlying mechanisms and evaluating the efficacy of YA-101.

Transplantation Of Gene-edited Hematopoietic Stem Cells Rescues Neuronal And Cardiac Complications In A Mouse Model Of Friedreich's ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Anusha Sivakumar [1], ***Ms. Alexis N. Corl*** [1], ***Ms. Angelyn B. Solis*** [1], ***Ms. Lilas Rony El-Hachem*** [1], ***Ms. Rishika Murthy*** [1], ***Dr. Rafael A. Badell-Grau*** [1], ***Ms. Rita Wan*** [2], ***Mr. Eric Thai*** [1], ***Dr. Veenita Khare*** [1], ***Prof. Stephanie Cherqui*** [1]

1. *University of California, San Diego,* ***2.*** *Univeristy of California, San Diego*

Friedreich's ataxia (FA) is an autosomal recessive, neurodegenerative disorder caused by homozygous GAA-repeat expansion mutation in intron 1 of frataxin gene (FXN), reducing FXN expression crucial for mitochondrial function. Symptoms begin between ages 5 and 15, leading to wheelchair dependence within 10-15 years of onset and mortality is predominantly caused by cardiomyopathy. Currently, there is no effective treatment for FA. We previously demonstrated that a single infusion of wild-type hematopoietic stem and progenitor cells (HSPCs) rescued neurological and cardiac complications in a FA mouse model via FXN transfer from engrafted HSPC-derived microglia/macrophages to neurons/myocytes. For autologous therapy, we utilized CRISPR/Cas9 to excise GAA repeat mutation from *FXN* in patient derived $CD34^+$ HSPCs, increasing *FXN* expression. This study investigates the *in vivo* safety and efficacy of this gene editing approach in the mouse model of FA, YG8s(GAA)>800 mice (YG8JR). Two-month-old, myeloablated, YG8JR mice transplanted with either *ex vivo* gene edited, analogous $Sca1^+$ HSPCs (GAAed) or mock-edited HSPCs were analyzed at 6-7 months post-transplant. Despite limited gene editing efficiency in the cell product (mean 15.7%), substantial engraftment of edited HSPC-derived cells was observed in hematopoietic tissues (~ 5.25% in bone marrow, ~ 8.3% in blood, 19% in spleen, 16.51% in thymus) and in primary FA-affected organs (2.4% in heart, 1.1% spinal cord, 0.69% cerebellum, 4.4% skeletal muscle). Increased FXN expression led to significant tissue preservation. Neurodegeration in cerebellar granular cell layer was prevented in mice transplanted with GAAed HSPCs compared to mock controls. In the heart, GAAed HSPCs preserved myofiber and mitochondrial architecture compared to mock controls. These promising findings demonstrate that single infusion of FXN-gene edited HSPCs engraft in bone marrow niche, creating a reservoir of healthy cells that can integrate in defective organs allowing their for long-term preservation and support the clinical translation of this approach for FA.

Assessing the Therapeutic Potential of Intermittent Fasting and Ketogenic Diets in a Mouse Model of Spinocerebellar Ataxia Type 3

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Cármen Vieira [1], Ms. Sara Guerreiro [1], Dr. Sara Duarte-Silva [1], Ms. Daniela Monteiro-Fernandes [1], Ms. Patrícia Araújo [1], Ms. Bruna Ferreira-Lomba [1], Prof. Patrícia Maciel [1], Dr. Andreia Teixeira-Castro [1]

1. *Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal*

Introduction and objective: Several studies reported that diet manipulations, such as caloric restriction (CR) and periodic fasting, can boost longevity and delay disease onset, likely due to a metabolic switch from glucose to ketone bodies as the primary fuel source. Spinocerebellar Ataxia type 3 (SCA3), a dominantly inherited disorder characterized by progressive motor impairments, results from an abnormal CAG repeat extension in the *ATXN3* gene. Currently, there is no effective therapy for SCA3. While short-term CR improved motor coordination and neuropathology of SCA3 mice, this approach may not be feasible for patients, who often experience weight loss across disease progression. Here, we ask whether prolonged metabolic switching without CR is sufficient to stall SCA3 progression in a transgenic mouse model of the disease.

Results: Time-restricted feeding (TRF) and ketogenic (KETO) dietary regimens successfully induced sustained ketosis, while maintaining normal caloric intake and without causing body weight loss in SCA3 mice. However, our evaluation of motor performance across disease progression revealed limited impacts of both diets on motor coordination and balance, indicating that these dietary approaches did not significantly modify disease progression. Moreover, KETO and TRF dietary interventions did not alter neuropathology in SCA3 mice. Gene expression analysis in disease-relevant brain areas showed no significant changes in genes associated with metabolism or neuroprotection, suggesting that the lack of impact at the molecular level may underlie the limited therapeutic effects observed.

Conclusion: Our study highlights the potential limitations of time-restricted feeding and ketogenic diet regimens in delaying SCA3 progression. These findings emphasize the need for further research to identify more effective dietary interventions for SCAs. Future studies should focus on evaluating the efficacy and safety of these dietary regimens by determining the optimal timing for treatment initiation, duration, and protocols to improve the design of pre-clinical and clinical studies.

Allele-specific silencing of ATXN7 to treat Spinocerebellar Ataxia Type 7.

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Linde Bouwman [1], ***Prof. Bart van de Warrenburg*** [2], ***Prof. Willeke van Roon-Mom*** [1]

1. *Leiden University Medical Center,* ***2.*** *Radboud university medical center*

Background:
Spinocerebellar ataxia type 7 (SCA7) is an autosomal dominantly inherited neurodegenerative disorder. Patients mainly suffer from cerebellar ataxia and blindness. SCA7 is caused by a CAG trinucleotide repeat expansion in exon 3 of the *ATXN7* gene resulting in an elongated polyglutamine (polyQ) tract in the ATXN7 protein that causes a toxic gain of function of the protein. At the moment, there is no therapy that can stop or delay disease progression. Previously, gapmer antisense oligonucleotides (AONs) targeting mouse Atxn7 reduced retinal degeneration in a SCA7 mouse model. However, as the ATXN7 protein might have an important physiological role, reducing both wild type and mutant ATXN7 protein levels could be harmful. Therefore, we aimed to design allele-specific gapmer AONs that can reduce SCA7 protein levels but retain wild type levels by targeting common single nucleotide polymorphisms (SNPs).

Methods:
On cDNA derived from a SCA7 patient, we identified heterozygosity for two common SNPs, and identified using PCR that both SNPs were located on the CAG repeat expanded allele but not on the wild type allele. For both SNPs that were present on the SCA7 allele three gapmers were designed. Next, from patient-derived blood cells pluripotent stem cells were generated, which were differentiated to neuronal progenitor cells (NPCs). The gapmers were transfected in NPCs using lipofectamine.

Results:
For the first SNP, the three gapmers could reduce the SCA7 ATXN7 transcript by 37-51%, while the wild type ATXN7 transcript was reduced by 18-41%. The gapmers targeting the second SNP reduced ATXN7 levels by 16-50% with no differences between the SCA7 and the wild type transcript.

Conclusion/Discussion:
Our results show that the gapmers can reduce *ATXN7* mRNA levels in patient-derived cells, however further optimizations are required to improve allele-specificity.

Funding:
This work was supported by the Dutch Brain Foundation (Nederlandse Hersenstichting).

Title: Antisense oligonucleotide targeting of a splicing defect in Friedreich's ataxia increases frataxin levels

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Selina Aguilar [1], ***Dr. Pouire Yameogo*** [1], ***Dr. David Lynch*** [2], ***Dr. Jill Napierala*** [1], ***Dr. Marek Napierala*** [1]

1. *Department of Neurology, University of Texas Southwestern Medical Center, Dallas, TX, USA,* ***2.*** *Department of Pediatrics and Neurology, The Children's Hospital of Philadelphia*

Friedreich's ataxia (FA) is a progressive neurodegenerative disorder most frequently caused by a homozygous trinucleotide GAA repeat expansion in intron 1 of the FXN gene. Pathological repeats range from 70 – 1500 units while unaffected individuals carry less than 60 GAAs. The expansion interferes with FXN transcription, leading to a reduction of frataxin, a mitochondrial protein involved in iron-sulfur (Fe-S) cluster assembly. Approximately 4% of patients with FA are heterozygous for the repeat expansion on one FXN allele but have a point mutation on the second allele. A newly reported point mutation, FXN c.165+5G>C, was identified in intron 1 of two FA patients. We hypothesize that this mutation affects canonical splicing of the FXN gene, leading to the production of an aberrant transcript and decreased FXN expression. This type of mutation may be amenable to antisense oligonucleotide (ASO)-mediated therapeutic intervention.

Methods:

Skin fibroblasts of FA patients harboring the FXN c.165+5G>C transversion were derived from a 3 mm punch biopsy. The mutation was confirmed using DNA sequencing and frataxin expression verified by qRT-PCR and western blot. A splicing defect was identified by ultra-deep short read RNA sequencing and validated by RT-PCR. A library of 2'-O-Methoxyethyl (2'-MOE) phosphorothioate (PS) ASOs were designed to target near the mutation and were transfected to FA fibroblasts using Lipofectamine. Frataxin expression was determined using qRT-PCR and western blot.

Result and conclusions:

FXN levels in patients carrying the FXN c.165+5G>C point mutation were found to be significantly lower than unaffected controls. RNA sequencing revealed an alternate transcript with partial retention of intron 1, expressed from the point mutant FXN allele. We identified two ASOs capable of increasing FXN expression upon transfection into patient cells. These results are paving a path for potential ASO treatment of rare mutations in FA and for the design of N=1 clinical trials.

Cardiac arrhythmias and omaveloxelone treatment in a mouse model of Friedreich's ataxia with severe cardiomyopathy

Wednesday, 13th November - 18:00: (Minories) - Poster

Mr. Francisco Figueroa [1], Dr. Phung Thai [1], Ms. Lili Salinas [1], Ms. Claire Montgomery [1], Dr. Nipavan Chiamvimonvat [1], Dr. Gino Cortopassi [1], Dr. Elena Dedkova [1]

1. *University of California Davis*

BACKGROUND: Friedreich's ataxia (FA) is a monogenic recessive disorder caused by the reduction of the mitochondrial protein frataxin (FXN). Most patients die from lethal cardiomyopathy in their 30's from progressive cardiac disease. The mechanisms leading to cardiomyopathy and arrhythmias in patients are poorly understood. This study aims to interpret electrical signal propagation in a mouse model that mimics cardiomyopathy in FA patients, treated with the first FDA-approved therapy omavolexolone (OMAV).

METHODS: Cardiac-specific McK-Cre FXN knockout (FXN-cKO) mice were used as model of FA cardiomyopathy. Animals were subject to *in-vivo* surface electrocardiogram (ECG) recordings. Gene expression was measured using RT-qPCR.

RESULTS: Heart rate variability (HRV) Poincare plot analysis showed that individual mice can experience disparate arrhythmias. OMAV effectively removed disparate arrhythmias by mostly presenting as a single cluster on Poincare plots. However, ion channel impairment and arrhythmias still persisted. Additionally, we found that OMAV had no significant therapeutic effect on electrical parameters when analyzed traditionally as averaged values of combined sex cohorts. Without treatment, ventricular electrical propagation intervals increased significantly in FA males (QTc, QRS), while FA females primarily displayed delayed atrial (P, PR) propagation. When data was aggregated as frequency distribution histograms, OMAV-treated FXN-cKO mice showed varying time durations with small improvements in atrial (P-wave) and ventricle-specific (QTc) waveforms.

CONCLUSIONS: Our study showed electrical propagation disturbances and ANS dysfunction, in addition to revealing a sexual dimorphism of electrical function in mice exhibiting late-stage cardiomyopathies in FXN-cKO hearts. OMAV-treatment has improved heart function by prevention of disparate arrhythmias seen in Poincare plots. This analysis of electrical parameters with OMAV-treatment highlights the importance of utilizing different mathematical approaches for drug testing in animal models that best represents the manifestation of cardiomyopathy seen in FA patients.

A Novel, Pleiotropic Nanozyme for Targeting Metabolic Deficits in Friedreich's Ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Uffaf Khan [1], ***Mr. Karthik Mouli*** [2], ***Dr. Anton Liopo*** [1], ***Mr. Elias Perli*** [2], ***Ms. Anh Vo*** [1], ***Dr. Paul Derry*** [1], ***Dr. Thomas Kent*** [1]

1. *Texas A&M University Institute of Biosciences and Technology,* ***2.*** *Texas A&M University School of Medicine*

Objective: This study investigates a novel therapeutic approach for Friedreich's Ataxia (FRDA) for diverse underlying pathologies resulting from frataxin gene mutation.

Background: To address multiple pathologies centered on mitochondrial dysfunction in FRDA, we tested a synthetic nano-sized enzyme, "nanozyme", with versatile enzymatic properties we term "Pleozyme". Pleozymes catalytically dismutate superoxide, facilitate electron transfer between mitochondrial components and oxidize hydrogen sulfide to polysulfides. They exhibit avid uptake, co-localize with mitochondria and improve outcomes in acute and chronic disease models. FRDA arises from the expansion of GAA repeats in the FXN gene, diminishing frataxin protein levels, leading to iron-sulfur cluster disruption, crucial for the mitochondrial electron transport and potentially ferroptosis. Here, we tested Pleozymes' protective effects on human FRDA-derived cells with/without the approved therapy, SKYCLARYS® (Omaveloxolone).

Methods: Pleozymes, were derived from acid oxidation of medicinal grade activated charcoal and PEGylated (PEG-OAC). Oxidation alters the chemical properties, yielding 3-8 nm graphene discs with broad redox potential within the range of several key metabolic steps. The iron chelator, deferoxamine, was covalently bonded (DEF-PEG-OAC). Pleozymes were tested in FRDA patient-derived fibroblasts, induced Pluripotent Stem Cells (iPSCs) and isogenic controls (Marek Napierala, UTSW). iPSCs were differentiated into cardiomyocytes. Metabolic activity was measured via extracellular flux analysis (Agilent). Lipid peroxidation, a ferroptosis indicator, was measured using C11-BODIPY (Dojindo). Nuclear Nrf2 was measured by quantitative immunofluorescence (Cell Signaling).

Results: Compared to isogenic cells, FRDA cardiomyocytes demonstrated reduced maximal respiration (74.5%) (n=8 technical replicates, $p<.01$), which was increased by 16% after a single 24-hour exposure to Pleozymes (n=4 technical replicates; $p=0.01$). Lipid peroxidation was reduced by 110%. Pleozyme-treated FRDA iPSC-CMs increased nuclear Nrf2 additively with Omavaloxone (n=3 technical replicates; $p=0.01$).

Discussion/Conclusions: Pleozymes demonstrated multiple beneficial effects in FRDA-derived cells, warranting testing in-vivo in FRDA models and comparison to approved therapy, currently planned.

Funding: NIHR01NS094535, Welch Foundation BE-0048.

Design and validation of cell based potency assay for frataxin supplementation treatments

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Jill Napierala [1], ***Dr. Shibani Mukherjee*** [1], ***Ms. Achisha Saikia*** [2], ***Dr. Jeon Lee*** [2], ***Dr. Maria Grazia Cotticelli*** [3], ***Dr. Robert Wilson*** [3], ***Dr. Marek Napierala*** [1]

1. *Department of Neurology, University of Texas Southwestern Medical Center, Dallas, TX, USA,* ***2.*** *Lyda Hill Department of Bioinformatics, University of Texas Southwestern Medical Center, Dallas, TX, USA,* ***3.*** *Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia*

Introduction: Friedreich's ataxia (FRDA) is a multisystem, autosomal recessive disease caused by a mutation in the frataxin (*FXN*) gene. Most frequently FRDA is caused by a bi-allelic GAA repeat expansion while point mutations are found in approximately 5% of patients. Irrespective of the genetic defect, low level of frataxin is the most typical molecular manifestation of the disease. As FRDA is considered a frataxin deficiency disorder, numerous therapeutic approaches currently in development or clinical trials aim to supplement FXN or restore endogenous expression of the gene. These include gene therapy, protein supplementation, genome editing or targeted upregulation of FXN transcription. To evaluate efficacy of these therapies, potency assays capable of quantitative determination of FXN biological activity, need to be established.

Methods: Here we evaluated suitability of mouse embryonic fibroblasts derived from the Fxn G127V knock-in mouse model (MUT MEFs) as a candidate for a cell-based potency assay. WT and MUT MEFs were immortalized using SV40 T antigen expression and cultured under standard (normoxic) conditions. Their growth rates were analyzed and cells were assayed for ATP and reactive oxygen species (ROS) production.

Results: We demonstrated that MUT cells, when immortalized, continue to express a minimal amount of frataxin and exhibit a broad range of phenotypes. Exogenous FXN supplementation via lentiviral transduction of a miniFXN gene reverses these phenotypic abnormalities.

Conclusions: Immortalized MUT MEFs are an excellent tool to be used for potency assays while testing novel therapies aimed at increasing FXN levels. Care must be exercised while utilizing these immortalized cell lines, as extended passaging results in molecular changes that spontaneously reverse FRDA-like phenotypes without increasing FXN expression. Based on comparative transcriptome analyses, aerobic glycolysis, or Warburg effect, was identified as the mechanism that allows cells expressing a minimal level of frataxin to thrive under standard cell culture conditions.

Gene editing strategies for spinocerebellar ataxia type 3

Wednesday, 13th November - 18:00: (Minories) - Poster

Prof. Nicole Deglon [1]

1. *Lausannne Universirty Hospital*

Background: Spinocerebellar ataxia type 3 (SCA3) is a neurodegenerative disease caused by a CAG expansion in exon 10 of the ataxin-3 gene (ATXN3). As a monogenic disease for which only symptomatic treatment is available, ATXN3 represents an attractive therapeutic target for gene editing.
Methods: We validated candidates in HEK cells and then employed AAV-mediated co-delivery of CRISPR/Cas9 system in the deep cerebellar nuclei of SCA3 transgenic mice (MJD84.2).
Results: We have explored three global gene-editing approaches, so that all SCA3 patients are eligible: 1) exon 10 deletion, 2) exon 9 truncation, 3) and an ablate and replace strategy.
For exon 10 deletion, sgRNAs candidates actively cleave the ATXN3 gene and induce a partial deletion of exon 10 in HEK293T cells, but most editing events do not lead to exon 10 deletion in SCA3 (MJD84.2) transgenic mice, as recently reported for other pathologies. For exon 9 truncation, a sgRNA inducing a premature stop codon has been identified and shown to generate a truncated but functional ataxin-3 protein in vitro; unfortunately, in vivo editing efficiency was very low.
Finally, for the ablate strategy, a sgRNA targeting the translational start site ATXN3 and inactivating both wild-type and mutant alleles was validated *in vitro* and *in vivo*. In parallel, we developed a CRISPR-resistant replacement vector encoding a wild-type human ATXN3-27Q gene. In a final proof-of-principle study, we evaluate the ablate and replace strategy combined or not with the self-inactivating KamiCas9 system. Two months post-injection, editing efficiencies were measured on cerebellar punches and reached 39±12%, and 39,9±4,7% in the ablate constitutive or KamiCas9 groups. The ablate and replace reached 29,5±6,0%, and 29,1±3,8%, for the constitutive and KamiCas9 mice.
Discussion and conclusions: Together, this study demonstrates the potency of CRIPSR/Cas9 for ATXN3 editing in the mouse cerebellum, underpinning a promising therapeutic option for future clinical applications.

Developing antisense oligonucleotide therapies for SCA3 using iPSC-derived neural models

Wednesday, 13th November - 18:00: (Minories) - Poster

Mr. Jacob Helm [1], Ms. Luisa Baraban [1], Ms. Laura Garcia Manzano [1], Ms. Melanie Kraft [2], Ms. Yvonne Schelling [1], Dr. Jeannette Hübener-Schmid [3], Dr. Nicolas Casadei [4], Prof. Ludger Schöls [1], Dr. Stefan Hauser [1]

1. *Department for Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research and Center for Neurology, University of Tübingen. German Center for Neurodegenerative Diseases (DZNE), Tübingen,* ***2.*** *Center for Neurology and Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany,* ***3.*** *Department of Medical Genetics, University of Tübingen,* ***4.*** *Institute of Medical Genetics and Applied Genomics, University of Tübingen, 72076 Tübingen, Germany*

Background and Objectives: Antisense oligonucleotides (ASOs) are short RNA sequences that bind to complementary (pre-)mRNA in the cytosol or nucleus and alter the processing of the target gene by regulating splicing or initiating degradation through RNase H recruitment. Current methods for developing ASO therapies for neurodegenerative diseases lack a human-relevant and scalable testing platform and rely heavily on animal models. We aim to establish a multimodal platform using iPSC-derived neurons and brain organoids to evaluate ASO efficacy, longevity, specificity, and toxicity using the genetic background of patients in a disease-specific context, focusing on spinocerebellar ataxia type 3 (SCA3).

Methods: We optimised a workflow to identify disease-associated SNPs using a multiplexed, targeted allele-specific sequencing approach. Gapmer ASOs targeting various disease-associated SNPs were screened in iPSC-derived neurons and further validated in cerebellar organoids from SCA3 patients.

Results: Several gapmer ASOs were identified that are able to specifically reduce mutant ataxin-3 protein levels in both iPSC-derived neurons and cerebellar organoids, while maintaining the expression of the wild-type form. Cerebellar organoids will be used for in-depth characterisation of promising ASOs, including acute and long-term toxicity assays and analysis of on- and off-target effects, to enable improved, personalised and patient-centric analysis of ASOs as a treatment for SCA3.

Discussion and Conclusion: iPSC-derived neural models provide a human-relevant platform to evaluate ASOs for neurodegenerative diseases. This platform allows for the assessment of ASO efficacy, off-target effects, acute and long-term toxicity using protein quantification, calcium imaging, NfL levels, and omics technologies. This approach is adaptable to other brain diseases and RNA-based therapeutics.

Funding: The development of allele-specific ASOs for SCA3 is supported by “Ataxia UK” and the “Deutsche Heredo-Ataxie-Gesellschaft (DHAG)”.

An AI-predicted chemical compound suppresses ATXN1 protein aggregation and rescues iNSC-derived SCA1 neurons from cell death

Wednesday, 13th November - 18:00: (Minories) - Poster

Ms. Katerina Pliatsika [1], Dr. Ioannis Gkekas [1], Mr. Stelios Mylonas [2], Dr. Sotirios Katsamakas [3], Ms. Evi Pechlivani [1], Dr. Lea Jessica Berg [4], Dr. Apostolos Axenopoulos [2], Prof. Bart van de Warrenburg [5], Prof. Konstantinos Xanthopoulos [3], Dr. Petros Daras [2], Dr. Michael Peitz [4], Dr. Spyros Petrakis [1]

1. *Institute of Applied Biosciences (INAB), Centre for Research and Technology Hellas (CERTH), Thessaloniki, Greece,* ***2.*** *Information Technologies Institute (ITI), Centre for Research and Technology Hellas (CERTH), Thessaloniki, Greece,* ***3.*** *Department of Pharmacy, School of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece,* ***4.*** *Institute of Reconstructive Neurobiology, Life & Brain Center, University of Bonn Medical Center, Bonn, Germany,* ***5.*** *Radboud university medical center*

Background and objectives: Spinocerebellar ataxia type 1 (SCA1) is a neurodegenerative disease caused by CAG repeat expansions in the *ATXN1* gene. The mutant ataxin-1 (ATXN1) protein forms toxic oligomers which slowly aggregate into larger insoluble inclusions within the nucleus. This process correlates with disease progression and is dependent on the age of the patient. The AXH domain of ATXN1 is suggested to play a critical role in the aggregation of its mutant isoform. Currently, there is no treatment for SCA1. Therefore, a reliable cell model is essential for understanding disease mechanisms and testing potential therapeutic interventions.

Methods/Results: Here, we present the direct trans-differentiation of peripheral blood mononuclear cells (PBMCs) from SCA1 patients into induced neural stem cells (iNSCs), using non-integrating Sox-2 and c-Myc Sendai viruses. The generated iNSCs exhibit neural stem cell properties, including the expression of relevant markers, self-renewal and differentiation potential into neurons. SCA1 neurons partially retain the age of the donor and accumulate insoluble polyQ inclusions as those observed in the brain of patients.

In parallel, using artificial intelligence (AI), we identified a compound which binds to the AXH domain of ATXN1, inhibits its dimerization and suppresses the aggregation of polyQ-expanded ATXN1 in cell-based assays. Most importantly, this compound also protects SCA1 neurons from aggregation-induced programmed cell death.

Discussion/Conclusions: We have successfully established human SCA1 neurons partially retaining the age of the donor. A novel chemical compound which suppresses ATXN1 aggregation also rescues iNSC-derived SCA1 neurons from cell death. By utilizing SCA1-iNSCs for disease modeling and compound testing, we provide a workflow for the development of a drug-based therapeutic intervention for SCA1.

Autologous haematopoietic stem cell gene therapy for people with Friedreich's Ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

Dr. Sian Baker [1], ***Ms. Nicole Jastrzebowska*** [1], ***Mr. Bruno Salomone Gonzalez de Castejon*** [1], ***Dr. Claire Rice*** [1], ***Prof. Neil Scolding*** [1], ***Dr. Oscar Cordero-Llana*** [1], ***Prof. James Uney*** [1], ***Dr. Kevin Kemp*** [1]

1. *University of Bristol*

Background

Pre-clinical studies have shown that allogeneic haematopoietic stem cell (HSC) transplantation may offer an effective treatment for Friedreich's ataxia (FA). However, when used clinically, allogeneic HSC transplantation carries the risk of significant morbidity and mortality. Additionally, using an allogeneic source of stem cells presents other notable limitations, such as the challenge of finding an appropriately HLA-matched donor. To address these limitations, we are investigating the therapeutic potential of lentiviral vector-mediated genetic modification of FA HSCs by inserting a functional frataxin gene (*FXN*) ex-vivo, followed by autologous HSC transplantation in FA mice.

Methods

Murine HSCs (lin^{neg}, Sca-1^{pos}, c-kit^{pos} cells) were isolated from FA transgenic mice (Fxnnull::YG8s(GAA)>800) and transduced ex-vivo with a self-inactivating lentiviral vector encoding *FXN* and an *EGFP* reporter. Following autologous transplantation of *FXN*-transduced HSCs into myeloablated FA mice, engraftment was quantified through EGFP expression in peripheral blood mononuclear cells. Motor behaviour was assessed at monthly intervals using the rotarod, beam walk, grip strength, and gait analysis. Six months post-transplant, mice were euthanized for gene, protein, and histological analysis of the heart, dorsal root ganglia, spinal cord, and cerebellum.

Results

Engraftment of *FXN*-modified FA HSCs averaged at 53% and remained stable over a 6-month period. Transplanted FA mice showed significant improvements in disease phenotype, including increased body mass, improved beam walk performance, and normalised gait. Widespread biodistribution of *FXN*-modified cells was observed, with EGFP-positive cells and transgene DNA detected throughout the heart and nervous system. Frataxin protein expression was significantly elevated in both heart and cerebellar tissues, and normalisation of FA-associated pathology was also evident.

Discussion and conclusion

Our results demonstrate that autologous ex-vivo HSC gene therapy, using self-inactivating lentiviral vectors, can achieve sufficient *FXN* gene delivery and biodistribution to rescue the FA phenotype. This approach therefore offers a highly translatable disease-modifying treatment for people with FA.

Sulforaphane targets multiple pathological processes in Friedreich ataxia patient induced pluripotent stem cell-derived sensory neurons

Wednesday, 13th November - 18:00: (Minories) - Poster

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Background and Objectives

Friedreich Ataxia (FRDA) is a neurodegenerative disease caused by a GAA expansion in the frataxin gene. Consequently, sensory neurons suffer from epigenetic dysregulation, frataxin deficiency, oxidative stress, and inflammation; thus, threatening cell survival. The only clinically-approved treatment, omaveloxolone, is an activator of the Nrf2 anti-oxidant pathway that was not designed to restore frataxin levels. Therefore, new therapies addressing frataxin deficiency and the above pathological processes are needed. Sulforaphane, a natural compound, was shown to target these processes in other disease settings. This study reveals its effects on the viability and molecular mechanisms-of-action in an FRDA cell model.

Methods

Sensory neurons derived from FRDA patient-induced pluripotent stem cells with low frataxin expression and 550/830 GAA repeats and its isogenic control, were treated with 100nM to 20μM sulforaphane for 24 hours. Dimethyl fumarate (DMF) at 30μM and 10nM to 100nM omaveloxolone were included as positive drug controls regulating inflammation and oxidative stress. Cell viability, gene expression and protein assays were conducted.

Results

None of the drugs reduced the viability of the isogenic control sensory neurons but sulforaphane increased the proportion of viable patient sensory neurons by up to 61% compared to the untreated control. Increased frataxin levels, reversal of aberrant gene expression levels of epigenetic enzymes *HDAC1/3/6* and *DNMT1*, diminished oxidative stress through upregulation of *NRF2*, *NQO1*, *HO-1*, *GCLM* and GSH/GSSG ratio, and reduced expression of inflammatory cytokines *TNF-α* and MCP-1 accompanied this protective effect of sulforaphane. However, DMF and omaveloxolone only targeted some of these biomarkers.

Discussion and Conclusion

Sulforaphane offers a multi-pronged approach to alleviating the different cellular events underlying the symptoms in FRDA.

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Omaveloxolone was donated by Reata Pharmaceuticals (acquired by Biogen in 2023)

Therapeutic activity of a haematopoietic stem cell delivered tissue penetrating peptide in a Friedreich's ataxia mouse model.

Wednesday, 13th November - 18:00: (Minories) - Poster

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INTRODUCTION

The autosomal recessive neurodegenerative disease Friedreich's ataxia (FRDA) is caused by intron 1 GAA-repeat expansion of the *frataxin* (*FXN*) gene and results in severe reductions in the production of the mitochondrial protein FXN. Diminished cellular FXN results in sensory neuron and cardiomyocyte death leading to motor deficits and cardiomyopathy respectively. Restoration of endogenous FXN levels within patient cells is a rational therapeutic approach for FRDA.

METHODS

Using lentiviral vectors, we generated genetically modified haematopoietic stem cells (HSCs) that secreted a cell-penetrating FXN fusion protein (FXN-APP). *In vitro* assessment to determine whether FXN-APP was mitochondrial localising, via immunofluorescence microscopy, as well as biologically active, via aconitase enzyme activity (aconitase assay) and cell viability post oxidative stress (MTS assay) analysis. *In vivo* therapeutic effects were subsequently studied by transplanting these HSCs into a FRDA transgenic mouse model (YG8XLR) and measuring blood FXN levels by ELISA, analysing cerebellar neuron size and their FXN contents by immunohistochemistry, assessing brain and heart mitochondrial function through aconitase activity assay and performing motor function tests (rotarod, beam walk and activity-breaker beam trial).

RESULTS

We show that the syngeneic transplantation of HSCs into the FRDA mice, resulted in increases in the blood FXN levels of recipient mice and impacted disease progression by preventing the manifestation of motor-coordination/sensory symptoms, maintaining brain and heart mitochondrial activity and impeding brain pathology.

CONCLUSION

Our study indicates a capacity for transplanted, FXN fusion protein secreting, HSCs to restore FXN in FRDA cells and promote phenotypic rescue in diseased mice, suggesting the potential of this therapeutic strategy.

Viral gene therapy for Christianson syndrome

Wednesday, 13th November - 18:00: (Minories) - Poster

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Objectives: Christianson syndrome is a rare x-linked neurodevelopmental and neurodegenerative disorder characterized by a number of severe symptoms, including intellectual disability, cerebellar degeneration, progressive ataxia, seizures, and autistic behavior. Christianson syndrome is caused by loss-of-function mutations in the *SLC9A6* gene, which encodes NHE6, a sodium/hydrogen exchanger that is highly expressed in early and recycling endosomes. In this work, we sought to preclinically evaluate a viral gene therapeutic strategy aimed at replacing missing *SLC9A6* in a rodent model of Christianson syndrome.

Methods: We developed a translatable construct, AAV9-CAG-SLC9A6, making use of the AAV9 serotype, a ubiquitous promoter, and the human *SLC9A6* gene. Taking advantage of high homology between rat *Slc9a6* and human *SLC9A6* in the Wistar Furth *shaker* rat, a spontaneous model of *Slc9a6* loss of function and Christianson syndrome. We administered doses as low as 8E9 viral genome copies via intracerebroventricular injection and quantified its effects on molecular, cellular, and motor dysfunction.

Results: When administered prior to the onset of Purkinje cell loss, AAV9-CAG-SLC9A6 yielded substantial improvements in cerebellar health markers and motor function at doses as low as 8E9 viral genome copies. Notably, we identified strong, significant relationships between NHE6 expression and motor function, NHE6 expression and CALB1 expression, and, in particular, CALB1 expression and motor function. Further, and quite interestingly, we found that AAV gene replacement had differential effects on tremor and ataxia.

Discussion: NHE6 loss of function leads to an overly acidified endosome, which may assist with viral transduction of target neurons, yielding strong expression and uptake at low doses. Underlying cerebellar health markers are highly predictive of motor coordination. Further, tremor and ataxia may arise from dissociable mechanisms in Christianson syndrome.
Conclusion: Gene therapy is a promising, potential treatment strategy for Christianson syndrome. Additional work is required in terms of non-motor symptoms and safety / toxicology.

Systemic AAV Gene Therapy with Next Generation Engineered Capsids for Treatment of CNS and Cardiac Symptoms in Friedreich's Ataxia

Wednesday, 13th November - 18:00: (Minories) - Poster

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1. *Capsida Biotherapeutics, Inc*

Friedreich's Ataxia (FA) is a rare, hereditary, form of ataxia with no approved disease modifying treatment. FA is caused by an intronic triplicate repeat expansion that diminishes the expression of the frataxin (FXN) protein causing progressive deficits in cellular respiration and cell death, with pronounced impact to functions controlled by non-regenerative cell types (neurons, cardiomyocytes). Leveraging our high throughput directed evolution engineering platform, Capsida has identified a systemically administered (IV) capsid that achieves NHP brain-wide biodistribution transducing large percentages of neurons in key FA-related brain areas in addition to delivering therapeutically significant levels of cardiac transduction. In an NHP study dosing a small pool of capsids simultaneously (N=3 NHP), the engineered capsid drives RNA expression levels ~100x higher than AAV9 in CNS, while maintaining similar RNA expression in cardiac tissue, and ~10x de-targeting in the liver. When administered IV as a single variant at a low to moderate dose (N=3 NHP), the engineered capsid delivering hFXN transduced more than 80% of cerebellar Purkinje cells, dentate nucleus neurons, motor neurons in the cortex and spinal cord, and nearly 30% of cardiac left ventricle tissue area, on average. Bulk protein levels in treated NHPs were 1.7x higher than endogenous levels in the left ventricle, and 8.2x higher than endogenous levels in the motor cortex by ELISA. Moreover, significant RNA expression levels were detected in the retina (~1E6 copies/ug RNA) by qPCR suggesting a potential benefit for sensory vision loss experienced by FA patients. Significant de-targeting of the liver and other non-target tissues contributed to the favorable safety profile characterized by no adverse immunogenicity, clinical pathology, and histopathology findings. Together, these data demonstrate that a drug product driven by Capsida's engineered capsid produces therapeutically meaningful FXN expression in CNS, cardiac, and sensory regions impacted by disease and has the potential to become a best-in-class targeted therapy for the treatment of FA.

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